
2.1 Introduction

Several groups of lipids have been shown to provide health benefits either through modification of tissue fatty acid composition or induction of cell signaling pathways. While some health benefits are derived from consumption of short- to medium-chain fatty acids, evidence suggests that the polyunsaturated fatty acids (PUFAs) are the most important bioactive lipids. PUFAs are found mostly in plant seed oils and are important substrates for the biosynthesis of cellular hormones (eicosanoids) and other signaling compounds that modulate human health. The beneficial health effects of PUFAs seem to be dependent on their isomer configuration as the *cis*-isomer is the predominant bioactive form. Moreover, fatty acids in the *cis*-configuration have a rigid nonlinear structure, which enhances membrane fluidity when incorporated into cells. Increased membrane fluidity enhances cell to cell communication and helps maintain normal homeostasis or prevent the development of metabolic disorders. Therefore, this chapter will focus mostly on PUFAs but with brief discussions on a short-chain fatty acid (butyric acid) and medium-chain fatty acids. Detailed descriptions of the

metabolic effects of short-chain fatty acids have been discussed in other parts of this book.

2.2 Butyric Acid

Butyrate is commonly found as part of the lipid component of dairy milk but is also one of the main by-products (others are acetate and propionate) of fiber fermentation in the colon and has been shown to induce various beneficial metabolic effects. Butyrate has been shown in vivo to be a stimulant of normal colonic cell proliferation but can also inhibit growth and proliferation of colon cancer cell lines. Other suggested health benefits of butyrate include:

- Substrate used for growth and regeneration of cells in large intestine.
- Anti-colon cancer properties probably through enhanced apoptosis of mutant colonic cells.
- Animal experiments also showed beneficial effect on the growth of cells in the small intestine.
- Increased thermogenesis to increase energy expenditure, which contributes to reduced body weight and other markers of metabolic syndrome.

2.3 Medium-Chain Fatty Acids

These are fatty acids that contain 8–10 carbon atoms, mainly caprylic (C8:0) and capric (C10:0) acids, which are metabolized differently when compared to long-chain fatty acids (14 or more carbon atoms). Medium-chain triglycerides (MCTs) contain medium-chain fatty acids (MCFAs) esterified to glycerol backbone and are usually completely hydrolyzed to yield the free fatty acids by lipases present in the gastrointestinal tract. When absorbed directly, MCTs enter the blood circulatory system through the portal vein and carried to the liver where they are oxidized to ketones. This is because in the mitochondria, transport of MCTs does not require carnitine palmitoyltransferase, a rate-limiting enzyme of β -oxidation. The mostly catabolic fate of MCFAs is evident by the fact that dietary MCTs reduce blood triglyceride levels during human intervention trials. Thus, dietary MCTs induce thermogenesis and do not contribute to weight gain since they are not deposited in the adipose tissue. This has been demonstrated in diet intervention trials involving hypertriglyceridemic human subjects where MCTs reduced body mass index, hip circumference, waist-hip ratio, total abdominal fat, visceral fat, body fat mass, and waist circumference. MCT diets also reduced blood levels of several types of LDL as well as LDL-cholesterol to greater extent than traditional oil that contained long-chain triglycerides. Therefore, MCTs may be used as a means of preventing and treatment of obesity, though the exact molecular mechanism of action has not been fully elucidated apart from the thermogenic effects. But MCTs were shown to activate hormone-sensitive lipase and down-regulate fatty acid synthase, which led to increased lipolysis and reduced fat accumulation, respectively, in white adipose tissue. And MCTs are able to upregulate expression of lipoprotein lipase, which is the major enzyme that is responsible for lipolysis. It should be noted that MCT oils are difficult to use as cooking oils because the presence of medium-chain fatty acids causes the oil to have lower smoke point than oils containing

long-chain fatty acids. One solution to this problem has been the development of oils that contain triglycerides with combinations of MCFAs and long-chain fatty acids (LCFAs) esterified to the glycerol backbone; such oils are called medium- and long-chain triglycerides (MLCT), which have received regulatory approval in Japan for use in human foods. In type 2 diabetes, diet supplementation with 7% (w/w of diet) MLCT (contains ~13% MCFAs and ~87% LCFAs) led to significant decreases in mesenteric fat weight and postprandial insulin levels. Increase in mesenteric fat weight has been associated with insulin resistance; hence, the effect of MLCT has potential health benefits for blood glucose management. The MLCT diet-induced higher plasma levels of adiponectin was inversely correlated with mesenteric fat weight and plasma insulin level. The observed increased levels of plasma adiponectin may be because MCFAs can suppress adipocyte hypertrophy. Adiponectin is known to increase AMPK, an enzyme that increases muscle sensitivity to glucose uptake.

2.4 Long-Chain Fatty Acids

These are fatty acids with 14 or more linearly arranged carbon atoms and may be saturated (no double bonds) or unsaturated (one or more double bonds). These fatty acids are found mostly as components of the triglycerides of edible oils and fats.

2.4.1 Monounsaturated Fatty Acids

Feeding monkeys with a diet rich in oleic acid (60% of the fatty acids) led to up to 17% reduction in plasma total cholesterol concentrations when compared to the group that was fed a diet that was predominantly rich in saturated fatty acids.

Substitution of dietary monounsaturated for saturated fatty acids resulted in a 28% decrease in the Apolipoprotein B (ApoB) levels, which is due to lower production rates of LDL ApoB. There were also less amounts of circulating LDL

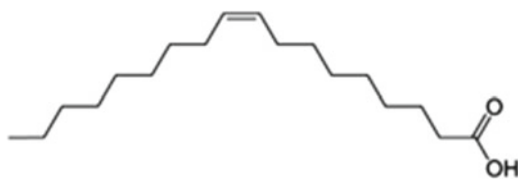


Fig. 2.1 Structural configuration of *cis*-oleic acid

particles in the plasma. Oleic acid (Fig. 2.1) has also been proposed to have a potential role in decreasing brain-related disorders such as dementia and Alzheimer's disease. Among unsaturated fatty acids tested, oleic acid had the highest *in vitro* inhibition of prolyl endopeptidase (PEP), an enzyme that is believed to have a role in amyloid formation in the brain. Affinities of fatty acids for PEP as measured by inhibition constants (inversely related to affinity) were ~27, 51, 89, 91, and 248 μM , respectively, for oleic, linoleic, docosahexaenoic, arachidonic, and eicosapentaenoic acids. PEP levels have been found to be upregulated in Alzheimer's disease patients, and rat experiments have shown improved cognitive functions when administered with PEP inhibitors. While *in vivo* studies are required to determine exact mechanisms of action, preliminary work suggests that consumption of oleic-acid-rich diets could have beneficial effects on brain functions by reducing activity of PEP.

Oleic acid has also been shown to have a potential role in the therapeutic management of colorectal cancer, one of the most common types of tumors, especially in western countries. The basic mechanism involves inhibition by oleic acid of the store-operated Ca^{2+} entry (SOCE) process that controls the Ca^{2+} influx pathway. Operation of the SOCE is believed to be involved in several cellular and physiological processes including cell proliferation. Therefore, attenuation of the SOCE process by oleic acid could reduce Ca^{2+} influx into cells and diminish or eliminate tumor cell proliferation. Oleic acid is able to block Ca^{2+} entry into cells, probably through free carboxylate-mediated metal chelation; this is because methylated oleic acid had no inhibitory effect on SOCE. Structural conformation of the fatty acid also seemed important contributory effect to inhibitory properties because

use of stearic acid (same carbon length but no double bond) had no effect on SOCE. Oleic acid blocks Ca^{2+} entry by binding to membrane molecules at the outer side of the membrane; therefore, it is possible that the structural conformation arising from the presence of a single double bond enhances interaction with the membrane surface. In contrast, conformation of the saturated stearic acid seems to be incompatible with the required binding protocol at the surface of the cell membrane.

2.4.2 Polyunsaturated Fatty Acids (PUFA)

Epidemiological studies have showed a low incidence of coronary heart diseases (CHD) in the Inuit population even though they consume a diet that is high in saturated fatty acid content. The low incidence of CHD was associated with the high levels of n-3 PUFAs that are also present in the mostly marine-product-laden diet of the Inuit. It is also known that a diet with high levels of linoleic and linolenic acids has better cholesterol-lowering effects when compared with a diet rich in saturated fatty acids. Unlike monounsaturated fats, a polyunsaturated-rich diet can decrease ApoB levels by a combination of reduced production and increased catabolism. ApoB is the main lipoprotein in low-density lipoproteins and is a marker of atherosclerosis and increased risk of cardiovascular damage because it is the main trafficker of cholesterol in the blood circulatory system. By decreasing blood circulating levels of ApoB, certain PUFAs can provide protection against certain cardiovascular diseases that arise from excess levels of vascular cholesterol. Increased dietary consumption of PUFAs is also associated with decreased blood levels of mediators of lipid-induced insulin resistance, increased insulin sensitivity, and enhanced leptin levels. The high plasma leptin levels reduces food intake due to appetite dampening effects. High levels of fish consumption (contains high PUFA levels) have been associated with improved immune response such as reduced risk of asthma-related symptoms and lower rate of allergic sensitization

in addition to decreased levels of proinflammatory compounds such as C-reactive proteins, interleukin-6, and prostaglandins (PG). In fact, lack of fish consumption during childhood has been linked to increased risk of asthma development.

In general, long-chain PUFAs act as antihypertensive agents and reduce the risk for adverse cardiovascular events by enhancing production of vasodilatory PGs such as PG_1 and PG_2 . For example, the metabolic products of linoleic acid (gamma-linolenic acid, GLA, and dihomo-GLA) are activators of PG_1 and PG_2 syntheses and have been found to prevent elevated blood pressure that is associated with consumption of saturated fatty acids. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are PUFAs that can reduce blood pressure and blood viscosity by enhancing formation of PGI_3 (a vasodilator and platelet anti-aggregator) and inhibiting thromboxane A₂ (TXA_2 , a potent vasoconstrictor and platelet aggregator). PUFAs also work as inhibitors of angiotensin-converting enzyme (ACE), a major enzyme responsible for increased formation of angiotensin II (a potent vasoconstrictor). The antihypertensive effects of PUFAs have also been shown to be associated with upregulation of endothelial nitric oxide production, and hypertensive patients have been shown to have low levels of PUFAs. The high level of PUFAs in human milk has been associated with reduced risk of developing hypertension in adulthood when compared to formula-fed infants that consume less amounts of PUFAs. By suppressing hypertension, PUFAs can also inhibit development of proteinuria (a marker of kidney damage) and prevent excessive proliferation of vascular smooth muscle cells through suppression of TGF- β synthesis. TGF- β is found in elevated concentrations in hypertensive patients, and interaction with angiotensin II leads to increased synthesis of extracellular matrix proteins within the kidney and aorta. Therefore, high levels of TGF- β promote renal scarring and pathological progression of end-stage renal disease in hypertensive and diabetic patients.

However, there are other various types of beneficial PUFAs, mostly the omega-3 (n-3) and omega-6 (n-6) fatty acids as well as the conjugated fatty acids.

2.4.3 Omega-3 and Omega-6 Fatty Acids

The simplest omega-6 fatty acid is linoleic acid (C18:2), while linolenic acid (C18:3) is the simplest omega-3 fatty acid. Both fatty acids have been reported to protect against cardiovascular and inflammatory diseases, though linolenic acid has greater health benefits. Typical examples of chronic diseases that have inflammation component and could benefit from increased dietary intake of omega-3 and omega-6 fatty acids include lupus, diabetes, psoriasis, obesity, Crohn's, rheumatoid arthritis, cystic fibrosis, Alzheimer's, and multiple sclerosis. While less effective than oleic acid, the omega fatty acids have been shown to reduce in vitro activity of PEP, an enzyme with potential role in the pathogenesis of brain diseases. It has been documented that blood levels of omega-3 fatty acids are inversely proportional to the risk of adverse cardiovascular events such as stroke and sudden death. In animal experiments, the ratio of omega-3 (n-3) to omega-6 (n-6) was an important determinant of ultimate health benefits. A higher ratio (more n-3 and less n-6) in the diet is more desirable as a means of improving human health such as reduced weight of intra-abdominal fat, adipocyte size, and normalization of heartbeat. This is because n-3 PUFAs are usually converted to anti-inflammatory eicosanoids while n-6 PUFAs are converted to proinflammatory eicosanoids. Thus, high levels of dietary n-3 PUFAs enhance the body's ability to reduce damaging inflammatory conditions that are known to be responsible for the initiation and growth of chronic diseases such as cancer, kidney malfunction, diabetes, and cardiovascular disorders. A human interventional trial involving >2,800 patients that survived a recent myocardial infarction (MI) showed that consumption of 1 g omega-3 fatty acid on a daily basis led to significant reduction in the cumulative rate of all-cause death and nonfatal MI. Specifically, combinations of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) with α -linolenic acid can lower the risk of fatal ischemic heart disease in older adults. Fish oil contains a high n-3:n-6 ratio and has been shown to decrease serum triglyceride and cholesterol

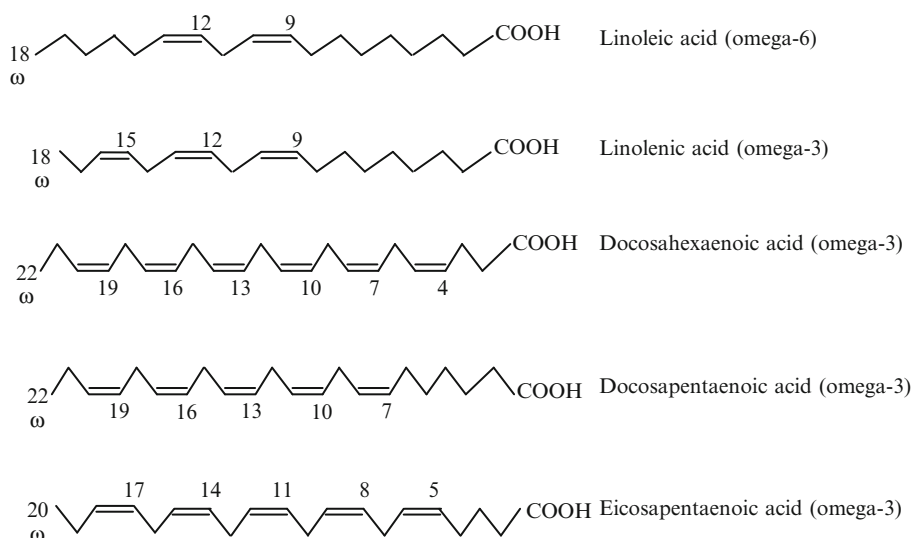


Fig. 2.2 Structural configurations of polyunsaturated fatty acids showing number of double bonds and position of the omega double bond (double bond nearest to the ω -carbon atom)

levels. However, during clinical trials, the total amount of fish oil consumed was a more important determinant of health benefits than the ratio of n-3 to n-6. Fish oil is also able to lower blood pressure in hypertensive patients. The health benefit of fish oil is due mostly to the n-3 PUFA level, especially EPA and DHA. α -Linolenic acid (ALA), which is present in vegetable sources such as rapeseed, walnuts, flaxseed, and green leafy products, may be desaturated and elongated within the human body to yield EPA, docosapentaenoic acid (DPA), and DHA. For example, daily consumption of 3 g ALA (given as flaxseed oil) resulted in 25% and 60% increases in plasma levels of DPA and EPA, respectively, but no effect on DHA level. Therefore, ALA may serve as a dietary precursor of EPA and DPA for vegetarians that do not consume fish oil products. However, ALA should not be used as the sole source of n-3 PUFAs since current evidence shows that it is only partially converted to EPA and DPA, but not DHA, especially in men. In women, partial conversion of ALA occurs probably because of less β -oxidation and role of estrogens in promoting fatty acid desaturation. The role of estrogens is evident from the fact that women using oral contraceptive pill that contains 17 α -ethynylloestradiol had a threefold higher rate of DHA synthesis

when compared to women not on the pill. Testosterone is also known to decrease DHA synthesis, which may be responsible for the reduced conversion of ALA to DHA in men. However, this effect (conversion of ALA to DHA) is not seen in lactating women, and therefore, DHA content of breast milk is influenced mostly by the fat storage during and before pregnancy, which reinforces the need for adequate nutrition in women of childbearing age. The lack of ALA to DHA conversion during lactation may be due to the action of prolactin (lactating hormone), which is a known estrogen suppressor. Preformed DHA is a critical component of a healthy diet and is recommended as a means of maintaining physiologically beneficial levels. Figure 2.2 shows the chemical structures of the most common omega-3 fatty acids.

DHA also has anti-inflammatory effects through its inhibitory effects on activation of nuclear factor kappa-B (NF- κ B). During high oxidative stress conditions within cells, the high levels of ROS activate NF- κ B by releasing it from the bound inhibitory protein (I κ B). Activated NF- κ B then moves into the nucleus where it upregulates gene expression for several proinflammatory cytokines (IL-2, IL-6, and IL-8) and nitric oxide (NO). DHA acts as an inhibitor of NF- κ B by

attenuating oxidative stress; for example, pretreatment of macrophages with DHA has been shown to reduce NO production. DHA can also inhibit NF- κ B activation through upregulation of intracellular antioxidants such as glutathione, which leads to reduction in oxidative stress. In addition to the direct effects, DHA can exhibit anti-inflammatory effects when it becomes oxidized to highly potent signaling molecules such as resolvins (resolution phase interaction products) and protectins (or neuroprotectin when present in the central nervous system), which are called docosanoids. DHA has four identified resolvins (D1–D4), while EPA has two (E1 and E2), all of which are generated through lipoxygenase-mediated oxidation of the fatty acids. Resolvins have anti-inflammatory activities; they act by downregulating activation of NF- κ B and removal of neutrophils from inflammatory sites. Protectins are DHA-oxidative products formed by peripheral blood mononuclear cells and CD4 cells in response to oxidative stress conditions. Protectins are present in peripheral blood, lung tissues, neurons, and astrocytes. Neuroprotectins have beneficial health effects such as enhanced nerve regeneration and reduced leukocyte infiltration during inflammation. Generally, neuroprotectins help to maintain homeostasis during aging by reducing proapoptotic and proinflammatory signaling. As an antioxidative agent, synthesis of neuroprotectins is enhanced during oxidative stress to protect retinal and neuronal cells from apoptosis, probably through inhibition of IL-1 β -induced induction of cyclooxygenases. These neuroprotective functions suggest that neuroprotectins (through increased dietary DHA) may be used for therapeutic management of neurodegenerative diseases, especially Alzheimer's as well as the normal cognitive decline associated with aging. Therefore, the anti-inflammatory effects of omega-3 fatty acids are mediated through various reactions such as reduced chemotactic responses of leukocytes and level of proinflammatory cytokines (via the NF- κ B route), as well as increased EPA-dependent formation of weakly inflammatory or anti-inflammatory eicosanoids. Other effects include increased DHA- and EPA-dependent formation of anti-inflammatory and inflammation-resolving

resolvins as well as reduced production of adhesion molecules (on leukocytes and endothelial cells) and proinflammatory arachidonic-acid-dependent eicosanoid mediators.

Proposed mechanisms for the cardioprotective effects of omega-3 (n-3) fatty acids:

1. Reduction in circulating plasma levels of triglycerides and bad lipoproteins.
2. Inhibition of thromboxane A2 synthesis, which leads to decrease in platelet aggregation.
3. Improved endothelial function (better control of arterial blood pressure) primarily through enhanced NO production. Secondary effects include improved vasodilation through blockage of calcium entry into vascular smooth muscle, suppression of vasoconstrictor prostanooids, and reduced plasma epinephrine level.
4. Upregulation and downregulation of genes involved in the synthesis of proteins responsible for lipid oxidation and lipid synthesis, respectively.
5. Prevention of arrhythmias and sudden death.
6. Decrease in plasma homocysteine levels, a known risk factor for cardiovascular diseases. Though mechanism is not fully understood, it is possible that omega-3 fatty acids modulate gene expression of enzymes involved in homocysteine metabolism. For example, omega-3 fatty acids are known to upregulate activity and mRNA expression of methionine adenosyl transferase (MAT), which increases cystathionine β -synthase activity that removes homocysteine from the methionine cycle.
7. DHA and EPA enhance NO (vasodilator) production by altering lipid composition such that endothelial NO synthase is displaced from its negative regulator (caveolin-1).

Some of the beneficial effects of omega-3 PUFAs are further discussed below.

(a) n-3 PUFAs and cardiac arrhythmias

- Arrhythmia: irregular or abnormal heartbeat.
- Most common fatal arrhythmia is known as ventricular fibrillation (VF).
- Studies have shown that VF can be prevented by n-3 PUFAs in cultured animal heart cells.

(b) Proposed mechanisms of anti-arrhythmic effect of n-3 PUFAs

- Incorporation and modification of myocyte cell membranes by n-3 PUFAs resulting in modulation of membrane ion channels
- Prevention of high accumulation of intracellular calcium
- Production of antithrombotic eicosanoids, which reduces the potential for plaque formation
- Influence on cell signaling mediated through phosphoinositides
- Induction of different antioxidant enzymes, which reduces level of reactive oxygen species

(c) Effects of n-3 PUFAs on blood lipid profile and atherosclerosis

- In hypertriglyceridemic patients, dietary n-3 PUFAs reduced blood triglycerides (TG) by up to 28% after 2 weeks. Longer trials (up to 16 weeks) resulted in up to 47% reduction in blood TG.
- Lower lipoprotein cholesterol, though evidence is stronger in animal studies than in human clinical trials. However, there is increase in HDL cholesterol and lowering of total cholesterol content.
- Anti-inflammatory effect, which leads to reduced platelet aggregation and inhibition of atherogenesis. Modulation of platelet aggregation is mediated through the eicosanoid pathway and is not as a result of direct effect of fatty acids on platelets. Reduced expression of cell-membrane-bound adhesion molecules also contributes to reduced potential for atherogenesis.
- Though the effect on blood pressure is minimal, DHA may be a more effective hypotensive agent than EPA through augmentation of the NO-dependent endothelium-dependent vasodilation. However, increased fish consumption leads to high levels of EPA and DHA in the blood, which favors blood pressure reduction.

(d) Effects of omega-3 (n-3) and omega-6 (n-6) PUFAs on cancer

The most commonly diagnosed type of cancer among men is prostate cancer,

which is believed to be associated with dietary factors. One of the dietary factors that have been associated with prostate cancer development is ratio of n-6/n-3 PUFAs. It is known from various in vitro and animal experiments that the two types of PUFA have different and opposite effects on cancer pathogenesis. This is because n-6 PUFAs (linoleic and arachidonic acids) promote tumor development while n-3 fatty acids (α -linolenic acid, EPA, and DHA) suppress tumor carcinogenesis. Evidence suggests that EPA and DHA have antiproliferative effects on cancer cells, but direct relationships to prostate cancer have been mixed with some researchers reporting negative while others reported positive associations. Some studies have shown that high levels of dietary linoleic acid (n-6 PUFA) are positively correlated with elevated risk of prostate cancer development. Most importantly, it is the balance of n-3 to n-6 PUFAs that is believed to be the main factor in tumor carcinogenesis. From epidemiological studies, it is known that high n-6/n-3 PUFA ratio is associated with prostate cancer risk in men, though the trend was dependent on race of the patient. In white men, high n-6/n-3 PUFA ratio was associated with risk of overall prostate cancer as well as risk of developing high-grade form of the tumor. However, in African-American men, high n-6/n-3 PUFA ratio was associated only with risk of developing high-grade form of prostate cancer tumor. The main mechanism for the beneficial effects of high dietary n-3 PUFAs on prostate cancer is believed to be through the competitive inhibition of conversion of n-6 PUFAs to proinflammatory eicosanoids. This is because n-3 and n-6 PUFAs compete for similar enzymes during eicosanoid synthesis; therefore, high levels of n-3 PUFAs will reduce catabolism of n-6 PUFAs but increase formation of anti-inflammatory eicosanoids. High oxidative state coupled with high levels of proinflammatory eicosanoids can cause damage to critical cellular components such as the DNA, which could lead to carcinogenesis.

By reducing the level of proinflammatory eicosanoids in prostate cells, the n-3 PUFAs have the potential to limit cellular damage and reduce the risk for carcinogenesis. Using adult mice colonocytes, it was shown that DHA in combination with butyrate was better than EPA/butyrate in inducing apoptosis.

Omega-3 fatty acid-containing fish oil has also been shown to induce apoptosis but confers resistance to oxidation-induced DNA damage in colonic cells. Using mice with insufficient activity of superoxide dismutase (SOD2), it was shown that fish oil can increase oxidative stress and lead to increase apoptosis of the colon cancer cells. SOD is one of the main antioxidant enzymes responsible for free radical scavenging; deficiency usually leads to increased mitochondria oxidative stress. In normal cells, high oxidative stress could cause health problems by damaging essential nutrients and cellular components. However, in abnormally growing cells like cancer cells, a high oxidative stress could be used to reduce growth and enhance apoptosis. This is because as the level of reactive oxygen species and lipoperoxides increases, eventually the mitochondria detoxification capacity is exceeded, and the resultant chronic oxidative stress triggers release of pro-apoptotic factors from the mitochondria into the cytosol. In this case, the high level of unsaturated omega-3 fatty acids coupled with the reduced SOD level can induce such pro-apoptotic condition of chronic oxidative stress.

(e) Omega-3 PUFAs, obesity, and kidney disease

Obesity continues to be an important risk factor for other chronic metabolic disorders such as insulin resistance (type 1 diabetes), hypertension, kidney function impairment, and cancer, all of which can lead to death. In a rat model (Han:SPRD-*cy*) of chronic kidney disease (CKD), supplementation of diet with omega-3-rich flaxseed oil (FO) resulted in increased whole-body bone mineral content (~1 g) and density (~0.5 g/cm²) as well as increased lean body mass (~12 g) in males

when compared to omega-3-deficient control diet that contained corn oil. The FO was only effective in increasing whole-body bone mineral content (~0.5 g) and density (~0.05 g/cm²) in the female Han:SPRD-*cy* rats. Thus, it seems that the effect of the FO differed according to gender of the rats. The results are important because decreases in bone mineral density and lean body mass as well as increase in adipose tissue mass are associated with chronic renal failure and renal transplant. The Han:SPRD-*cy* is an autosomal-dominant inheritance that is characterized by epithelial proliferation, progressive dilatation of nephrons, interstitial inflammation, oxidative injury, and fibrosis. FO also reduced some of these renal disease markers such as numbers of macrophage cells and proliferating cell nuclear antigen (inflammation) as well as oxidized LDL (oxidative injury) content. Thus, FO may serve as a suitable therapeutic intervention tool to reduce the degree of renal inflammation, oxidative injury, and severity of lean body mass losses associated with CKD. It has also been demonstrated that dietary intervention through maternal nutrition may be effective in reducing pathological intensity or progression of genetically inherited CKD. For example, supplementation of adult female Han:SPRD-*cy* rat diets with FO led to a 15% decrease in renal cyst growth, 12% decrease in cell proliferation, and 15% decrease in oxidative injury in the offsprings that inherited the disease and maintained on FO-free diet. However, the renal health benefits of FO were increased when the offsprings from FO-fed mothers were also maintained on FO diet, postweaning. FO-fed Han:SPRD-*cy* offspring rats from FO-fed mothers had reduced proteinuria (13%), creatinine clearance rate (30%), renal fibrosis (34%), and glomerular hypertrophy (23%). FO also mitigated the detrimental effects of a high-fat diet on renal fibrosis in polycystic kidney disease mice. The mechanism involved in the renoprotective effects of FO may be due to

modulation of eicosanoid synthesis in favor of less inflammatory agents. In FO-fed rats, the tissues, organs, and plasma contain higher levels of omega-3 fatty acids, especially α -linolenic acid (ALA), which is a precursor for synthesis of longer-chain omega-3 fatty acids, especially EPA. The high plasma concentrations of ALA inhibit conversion of linoleic acid to arachidonic acid (AA); therefore, concentration of EPA increases while that of AA decreases. EPA is converted slowly to the less vasoactive thromboxane A₃, while AA is converted faster to thromboxane A₂, a strong vasoconstrictor, and both fatty acids compete for the same metabolic (conversion) enzymes. Therefore, the high levels of EPA lead to competitive inhibition of AA conversion, which alters metabolic products in favor of EPA-derived eicosanoids and reduction in renal injury. Thus, FO may be used in maternal diet to alter eicosanoid production during pregnancy, which can then attenuate disease symptoms associated with inherited CKD in the offsprings.

Various experiments using nondiabetic animals have confirmed the potential use of omega-3 fatty acids as therapeutic agents to reduce plasma triglycerides and prevent (or treat) excessive accumulation of body fat and even weight gain. In diet-induced obesity, omega-3-supplemented rat diets led to a decrease in epididymal fat in addition to attenuation of the increase in retroperitoneal fat mass, which were attributed to reductions in number of mature adipocytes and adipocyte hypertrophy but not adipocyte number. It is important to note that anti-obesity of omega-3 oil-supplemented diets may be due to the content of DHA because low ratio of EPA/DHA has been shown to promote reduced accumulation of subcutaneous fat. However, canola oil has also been shown to reduce accumulation of intra-abdominal fat mass, which was associated with less adipocyte surface area. Human studies have also shown that abdominal obesity and visceral abdominal fat area are inversely related to omega-3 fatty acid content (especially DHA) of perivisceral and omental adipose tissues.

Similarly, the size of adipocyte present in the subcutaneous adipose tissue was inversely related to the content of omega-3 fatty acids. In non-obese, healthy adults with BMI of 20–40 kg/m², plasma omega-3 fatty acid levels were found to be inversely proportional to waist and hip circumferences as well as BMI, which indicates a protective role against obesity for this group of fatty acids. However, supplementation of diabetic mice diet actually exacerbated weight gain, suggesting that impaired glucose control may nullify the weight-reducing effects of omega-3 fatty acids. Apart from weight gain prevention, there has been a limited study on the role of omega-3 fatty acids in established obesity. Dietary omega-3 fatty acid was shown to reduce body fat mass in mice that were made obese through intake of high-fat diet. The loss in weight was attributed to reduced metabolic efficiency because of the decreased food efficiency (lowest weight gain per unit energy intake) in obese mice that whose diet was switched to omega-3-supplemented feed. In humans, the mechanism involved in omega-3 fatty acid-induced weight loss has been shown to involve decreased appetite, modulation of lipogenic gene expression, and tissue metabolism. Human subjects on omega-3-supplemented diets have been shown to consume less amount of food than equivalents on control diets. It is believed that omega-3 fatty acids can increase postprandial satiety in overweight and obese individuals, which reduces food intake and calories and which enhances body weight loss and improved body composition. Increased β -oxidation of fatty acids through upregulation of mitochondrial carnitine palmitoyl transferase 1 (CPT-1) has been shown to be associated with dietary omega-3 fatty acids. CPT-1 exchanges coenzyme A for carnitine, which then facilitates movement of fatty acids into the mitochondria for β -oxidation. Mitochondrial expression of CPT-1 is regulated by peroxisome proliferator-activated receptors (PPARs) and by AMP-activated protein kinase (AMPK). AMPK is activated by EPA in adipose tissue and skeletal muscle, which then leads to upregulation of CPT-1 expression and increased fatty acid oxidation in the mitochondria. This metabolic regulation of

fatty acid oxidation has been demonstrated in rat feeding experiments where supplementation of the diet with EPA-rich fish (menhaden) oil led to significant increase in skeletal muscle mitochondrial CPT-1 activity and reduced sensitivity to inhibitors when compared to low EPA types of oils. The lower sensitivity of CPT-1 ensures increased lipid oxidation even in the presence of other dietary factors that may act as inhibitors.

Another mechanism proposed for the adipose tissue effects (decreased fat deposition) of omega-3 fatty acids is through increased expression of uncoupling protein 3 (UCP-3) mRNA in the skeletal muscle coupled with increased expression of peroxisomal acyl-CoA oxidase (PACO) in liver, heart, and skeletal muscle. However, the PACO pathway is less efficient for energy production because it produces 30–40% more heat and 30% less ATP when compared to mitochondrial β -oxidation. The UCP-3 reduces mitochondrial oxidative phosphorylation efficiency because it induces leakage of protons from the mitochondria, which leads to less ATP formation but more heat production. Thus, the combined effects of PACO and UCP-3 lead to overall reduction in metabolic efficiency and contributes to decreased stored fat energy (due to decreased availability of fatty acids) but increase energy losses in the form of heat. The ability of omega-3 fatty acids to reduce adipose tissue weight can also be linked to upregulation of intestinal lipid oxidation. This is because dietary omega-3 fatty acids have been shown to increase intestinal expression of mRNAs for lipid-oxidizing agents such as CPT-1a, cytochrome P450 4A10, and malic enzymes. In addition to the indirect effects on skeletal muscle, intestinal tissue, heart, and liver, there is evidence for the direct effects of omega-3 fatty acids on adipose tissue in the form of increased fat oxidative and decreased fat synthesis capacities in visceral fat depots. The increased fat oxidation in visceral fats resulted from upregulated levels of peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1 α) and nuclear respiratory factor-1 (NRF-1) that regulate mitochondria biogenesis, as well as CPT-1 that regulates fatty transfer into the mitochondria for β -oxidation.

There was also reduced mRNA expression level of stearoyl-CoA desaturase (a lipogenic enzyme), which led to reduced fat synthesis that was associated with the omega-3-supplemented diet. It has been hypothesized that the increased flow of fatty acids into the skeletal muscle for mitochondrial oxidation could have been due to the fact that omega-3 fatty acids enhance vasodilation and blood flow. The increased vascular blood flow enhances nutrient delivery to the skeletal muscles, where the nutrients are utilized for energy production, but reduces availability of nutrients for fat synthesis and storage in the adipose tissue.

Animal studies have shown the ability of omega-3 fatty acids to alter the metabolic pathways in skeletal muscle and promote protein synthesis to maintain lean muscle mass. Increased protein synthesis required higher metabolic rate and fatty acid utilization (to produce ATP), which could indirectly contribute to reductions in adipose tissue mass. For example, EPA is a known indirect suppressor of the ubiquitin-proteasome pathway that is critical for muscle proteolysis to occur. This is because EPA attenuates activation of the transcription factor, NF- κ B, a positive modulator of the ubiquitin-proteasome pathway. Increased dietary omega-3 levels promote muscle protein synthesis by activating key protein synthesis regulatory kinases such as mammalian (mechanistic) target of rapamycin (mTOR) and S6K. While the specific effects of DHA have been reported, the anti-obesity activity of individual omega-3 fatty acids is not fully elucidated. Therefore, the observed beneficial effects of omega-3 oils on weight reduction could be due to summation of individual effects or synergistic interactions. Overall, the net effect of the inhibition of protein degradation and promotion of protein synthesis is decreased substrate for building adipose tissue mass and hence reduced weight gain.

Potential adverse effects of omega-3 fatty acids

1. At very high dietary doses (>20 g/day) of omega-3 fatty acids, there is the potential for increased bleeding times that is not seen with moderate (2–5 g/day) consumption.

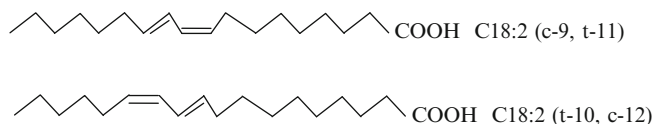


Fig. 2.3 Structures of the two main forms of conjugated linoleic acid (CLA) present in food product

2. High dietary doses may also lead to increase (<5%) in plasma LDL concentration. It is recommended that administration of an HMG-CoA reductase inhibitor (e.g., statins) could be used to offset the increase in LDL concentration. Inhibition of HMG-CoA reductase leads to upregulated expression of liver LDL receptors, which enhances uptake of LDL from the plasma and subsequent catabolism.
3. In some people, consumption of oils or foods that are rich in omega-3 fatty acids may induce gastrointestinal discomfort (bloating, belching, stomach upset) and nausea.

2.4.3.1 Conjugated Linoleic Acid (CLA)

CLA is a mixture of positional and geometrical isomers of linoleic acid formed by rumen micro-organisms. CLA occurs naturally and can be found at low levels in ruminant fats such as beef tallow and milk fat. CLA can also be synthesized from linoleic acid or vegetable oils that have high levels of linoleic acid such as corn, canola, soybean, safflower, and sunflower. The principal bioactive dietary CLA is *cis(c)*-9, *trans(t)*-11 isomer, which is present at 73–94% content of the total CLA in milk, dairy products, meat, and processed meat products of ruminant origin. The following 17 natural CLA isomers have been detected in food products, though *c*9, *t*11 and *t*10, *c*12 isomers (Fig. 2.3) are the predominant forms found in food products:

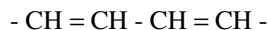
*c*9, *t*11; *t*10, *c*12; *c*9, *c*11; *c*7, *t*9; *c*11, *t*13; *c*8, *t*10; *c*10, *t*12; *c*11, *c*13; *c*12, *t*14; *t*9, *t*11; *t*8, *t*10; *t*7, *t*9; *t*7, *c*9; *t*12, *t*14; *t*11, *t*13; *t*6, *t*8; *t*11, *c*13

The main structural features and potential physiological functions of CLAs are as follows:

- In linoleic acid, the two double bonds are separated by two single bonds:



- In conjugated linoleic acid, the two double bonds are separated by one single bond:



- CLA is an essential fatty acid found mostly in animal products such as milk and meat.
- Meat of ruminants – cows, sheep, and other animals – that chew the cud contains more CLA than nonruminant meats such as turkey, chicken, and pork.
- This is because bacteria in the stomach of ruminants convert linoleic acid to CLA, which is absorbed into the animal tissue.
- Nonruminants do not have this type of bacteria, so they cannot produce CLA.
- Has been found to suppress atherosclerosis (plaque formation that leads to heart attack) in blood vessels.
- Potential anticarcinogenic effect has been shown in rats.
- A year 2000 survey determined that women with the most CLA in their diets had a 60% reduction in the risk of breast cancer.
- Has been shown to reduce fat mass and preserve muscle tissue in rats. Human benefit for weight loss has not been completely demonstrated.
- Lowers insulin resistance which may help prevent adult-onset of diabetes.
- Enhances immunity and resistance to infections.
- Milk fat contains 5–7 mg/g of CLA.
- CLA has been shown to inhibit growth of tumors in experimental rats.
- The 9-*cis*,11-*trans* isomer is believed to be the most biologically active.
- Tumor growth inhibition may be due to ability of CLA to inhibit protein and nucleotide biosynthesis.

Proposed mechanisms of CLA action: there are contradictory reports in literature on the mode of action of CLA, though the following seem to be the most plausible among several others:

- Indirect antioxidant property through direct scavenging of free radicals and inhibition of lipid peroxidation. CLA also upregulates vitamin E level, a potent antioxidant. Thus, CLA protects membranes and tissues from destructive oxidative stress by maintaining structural integrity of essential fatty acids, which also enhances membrane fluidity.
- Inhibition of carcinogen-DNA adduct formation.
- Induction of apoptosis.
- Modulation of tissue fatty acid composition and eicosanoid metabolism; CLA inhibits activities of lipooxygenase (leads to reduced formation of leukotrienes) and cyclooxygenase (leads to reduced formation of prostaglandins). This leads to reductions in signal transduction and cellular activities.
- Inhibition of hepatic 3-hydroxyl 3-methyl glutaryl CoA (HMG-CoA) reductase activity, which attenuates cholesterol synthesis with beneficial effects on plasma cholesterol content.
- Increases activity of enzymes involved in fatty acid oxidation (e.g., carnitine palmitoyl transferase) while decreasing fatty acid synthesis through inhibition of fatty acid synthase activity.
- Affects expression and action of cytokines and growth factors.
- Regulation of certain nuclear receptors involved in the control of body weight and adiposity either by reducing level of expression or translational ability of the genes that code for these receptors.

However, the potential modulation of chronic kidney disease by CLA isomers has received considerable attention, and promising effects have been demonstrated. In adult male Han:SPRD-cy rats with advanced kidney disease, diet supplementation with 1% CLA mixture (52% c9, t11: 3% t10, c12: 40% other geometrical isomers) led to significant decreases in oxidative damage (30%), proliferating cells (28%), inflammation

(42%), and fibrosis (28%). There was also a significant decrease in production of parathyroid hormone (PTH), which is normally elevated in this rat model of CKD. PTH is known to induce bone loss during kidney disease. However, the CLA diet did not produce any significant effect on renal function, which suggests that reductions in inflammation and oxidative damage markers alone may not be sufficient to prevent deterioration of renal functions. In obesity-associated kidney disease, dietary CLA reduced pathological symptoms such as kidney weight (7%), glomeruli size (20%), and COX-2 protein levels (39%). In the obese rats, dietary CLA was also effective in preserving pancreatic islets, improving peripheral utilization of glucose, and reducing level of inflammatory agents. CLA reduced adipocyte size, hepatic steatosis, urinary albumin, and plasma lipids, but liver function was improved.

2.4.3.2 Conjugated Eicosapentaenoic Acid (CEPA)

CEPA can be prepared by alkaline treatment of eicosapentaenoic acid (EPA) and has been shown to induce strong and selective in vitro apoptosis of tumor cells through a lipid peroxidation mechanism. In animal experiments, CEPA had stronger antitumor effects, especially by preventing development of new blood vessels (angiogenesis) when compared to CLA and EPA. Through this mechanism, CEPA acts by cutting off the flow of nutrients into the developing tumor cells; the malnourished cells will eventually die off and scavenged by macrophages. In order to prevent angiogenesis, CEPA inhibits secretion and mRNA expression of matrix metalloproteinases (MMP), in particular MMP2 and MMP9 as shown in tissue culture experiments. This is because MMP are a group of enzymes that cause degradation of sub-endothelial basement membrane and surrounding extracellular matrix, which is then followed by migration and proliferation of the endothelial cells to form new vessels. Since MMP are key factors in angiogenesis, their inhibition by CEPA limits cell migration and provides a therapeutic approach to preventing growth and metastasis of tumors. It has also been suggested that CEPA may act as an antitumor agent by

promoting increased lipid peroxidation in tumor cells. These tumor cells are known to have less antioxidant defense than normal cells; therefore, CEPA can cause accumulation of high levels of lipid peroxides, which eventually becomes toxic and lead to apoptotic cell death in the tumor. This is evident by the fact that CEPA had no effect on the proliferation of normal cells. Another potential mechanism for the anticancer activity of CEPA is through direct inhibition of topoisomerases, enzymes that are involved in DNA replication. Using in vitro methods, it has been shown that CEPA binds to topoisomerases I and II, which prevents interaction of the enzymes with DNA strands and hence reduced ability for cell division and proliferation. CEPA also caused arrest of cell replication at the G1/S-phase and prevented incorporation of thymidine into the cells, thus blocking the primary step of DNA replication by inhibiting activity of DNA polymerases. The arrest of G1/S-phase cell replication is due to CEPA-dependent enhanced levels of cyclins A and E; excessive cyclin levels may be due to inhibition of polymerase activity by CEPA. These anti-replication effects of CEPA led to increased apoptosis of the tumor cells.

CEPA has also been investigated for potential anti-obesity effects with promising data on total lipid reduction and attenuated adipose tissue growth. In rats, dietary CEPA was associated with reduced body weight and epididymal adipose tissue mass, a visceral white adipose tissue. Plasma levels of free fatty acid (FFA), triglycerides, total cholesterol, and TNF- α (a known inducer of insulin resistance) were significantly lowered by dietary treatment of rats with CEPA. Adipocytes grow in size by increasing intracellular accumulation of lipids in addition to secreting large amounts of TNF- α and FFA; therefore, CEPA reduced adipose tissue weight by attenuating cellular activities of adipocytes. Analysis of hepatic enzymes showed that rats that consumed CEPA diets had higher levels of lipid-catabolizing enzymes but reduced levels of lipid-synthesizing enzymes. For example, activity of fatty acid synthase and malic acid was significantly reduced, while activity of acyl-CoA oxidase (rate-limiting enzyme in hepatic fatty acid β -oxidation) was

significantly increased. However, further tests with humans are required to confirm efficacy, required dosage, and safety levels of CEPA with respect to obesity prevention.

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