A toxicity/safety assessment of dietary palmitoleic acid (POA).

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Table of Contents

1.0 Summary……3
2.0 Introduction……3
3.0 Palmitoleic acid……...3
4.0 Metabolism of palmitoleic acid……...4
4.1 Diet derived POA……4
4.2 Endogenously synthesized POA……4
5.0 Dietary consumption of palmitoleic acid……4
5.1 Fish oils……..……4
5.2 Plant oils……5
5.3 Other food sources……..5
5.4 Consumption in Unique Populations……..5
5.4.1 Greenland Inuit……..5
5.4.2 Infants……6
5.4.3 Those with food allergies……..7
6.0 Scientific Studies……7
6.1 Toxicology studies……7
6.2 Preclinical intervention studies……7
6.2.1 In Vitro Studies……..……8
6.2.2 In Vivo Studies……9
6.3 Human Epidemiological studies……9
6.3.1 Blood fatty acid composition studies linking POA acid to health risk factors or disease……..9
6.3.2 Impact of carbohydrate intake on blood POA concentration……....11
6.3.3 Impact of other contributing factors concurrent with POA on poor health outcomes or disease……11
6.3.4 Impact of higher POA intake on blood POA concentration and health risk factors/disease……12
6.3.4.1 Dyerberg and Bang Study……12
6.3.4.1 More recent studies……...12
6.4 Human intervention trials……13
6.4.1 Fish Oils……13
6.4.2 MLCT……13
6.4.3 Macadamia nut oil……..14
6.4.4 Sea buckthorn oil……..……14
6.4.5 Purified POA ……...…14
7.0 Current regulatory status of POA……14
7.1 Relevant GRAS Notifications ……14
7.2 Independent GRAS determinations……14
8.0 Conclusion……...……15
9.0 References……15

Figures and Tables

Figure 1. Chemical structure of the free fatty acid, *cis*-palmitoleic acid…….3

Table 1. Main fatty acids in various fish oils……..…25

Table 2. Predominant fatty acids of commonly consumed edible oils……..….26

Table 3. Fatty acid composition of whale and seal oil expressed as a percentage of total fatty acids……...…...27

Table 4. Calculated daily production of POA in breast milk and POA intake in infants from breast milk……..…..28

Table 5. Summary of preclinical studies investigating the effects of POA in various models…….…..29

Table 6. Blood POA concentration (% of total fatty acids) in various patient populations and association with health outcomes……..……..37

Table 7. Summary of POA human intervention trials reporting anti-inflammatory effects…….…….40

Table 8. Summary of POA human intervention trials reporting lipid lowering effects.……...42

Table 9. Relevant GRAS Notifications……..…44

1.0 Summary

Palmitoleic acid (POA) has potential as an oral therapeutic agent, but its safety has been questioned due to correlation studies showing that higher blood POA concentrations are linked to some poor health outcomes and disease. No formal toxicology studies have been reported for highly purified POA. However, studies on oils containing POA found no significant toxicity and an algal oil containing 20-25% POA had an LD50 of >2000 mg/kg bw. About 2 g/day(d) are typically consumed as part of a normal modern day diet and up to 6.54 g/d of POA had no negative effect on cardiovascular health or common cancer risk in Greenland Inuit. Preclinical studies do not expose any significant adverse effects associated with POA treatment within a variety of cell culture and animal models. Correlation studies in humans showing that elevated blood POA concentration is associated with poor health outcomes or disease, are a poor indicator of safety concerns attributed to dietary POA. Within human intervention trials up to 15.3 g of POA/d have been consumed for up to four weeks, without any reported serious adverse events. Purified POA as Provinal® has a positive effect on blood lipids and inflammation at a therapeutic dose of 220.5 mg/d for 30 days without any significant side effects. For the average user, up to 5 g POA/d (as 10 g of either Provinal® EE or TG), and for a 90th percentile user up to 10 g POA/d (as 20 g of either Provinal® EE or TG) is Generally Recognized as Safe (GRAS) in food. In addition, various GRAS affirmations provide substantial evidence of the safety of POA intake in humans. Based on the totality of evidence, dietary POA has no significant detrimental effects in humans under the conditions and at the intake levels/dosages described.

2.0 Introduction

Preclinical and some human epidemiological study outcomes suggest that the naturally occurring omega-7 fatty acid commonly referred to as POA, has potential as a therapeutic agent against metabolic syndrome including cardiovascular disease and insulin resistance associated with diabetes and obesity [1]. However, a large number of human correlation studies report that higher concentrations of blood POA acid are associated with a variety of illnesses including Type 2 diabetes, cardiovascular disease, and cancer [2]. As a consequence, concerns have been expressed regarding the safety of dietary POA.

POA is present in modest amounts in some plant and marine based foods, but it is particularly concentrated in macadamia nut (*Macadamia integrifolia)* and sea buckthorn (*Hippophae rhamnoides*) oils, where it accounts for roughly 17% and up to 29% respectively of fatty acids [3]. Although our dietary intake of POA accounts for <4% of total energy, it is the second most plentiful monounsaturated fatty acid in most blood lipid pools and is concentrated in adipose tissue. This discrepant abundance, compartmentalization and tissue-specific formation and or storage indicates its content in lipid pools is influenced mostly by endogenous synthesis rather than dietary intake [3]. As a result, it is inappropriate to assume that results of studies correlating higher proportions of POA within blood lipids with metabolic syndrome [4, 5, 6], type 2 diabetes [7, 8, 9], cardiovascular disease [10, 11, 12] and cancer [13] are a definitive measure of *cause* and *effect* where higher dietary intake of POA would contribute to either disease commencement or progression. This review presents evidence pertaining to the toxicity/safety of dietary POA.

3.0 Palmitoleic acid

POA [(9Z)-hexadec-9-enoic acid or C16:1Δ7 or 16:1 n-7] was first isolated in 1906 from cod liver and herring oils [14], where in the latter case, it accounts for up to 12% of the total fatty acids (Table 1) [15]. It is a sixteen carbon chain fatty acid with one double bond located 7 carbons from the methyl terminus of the molecule. Both *cis* (*cis*-C16:1n-7) and *trans* (*trans*-C16:1n-7) isomers occur naturally, but only the cis form (Figure 1) is typical supplied in dietary supplements/natural health products.

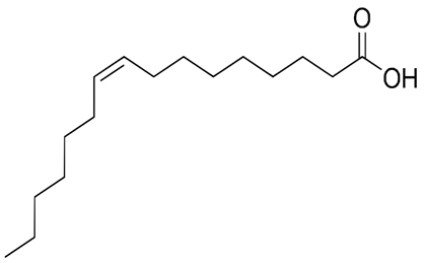

Figure 1. Chemical structure of the free fatty acid, *cis*-palmitoleic acid

4.0 Metabolism of palmitoleic acid

POA is found in blood and other body tissues in most, if not all, mammals [7, 16, 17, 18] in a variety of lipid farctions including phospholipids (PL), triglycerides (TG) , wax esters, cholesteryl esters (CE) and free fatty acids (FFA). It is also present in human breastmilk where it makes up about 2% of the total fatty acid composition [19]. POA within our bodies originates from two sources; either diet derived or endogenously produced. We typically eat only about 2 g of POA daily, therefore most of the POA within our bodies is endogenously produced [3].

4.1 Diet derived POA

Dietary POA is readily absorbed, and consuming foods rich in POA causes a significant increase in plasma POA. One study reported a 4% increase in dietary POA (as calories) resulted in a 60% increase in plasma POA [20]. Diet derived POA is found primarily in TG, composed of one glycerol molecule esterified to three fatty acids [21]. Once ingested, the fatty acids are hydrolyzed from the glycerol backbone by pancreatic lipase (which has a high specificity for the *sn*-1 and *sn*-3 positions of TG) in the small intestine, thereby forming a monoglyceride (2-monoacylglycerol) and two FFA [21]. These are absorbed into enterocytes and subsequently re-esterified to form TG, incorporated into chylomicrons and ultimately serve as a source of fatty acids for a variety of biochemical pathways, including beta-oxidation in the mitochondria, conversion into other fatty acids, and incorporation into cell membranes [22].

4.2 Endogenously synthesized POA

POA is primarily synthesized in the liver where it is used in the formation of TG, packaged in very low-density lipoprotein (VLDL) and secreted into the blood [23]. Adipose and liver tissues exhibit the highest concentration of POA found in the human body, and it appears to be a highly regulated fatty acid in adipose tissue [24].

POA can be synthesized from dietary palmitic acid via steroyl-coenzyme A desaturases (SCDs), including SCD1 primarily found in adipose tissue and liver and SCD5 observed in brain and pancreas [23]. However, a radiolabeled tracer study reported that <2% of a 28-32 mg dose of palmitic acid was rapidly converted to POA [25]. Instead, endogenously derived POA primarily originates via de novo synthesis from surplus dietary carbohydrate [3] which is also dependent on SCD1 activity to convert palmitic acid generated from carbohydrate through lipogenesis to POA. POA can be further metabolized to several other members of the omega-7 family, including palmitolinoleic acid (16:2n7), rumenic acid (18:2n7) and cis-vaccenic acid [18:1 cis-11, (Z)-11-octadecenoic acid] [26].

5.0 Dietary consumption of palmitoleic acid

POA is the second most abundant monounsaturated fatty acid within the standard American diet (oleic acid being the first) [21, 23, 27], although it is still considered to be a minor dietary constituent since only about 2 g/d are typically consumed [1]. It is found in a wide variety of modern human food products derived from marine life, nuts and seeds [28, 29] and oils derived from them (see Tables 1 and 2) [30, 31, 32, 33]. It is particularly rich in fish (including anchovies and menhaden), macadamia nuts, and their respective oils, which are widely available in commerce and commonly consumed. An Internet search for these food items, or their oils, reveals thousands of recipes from around the world, suggesting that dietary intake of these fatty acids in the normal diet is extensive. Variations in fatty acids levels within these products are not known to pose any health concerns.

5.1 Fish oils

Fish is one food that is uniquely rich in POA and that has an extensive history of human consumption dating back to as early as 4000 B.C., according to ancient tomb and cave drawings and engravings [29, 34]. Today, aquatic food products are still a major component of the diet for many people within the world. Moffat and McGill 1993 reported that “virtually all fish oils, can be described by reference to eight fatty acids, one of which is POA” [35]. Sardines, followed by menhaden, anchovies, pollock and herring, contain the highest amount of POA among commonly consumed fish, although oils from other marine life that are less commonly consumed, such as sheepshead (*Semicossyphus pulcher*), sperm whale (*Physeter macrocephalus*) and harbor seal (*Phoca vitulina concolor*), contain even higher levels [15]. No toxicity issues associated with any fish or fish oil product have been ascribed to its POA content.

5.2 Plant oils

In 2010, cooking oil consumption was reported within the USDA Oil Crop Yearbook to reach 52.5 pounds per capita [36]. No toxicity issues associated with any cooking oil product have been ascribed to its POA content. Rather, monounsaturated fatty acid (MUFA) rich cooking oils that can contain POA (see Table 2), offer significant health benefits. Although the intake recommendations from health organizations vary, the majority suggest between 10–20% of our total energy intake should be derived from MUFAs [27, 37-43**].**

Macadamia nut oil contains by far the most POA of all typically consumed food oils, where one tablespoon (14 g) of macadamia nut oil contains approximately 2.38 g of POA, and a serving of macadamia nuts (10–12 nuts; 1 oz.; 28.35 g) contains approximately 3.7 g of POA [30, 44]. The United States is the world's largest consumer of macadamia nuts. In 2001–2002 domestic consumption was expected to reach 44,069 tons and according to the U.S. Department of Agriculture, macadamia nut consumption per capita in the United States rose from 0.07 lbs in 1980–1981 to 0.13 lbs (58.97 g) in 2006–2007 [45, 46]. Therefore, the average US person consumed roughly 7.7 g of POA in 2006-2007 from macadamia nuts alone. Despite this widespread use, there is no history of adverse events associated with macadamia nut or macadamia nut oil consumption other than a rare allergy to the nut protein.

5.3 Other food sources

Although most of the POA consumed by humans comes from the fish and plant oils mentioned in Tables 1 and 2, it is found in other foods and medicinal products. The total fatty acid content of plants within the *Brassica* family (cabbage, turnip, mustard) contain 0.06% to 0.31% POA [26]. High concentrations of POA are also found in some yeasts, algae and cyanobacteria that are consumed by humans, and represent up to 56.9% of their total fatty acid content [47, 48]. As well, sea buckthorn oil (*Hippophae rhamnoides*), a common Chinese herbal medicine, whose medicinal use dates back to 618 AD, can contain up to 39% POA in its pulp oil [49, 50, 51].

Numerous dietary supplements/natural health products provide POA. One such product, Provinal® is a purified blend of edible fatty acids from anchovy and menhaden oil containing not less than 50% POA and not more than 1% palmitic acid. It is available in either TG or ethyl ester (EE) form. It reportedly has a therapeutic effect on blood lipids and inflammation at a dose of 220.5 mg/d for 30 days without any significant side effects [52]. To the author's knowledge, there are no other commercially available comparable POA products. To date, at least 720 Kgs of Provinal® TG representing 1,714,285 doses, and 16,000 Kgs of Provinal® EE equating to over 38 million doses, at a suggested daily intake of 420 mg/d (210 mg POA/d), have been consumed with no reported adverse events [53].

5.4 Consumption in Unique Populations

When evaluating the safety of POA consumption, it is important to consider unique populations that routinely consume large quantities of POA compared to other populations (historical Greenland Inuit), as well as vulnerable populations (infants and those with allergies).

5.4.1 Greenland Inuit

Greenland Inuit eating a traditional diet are a unique population within the world due to their high dietary intake of marine based food. In the mid-1970s, two researchers in Denmark, Jorn Dyerberg and Hans Olaf Bang embarked upon a mission to discover why Greenland Eskimos (Inuit) living on a traditional high fat diet had a lower than expected prevalence of cardiovascular disease [54]. They reported that coronary atherosclerosis was almost non-existent [54] and that only 3.5 % of all deaths was due to ischemic heart disease in that population despite a life expectancy of greater than 60 years [55]. Since it was already known that the incidence of coronary atherosclerosis was lower in people with relatively lower plasma lipids, they first investigated and reported significantly lower blood cholesterol, TG, β-lipoproteins and pre-β-lipoprotein concentrations in Eskimos compared to the Danish population that also had a comparable high fat diet (rough 40% of calories) [54]. They subsequently proposed that the fatty acid composition of the Eskimo diet, rich in marine derived protein and relatively low in carbohydrate, may produce altered lipid profiles that could confer cardio-protective effects [54].

The traditional Eskimos diet, as well as including fish, contained large amounts of seal and whale meat and blubber [56], with the average intake being about 400 g/person/d [55]. Whale and seal oil contains up to three times more POA than fish oil (Table 3) [30, 57] and based on the dietary analysis conducted by Dyerberg and

Bang, the quantity of POA in the Eskimo diet was more than 2.5 times that measured in the Danish diet (i.e. the average content of POA as a percentage of the total fatty acids in the Eskimo diet was 9.8% as compared to 3.8% in the Danish diet). The Eskimos consumed 39% of their average daily 1541 kcal (calories) as fat and their POA intake averaged 6.54 g/d. That is more than 3 times the quantity typically consumed from a Westernized diet today [1, 58], and yet there was no apparent negative impact on cardiovascular health. As well, the incidence of various cancers common in Western countries affecting the breast, skin, prostate and the hematologic system were found to be extremely low in the Inuit population in surveys conducted around the same time as the Dyerberg and Bang study (from 1969-1988 [59] and from 1953-1985 [60]). These observations suggest that consuming up to 6.54 g/d of POA within the typical Eskimo diet has no negative effect on cardiovascular health, nor does it increase the risk of breast, skin, prostate or hematologic cancers.

One could argue that other components within the Inuit diet (i.e. eicosapentaenoic acid (EPA) and other nutrients rich in marine based -foods) were providing protective effects against the believed toxic effects of dietary POA. However, it is unlikely given that within human intervention trials, up to 15.3 g of POA/d have been consumed for up to four weeks, without any reported serious adverse events [40]. This quantity was being consumed as 40-90 g/d of macadamia nuts (depending on the subject's energy intakes) within a standard Australian diet in the mid-2000s, by roughly 54 year old hypercholesterolemic men with an average body mass index of 26.54 9 (i.e. over weight). It is unlikely that any components derived from the marine foods within the Inuit diet were present at the same level in the Australian diet, given the low omega-3 content of the Australian diet around the time that the study was completed [61]. Therefore, it is unlikely that the participants were being protected from any believed toxic effects of dietary POA by other components within the Australian diet that would also be present in the Inuit diet. It is more likely that the high dietary intake of POA was not significantly harmful for the duration of the study. Although this is not definitive proof of the safety of high routine POA intake throughout life, it provides some assurance that high doses are tolerable without immediate ill effects.

5.4.2 Infants

There is a large body of published research showing that POA is a normal constituent of human breast milk regardless of what country of origin or patient population [62-64]. It is possible to calculate the average and maximum daily intake of POA within infants based on breast milk fatty acid composition (Table 4).

The fat content in breast milk typically ranges from 2-6 g/dL and the total quantity of milk produced per mother in 24 hours is 440-1220 g/d [65]. Using a maximum fat content of 6 g/dL and maximum milk production of 1220 g/d, the highest quantity of POA that could be expressed in a mother's milk was found to be 3.36 g/d based on the fatty acid composition of breast milk from mothers in the Philippines [62].

The average intake of breast milk within breastfed babies aged 1-6 months is 750 mL/d and the maximum intake is 900 mL [66]. Based on these milk intakes, the average POA intake in the countries and populations presented in Table 4 ranged from 168- 689 mg/d. The highest maximum intake of 2.479 g/d was found to be in the Philippines [6]. This intake is higher than the 2 g typically consumed by adults eating a Westernized diet [1]. Given the vulnerable nature of growing infants to any toxic influence, if POA were inherently toxic, one might expect to see some health issues within the Philippine infants that potentially could be consuming significant quantities of POA relative to their size. However, these infants were reported to be exclusively breastfeeding single-birth, full-term, healthy infants. In addition, if POA were inherently toxic, one might expect that the mothers of these infants, who could be producing and excreting up to 3.36 g of POA/d, to also be unhealthy. However, these mothers were also reported to be healthy [62]. Based on these results, it is unlikely that dietary POA up to about 2.5 g/d, poses any significant risk to growth in infants. Given the vulnerability of this population, it is likely that adults could tolerate considerably higher routine intakes without ill effects.

It is worth noting that the preterm infants from Serbia that were small for their gestational age, consumed the least amount of POA relative to infants from all countries and patient populations reported. In addition, the preterm infants from Serbia that were small for their gestational age, consumed less POA than preterm infants from that country that were of appropriate size for their gestational age [64]. If correlational studies were a definitive measure of cause and effect, one could argue that low POA exposure of infants in utero causes growth retardation, since fatty acid composition of breast milk reflects maternal fatty acid status and therefore fetal exposure [65]. However, it is unjustifiable to draw such a conclusion, just as it is unjustifiable to similarly conclude that high blood levels of POA cause metabolic syndrome, type 2 diabetes, cardiovascular disease and cancer.

5.4.3 Those with food allergies

No reports of allergies to POA or POA containing oils were found in the public domain. However, those with macadamia nut and fish allergies should exercise caution when consuming POA containing oils since they are derived from these sources. Although, these oils are unlikely to cause allergic response since allergic reactions are typically launched against *proteins* that are generally not present in concentrated *oil* preparations.

6.0 Scientific Studies

6.1 Toxicology studies

Formal toxicology studies on purified POA are not found in the public domain. However, a few studies have included edible oils which contain POA as follows:

An Ames mutagenicity test reported that unsaturated fatty acids in the range of C16–24 (including POA) showed inhibition of mutagenicity evoked by the food pyrolysate mutagen, Trp-P-1 (1.5 nmols per plate). POA required a concentration of 0.06 μmol/plate to achieve 50% inhibition, and 0.20 μmol/plate to achieve >95% inhibition [67].

A similar study assessing the impact of sea buckthorn oil on cyclophosphamide, farmorubicin and dioxadet mutagenicity, reported the oil significantly decreased the cytogenetic action of cyclophosphamide and farmorubicin, but not of dioxadet [68].

A novel cooking oil composed of medium and long-chain TG that included POA was the subject of a battery of studies to evaluate its safety [69]. The studies included a medium- and long-chain TG oil (MLCT) and a control of long-chain TG oil (LCT). The MLCT oil included rapeseed oil, that can have up to 3% POA as one of its components, and the LCT oil was a mixture of rapeseed and soybean oils containing approximately 0.2% POA.

- The MLCT oil was found to have no genotoxic potential in an Ames assay when tested at levels up to 5000 μg/plate.
- In a 14-day, repeated-dose oral exposure study, two groups of five-week-old Wistar rats (5/sex/group) were administered either 5000 mg/kg bw mixed rapeseed and soybean oils, or MLCT by gavage daily. No deaths, abnormal clinical signs or gross pathological abnormalities were noted and no significant differences were noted between the MLCT and control groups throughout the study. The LD_{50} value for MLCT was determined to be greater than 5000 mg/kg bw.
- A 6-week repeated-dose oral exposure study was also conducted on six-week-old male Wistar rats (20/group), although it did not appear to follow international guidelines for examinations for a repeated dose oral toxicity study. Rats were administered 7% of the diet (approximately 3500 mg/kg bw/d) of either LCT or MLCT for six weeks. The weights of the mesenteric adipose tissue, and the adipose tissue surrounding the epididymis and kidneys, as well as the mesenteric adipose tissue were measured. Liver weight, subcutaneous fat weight, and carcass protein were also determined for each rat. No adverse effects were noted and no overt signs of toxicity were noted. The MLCT fed rats exhibited significantly increased food and energy intake however total body fat and body fat ratios were significantly decreased compared to controls. The MLCT group also exhibited significant increases in total carcass protein levels and significantly lower serum cholesterol levels. No other significant differences between groups were noted. The NOAEL was concluded to be 3500 mg/kg bw/d, the highest dose tested.

A toxicological evaluation performed on a *Nannochloropsis* algae oil (Algal-EE), a proprietary ingredient derived from algal oil and standardized to contain 20–25% POA as an EE found no evidence of genotoxicity or mutagenicity as determined by Ames bacterial reverse mutation, in vitro mammalian chromosomal aberration and in vivo mouse micronucleus studies. A 14-day single-dose oral toxicity study determined an LD50 of >2000 mg/kg bw [70].

6.2 Preclinical intervention studies

Highly purified POA (up to >98.5%) has been tested in a number of in vitro and in vivo models to decipher mechanism of action. The highly purified POA studies can also uncover evidence of toxic effects if present and so are summarized in Table 5. Given the significant body of research on highly purified POA, it was deemed

unnecessary to include literature on preclinical studies accessing the effects of other POA test products including macadamia nut and sea buckthorn oils.

6.2.1 In Vitro Studies

Results of in vitro studies indicated that POA:

- had no negative effects on β-cell function and instead protected them from the toxic effects of palmitic acid [71, 73]
- enhanced glucose uptake by adipocytes more effectively than oleic acid [74] and counteracted palmitic acid-mediated insulin resistance [75]
- enhanced insulin sensitivity [75 , 76] and worked half as well as the anti-diabetic drug rosiglitazone [76]
- reduced desaturation and lipogenesis that contributes to fat synthesis and storage [77]
- altered gene expression thereby decreasing lipogenesis and increasing β-oxidation [78]
- reduced inflammatory gene expression while PA increased it [79, 80]
- increased adipocyte lipolysis and lipase content [81]
- reduced the proinflammatory effect of palmitic acid by suppressing mitochondrial dysfunction [82]
- had no negative impact [83] and prevented palmitic acid induced endoplasmic reticulum stress and apoptosis [84, 85]

These results, rather than exposing toxic effects, instead highlight the potential health benefits that could potentially be derived from POA supplementation including:

- enhanced glucose metabolism [71-76] that positively impacts diabetes
- enhanced fat metabolism [77-79, 81] that could help to prevent obesity
- reduced inflammation [79, 80] that could improve a multitude of health issues including cardiovascular disease, obesity, arthritis and dry eye syndrome
- enhanced cellular function [82, 84, 85] that could help prevent cell death which may have positive implications in cancer growth

As well, the fact that POA attenuates saturated FFA-induced cell death in hepatocytes implies that POA and/or its analogues could prove beneficial in human non-alcohol fatty liver disease [85].

Seemingly opposite to these benefits of cell exposure to POA, POA and its ethyl ester, POAEE are well known to cause pancreatic cell death that is believed to lead to pancreatitis [86-91]. However, it is important to recognize that this POA and POAEE is not *diet derived POA*, but is instead *endogenously synthesized* through non-oxidative metabolism of excess ethanol (alcohol) consumption and the resulting pancreatitis is described as *alcohol-induced pancreatitis*. The cellular damage produced by this process is specific to the pancreas and does not occur in liver, lung, myocardium, skeletal muscle or subcutaneous fat [91]. In addition, in vitro studies have shown that the ethanol by-products that cause the damage are not specifically POA and POAEE. All other fatty acid and EE non-oxidative metabolites derived from ethanol that have also been evaluated, including palmitic acid, arachidonic acid-EE and arachidic acid-EE, also cause pancreatic cellular necrosis [87]. All these by-products cause calcium-ATP pump failure that leads to cell death [86, 87]. Results of these studies, rather than being exploited to substantiate the toxicity of POA, should be used to highlight the detrimental effects of excess alcohol consumption with the understanding that they are not necessarily indicative of *dietary* POA effects. In addition, awareness of a study in pancreatic acinar cells where treatment with insulin effectively abolished the POA-induced calcium-ATP pump failure and necrosis caused by excess alcohol exposure, is relevant [92].

Similar awareness should be applied to POA in relation to cardiac cell death. Metabolic syndrome and insulin resistance, that are risk factors for cardiovascular disease, are frequently accompanied by high serum FFA levels [93]. In addition, sudden cardiac death is associated with excess FFA, even though fatty acids are the major exogenous energy substrate in the healthy heart. As well, arrhythmias, cardiac apoptosis and mitochondrial damage within the heart have all been associated with excess FFA [93]. An overload of FFA, specifically long chain saturates such as palmitic acid, affect mitochondrial function and induce cardiomyocytes apoptosis [92]. Palmitic acid can be endogenously converted to POA and both can be further metabolized to their coenzyme A (CoA) ester derivatives, palmitoyl-CoA and palmitoleoyl-CoA, respectively. These CoA derivatives are normally taken up and processed by mitochondria. However accumulation of excess palmitoyl-CoA and palmitoleoyl-CoA may have a

negative impact on mitochondrial function under physiological conditions that could contribute to development of cardiac disease [93, 94].

Contrary to the negative effects of endogenously produced POA on pancreas and heart cells, *supplemental (i.e. dietary) POA* can restore some cells' function based on results of an in vitro study, where POA induced cell proliferation and restored the proliferation rate of cells whose POA biosynthesis was blocked by inhibition of SCD-1. In addition POA more effectively restored cell proliferation than palmitic acid [95]. Therefore, it is important to make the distinction between *endogenous POA* and *dietary POA* when discussing the impact of POA on cell status.

6.2.2 In Vivo Studies

Results of in vivo studies indicated that POA:

- reduced insulin resistance, hyperglycemia [96] and fasting glucose and insulin levels [97] while stimulating insulin secretion [98]
- improved the insulin-signaling pathway, glucose transport into skeletal muscle, glycemic control and insulin resistance while palmitic acid did not [96]
- reduced lipogenic gene expression, food intake [96], fat metabolism [99], and appetite [100] and body weight gain [96, 99]
- reduced blood TG [43, 96] and non-HDL cholesterol while raising HDL cholesterol levels [43]

These results, rather than exposing toxic effects, instead highlight the potential health benefits that could be derived from POA supplementation including:

- enhanced diabetic control [96-98]
- improved control of body weight [96, 99, 100]
- enhanced blood lipid profiles [43, 96]

Only two studies reported results that could be adversely construed as follows:

- Hamsters fed macadamia nut oil, had higher than baseline liver free cholesterol levels, but it was equivalent to levels observed in canola oil treated animals and deemed to be of no health consequence [43].
- Obese lambs given one jugular infusion of POA had increased blood glucose concentration [98], that repeatedly and over a prolonged period of time could lead to insulin resistance. However, repeat dosing over a 28 day period in obese sheep produced no change in blood glucose concentration and reduced blood insulin concentration, improved insulin resistance and positively altered gene expression for those regulating glucose uptake [99], indicating no adverse effects of repeat POA treatment on blood glucose concentration. These seemingly opposite results were explained by measuring insulin production where it took repeat glucose dosing over time to upregulate insulin producing genes to achieve sufficient blood insulin to maintain moderate and stable glucose concentration.

Overall, preclinical studies do not expose any significant adverse effects associated with POA treatment of cell cultures or when administered orally or intravenously to animals.

6.3 Human Epidemiological studies

6.3.1 Blood fatty acid composition studies linking POA acid to health risk factors or disease

There are a large number of human correlation studies reporting that higher concentrations of blood POA acid are associated with metabolic syndrome, type 2 diabetes, cardiovascular disease, obesity, hypertriglyceridemia, hyperglycemia, inflammation, and incidence and aggressiveness of prostate cancer [2], although the significance of its presence in specific lipid pools (i.e. PL, TG, CE, FFA, etc.) at present is not fully understood [58]. As well, scrutinizing of particular lipid fractions may be more relevant to specific conditions, for example Patel et al. 2010 found that fatty acids measure in the plasma PL fraction are more clearly associated with diabetes incidence than

RBC PL [101]. Therefore, interpretation of the existing data is difficult due to poor understanding of the mechanisms whereby POA may be present within a given fraction.

None of the population groups identified in the correlation studies reported since 1982 (Table 6) have blood POA concentrations that are as high as those reported for the Greenland Inuit living in Greenland in the mid-1970s [54]. The highest reported levels were in a group of men who did not experience myocardial infarctions during the course of the study [102]. The POA concentration in various lipid fraction (as a % of total fatty acids) was 1.3 in PL, 4.85 in TG and 5.06 in CE while those reported for the Greenland Inuit living in Greenland were 2.7 in PL, 9.5 in TG and 9.2 in CE [54].

In general, conclusions drawn from these correlation studies so far have been mixed. Some have reported that higher plasma POA is associated with poorer health markers or outcomes including:

- Higher liver fat content [103]
- Increased risk of cardiovascular disease [11, 12, 104] and stroke [10]
- Higher blood TG levels [4, 7, 23, 104]
- Higher de novo lipogenesis [103]
- Insulin resistance [7, 9, 103]
- Higher SCD1 index measured as the ratio of C16:1 to 16:0 [103]
- Fatty liver disease [103]
- Metabolic syndrome [4, 6, 105]
- Lower HDL cholesterol [4]
- Elevated blood pressure [4]
- Various markers of inflammation [106]
- Worsening of hyperglycemia [8]
- Higher incidence of Type 2 diabetes [8, 107-110]
- Higher cancer death in men (the author indicated that this association was with endogenously synthesized POA [13]
- Less successful weight maintenance after weight loss [111]
- Higher incidence of prostate cancer and high-grade tumors [112]

Others have reported that higher plasma POA is associated with indicators of better health or outcomes including:

- Lower LDL cholesterol, higher HDL cholesterol and lower total:HDL-cholesterol [7]
- Higher insulin sensitivity in non-diabetics (but not correlated in Type 1 diabetics) [113]
- Higher insulin sensitivity in subjects at risk of Type 2 diabetes [114]
- Lower breast cancer risk [115]
- Lower fibrinogen [7]

In addition, some studies have reported no association between blood POA concentration and health indicators or outcomes including:

- Risk of Type 2 diabetes. However, there was an inverse association for the POA metabolite vaccenic acid. In the same study, palmitic acid was positively associated with adiposity, TGs, inflammatory biomarkers and insulin resistance [116].
- Heart failure [117]
- Cardiac infarct [102]
- Type 2 diabetes [101]
- Coronary heart disease [118, 119]

Within these correlational studies, it is impossible to distinguish between *diet derived* and *endogenously produced* POA within blood. Therefore, based solely on the results of these studies, it is unjustifiable to attribute any positive or negative health outcomes to *dietary POA*.

A number of these studies reported poorer health markers or outcomes with higher lipogenic enzyme activity including SCD1 [8, 11, 109, 107, 105], delta-6-desaturase (D6D) [6, 8, 9, 13, 107, 105], D9D [9] and elongase [109]. All of these studies also reported higher blood POA levels associated with poorer health markers or outcomes. As well, some of these studies reported elevated levels of elongation products in addition to POA including dihommo-gamma-linolenic acid [6, 8, 9, 101, 106, 108, 110] while one reported no association with POA, but significant elevation of dihommo-gamma-linolenic acid [119]. Two studies reported altered D5D activity [105, 119]. One had elevated D5D activity associated with a higher incidence of coronary heart disease where there was no association between POA and risk of the condition [119]. The other had lower D5D activity that predicted the development of metabolic syndrome where high POA was also implicated [105].

All of these studies point to altered gene expression and/or the presence of genetic polymorphisms, and at least one study investigated the impact of the later on disease outcome [112]. Within that study, five tagging SNPs polymorphisms in the fatty acid synthase (FASN) gene (rs1127678, rs6502051, rs4246444, rs12949488, and rs8066956) were related to blood fatty acid levels. Two of these, rs6502051 and rs4246444, were associated with lower blood POA while elevated POA was directly associated with higher incidence of prostate cancer and high-grade tumors. Based on these findings, higher activity of enzymes involved in de novo lipogenesis, as reflected in higher blood levels of POA could be involved in the development of prostate cancer. Therefore a genetic predisposition within the individuals may be responsible for the development (potential cause) of the cancer, rather than exposure to POA. In addition, high POA is a consequence (effect) of the genetic predisposition and reflects *endogenous synthesis* rather than *dietary intake*. As well, based on these study results, it is not possible to reliably speculate on the impact that *dietary POA* may have on prostate cancer development or potentially any other poor health outcome or disease where genetic anomalies are present.

6.3.2 Impact of carbohydrate intake on blood POA concentration

It general, circulating fatty acids reflect dietary intake, but those associations are weak, in particular for saturated and monounsaturated fatty acids. Typically during low calorie diets, when dietary carbohydrate is reduced, blood levels of lipogenic fatty acids including POA, palmitic and total saturates, consistently decrease, despite higher saturated fat intake. POA appears to be most affected by carbohydrate intake and its levels rapidly drop when carbohydrate is limited to less than 50 g/d. Therefore, POA levels within blood may partly be reflective of carbohydrate intake and metabolism. Since there is little POA in common dietary fat, the presence of elevated blood POA, which indicates de novo fatty acid synthesis, may be indicative of carbohydrate metabolism through nonoxidative disposal pathways that lead to adverse clinical outcomes [2].

Results of correlation studies supporting this include:

- A low calorie diet followed by five different diet plans where higher adipose tissue triglyceride POA was associated with higher alcohol and carbohydrate intake. In addition, POA was significantly lower during the low calorie and maintenance phases of the trial than at baseline [111]
- A population where high adipose tissue POA was associated with greater obesity. However, the association was reduced by low carbohydrate intake indicating that elevated POA within adipose tissue was the results of excess carbohydrate intake [23]
- Six 3-week diets progressively increasing carbohydrate from 47 to 346 g/d where plasma TG and CE POA dropped as carbohydrate intake decreased and then increased with reintroduction of carbohydrate. Within this study, the highest POA achieved in plasma TG was about 7.2% in one patients when consuming the highest quantity of carbohydrate daily (i.e. 346 g) [2].

Essentially, increases in blood POA with increased carbohydrate intake may be signaling impaired carbohydrate metabolism, even during conditions of negative energy balance and significant weight loss [2]. Therefore, studies reporting high blood POA in association with poor health outcomes or disease may be partly reflective of excess carbohydrate/calorie intake where high POA is an *effect* of this rather than the *cause* of the outcomes.

6.3.3 Impact of other contributing factors concurrent with POA on poor health outcomes or disease

Similar to the observed association of excess carbohydrate intake with elevated POA and its contribution to poor health, many studies reporting high blood POA associated with poor health outcomes or disease, also report positive associations with other factors contributing to compromised health including:

- A case-control study in coronary heart disease patients where high POA was associated with coronary heart disease risk. But high POA was also associated with higher BMI, higher energy intake, prevalence of hypertension, atrial fibrillation, and hypercholesterolemia, lower concentration of RBC marine omega-3 fatty acids, and less exercise. Given that so many of the other factors that correlated with POA are known to contribute to poor cardiovascular health, it is unlikely that POA was solely responsible for the coronary heart disease. Interestingly, higher vaccenic acid (derived from POA) was inversely related to the condition, suggesting that further elongation of POA may provide protective effects [11].
- In subjects with risk of Type 2 diabetes that also had low insulin sensitivity at baseline, improved lifestyle habits including weight loss and increased exercise intensity had a positive stronger impact on insulin sensitivity than POA levels [114].
- A case control study, where higher incidence of diabetes was also associated with higher blood stearic acid and saturated fatty acid levels [109].
- Two cohort studies where high saturates [108] and palmitic acid [101, 108] were directly associated with incidence of diabetes
- A cohort study where elevated palmitic acid was associated with metabolic syndrome [6]

In these instances, it is not possible to ascribe poor health outcomes or disease solely to elevated blood POA.

As well, other studies reported no association between blood POA levels and poor health outcomes or disease, but instead positive associations with other factors contributing to compromised health including:

- A case control study where high blood palmitic acid was directly associated with coronary heart disease [118]
- A population based study where elevated saturates and lower polyunsaturates where associated with coronary heart disease [119]
- A population study where elevated saturates were associated with incident of heart failure [117]
- A cohort study where elevated palmitic acid and stearic acid predicted myocardial infarction [102]

In essence, in all these studies, a multitude of factors may be contributing to poor health outcomes or disease and it is not possible to say with certainty whether or not elevated blood POA is a significantly contributor. In addition, based on these studies, it is not possible to ascertain the impact that *dietary POA* may have.

6.3.4 Impact of higher POA intake on blood POA concentration and health risk factors/disease

6.3.4.1 Dyerberg and Bang Study

The Greenland Inuit study reported in the mid-1970s by Dyerberg and Bang found significantly higher dietary intake was concurrent with higher blood POA content in Greenland Inuit living in Greenland compared to Greenland Inuit living in Denmark and Caucasians living in Denmark [54, 55]. They also compared the fatty acid composition of various blood lipids fractions within Greenland Inuit living in Greenland to the other two groups mentioned previously as well as various other patient populations from six other studies including normal controls from westernized countries, diabetics, and those with atherosclerosis, hypertriglyceridemia and myocardial infraction [54]. The POA content of plasma CE (which mainly reflects liver enzymatic activity [120]) within the Greenland Inuit living in Greenland was 9.2% of the total fatty acids compared to 3.2-7.2% for all other groups reported. Similarly, POA was much higher in plasma TG from Greenland Inuit living in Greenland (9.5%) compared to other groups (3.1-7.7%). As well, POA was higher in plasma PL from Greenland Inuit living in Greenland (2.7%) compared to all other groups (0.7-2.0%), except in one study (3.6%).

If high dietary intake and resulting blood levels of POA cause poor health markers or outcomes, then one would expect that the Greenland Inuit living in Greenland to be significantly less healthy than the other population groups reported. However, as mentioned in Section 5.4.1, Greenland Inuit living in Greenland in the mid-1970s were relatively unaffected by diseases typical of Westernized society, including cardiovascular disease and cancer.

6.3.4.1 More recent studies

Studies associating POA intake to disease incidence are scarce. Only a few studies published during the last two decades have reported both POA intake and blood POA concentration [103, 107, 101, 109]. The highest recorded intake of POA in any of these studies was 2.2 (Range 2.0-2.3) g/d in a case-cohort of diabetic [107]. This quantity is

much lower than the 6.54 g/day reportedly consumed by Greenland Inuit living in Greenland [55] and roughly equivalent to typical intakes today [58].

Only one of these studies associated POA intake with disease where high dietary intake of POA as well as total fat, total monounsaturated fats, oleic acid, total polyunsaturated fats, n-6 fats, linoleic acid, arachidonic acid, n-3 fats, alpha-linolenic acid, and trans fats and a lower intake of pentadecanoic acid (15:0) at baseline was found in diabetics than in those who did not develop diabetes [109]. This study also reported that elevated blood POA as well as a number of other fatty acids were associated with higher incidence of diabetes. Clearly based on these results, it is not possible to ascribe any poor health outcomes solely to *dietary POA*.

One study including 60 patients classified as overweight, obese or morbidly obese found a decreasing intake of POA with increasing level of obesity (i.e. 2.5% of total dietary fat in overweight compared to 1.17% total dietary fat in morbidly obese patients) [121].

One study including patients with metabolic syndrome, rather than reporting POA, instead controlled carbohydrate intake and measured blood POA levels. The highest POA achieved in plasma TG was about 7.2% in one patient when consuming the highest quantity of carbohydrate daily (i.e. 346 g) [2]. That quantity was less than the 9.5% observed in plasma TGs from Greenland Inuit living in Greenland [54] that were not reported to be suffering from metabolic syndrome.

Based on these studies, it is not possible to ascribe any specific poor health outcomes or disease to elevated *dietary POA intake* or its associated rise in blood POA levels.

6.4 Human intervention trials

The main POA sources that have been tested in human intervention trials are fish oil, MLCT, macadamia nut oil, sea buckthorn oil and purified POA. Therefore, review of the safety details provided within each of these studies has been undertaken. Human interventions trials assessing the health implications of POA containing products including macadamia nut and sea buckthorn oils and the purified POA Provinal®, have been previously summarized [122]. These studies have been categorized as those investigating anti-inflammatory effects (Table 7) and lipid lowering effects (Table 8). None of these studies reported significant adverse effects and instead many described treatment responses that contribute to health including lipid lowering and anti-inflammatory effects. Any reported safety issues and adverse effects are discussed below.

6.4.1 Fish Oils

A wealth of long-term human clinical trials support the safety fish and fish oils consumption, with little to no adverse effects noted, suggesting that the fish oil fatty acids including POA are without toxicological concern, even in pregnant women and infants [123- 128]. There is little clinical evidence to support the concern that fish oil concentrates may increase the risk of bleeding, may reduce glycemic control in diabetics or increase the risk if cancer or serious infection associated with modified immune responses [129]. Intake of 2 g/d of combined EPA and docosahexaenoic acid (DHA), is similar to that seen in large sectors of the Japanese population and well below that of Greenland Inuit, both of whom suffer no ill effects from this routine consumption. Moderate increases in bleeding times, that are lower than those seen with acetylsalicylic acid (ASA) therapy, have been observed in individuals taking 3-4 g/d [130]. There are no reports of POA within fish oil as a contributing factor to any side effects of fish oil consumption described.

6.4.2 MLCT

Matulka et al. evaluated the safety MLCT and LCT in humans in a placebo-controlled, double-blind study in 20 subjects for 4 weeks [69]. Each group, including 10 healthy Japanese men and women (aged 21–39 years), consumed 42 g of either MLCT or LCT daily via a bread source. Anthropometric measurements, hematological analysis and urinalysis were conducted at study initialization and completion. A slight decrease in anthropometric measurements was noted in both groups with no significance reached between the groups. No significant changes were found in liver and renal function, urinalysis parameters, or hematology parameters and no adverse effects were reported. Several other studies have indicated that MLCT and LCT oils are well tolerated in humans [131]. There are no reports of POA within MLCT as a contributing factor to any side effects of MLCT described.

6.4.3 Macadamia nut oil

As a component of macadamia nuts or oil, humans have consumed up to 15.3 g of POA/d for up to four weeks, without any reported serious adverse events [40](see Tables 7 and 8).

6.4.4 Sea buckthorn oil

As a component of Sea buckthorn oil, up to 1.25 g of POA/d has been consumed by humans for 4 months without any reported signs of toxicity [51].

6.4.5 Purified POA

Only one randomized, double-blind, placebo controlled trial included purified POA as Provinal® at a dose of 220.5 mg POA/d for 30 days. Reported minor side-effects included mild gastrointestinal distress in 10% and headache in 3% of patients [52].

7.0 Current regulatory status of POA

A number of GRAS affirmations and food additive regulations confirm the safe use of POA as follows:

- Rapeseed oil (0.21% POA) is considered GRAS per *21 CFR 184.1555* [132]
- Menhaden oil (10.48% POA), prepared from fish of the genus Brevoortia, is GRAS per 21 CFR 184.1472. "This ingredient may be used in food only within the following specific limitations to ensure that total intake of EPA and DHA does not exceed 3.0 g/person/d". Furthermore, in the Federal Registrar 62 Issue 74, FDA states: "Specifications for the notified (GRAS) substance may be the same as, or similar to, specifications for an oil that is substantially similar to hydrogenated and partially hydrogenated menhaden oil, which FDA has affirmed as GRAS for use as an edible fat or oil, could be based on the specifications in 21 CFR 184.1472. [132]
- Anchovies (oil is 9.42% POA), are considered "food for human consumption" by FDA and defined in 21CFR 123.3—"fish means fresh or saltwater finfish, crustaceans, other forms of aquatic life (including but not limited to, alligator, frog, aquatic turtle, jellyfish, sea cucumber, and sea urchin and the roe of such animals) other than birds or mammals, and all mollusks, where such animal life is intended for human consumption

7.1 Relevant GRAS Notifications

The most significant GRAS notification relevant to the safety of POA, is GRAS notification (GRN 494) submitted to the FDA by Tersus Pharmaceuticals LLC for Provinal® EE, a blend of fatty acid EE from menhaden or anchovy oil, standardized to approximately 50% POA. It states that 'Taking into account organoleptic properties as well as the lipid nature of the notified substance, the notifier estimates that approximately 10% of fats and oils in the diet of a consumer at the 90th percentile level could be provided by FAEE, resulting in an intake of approximately 20 g FAEE /person/day, or 10 g POA acid/person/d'. This GRAS notification was filed by the FDA on December 24, 2013 and received a no objection letter on December 16, 2014 [133].

There are several other GRAS notifications in the FDA's GRAS Notice Inventory database that were filed by FDA without question and attest to the safety of ingredients containing POA (Table 9) [132].

7.2 Independent GRAS determinations

An independent GRAS determination was completed on behalf of Tersus Life Sciences LLC for Provinal® TG, a blend of purified edible fatty acids derived from anchovy or menhaden oil, in their natural TG form, with POA comprising approximately 50% of the fatty acids. This report includes a detailed description of Provinal® TG, including its manufacturing process, quality control, and history of exposure, as well as a discussion of toxicological studies performed on the components of Provinal® TG, establishing the safety of this ingredient. The report was prepared by a panel of experts ("Expert Panel") who are qualified by training and experience to evaluate the safety of food ingredients. The data and information used for this GRAS determination was widely published and available in the public domain. It included FDA regulatory information, as well as information from scientific peer-reviewed

articles and texts. The reference section of this notification cites all published studies. The Expert Panel determined through scientific procedures, and corroborated by a history of safe use (exposure), that Provinal® TG (containing fatty acids that are endogenously produced and ubiquitously found in the diet) is GRAS for its intended use and, therefore, exempt from pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act (FFD&C). They estimated that approximately 10% of fats and oils in a high consumer's diet might come from Provinal® TG daily over a lifetime. This is equivalent to approximately 10 g of Provinal® TG, or 5 g of POA/d for an average user, and 20 g of Provinal® TG, or 10 g of POA/d for a 90th percentile user [132].

Overall, these GRAS affirmations, notifications and determinations provide substantial evidence of the safety of POA use in humans.

8.0 Conclusion

There is no evidence to suggest that POA is toxic in humans based on the intake levels/dosages deemed acceptable through history of routine dietary consumption and the toxicology, preclinical and human intervention trials reviewed and presented. Current regulatory status of POA concurs with this conclusion.

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Table 1. Main fatty acids in various fish oils

	Fatty Acid Composition of Fish Oils (expressed as percentage of total fatty acids)*									
	Anchovy	**Sardine**	**Wild Salmon**	**Men-haden**	**Tuna**	**Krill**	**Squid**	**Pollock**	**Herring**	**Cod Liver**
C14:0	5.0–11.5	4.0–21.5	2.0–4.5	6.5–12.5	2.0–5.0	ND–9.5	1.0–6.0	4.0–5.5	3.0–10.0	2.0–6.0
C16:0	14.0–22.0	9.0–25.5	12.0–13.5	14.0–23.0	14.0–24.0	6.0–18.5	10.0–20.0	8.0–11.0	8.0–25.0	4.0–14.0
C16:1 (*n*-7)	5.0–12.0	5.5–17.5	4.5–5.0	7.5–15.5	1.0–12.5	ND–5.5	1.0–8.0	9.0–12.0	3.5–12.0	4.5–11.5
C18:1 (*n*-9)	5.0–17.0	4.0–17.5	16.0–17.5	3.5–16.0	10.0–25.0	2.5–11.0	6.0–25.0	7.0–13.5	4.0–22.0	12.0–21.0
C20:1 (*n*-11)	ND–4.0	3.0–4.0	4.5–6.0	0.5–2.0	ND–3.0	ND–3.5	ND–13.0	10.0–16.0	NA	1.0–5.5
C22:1 (*n*-11)	ND–5.0	ND–4.12	4.0–6.0	ND–0.5	ND–1.0	ND–2.0	2.0–10.0	11.5–15.5	11.0–27.0	1.0–5.5
C20:5 (*n*-3)	5.0–26.0	8.0–25.0	8.5–9.5	11.0–18.5	2.5–9.0	>9.0	7.0–15.0	9.5–11.0	4.0–15.0	7.0–16.0
C22:6 (*n*-3)	4.0–23.0	2.5–15.5	10.5–11.0	4.0–14.5	21.0–42.5	>4.0	12.5–34.5	4.5–5.5	2.0–12.0	2.5–11.0

* Reference 15 and 132

Table 2. Predominant fatty acids of commonly consumed edible oils

	Fatty Acid Composition of Commonly Consumed Edible Oils (expressed as percentage of total fatty acids)*								
	Palm stearin	**Rapeseed**	**Rice bran**	**Safflower**	**Sesame**	**Soy-bean**	**Sun-flower**	**Macadamia**	**Olive**
C14:0	1.0–2.0	ND–0.2	0.1–0.7	ND–0.2	ND–0.1	ND–0.2	ND–0.2	1.0	0
C16:0	48.0–74.0	1.5–6.0	14–23	5.3–8.0	7.9–12.0	8.0–13.5	5.0–7.6	7.9–8.3	11.3
C16:1 (*n*-7)	ND–0.2	ND–3.0	ND–0.5	ND–0.2	ND–0.2	ND–0.2	ND–0.3	17–18.1	1.3
C18:0	3.9–6.0	0.5–3.1	0.9–4.0	1.9–2.9	4.5–6.7	2.0–5.4	2.7–6.5	3.3–3.9	2.0
C18:1 (*n*-9)	15.5–36.0	8.0–60.0	38–48	8.4–21.3	34.4–45.5	17–30	14.0–39.4	57.7–60.9	71.3
C18:2	3.0–10.0	11.0–23.0	29–40	67.8–83.2	36.9–47.9	48.0–59.0	48.3–74.0	1.6–1.7	9.8
C18:3	ND–0.5	8.0–25.0	5.0–13.0	11.0–18.5	0.1–2.9	ND–0.1	0.2–1.0	0.1	0.8
C20:0	ND–1.0	ND–3.0	ND–0.9	0.2–0.4	0.3–0.7	0.1–0.6	0.1–0.5	<1–2.7	0.4
C20:1	ND–0.4	3.0–15.0	ND–0.8	0.1–0.3	ND–0.3	ND–0.5	ND–0.3	2.3	0.3
C22:0	ND–0.2	ND–2.0	ND–0.5	ND–0.1	ND–1.1	ND–0.7	0.3–1.5	0.7	0.1

* Reference 30, 32, 33, 132

Table 3. Fatty acid composition of whale and seal oil expressed as a percentage of total fatty acids

Fatty Acid	Blue* Whale	Typical Seal*	Bearded Seal**	Gray Seal**	Harbor Seal**	Harp Seal**	Hooded Seal**	Ringed Seal**
14:0	5	4	3.05	3.83	4.52	4.66	4.40	3.36
16:0	8	7	10.14	6.61	8.03	6.24	9.81	4.82
16:1ω7	9	16	17.77	12.77	19.26	14.93	10.09	23.12
18:0	2	1	2.15	0.94	0.85	0.95	1.83	0.42
18:1ω9	29	28	16.76	24.5	18.61	18.59	22.77	19.72
18:1ω7			9.49	4.95	5.16	3.57	3.75	5.03
18:2ω6	2	1	2.3	1.28	1.27	1.36	1.63	2.58
18:3ω3	0.5							
18:4ω3	0.4							
20:1ω9	22	12	5.08	12.5	9.06	12.56	13.0	6.71
20:4ω6	0.5		0.94	0.51	0.44	0.36	0.31	0.3
20:5ω3	2.5	5	8.28	4.85	9.31	6.82	5.21	8.72
22:0			0.63	<0.3	1.19	<0.3	<0.3	0.75
22:1ω11	14	7	0.27	0.62	0.31	0.77	0.86	0.34
22:5ω3	1.5	3	4.26	5.06	4.22	4.78	2.29	5.46
22:6ω3	3	6	7.22	8.91	7.76	10.48	9.56	9.45

* Reference 57

** Reference 31

Table 4. Calculated daily production of POA in breast milk and POA intake in infants from breast milk

Country of origin (Number of women)	Reference	Lowest POA (mg/d) Excreted in Breast Milk*	Highest POA (mg/d) Excreted in Breast Milk**	Average POA Intake (mg/d) in Infants between 1-6 months of age#	Highest POA Intake (mg/d) in Infants between 1-6 months of age##
Australia (48)	62	260	2174	446	1604
Canada (48)	62	246	2042	419	1507
Chile (50)	62	238	1976	405	1458
China (50)	62	165	1376	282	1015
Japan (51)	62	225	1874	384	1382
Mexico (46)	62	232	1932	396	1426
Philippines (54)	62	404	3360	689	2479
United Kingdom (44)	62	251	2086	428	1539
United States (49)	62	232	1932	396	1426
Coastal China (20)	63	214	1779	365	1312
Inland China (82)	63	143	1186	243	875
Serbia (12 bearing preterm infants of appropriate size for gestational age)	64	139 at birth	1157	237	853
Serbia (11 bearing preterm infants small for gestational age)	64	99 at birth	820	168	605

For simplification, it is assumed that the specific gravity of milk is equivalent to water.

* Calculated based on a fat intake of 8.8 g/d from 440 g/d ~ 440 mL/d of breast milk with a lipid concentration of 2 g/dL [65]
** Calculated based on a fat intake of 73.2 g/d from 1220 g/d ~ 1220 mL/d of breast milk with a lipid concentration of 6 g/dL [65]
# Calculated based on a fat intake of 15 g/d from 750 mL of breast milk [66] with a lipid concentration of 2 g/dL [65]
Calculated based on a fat intake of 54 g/d from 900 mL of breast milk [66] with a lipid concentration of 6 g/dL [65]

Table 5. Summary of preclinical studies investigating the effects of POA in various models

Study	Cell culture/ Animal Model	Study Design	POA Dose	Outcome	Adverse Events/Outcome
In Vitro Studies					
71	Adult rat pancreatic islets	Parallel, placebo controlled against 0.5 mmol/L palmitic acid	0.5 mmol/L POA alone and in combination with 0.25 or 0.5 mmol/L palmitic acid	● POA increased glucose stimulated β-cell proliferation while palmitic acid decreased it ● POA increased β-cell insulin content while palmitic acid decreased it ● POA increased β-cell secretory function while palmitic acid reduced it ●POA had no effect on β-cell death while palmitic acid increased it	POA had no negative effects on β-cell function and instead protected them from the toxic effects of palmitic acid
73	Rat BRIN-BD11 β-cells (have similar viability regulation to human β-cells)	Parallel, placebo controlled against vehicle [ethanol and bovine serum albumin (BSA)]	0.5 mM POA and 0.5 mM palmitic acid alone and combined for up to 18 hours, and 0.5 mM palmitic acid in the presence or absence of POA up to 100 μM POA.	●Palmitic acid caused cell death while POA had no effect and prevented palmitic acid induced apoptosis dose dependently ● POA added up to 6 h after palmitic acid, was still effective at blocking the increased apoptosis normally seen in response to palmitic acid ● POA promotion of cell viability was not associated with reduction in nitric oxide formation during incubation with the cytokines IL-1L and IFNQ revealing it impacts a distal step in the apoptosis pathway ● POA prevented loss of cell viability caused by removal of cell culture medium over 48 hours even in the presence of carnitine palmitoyltranferase-1 inhibitor etomoxir, confirming that mitochondrial oxidation of the fatty acid was not required for this response	POA had no negative effects on β-cell function and instead protected them from the toxic effects of palmitic acid and removal of culture medium even during exposure to a carnitine palmitoyltransferase-1 inhibitor

72	Rat BRIN-BD11 β-cells	Parallel, placebo controlled against vehicle (ethanol and BSA)	0.25 mM POA and 0.25 mM palmitic acid alone and combined for up to 72 hours	● Palmitic acid caused a significant decrease in total cell viability over 18 h accompanied by increased caspase activation consistent with induction of apoptosis while POA failed to induce cell death and caused no activation of caspases ● POA completely prevented loss of viability caused by palmitic acid and abolished the induction of caspase activity ● POA did not alter cAMP levels	POA had no negative effects on β-cell function and instead protected them from the toxic effects of palmitic acid
74	Differentiated 3 T3-L1 cells (programmed to become adipocytes)	Parallel, placebo controlled against vehicle (ethanol)	200 μM POA or palmitic acid for 18 hours	Cells treated with POA, but not PA had increased: ● Non-insulin stimulated glucose uptake by 51% ● Insulin- stimulated glucose uptake by 36% ● GLUT 4 transcription by 34% ●GLUT4 protein levels by 78%	POA had no reported negative effects POA enhanced glucose uptake by adipocytes
74	Adipocytes from wild type mice	Parallel, placebo controlled against vehicle (water)	300 mg/kg/d POA or oleic acid for 10 days	Increased: • Non-insulin stimulated glucose uptake by 3-fold relative to water treated control mice • Insulin- stimulated glucose uptake by 1.8-fold relative to water treated control mice • Increased GLUT4 transcription by 86% ● POA was more effective than oleic acid	POA had no reported negative effects POA enhanced glucose uptake by adipocytes
75	Conditioned medium from treated Macrophages on C2C12 myotubes	Parallel, placebo controlled against lipopolysaccharide as positive control.	0.75 mM POA or palmitic acid alone or in combination for 8 hours	POA improved muscle insulin sensitivity and counteracted the palmitic acid -mediated insulin resistance through macrophage activation	POA had no reported negative effects POA enhanced insulin sensitivity
76	Primary human adipocytes from normal weight and obese patients and those with and without Type 2 diabetes	Parallel, placebo controlled against ethanol and BSA	1 and 10 μM of a variety of fatty acids including POA	Peroxisome proliferator-activated receptor-gamma response element (PPRE) transcriptional activity was increased when cells were treated with palmitic and stearic acids. Linoleic acid, γ linolenic acid, and docosahexaenoic acid did not induce any statistically significant changes in PPRE activity. But POA increased basal PPRE activity by 35% at 1 μM.	POA had no reported negative effects POA was half as effective as rosiglitazone (a diabetic medication) to enhance insulin sensitivity

77	Stromal vascular cells	Parallel, placebo controlled against vehicle	150 µM palmitic acid, POA or cis-vaccenic acid for 4 days followed by exposure to radiolabeled fatty acid on day 6	●Only POA inhibited desaturation ●POA and its elongation product cis-vaccenic acid both inhibited lipogenesis	POA had no reported negative effects POA reduced desaturation and lipogenesis that contributes to fat synthesis and storage
78	Stromal vascular cells	Parallel, placebo controlled against vehicle	0, 50, 150, 300 µM POA	● POA reduced mRNA for stearoyl-CoA desaturase (SCD1), fatty acid synthase (FASN) and elongase protein 6 (ELOVL6) genes ● POA upregulated carnitine palmitoyltransferase 1A (CPT1A) dose dependently	POA had no reported negative effects POA altered gene expression thereby decreasing lipogenesis and increasing β-oxidation
79	Differentiated 3 T3-L1 cells	Parallel, placebo controlled against vehicle	250 µM palmitic, stearic, or oleic acid or POA for 48 hours	●Palmitic acid upregulated the extracellular matrix (Focal adhesion and ECM-receptor interaction) and the Toll-like receptor (TLR) signaling pathways, and downregulated the ABC transporters and Antigen processing and presentation pathways while POA had the opposite effect. ● POA also downregulated Chemokine signaling and Cytokine-cytokine receptor interaction pathways	POA had no reported negative effects PAO reduced inflammatory gene expression that would contribute to obesity while PA increased it
81	Differentiated 3 T3-L1 cells	Parallel, placebo controlled against vehicle (ethanol)	200 µM POA or palmitic acid for 18 hours	● POA, but not palmitic acid increased lipolysis, mRNA levels of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) and protein content of ATGL and pSer(660)-HSL as associated with higher rates of PPARα binding to DNA.	POA had no reported negative effectsPOA increased adipocyte lipolysis and lipase content
81	Differentiated 3 T3-L1 cells from treated wild type and PPARα-deficient mice	Parallel, placebo controlled against vehicle (water)	300 mg/kg/d POA or oleic acid for 10 days	● POA increased primary adipocyte basal and stimulated lipolysis and ATGL and HSL mRNA levels and increased fatty acid incorporation into TAG and glycerol 3-phosphate synthesis from glucose in both wild-type and PPARα-deficient mice.	POA had no reported negative effects POA increased adipocyte lipolysis and lipase content
82	L6 muscle cells	Parallel, placebo controlled against vehicle	0.75 mM POA or palmitic acid alone or in combination for 16 hours	●Palmitic acid, but not POA, induced phosphorylation/activation of the MEK-ERK-IKK axis and pro-inflammatory cytokine (IL-6, CINC-1) expression	POA had no reported negative effects POA reduced the pro-inflammatory effect of palmitic acid by suppressing mitochondrial dysfunction.
80	Macrophages from low-fat and high-fat diet fed mice	Parallel, placebo controlled against vehicle (BSA)	0.5 mM POA or BSA for 6 hours	●POA significantly prevented high-fat induced pro-inflammatory gene expression and cytokine production	POA had no reported negative effects POA prevented pro-inflammatory gene expression

80	Macrophages from low-fat diet fed mice	Parallel, placebo controlled against vehicle (BSA)	0.5 mM Palmitic acid or 0.5 mM POA singly and in combination for 18 h.	●Palmitic acid elevated pro-inflammatory gene expression ●POA increased anti-inflammatory genes expression and oxidative metabolism ●Co-incubation with both fatty acids prevented a multitude of palmitic acid induced pro-inflammatory events.	POA had no reported negative effects POA ●prevented inflammation while palmitic acid caused it ● prevented palmitic acid-induced inflammation
80	Macrophages from low-fat diet fed mice	Parallel, placebo controlled against vehicle (BSA)	0.5 mM Palmitic acid or 0.5 mM POA singly and in combination for 18 h.	● Palmitic acid decreased AMPK phosphorylation while POA increased it significantly, and prevented the inhibition by palmitic acid ● An AMPK inhibitor significantly diminished the ability of POA to prevent a number of palmitic acid-induced inflammatory events . A similar trend was seen in AMPKβ1 knockout macrophages.	POA had no reported negative effects POA prevents inflammation via AMPK activation
84	Wild type (WT) macrophages	Parallel, placebo controlled against vehicle	300-500 µM POA and palmitic, stearic and oleic acid	● Palmitic and stearic acid, but not oleic or POA lead to endoplasmic reticulum (ER) stress as judged by the robust phosphorylation of PERK and eIF2–α, activation of JNK, and induction Ddit3 and sXBP-1 expression ●WT macrophages pretreated with POA become resistant to Palmitic acid-induced ER stress and apoptosis	POA had no reported negative effects POA prevented palmitic acid induced endoplasmic reticulum stress and apoptosis

85	Human and mouse primary hepatocytes, and the human hepatocellular carcinoma Huh-7 and Hep 3B cell lines	Parallel, placebo controlled against vehicle (isopropanol)	200–800 μM POA or palmitic or stearic acid (These concentrations are similar to the fasting total FFA plasma concentrations observed in human nonalcoholic steatohepatitis.)	●POA significantly reduced lipoapoptosis by palmitic and stearic acid in both primary cells and cancer cell lines ●POA accentuated palmitic acid-induced steatosis in Huh-7 cells excluding inhibition of steatosis as a mechanism for reduced apoptosis ●POA inhibited palmitic acid induction of the endoplasmic reticulum stress response as demonstrated by reductions in CHOP expression, eIF2-α phosphorylation, XBP-1 splicing, and JNK activation ● Palmitic acid increased expression of the BH3-only proteins PUMA and Bim, which was attenuated by POA ● POA prevented activation of the downstream death mediator Bax	POA had no reported negative effects POA prevented palmitic acid induced endoplasmic reticulum stress and apoptosis.
83	Human umbilical vein endothelial cells	Parallel, placebo controlled against a vehicle	0, 0.25, 0.5 0.75 and 1.0 mM Palmitic acid or POA for 0, 4, 8, 12, 16, 20 and 24 h	●Palmitic acid, but not POA, inhibited insulin-induced Akt activation by a mechanism involving alterations in SERCA2 expression and enzymatic activity that results in loss of endoplasmic reticulum homeostasis followed by maladaptive endoplasmic reticulum stress response.	POA had no reported negative effects Palmitic acid induced endoplasmic reticulum stress while POA did not.
86	Mouse pancreatic acinar cells	Parallel, placebo controlled against vehicle (isopropanol)	10-100 μM POA and POAEE	● POAEE increased calcium ion concentration through inositol trisphosphate receptors and, following hydrolysis, through calcium-ATPase pump failure from impaired mitochondrial ATP production. Inhibition of ester hydrolysis markedly reduced its calcium-releasing effect and consequent toxicity.	POAEE causes calcium-ATP pump failure in pancreatic cells
87	Isolated pancreatic acinar cells and acinar cell clusters of two or three cells were prepared from the pancreas of adult CD1 mice	Parallel, placebo controlled against vehicle	Incubated for 1 h at room temperature with various concentrations of ethanol and selected ethanol metabolites including 5-100 μM Palmitic acid, POA, POAEE, arachidonic acid EE and the saturated arachidic acid EE	●POAEE, a non-oxidative metabolite of ethanol, induced a concentration-dependent and sustained increase in calcium concentration similar to all other fatty acids tested●100 μM POA for 1 h induced significant cellular necrosis as did other fatty acid products	Endogenously synthesized POA through non-oxidative metabolism of ethanol, induces cellular necrosis through its effects on calcium concentration in the same way as other non-oxidative fatty acid metabolites of ethanol

92	Rat pancreatic acinar cells from Sprague-Dawley rats	Parallel, placebo controlled against vehicle	50-100 μM POA, POA-ethyl ester (POAEE) or ethanol	● POA (50–100 μM) induced a robust Ca2+ overload, ATP depletion, inhibited ATP-driven plasma membrane Ca2+-ATPase (PMCA) activity and consequently induced necrosis ● POAEE (100 μM) induced a small but irreversible Ca2+ overload response but had no significant effect on PMCA activity ●Insulin pretreatment (100 nM for 30 min) prevented the POA-induced Ca2+ overload, ATP depletion, inhibition of the PMCA, and necrosis	●Endogenously synthesized POA and POAEE within the pancreas, derived from excess alcohol consumption, leads to alcohol-induced pancreatitis ● Insulin treatment effectively abolished the POA-induced ATP depletion, inhibition of the PMCA, Ca2+ overload, and necrosis
95	Mouse Swiss 3T3 fibroblasts, NIH-3T3 fibroblasts, and 3T3-L1 pre-adipocytes	Parallel, placebo controlled against various vehicles and in combination with numerous inhibitors and activators	●To compare the cellular effects of exogenous fatty acids, 5 or 10 μM POA or palmitic acid for 4 h ● To determine cell viability, starved cells were treated with 5 μM POA or palmitic acid for 24 h ●To investigate cell proliferation treated with 50 μM POA or palmitic acid for 48 h at 37 °C and 5% CO2	●POA is synthesized upon stimulation with growth factors, induces cell proliferation, and is selectively incorporated into phosphatidylinositol (PI) through de novo PI biosynthesis ●Supplemented POA is channeled specifically into PI even in absence of growth factors ●Metabolites of POA including 16:1-PI and 18:1-PI may be partly responsible for the biological effects of POA ● POA supplementation induced cell proliferation, and restored the proliferation rate of cells whose POA biosynthesis was blocked by inhibition of SCD-1	POA had no reported negative effects POA more effectively restores cell proliferation than palmitic acid
In Vivo Studies					
96	KK-Ay mice (genetically diabetic/obese)	Parallel, placebo controlled against vehicle	Orally 300 mg/Kg/d of POA or palmitic acid for 4 weeks	POA ● reduced the expression of genes that are responsible for decreased insulin sensitivity ● increased pancreas weight ● decreased plasma glucose and insulin levels ● improved insulin-signaling pathway while Palmitic acid did not ● increased glucose transport into skeletal muscle while Palmitic acid did not ● improved glycemic control and insulin resistance while Palmitic acid did not ● suppressed lipogenic & inflammatory genes ● dramatically Improved their diabetic condition ● reduced food intake and body weight	POA had no reported negative effects POA reduced body weight increase, ameliorated the development of hyperglycemia and hypertriglyceridemia and improved insulin sensitivity.

97	C57BL6 wild-type (WT) and PPAR- alpha-knockout (KO) mice (have a reduced ability to use fat as an energy source)	Parallel, placebo controlled against vehicle	Orally 300 mg/Kg/d of POA or oleic acid for 2 weeks	PPARα knock out mice ● Reduced fasting glucose levels ● Reduced insulin resistance Wild Type Mice ● Reduced fasting glucose levels ●Reduced insulin resistance ● Increased glucose incorporation into muscle ● Improved glucose tolerance	POA had no reported negative effects POA improved the diabetic condition
98	Southdown yearling wethers (obese lambs)	Parallel, placebo controlled against vehicle	Exp #1 - Jugular infused 0, 2 and 5 mg/Kg BW U-13CPOA Exp #2 - Jugular infused0 or 5 mg/Kg BW POA	●POA was rapidly taken up in the blood stream and returned to baseline levels by 30 minutes post dosing ● POA increased circulating glucose levels and insulin levels during the insulin challenge and appeared related to stimulation of insulin release from pancreas islets	A single infusion of POA increased blood glucose levels, but also stimulated insulin excretion
99	Southdown (obese) sheep	Parallel, placebo controlled against vehicle	Intravenously infused 0, 5 or 10 mg /Kg BW/d POA twice daily for 28 days	The highest dose ● reduced blood insulin ●improved insulin resistance ● altered gene expression for those regulating glucose uptake and fatty acid oxidation ● reduced weight gain by 77% ● reduced intramuscular adipocyte (fat cell) size ● reduced lipid (fat) content within adipocytes ● these results occurred dose dependent as POA & vaccenic acid increased	POA had no reported negative effects POA improved glucose and fat metabolism in a dose dependent manner resulting in less weight gain
100	Male Sprague Dawley rats	Parallel, placebo controlled against vehicle	Gavage delivered 0, 50, 150 or 500 mg/10 mL/Kg POA, Oleic acid or palmitic acid as either FFA or TG	POA ● accumulated within the small intestine in a dose dependent fashion ●reduced food intake ● elevated levels of the satiety hormone cholecystokinin as evidenced by reduced protein and mRNA levels	POA had no reported negative effects POA increased satiety thereby reducing food intake

43	F1B golden Syrian hamsters	Parallel, placebo controlled	Orally within the diet - 10% fat + 0.1%cholesterol, wt:wt enriched with macadamia (POA rich), palm (Palmitic acid rich), canola (oleic acid rich), coconut (short chain saturate rich) or safflower (linoleic acid rich) oils for 12 and 18 weeks	●POA-fed hamsters had lower non-HDL cholesterol and triglyceride concentrations compared with the palm oil (palmitic acid rich) and coconut oil hamsters and higher HDL-cholesterol compared with the coconut, canola, and safflower oil-fed hamsters. ●The aortic cholesterol concentration was not affected by dietary fat type. ● Total free cholesterol concentration was higher in the POA and canola oil groups than in the palm oil group	POA does not adversely affect plasma lipoprotein profiles or aortic cholesterol accumulation

Table 6. Blood POA concentration (% of total fatty acids) in various patient populations and association with health outcomes

Reference	Patient Population	Plasma PL POA	Plasma TG POA	Plasma CE POA	Plasma FFA POA	Plasma Total Lipid POA	Red Blood Cell PL POA	Whole Blood POA	VLDL-TG POA	Adipose Tissue POA	Author Conclusion/Health Outcome
9	437 Japanese employees aged 21-67 y	NR	NR	1.9-3.5 *	NR	NR	NR	NR	NR	NR	High POA was associated with high insulin resistance. Also higher D9D and D6D were associated with higher insulin resistance
23	1926 Control subjects in Costa Rica 58-59 +/- 11 y	NR	NR	NR	NR	NR	NR	NR	NR	3.58-9.63*	High POA was associated with greater obesity However, the association was reduced by low carbohydrate intake indicating that elevated POA within adipose tissue was the results of excess carbohydrate intake
104	134 healthy men aged 28-70 y	NR	NR	NR	NR	2.79-3.78**	NR	NR	NR	NR	High POA was associated with hypertriglyceridemia and obesity
103 Dietary POA intake reported to be 1.6-1.8 g/d	24 overweight adults likely to have elevated de novo lipogenesis based on elevated liver enzymes	NR	NR	NR	Up to ~ 3.0 mol%	NR	NR	NR	Up to ~3.5 mol%	NR	High POA in VLDL-TG was associated with high liver fat content
7	3630 US men and women from the Cardio Health Study	0.49 +/- 0.20% (range: 0.11–2.55	NR	NR	NR	NR	NR	NR	NR	NR	High POA was associated with lower LDL-chol, higher HDL-chol, lower total:HDL-chol, lower fibrinogen, higher TGs and greater insulin resistance. It was not associated with incident diabetes
12	788 matched pairs of controls and heart failure (HF) patients aged 40-82 y	Controls 0.32 (range 0.04 to 2.22) HF patient range 0.039-2.217	NR	NR	NR	NR	NR	NR	NR	NR	High POA was associated with higher incidence of heart failure.
11	1000 patients of coronary heart disease and 1000 matched controls from the Physician Health Study, Average 68.7y	NR	NR	NR	NR	NR	Controls 0.49 (Range 0.37-0.65) Patients average of lowest & highest quintile 0.28 & 0.93	NR	NR	NR	High POA was associated with coronary heart disease risk, but higher vaccenic acid (derived from POA) was inversely related to the condition.
4	3107 men and women 50-70 y		NR	NR	NR	NR	0.41 +/- 20%	NR	NR	NR	High POA is associated with an adverse profile of adipokines and inflammatory markers and increased risk of metabolic syndrome
116	3004 free of diabetes in 1992 reassessed in	0.29-0.73 (Average of the lowest & highest quintile range)	NR	NR	NR	NR	NR	NR	NR	NR	There was no association between POA and diabetes risk, but high vaccenic acid was associated with lower risk. However, higher palmitic acid stearic acid were associated with higher diabetes risk.
6	427 participants 55-80y in the PREDIMED study	NR	NR	NR	NR	Without metabolic syndrome Men 1.15, Women 1.3 With Metabolic syndrome Men 1.31 Women 1.46	NR	NR	NR	NR	High POA was associated with increased prevalence of metabolic syndrome. High D6D and palmitic were associated with Met Syn
113	25 subjects with diabetes and 25 controls rough 45 y	NR	NR	NR	NR	15.8 ± 2 µmol/liter ; type 1 diabetes,	NR	NR	NR	NR	High POA was not related to insulin sensitivity in Type 1 diabetics, but it was in normal controls

						11.5 ± 2 µmol/liter					
106	965 healthy young Canadian adults, 22.6 +/-0.1 y					1.81+/- 0.02					High POA was associated with higher markers of inflammation
8	1346 Finnish men 45-73 y, nondiabetic at baseline and followed for 5 year	NR	NR	NR	NR	NR	Mean 0.4% Range 0.4-0.49	NR	NR	NR	High POA was associated with worsening hyperglycemia and predicted incidence of Type 2 diabetes. Also higher SCD1 (ratio of 16:1:16:0) and D6D activity.
115	250 cases and one matched control/case of first incident cancer cases in the SU.VI.MAX study	NR	NR	NR	NR	Control 2.23 +/- 0.79 Overall cancer 2.15 +/- 0.84 Breast cancer 2.06 +/- 0.68	NR	NR	NR	NR	High POA was not associated with higher cancer risk. High POA reduced cancer risk in the absence of anti-oxidant supplementation
13	1981 community based cohort of 50 year old men followed for >40 y	NR	NR	Cancer 3.87 +/- 1.38 No cancer 3.85 +/- 1.27	NR	NR	NR	NR	NR	NR	High POA was associated with increased cancer death (based on quintile analysis) Also higher SCD1 activity (ratio of 16:1:16:0)
114	100 subjects at risk for Type 2 diabetes	NR	NR	NR	At baseline 4.79+/- 0.12 At follow up 5.42 +/- 0.13	NR	NR	NR	NR	NR	Higher baseline POA predicted better insulin sensitivity independent of gender, age and body fat POA was lower in males and not associated with age, adiposity as body weight, BMI, waist circumference, total or visceral fat after adjusting for gender and age.
2	16 adults with metabolic syndrome, 44.9 +/-9.9 y fed six 3-wk diets that progressively increased carbohydrate from 47-346 g/d	NR	Baseline 3.87 +/- 0.90	Baseline 3.10 +/- 1.17	NR	NR	NR	NR	NR	NR	POA dropped as carbohydrate intake decreased and then increased with reintroduction of carbohydrate.
112	476 incident prostate cancer cases and an equal number of controls	NR	NR	NR	NR	NR	NR	0.94-1.10	NR	NR	High POA was associated with higher incidence of prostate cancer and higher incidence of high-grade tumor. There was no association with low-grade tumors.
117	3592 white participants from the ARIC study, 45-64 y at baseline, followed for 14.3 y.	Baseline 0.64+/-0.18	Baseline 2.56+/- 1.24	NR	NR	NR	NR	NR	NR	NR	There was no association between POA and heart failure.
107 Dietary intake of POA was 2.2 (2.0–2.3) g/d	2724 case-cohort including diabetic cases within the EPIC-PtsdamStudy followed for 7 y	NR	NR	NR	NR	NR	0.45 (0.36–0.57)	NR	NR	NR	High POA was associated with greater diabetic risk. SCD1 and D6D activity were also associated with increased risk of diabetes.
102	33 middle aged men initially free of coronary heart disease that sustain fatal or non-fatal myocardial infarction of died suddenly during a 5-7y follow up	**No infarct 1.30 ±0.06** Infarcts 1.18±0.07	**No infarct 4.85 ±0.15** Infarct 4.84±0.19	**No infarct 5.06±0.25** Infarct 4.98±+0.30	NR	NR	NR	NR	NR	NR	There was no association between POA and infarcts. However, palmitic acid and stearic acid were significantly higher in infarct subjects.
101 Dietary intake of POA was 1.26	184 non-cases, 40-79 y at baseline from the EPIC study	Controls 0.75(1.11-1.44) Cases	NR	NR	NR	NR	Control 0.51 (0.43-0.58) Cases 0.57(0.49-0.68)	NR	NR	NR	There was no association between POA and risk of Type 2 diabetes

(1.11-1.40) g/d		0.87(0.69-1.09)									
108	2909 adults, 45-64 y followed for 9 y	Non-Diabetic 0.63 ± 0.18 Diabetics 0.66 ± 0.18	NR	Non-diabetic 2.51 ± 1.21 Diabetic 2.90 ± 1.21	NR	NR	NR	NR	NR	NR	High POA in the CE was associated with incidence of diabetes as was palmitic acid in the PL fraction.
109 Dietary intake of POA was 1.75 +/- 0.69 g/d in Controls and 1.89 +/- 0.80 g/d in Cases (P= 0.001)	Case-cohort of 3737 adults, 36-72 y followed for 4 y	Non-diabetics 0.43 +/- 0.19 Cases 0.49 +/- 0.20	NR	NR	NR	NR	NR	NR	3.4 +/-1.67	NR	High POA was associated with higher incidence of diabetes as were a number of other fatty acids. SCD1 and elongase activity were also associated with increased risk of diabetes.
118	Case-control 94 men with coronary heart disease and 94 without, 35-57 y	Control 0.69 +/-0.42 Case 0.71 +/-0.38	NR	Control 3.4 +/- 1.67 Case 3.84 +/-1.98	NR	NR	NR	NR	NR	NR	There was no association between POA and coronary heart disease. There was no significant difference between case and control POA.
105	Population based cohort of 1558 men, 50 y followed for 20 years	NR	NR	Control 3.5±1.1 Case 3.8±1.1	NR	NR	NR	NR	NR	NR	High POA at baseline was associated with development of metabolic syndrome. High SDC1 and D6D, and low D5D activity predicted the development of metabolic syndrome
119	3591 white participants of the ARIC study, 45-64 y followed for 10.7 y	Control 0.64 +/-0.2 CHD 0.62 +/-0.2	NR	Control 2.58 +/- 1.2 CHD 2.53 +/- 1.2	NR	NR	NR	NR	NR	NR	There was no association between POA and risk of coronary heart disease (CHD). There was no significant difference between CHD and control POA. Higher D5D activity was associated with higher incidence of coronary heart disease.
110	1828 males, aged 50 y followed for 10 y	NR	NR	Normo-glycemic 3.76 ± 1.26 NIDDM 4.86 ± 1.78	NR	NR	NR	NR	NR	NR	High POA was associated with development of non-insulin-dependent diabetic mellitus (NIDDM)
111	195 subjects that participated in a 8 week low calorie diet (LCD) followed by 5 different maintenance plans (MP)	NR	NR	NR	NR	NR	NR	NR	NR	Before 4.57 +/-0.09 LCD 4.23 +/-0.09 MP 4.25 +/- 0.09	High baseline POA was associated with weight gain during the following maintenance plan (i.e. predicts less successful weight maintenance) POA was significantly lower than baseline when on the low calorie diet as well as during the maintenance plan.

NR = Not reported in the study

* Average per quintile

** Average per percentile

Groups with the highest percentage of POA within their corresponding lipid fraction are bolded.

Table 7. Summary of POA human intervention trials reporting anti-inflammatory effects.

Reference	Subjects	Design	Intervention	POA Dose	Duration		Assay Results	Treatment Outcomes	Adverse Events
MACADAMIA NUT OR NUT OIL									
134	N=17 M, Mean 54y, HC	O	Macadamia nut	40-90 g/d providing ~15% TE	4 wk	Pre	LTB_4 – 876 pg/mL, 8ISOP – 1353 pg/mL, TXB_2 – 122 pg/mL, PGI_2 192 pg/mL	Plasma markers for inflammation including LTB^4 and 8ISOP were significantly lower within 4 weeks following as well as a non-significant (23.6%) reduction in TXB_2/PGI_2 in the macadamia nut intervention.	Serum POA levels significantly increased after consuming the test product. There were no adverse events reported in the publication.
						Post	LTB_4 – 679*pg/mL, 8ISOP -1030*pg/mL, TXB_2 – 90 pg/mL, PGI_2 177 pg/mL		
SEA BUCKTHORN OIL									
51	N=49 M & F, atopic dermatitis	R, DB, PC, P	SB seed oil	5 g oil providing 220 mg POA/d	4 m	Pre	IgE – 1767 IU/L	Dermatitis improved in the pulp oil group, but improvements in the seed oil group were not significant.	Pulp oil significantly increases POA in the plasma PL and neutral lipids. There were no adverse events reported in the publication.
						Post	IgE – 3027 IU/L		
			SB pulp oil	5 g oil providing 1.25 g POA/d		Pre	IgE – 2601 IU/L		
						Post	IgE – 2672 IU/L		
			Paraffin oil placebo	5 g oil providing no POA		Pre	IgE – 2485 IU/L		
						Post	IgE – 1997 IU/L		
135	n=110 Mean 44.2y F, overweight	X, Wash-out 30-39 days	Frozen Bilberries	NR	33-35 d	Pre	IL-6 – NR, CRP – NR, ICAM – NR, VCAM 872 ng/mL, TNFα - 4.9 pg/mL	There was a significant decrease in waist circumference following Frozen berries and SB berries and a small decrease in weight after frozen berries. Vascular adhesion molecules decreased after frozen berries and SB berry oil and intercellular adhesion molecule decreased after SB seed oil. Therefore, different berry fractions have various but slightly positive effects on the associated variables of metabolic disease.	Compliance was good. There were no adverse events reported in the publication.
						Post	IL-6 – NC, CRP – NC, ICAM – NC, VCAM 820* ng/mL, TNFα – 4.7* pg/mL		
			SB berries	NR		Pre	IL-6 – NR, CRP – NR, ICAM – NR, VCAM – NR, TNFα pg/mL		
						Post	IL-6 – NC, CRP – NC, ICAM – NC, VCAM – NC, TNFα – 4.5* pg/mL		
			SB berry oil	NR		Pre	IL-6 – NR, CRP – NR, ICAM 184.0 ng/L, VCAM – NR, TNFα- 4.8 pg/mL		
						Post	IL-6 – NC, CRP – NC, ICAM 178.3* ng/L, VCAM – NC, TNFα - 4.5* pg/mL		
			SB seed oil	NR		Pre	IL-6 – NR, CRP 2.0 mg/L, ICAM – NR, VCAM – 882.1 ng/mL, TNFα - NR		
						Post	IL-6 – NC, CRP 2.4* mg/L, ICAM – NC, VCAM 814.8* ng/mL, TNFα - NC		
136	n=45 Mean 62y M & F, hemodialysis patients	R, DB, PC, X, Wash-out 4 weeks	SB oil	2 g oil providing 388 mg/d POA	8 w	Pre	CRP 6.7 mg/L, Antitrypsin 1.5 g/L, Oroso-mucoid 1.0 g/L, Leukocytes 7.2 X 10^9/L	Supplementation did not protect against oxidative stress, nor improve oral health or inflammation status in hemodialysis patients. There were no significant changes in DNA breaks, oxidative DNA lesions, salivary flow rates, or inflammation, typical of the condition, following supplementation.	Plasma levels of phosphate and sodium increased and plasma levels of iron decreased. Reduced iron levels can be harmful because iron deficiency might lead to anemia. Higher levels of phosphate can influence vascular calcification, which is a risk factor for developing atherosclerosis and cardiovascular disease. No other blood markers were affected. There were 18 drop outs (2 deaths, 3 noncompliant patients and 13 cases of acute illness) in this study that limited the outcome.
						Post	CRP 9.4 mg/L, Antitrypsin 1.5 g/L, Orosomucoid 1.0 g/L, Leukocytes 7.1 X 10^9/L		
			Placebo	2g Coconut oil		Pre	CRP 10.1 mg/L, Antitrypsin 1.5 g/L, Orosomucoid 1.1 g/L, Leukocytes 7.1 X 10^9/L		
						Post	CRP 9.0 mg/L, Antitrypsin 1.5 g/L, Orosomucoid 1.0 g/L, Leukocytes 7.1 X 10^9/L		

140	N= 16	R, P, PC	SB seed oil	5 g/d	4 m	Seed oil treatment increased the proportion of docosapentaenoic acid and decreased the proportion of palmitic acid in skin glycerophospholipids almost significantly. Pulp oil treatment slightly increased the proportion of stearic acid. A small increase in the proportion of linoleic acid and stearic acid was also observed in the placebo group. A significant increase in the proportion of linoleic acid was found in plasma glycerophospholipids of patients in the seed oil group.			There were no adverse events reported in the publication.
			SB pulp oil 5	5 g/d					
			Paraffin oil placebo	5 g/d					
PALMITOLEIC ACID									
52	n=60 Mean 45y M & F, with elevated CRP	R, DB, PC	POA ethyl ester concentrate	420 mg of oil providing 220.5 mg/d POA	30 d	Pre	CRP 4.3 mg/L	Purified POA reduced CRP by 44%.	Two to 3 participants (6.7-10%) in the intervention group developed gastrointestinal distress and one developed a headache during the study. There were no reported side effects or adverse effects in the control group.
						Post	CRP 2.1 mg/L #		
			Placebo	1 g medium chain triglyceride		Pre	CRP 4.3 mg/L		
						Post	CRP 4.0 mg/L		

F = female; M = male; y = years; HC = hypercholesterolemic; O = open label; d = days; TE = total energy; wk = weeks; LTB_4 = leukotriene B4; 8ISOP = 8-isoprostane; TXB_2 = thromboxane; PGI_2 = prostacyclin I_2; IgE = immunoglobulin E; IL6 = interleukin 6; CRP = C-reactive protein; ICAM = intercellular adhesion molecules; VCAM = vascular adhesion molecules; TNFα = tumor necrosis factor α; R = randomized; DB = double-blind; PC = placebo-controlled; NR = measured but not reported; NC = not reported.

* P <0.05 compared with pre-intervention value
# P< 0.0001 compared with baseline

Table 8. Summary of POA human intervention trials reporting lipid lowering effects.

Reference	Subjects	Design	Intervention Group	POA Dose	Duration		Tot Chol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	TAG (mmol/L)	Adverse Events
MACADAMIA NUT OR NUT OIL											
39	n=30 18-53y M & F with Chol above 150 mg/dL & TAG below 400 mg/dL	X, No wash-out	AAD	NR	30 d		5.2	1.43	3.37	0.87	No important side effects of consistent ingestion of large amounts of macadamia nuts were noted. A pilot study was reported to have no side effects (other than gastrointestinal discomfort consistent with radical shifts in dietary fat content). There was no difference in serum cholesterol level between the groups in the pilot study, although the high-dose macadamia nut group ate 50% of their energy as fat.
			AHA Step 1	NR			4.99*	1.34*	3.21*	0.94*	
			Macadamia nut	NR			4.95*	1.37*	3.22*	0.79*	
40	n=17 Mean 54y M, HC	O	Macadamia nut	40-90 g of nuts/d providing 15% TE or 6.8 to 15.3 g POA/d§	4 wk	Pre	6.51	1.20	4.49	1.79	Serum POA levels significantly increased after consuming the test product. There were no adverse events reported in the publication.
						Post	6.30**	1.28**	4.22**	1.74	
42	n=71 19-23y F, H	P	Enriched bread-coconut	0.04 g/100g or 0.08 g POA/d¥	3 wk	Pre	4.66	1.84	2.67	0.60	Compliance was good with no significance difference between groups. Blood POA significantly increased in the macadamia nut enriched bread group. There were no adverse events reported in the publication.
						Post	4.38**	1.66	2.43**	0.70	
			Enriched bread – butter	0.34 g/100g or 0.68 g POA/d¥		Pre	4.58	1.84	2.53	0.70	
						Post	4.53	1.79	2.49	0.78	
			Enriched bread – macadamia nut	2.85 g/100g or 5.7 g POA/d¥		Pre	4.66	1.94	2.51	0.66	
						Post	4.38**	1.79	2.33**	0.62	
41	n=25 25-65y M & F, mHC	X, Wash-out 2 weeks	AAD	0.4% TE or 933 mg POA/d#	5 wk		5.45	1.20	3.44	1.59	Serum POA significantly increased after consuming the test article. There were no adverse events reported in the publication.
			Macadamia nut	2.5% TE or 5.83 g POA/d#			4.94*	1.11*	3.14*	1.55	
20	n=34 Mean 49y M, mHC	X, No wash-out	Foods enriched with high PA	0.32% TE	3 wk		5.78	1.14	4.05	1.3	Plasma POA increased by approximately 60%. Two participants complained of gastrointestinal discomfort, but the effect was transient.
			Foods enriched with macadamia nut oil (POA)	4.13% TE or 10.51 g POA/d+			5.73	1.10	4.06	1.28	
			Foods enriched with high oleic acid oil	0.31% TE			5.58€	1.12€	3.89€	1.27	
139	N=9 men 23 y+/-2	P, PC, Cross-over	Oleic acid as FFA	40 g	One time dose	FFAs empty from the stomach more slowly than TGs, but stimulate plasma cholecystokinin and peptide-YY and suppress appetite more potently than TGs in healthy human beings.					There were no adverse events reported in the publication.
			Macadamia nut oil as TG	40 g = 6.8 g/d POA§							
			Control -Milk protein	600 mL of 4%							
SEA BUCKTHORN OIL											
51	n=49 M & F, atopic dermatitis	R, DB, PC, P	SB seed oil	5 g oil providing 220 mg POA/d	4 m	Pre	4.34	1.36	2.38	1.39	Pulp oil significantly increases POA in the plasma PL and neutral lipids. There were no adverse events reported in the publication.
						Post	4.41	1.35	2.38	1.48	
			SB pulp oil	5 g oil providing 1.25 g POA/d		Pre	4.63	1.38	2.83	0.96	
						Post	4.80	1.53**	2.83	1.01	

			Paraffin oil placebo	5 g oil providing no POA		Pre	4.81	1.40	2.91	1.14	
						Post	4.95	1.49	2.94	1.14	
137	n=12 20-59y M, normolipidemic	R, DB, PC, X, Wash-out 4-8 weeks	SB oil	5 g oil providing 865 mg POA/d	4 wk	Pre	4.66	1.17	3.43	1.37	There were no adverse events reported in the publication.
						Post	4.71	1.13	3.51	1.43	
			Coconut oil	5 g oil providing no POA		Pre	4.79	1.12	3.41	1.52	
						Post	4.85	1.10	3.61	1.61	
135	n=110 Mean 44.2y F, overweight	X, Wash-out 30-39 days	Frozen Bilberries	NR	33-35 d	Pre	NR	NR	NR	NR	Compliance was good. There were no adverse events reported in the publication.
						Post	NC	NC	NC	NC	
			SB berries	NR		Pre	NR	NR	NR	NR	
						Post	NC	NC	NC	NC	
			SB berry oil – phenolic extract	NR		Pre	NR	NR	NR	NR	
						Post	NC	NC	NC	NC	
			SB seed oil	NR		Pre	NR	NR	NR	NR	
						Post	NC	NC	NC	NC	
138	n=80 Mean 44.2y F, overweight	X, Wash-out 30-39 days	Dried SB berries	20 g/d	30 d	Pre	NR	NR	NR	NR	There were no adverse events reported in the publication.
						Post	NC	NC	NC – decreasing trend	**	
			SB oil	4 g oil providing 960 mg POA/d		Pre	NR	NR	NR	NR	
						Post	**	NC	NC- decreasing trend	**	
			SB phenolic extract	14.6 g Extract		Pre	NR	NR	NR	NR	
						Post	NC	NC	NC- increasing trend	NC – increasing trend	
PALMITOLEIC ACID											
52	n=60 Mean 45y M & F, with elevated CRP	R, DB, PC	POA ethyl ester concentrate	420 mg of oil providing 220.5 mg/d POA	30 d	Pre		45.7 mg/dL	114.1 mg/dL	202.4 mg/dL	Two to 3 participants (6.7-10%) in the intervention group developed gastrointestinal distress and one developed a headache during the study. There were no reported side effects or adverse effects in the control group.
						Post		47.1 mg/dL #	105.8 mg/dL #	170.3 mg/dL #	
			Placebo	1 g medium chain triglyceride		Pre		43.3 mg/dL	119.6 mg/dL	210.6 mg/dL	
						Post		42.7 mg/dL	119.2 mg/dL	207.2 mg/dL	

Tot Chol = total cholesterol; LDL = low density lipoprotein cholesterol; HDL = high density lipoprotein cholesterol; TAG = triglycerides; F = female; M = male; y = years; X = randomized cross-over; AAD = average American diet; AHA = American Heart Association; NR = not reported; d = days; HC = hypercholesterolemic; O = open label; TE = total energy; wk = weeks; H = healthy; P = parallel; mHC = moderately hypercholesterolemic; R = randomized; DB = double-blind; PC = placebo-controlled; SB = sea buckthorn; m = months; NC = no significant change compared to pre-intervention value; CRP = C-reactive protein

* P<0.05 compared with AAD ** P <0.05 compared with pre-intervention value € P< 0.05 compared with POA intervention
# P< 0.0001 compared with baseline + Calculated based on the fatty acid composition of foods provided within the publication
§ Calculated assuming that macadamia nut oil contains 17% of total fat as POA

Table 9. Relevant GRAS Notifications

GRAS Notice GRN No.	Subject	Intended Level of Use
102	Small planktivorous pelagic fish body oil (SPPFBO) (8.3% POA)	Levels of use not to exceed two thirds of the levels of use described in 21 CFR 184.1472 and a combined intake of EPA and DHA from all added sources does not exceed 3 g/p/d.
105	Fish oil concentrate (~4% POA)	Maximum use levels are 57 percent of those specified in that regulation listed in 21 CFR184.1472(a)(3) (menhaden oil).
109	Tuna oil (4.5% POA)	Levels of use are 62 percent of the maximum levels of use specified in 21 CFR 184.1472(a)(3).
138	Fish oil (from anchovy, sardine, jack mackerel, Pacific mackerel and other occasional species) (8.46% POA)	Levels of use are 67 percent of the levels specified in listed in 21CFR184.1472(a)(3).
146	Salmon oil (POA not specified)	Level of use ensures that components of salmon oil (i.e., EPA and DHA) would not exceed 3 g/person/d (g/p/d) as the sole source of EPA and DHA in any given food category.
193	Fish oil (predominately sardine and anchovy) and Tuna Oil (6-8% POA)	Levels of use are 67 percent of the levels specified in that regulation listed in 21 CFR184.1472(a)(3)
200	Tailored triglycerides enriched in omega-3 fatty acids from fish oil (1.5% POA) (55% EPA+DHA)	Levels of use are 36 percent of the levels specified in listed in 21 CFR 184.1472(a)(3).
217	Tailored triglyceride oil containing approximately 12% medium chain fatty acids (0.1-0.2% POA)	Levels not to exceed a maximum daily intake of 31 g/person/d
332	Refined pine nut oil (POA 0.2%)	Levels not to exceed 3.0 g per serving; an estimated mean of 8.9 g/person/d and 90th percentile of 17.8 g/person/d.
371	Krill oil (4.9% POA)	Levels of EPA and DHA estimated from krill oil not to exceed 3 g/person/d set for EPA and DHA for menhaden oil in 21 CFR 184.1472. Average EDI of 4.1 g per person/d (g/person/d) and an approximated 90th percentile EDI of 8.3 g/person/d.

Reference 132