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STRUCTURED LIPIDS: SYNTHESIS AND APPLICATIONS

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ABSTRACT

Structured lipids (SL) are defined as triacylglycerols (TAG) restructured or modified to change the fatty acid composition and/or their positional distribution in glycerol molecules by chemical or enzymatic processes. SL may provide the most effective means of delivering desired fatty acids for nutritive or therapeutic purposes. In this review, the synthesis and applications of structured lipids, nutritional/clinical aspects of fatty acids, immobilization, and characteristics of lipases are discussed.

WHAT ARE STRUCTURED LIPIDS?

Structured lipids (SL) can be defined as triacylglycerols (TAG) restructured or modified to change the fatty acid composition and/or their positional distribution in glycerol molecules by chemical or enzymatic processes. SL may provide the most effective means of delivering desired fatty acids for nutritive or therapeutic purposes, targeting specific diseases and metabolic conditions (1). SL can also be synthesized to improve or change the physical and/or chemical characteristics of

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TAG such as melting point, solid fat contents, iodine, and saponification number. The concept of SL as a new type of lipids for nutritional and medical purposes in patients was introduced by Babayan (2). SL, sometimes referred to as "nutraceuticals" or "pharmafoods," can be produced through biotechnology by lipase-catalyzed transesterification reactions to incorporate desirable fatty acids for nutritional, medical, and food applications. Because a simple physical mixture results in retention of the original absorption rates of the individual TAG, the different structural composition of TAG between SL and simple physical mixture may lead to different hydrolysis and absorption rates, resulting in different metabolic fates (3,4).

There is a need for the enzymatic production of these specialty lipids. They can be produced from short-chain, medium-chain, long-chain TAG, any vegetable or animal fats, through biotechnology by lipase-catalyzed reactions with desirable acyl moieties or their esters, for potential use in food and nutrition. Enzyme modified lipids are currently receiving industry and research attention.

Modification with lipase provides a useful way to improve the properties of TAG. Through enzymatic transesterification, it is possible to incorporate a desired acyl group onto a specific position of the TAG, whereas chemical transesterification does not possess this regiospecificity due to the random nature of the reaction. Thus, lipase-catalyzed transesterification can provide regio- or stereospecific SL for nutritional, medical, and food applications (1,5).

SYNTHESIS OF STRUCTURED LIPIDS

SL can provide medium-chain fatty acids (MCFA) as a quick energy source and long-chain fatty acids (LCFA) as essential fatty acids to hospital patients (Fig. 1) (6).

To produce SL, chemical or enzymatic reactions such as direct esterification, acidolysis, alcoholysis, or interesterification can be used depending on the types of substrates available.

Direct esterification:

$$R_1\text{-CO-OH} + \text{R-OH} \rightarrow R_1\text{-CO-OR} + H_2O$$

Acidolysis:

$$R_1\text{-CO-OR} + R_2\text{-CO-OH} \rightarrow R_2\text{-CO-OR} + R_1\text{-CO-OH}$$

Alcoholysis:

$$\text{R-CO-OR}_1 + R_2\text{-OH} \rightarrow \text{R-CO-OR}_2 + R_1\text{-OH}$$

Interesterification (ester-interchange):

$$R_1\text{-CO-OR}_2 + R_3\text{-CO-OR}_4 \rightarrow R_1\text{-CO-OR}_4 + R_3\text{-CO-OR}_2$$

SL can be produced with lipases in organic solvent, where substrates are soluble, and hydrolysis (reverse reaction) can be minimized. It can also be produced

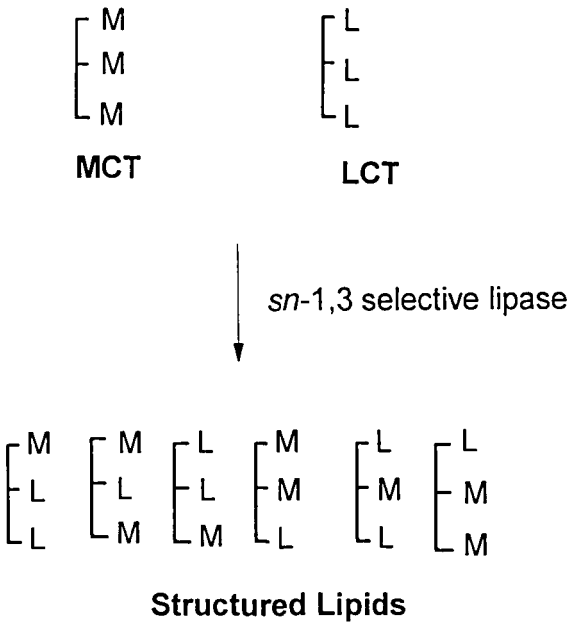


Figure 1. Scheme for producing structured lipids from a long-chain and medium-chain TAG. Where M = medium-chain fatty acid; L = long-chain fatty acid; MCT = medium-chain triacylglycerol; LCT = long-chain triacylglycerol.

in the absence of any solvent with lipases. Transesterification using *sn*-1,3 specific lipase results in the *sn*-2 fatty acids remaining almost intact in the resulting TAG. This is significant from a nutritional point of view because the 2-monoacylglycerol (2-MAG) produced by pancreatic lipase digestion are the main carriers of fatty acids through the intestinal wall (7).

SL can be produced with immobilized lipase, IM 60 (Novo Nordisk Biochem North America, Franklinton, NC), which is *sn*-1,3 specific. Soybean oil contains linoleic acid as a major fatty acid and the major TAGs are LLL (trilinolein), LLO (1,2-dilinoleoyl-3-oleoyl glycerol), and LLP (1,2-dilinoleoyl-3-palmitoyl glycerol) where linoleic acid, an essential fatty acid, is positioned at the *sn*-2 position. Soybean oil, caprylic acid, and immobilized enzyme from *Rhizomucor miehei* (IM 60) were mixed with solvent and transesterified at 55°C in a shaking water bath. After the reaction, solvent was evaporated and products were distilled to obtain synthesized SL (Fig. 2) (Lee and Akoh, 1997, unpublished data). The stereospecific positions of fatty acids in glycerol molecules are important for metabolic as well as physical properties of SL. In the study by Ikeda et al. (3), the lymphatic absorption of 2-octanoyl-1,3-dilinoleoyl-glycerol (18:2/8:0/18:2), 2-linoleoyl-1,3-dioctanoyl-glycerol (8:0/18:2/8:0), and tricaprylin (8:0/8:0/8:0) were compared and

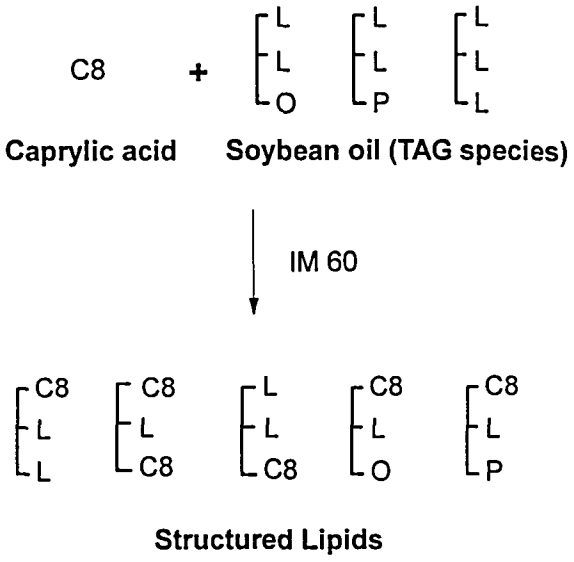


Figure 2. The possible structures of the major TAG molecules in soybean oil and structured lipids of soybean oil containing medium-chain fatty acid. Where C8 = caprylic acid; L = linoleic acid; O = oleic acid; P = palmitic acid. The reaction was performed with immobilized lipase (IM 60) from *Rhizomucor miehei* at 55°C for 24 h.

the lymphatic absorption of caprylic acid (8:0) were in the following order: 18:2/8:0/18:2 > 8:0/18:2/8:0 > 8:0/8:0/8:0, which may suggest that most 8:0 from tricaprylin was transported directly to the liver, not through the lymphatic system. However, 8:0 in SL (18:2/8:0/18:2) were transported via the lymphatic system as *sn*-2 MAG. When the rate of hydrolysis was compared between 2-oleoyl-1,3-dioctanoyl (MLM) and long-chain triacylglycerol (LCT) (soybean oil), MLM was more rapidly hydrolyzed by rat pancreatic lipase. Furthermore, when the rate was compared between MLM and MCT (Captex 810), a higher hydrolysis rate was observed in MLM, where the MCFAs were positioned at *sn*-1,3 and long chain (oleic acid) was at the *sn*-2 position. It was also suggested that 2-MAG is a well-absorbed form of most fatty acids since it readily forms mixed micelles with bile salts (8).

According to Mattson and Volpenhein (9), the fatty acid at the *sn*-2 position seemed to be conserved within the 2-MAG during fat absorption. Kodali et al. (10) suggested that this conservation may be due to slow acyl migration at 37°C, i.e., body temperature. Thus, after absorption into enterocytes, fatty acid in 2-MAG were predominantly maintained in TAG in chylomicron (11). The hypocholesterolemic effects of mono- or polyunsaturated fatty acids have been demonstrated. But these effects were significantly reduced when these fatty acids were not occupying the *sn*-2 position (11). Therefore, the positional distribution in

TAG molecule as well as saturation and chain length of fatty acids are important considerations regarding the metabolic effects of lipids.

Chemical interesterification, which is usually conducted at relatively high temperature, and the presence of catalyst do not yield regiospecific products because of the random nature of the reaction. Ray and Bhattacharyya (12) reported that lipase-catalyzed interesterified lipids, performed at 60°C showed a different distribution of fatty acids at the *sn*-2 position from the chemically interesterified lipids. The chemical interesterification was conducted at 90°C in nitrogen atmosphere and with 0.2% sodium methoxide as catalyst.

Lipases were used to change the properties of tallow with sunflower oil (13) and rapeseed oil (14). Enrichment of n-3 fatty acid (EPA or DHA) or other desirable acyl group in fish oil (15), phospholipids (16,17), and vegetable oils (18,19) have been reported.

LIPASES IN LIPID MODIFICATION

Lipases can be used for the modification of lipids. Lipases occur widely in nature and are active at oil/water interface in heterogenous reaction system. Most lipases have their substrate selectivity according to chain length, unsaturation, and positional distribution. Lipases can also transesterify lipids or fats to synthesize new compounds under certain conditions (20,21). Macrae (22) suggested that lipase can catalyze lipid modification reaction instead of hydrolysis when the concentration of water is maintained at levels less than 1%. Immobilized lipase from *Rhizomucor miehei* (IM 20) had the highest activity for esterification at water activity 0.12, suggesting that an essential amount of water is needed for catalytic activity (23). Derewenda et al. (24) reported the presence of 230 water molecules in lipase from *Rhizomucor miehei* using x-ray crystallographic methods. However, there is a limit to water concentration in reaction systems and no further increase in initial interesterification reaction rate or even reverse reaction (hydrolysis) can occur beyond that limit (25,26).

Lipases are enzymes that preferentially catalyze the hydrolysis and synthesis of esters and TAG. Some lipases exhibit substrate selectivity. The interesterification rate of heptadecanoic acid with TAG of ucuhuba seeds was about 40% higher than that of the methyl ester (methyl heptadecanoate) after an 8-h reaction with lipase from *Rhizomucor miehei* (27). Kuo and Parkin (28) reported the order of initial rate of acyl exchange in butteroil catalyzed by pancreatic lipase as: glycerol esters > fatty acid methyl esters > fatty acids. Lipase from *Penicillium camembertii* U-150 can hydrolyze mono- and diacylglycerols but not TAG (29).

TAG with lower molecular weight fatty acids such as tributyrin, tricaproin, tricaprin, and trilaurin were hydrolyzed more easily with lipase from *Penicillium caseicolum* than those with higher molecular weight fatty acids (30). Similar results were reported when monoacylglycerols (MAG) with different chain lengths

were hydrolyzed with lipoprotein lipase (31). In this study, the preferential reactivity for lipoprotein lipase was C4 > C6 > C8 when tributyl-, trihexanoyl-, and trioctanoylglycerol were used as substrates. It was suggested that observed preferential reactivity may be partly attributed to the accessibility of long-chain triacylglycerol (LCT) to enzyme active site. Also, increased acyl-chain length may result in the restriction of the movement of substrate due to the interaction between hydrophobic surface of active site pocket and hydrocarbon tail of fatty acid, resulting in strong substrate binding with lipase accompanied by a lower Km (31).

Lipase from *Geotrichum candidum* have shown preference to the unsaturated substrates with a double bond at the 9-position but there was no preference between 2-oleoyl-1,3-dipalmitoylglycerol and 1-oleoyl-2,3-dipalmitoylglycerol to hydrolyze 18:1, suggesting that this lipase does not have positional selectivity (32). When cis- and trans- form of 18:1 in 1-elaidate-2,3-dioleate were compared for lipolysis, lipase from *Geotrichum candidum* preferentially hydrolyzed the cis-form to free fatty acid (33). Substrates with too short acyl-chain length and too few double bonds cannot release enough energy, which may be used for conformational change of lipase to form an efficient structure so that substrates can be properly oriented to the active site of lipase. It was reported that lipases from *Rhizomucor miehei* showed bell shape distribution for acyl-chain length in ester synthesis reaction with maximum around C4–C6 (34). Recently, Villeneuve and Foglia (35) discussed lipase specificities to substrates, regioselectivities or stereospecificities and their potential applications in their review article.

IMMOBILIZATION OF LIPASES

Immobilization of lipase provides some benefits for their industrial application. Immobilization could increase their stability against pH and heat (36). It also allows for easy recovery and reuse of the lipase, resulting in reduced production cost. Immobilized enzymes are easily removed from the reaction mixture without any heat or conditions where denaturation of enzyme may occur. In addition, enzymatic reactions occur at mild conditions. However, it may lead to some loss of enzyme activity compared to native form due to structural perturbations of enzyme and decreased accessibility of substrates to the enzyme active site. Ogiso et al. (37) used a lipase from *Mucor javanicus* immobilized on various carriers such as amino ethyl (AE)-cellulose, DEAE-cellulose, and agarose to compare the pH and temperature stability, and substrate specificity with the soluble enzyme. Among the immobilized lipases, the lipase on DEAE-cellulose (specific activity; 11.4 units/mg of enzyme) had the highest activity even though the soluble lipase had 331.6 units/mg of enzyme. Immobilization on DEAE-cellulose was the most stable, active at a high temperature, wide range of pH, without change in substrate specificity. When the lipoprotein lipase was immobilized on polyacrolein micropheres,

the immobilized lipase showed a greater stability at various pH values (pH 5–10) than unimmobilized lipase even after 11 repeated reactions (38).

According to Kimura et al. (39), lipases immobilized on hydrophobic materials showed higher hydrolysis activity than those immobilized on hydrophilic materials. Generally, high losses of lipase activity are observed with adsorption on hydrophilic supports (e.g., silica gel, cellulose, ethyl cellulose, etc.) due to a conformational change of lipase, stearic hinderance by the carrier, or decreased access of hydrophobic substrates to the active site of lipase (40). Thermal and chemical stability, internal structure, surface area, pore size, and high affinity of supports should always be considered during immobilization. The selection of immobilization techniques as well as supports are important. Physical adsorption, ionic binding, cross linking, covalent binding, entrapment, and microencapsulation are available methods for lipase immobilization. Among them, physical adsorption is the easiest and the procedure is relatively simple. Adsorption of a lipase onto a carrier is achieved through relatively weak binding forces such as hydrogen bonds, Van der Waals forces, and/or hydrophobic interactions, etc. There is a little or minimal chance of a structural change in enzymes on immobilization. However, weak binding between protein and support is reversible and can cause the desorption of enzymes from the support during the process. Ionic binding occurs between charged groups of enzyme and ion-exchange resin containing counter ions as supports. Ionic binding is much stronger than physical adsorption and the immobilization procedure is as simple as in physical adsorption. Conformational change of enzyme can also be minimized with this method. DEAE-cellulose, DEAE-sephadex, Duolite, carboxylic acid ion-exchange resin, and macroporous ion-exchange resins can be used as supports. Covalent binding of functional groups of enzymes to an insoluble support is another method. The reaction may involve alkylation, arylation, amide bond formation, Schiff's base formation, and Ugi reaction, etc. Even though desorption can be minimized by relatively strong binding between enzyme and support, some activity of enzyme can be lost because binding procedures are usually complicated, require chemicals and, are conducted in less mild conditions where loss of catalytic site that is essential for enzyme activity or change of conformation, denaturation, can be occurring. Enzymes can be cross-linked with bifunctional reagents such as glutaraldehyde, forming relatively strong binding. However, cross-linking may not be suitable for large-scale industrial applications because mechanical stability may not be achieved (40–42).

LIPASES IN ORGANIC SOLVENT AND THEIR STRUCTURE

Because water participates in bonding to maintain the conformation of enzyme molecule, removal of too much water may lead to loss of activity. Also, it seems that enzyme can function in nonaqueous or low water environment if the essential water layer around enzyme is not stripped off. Such essential layer may act as the

primary component of the enzymic microenvironment and as a buffer between the enzyme surface and the reaction medium. Thus, nonpolar solvents are better than polar solvents because polar solvents can be water miscible and strip off the essential water layer from the enzyme (43). As the temperature is increased, the enzyme molecule unfolds reversibly by destruction of bonds, such as S-S linkages, and may lead to the hydrolysis of peptide bonds and deamidation of asparagine (A*sn*) and glutamine (Gln) residues. These processes require water. Thus, these processes can be avoided in water-free environment, resulting in stability of enzyme in organic solvent. It is generally known that less polar solvents give higher synthetic activity. Partition coefficient, P, between octanol and water can be used as parameter for selecting the organic solvents based on their hydrophobicity and correlated with enzyme activity in organic solvent. Solvents with high log-P values (3.5–4.5) are more hydrophobic and exhibit high enzyme activity while solvents with log-P values less than 2 exhibit little activity. Log-P values of selected organic solvents are: iso-octane (4.5), n-hexane (3.5), benzene (2.0), and acetonitrile (–0.03) (44).

Extracellular lipase from a fungus, *Rhizomucor miehei*, and human pancreatic lipase contain mixed β-pleated sheets (predominantly parallel) and their active centers were found to be buried. Human pancreatic lipase consists of a single polypeptide of 449 amino acids. When Ser 152, which is part of the Asp-His-Ser triad was chemically modified, the lipase lost its activity, indicating that Ser 152 is an essential component of the active site. By x-ray crystallography, it was found that active site was covered by a surface loop structure which acts as a lid, and the active site is exposed when enzyme is in contact with oil–water interface, leading to conformational change of enzyme and of lid structure position (24,45).

Lipase from *Rhizomucor miehei* consists of a single polypeptide chain of 269 amino acids and three disulfide bonds to stabilize the molecule. A short helix (referred to as lid structure) covers the active site and its displacement during activation occur in response to contact with lipase and oil/water interface. This conformational change was also found in human pancreatic lipase.

The catalytic center of the lipase from *Rhizomucor miehei* is made up of three amino acids, Asp-His-Ser, and this structure is responsible for the nucleophilic attack on the ester carbonyl carbon for activation by forming enzyme-substrate intermediate when the substrate is bound to the active site of lipase (46). Once this intermediate form is made, the subsequent hydrolysis of ester bond will occur followed by the release of glyceride residue and formation of a mixed acyl complex between hydrolyzed fatty acid residue and lipase. Then, hydrolyzed fatty acid is released and another fatty acid binds to the lipase for new ester bonds with glyceride residue. The final step is a release of newly formed glyceride from lipase. During the reaction, water may be required for hydrolysis of TAG at the initial stage and the mechanism involved in this reaction is a ping-pong mechanism (Fig. 3) (26). Reyes and Hill (26) also reported that high concentration of free fatty acid used as initial substrate for acidolysis reaction may inhibit the rate of

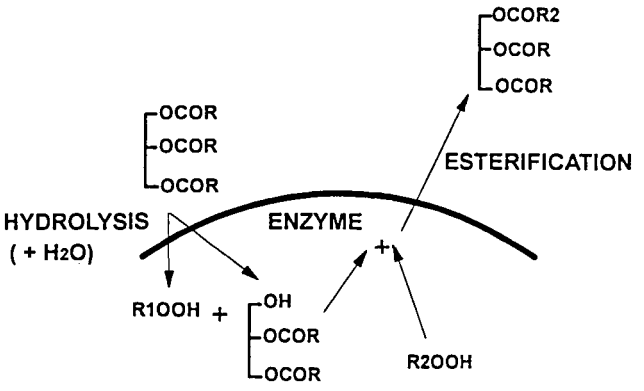


Figure 3. Ping-pong mechanism for transesterification.

hydrolysis of TAG at initial stage and higher concentration may not increase the rate of reaction.

MCFA

After hydrolysis by pancreatic lipase, as well as lingual and gastric lipases, MCFA are predominantly transported via the portal vein to the liver rather than through the lymphatic system, although there are some reports about their transportation through the lymphatic system under certain conditions; such as consumption of high levels of MCT or if MCFA is present at the *sn*-2 position (Fig. 4). There are some reports that MCFA can be incorporated into chylomicron and adipose tissue under relatively high content of MCFA in the diet (3,4,47). In the liver, MCFA are readily β-oxidized to form acetyl coA end products which are further oxidized to produce CO_2 in the Krebs cycle. Acetyl coA are used for the synthesis of ketone bodies for energy. Therefore, MCT may enhance ketogenesis and thermogenesis, causing energy dissipation as heat (48) and leading to reduction of fat deposition (49).

Because MCFA have unique characteristics, such as complete saturation, one might expect them to increase serum cholesterol concentration like other saturated fatty acids (palmitic or myristic which are hypercholesterolemic) or decrease serum cholesterol concentration due to their rapid oxidation and different transport system (50). Indeed, effects of MCFA on cholesterol concentration are debatable.

Rubin et al. (51) noted significantly increased total plasma cholesterol concentration in infants after administering by total parenteral nutrition (TPN), a 1:1 mixture of MCT and LCT compared to LCT. In the study by Cater et al. (50),

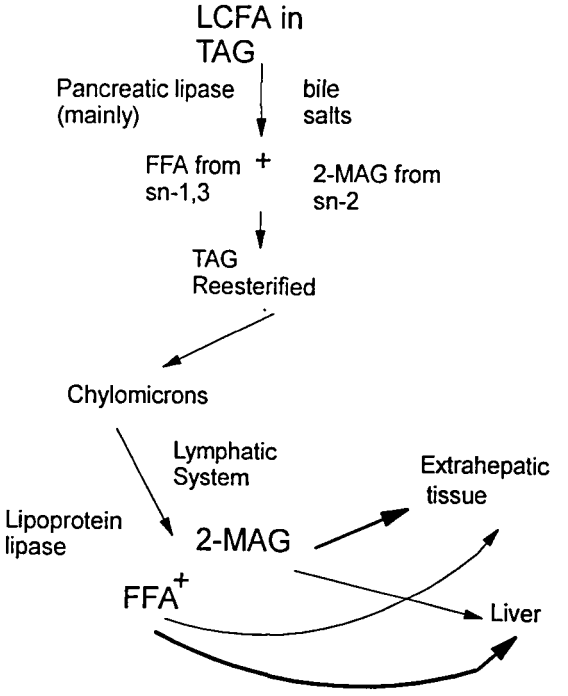
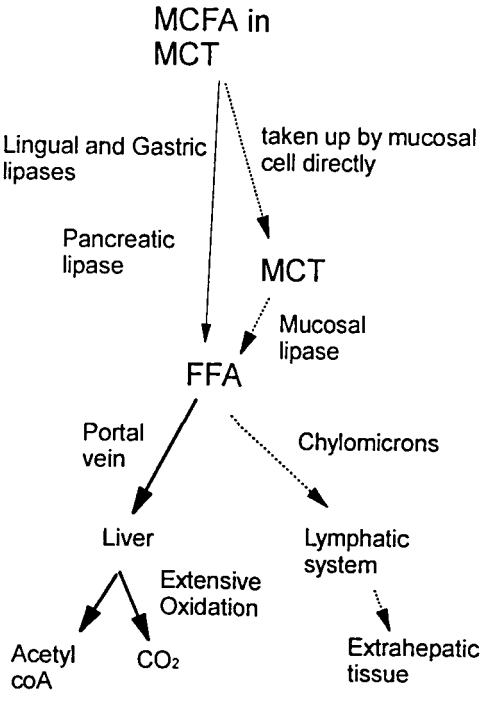


Figure 4. Digestive pathways for long-chain (a) and medium-chain (b) fatty acids. Where LCFA = long-chain fatty acid; TAG = triacylglycerol; FFA = free fatty acid; 2-MAG = 2-monoacylglycerol; MCT = medium-chain triacylglycerol; MCFA = medium-chain fatty acid.

MCT increased the concentration of total (11.1%) and LDL (12.8%) cholesterols when humans were fed fat supplements (diet with 10% of the total daily energy from fat) of palm oil (16:0, 48.3%; 18:1, 35%), sunflower oil (18:1, 87%) or MCT (8:0, 67.6%; 10:0, 32.2%). In that study, they explained that acetyl coA from MCFA oxidation are resynthesized into LCFA which behave like dietary LCFA. Further, they found elevated concentrations of 16:0 in plasma TAG in MCT-fed group, although MCT supplements did not contain much 16:0, suggesting that part of 16:0 in plasma TAG might be synthesized from MCFA. When diets (40% fat) containing MCT (8:0, 61%; 10:0, 32%) or LCT (18:1, 32%; 18:2, 51%) were provided to humans for 6 d, total serum cholesterol and TAG were high in the MCT-fed (47).

In the study by Swift et al. (52), diets containing different types of fat, MCT (8:0, 61%; 10:0, 32%), LCT (18:1, 32%; 18:2, 51%), and SL (8:0, 23.5%; 10:0, 21.2%; 18:2, 10%; 18:1, 6.7%; 22:0, 26.3%) were provided to human subjects and their effects on serum lipids were studied for 6 d. The concentration of plasma cholesterol was not significantly altered by any of the three diets but the



(b)

Figure 4. Continued.

concentration of plasma TAG was significantly increased by the MCT diet, but not changed by either SL or LCT. They explained that elevated acetyl coA from β-oxidation of MCFA may stimulate hepatic fatty acid synthesis, resulting in increased plasma TAG concentrations in MCT diet group. But, effects of MCFA on serum lipids may be offset by LCFA in SL diet group. Even though several studies are in agreement with the increase in blood TAG by MCT, other studies reported lower serum TAG concentration when the MCT or MCT + LCT mixture were provided (53).

In an earlier study, Hashim et al. (54) reported that a diet containing MCT (40% of energy from MCT) lowered total cholesterol concentration when compared to a diet containing butter in humans. Feeding of MCT to rats resulted in significantly lower serum cholesterol levels than in rats fed lard (55). In calves, MCT lowered plasma cholesterol when 30% of total dietary calories were provided from soybean oil, tallow, or MCT (56). In the study by Ball (57), patients requiring TPN were given an infusion of either Lipofundin® MCT+LCT (8:0, 27%, 10:0, 18%) or Lipofundin S® (LCT; B. Braun, Germany). There was no

significant difference on plasma TAG, but lower cholesterol levels in the blood were observed. As noted by Moyer et al. (58), pure MCT emulsion would be toxic. However, adverse effects could be alleviated by the addition of LCT. Thus, it seems that the ratio of MCT to LCT should be considered in emulsion formula. MCT may enhance ketogenesis and thermogenesis, causing energy dissipation as heat (48) which leads to a reduction of fat deposition (49). In the calorimetry study, unusual high respiratory quotient (RQ; the ratio of carbon dioxide production to oxygen consumption) was observed when MCT was used as an energy source, resembling carbohydrate utilization (59). If all energy comes from carbohydrate, RQ is near 1 and 0.7 if fat was the source of all energy. Reduced glucose level with MCT is a frequently observed phenomenon and this may be due to a decrease in glucose synthesis in the liver (11,60,61).

Clinically, MCT have some benefits. Sedman et al. (62) reported that Intralipid 20% (Kabi Vitrum, Stockholm, Sweden) and Lipofundin S 20% lipid emulsions (mainly composed of LCFA) inhibited interleukin-2 (IL2) dependent lymphocyte proliferation in vitro, more than Lipofundin MCT/LCT 20% emulsion in which MCT contributed 50% of the calories. Patients administered TPN containing 100% LCFA emulsion had significantly decreased ratio of $T_{helper}/T_{suppressor}$ (T_H/T_S) cells compared to MCT/LCT emulsion in a 1:1 ratio (63). The possible explanation of these benefits is that when long-chain polyunsaturated fatty acids (PUFA) are incorporated into cell membrane, membrane rigidity changes, resulting in downregulation of the IL2 dependent signaling (IL2 is a T-cell growth factor that regulates the immune response); or it could be due to the different metabolic pathways of MCFA and LCFA. n-6 LCFA produce prostaglandin E2 through the arachidonic acid pathway and may decrease IL2 which is believed to impair the immune response (64–66).

MCT are non-tumor promoting and anti-tumor fats (60,67). They did not show tumor-promoting effects when induced by the carcinogen, *N*-methylnitrosourea (67). In a study by Burton (68), caprylic acid showed oncolytic effects in liver of mice and rats. Currently, several commercial lipid products containing MCFA have been developed for medical and nutritional applications. For example, Advera (Ross/Abbott, Columbus, OH), NuBar Hi-Cal VM (NCI Medical Foods, Irwindale, CA), Scandishake (Scandipharm, Birmingham, AL), and Lipisorb (Mead Johnson, Evansville, IN) can be used by HIV/AIDS patients and for treatment of diverse medical conditions. Choice dm (Mead Johnson) for diabetes, Glytrol (Nestlé, Deerfield, IL) for hyperglycemia, and Impact (Sandoz, Minneapolis, MN) for trauma are commercially available (69).

n-3 FATTY ACIDS

n-3 fatty acids such as EPA (5,8,11,14,17-eicosapentaenoic acid), DHA (4,7,10,13,16,19-docosahexaenoic acid), and α-linolenic acid (9,12,15-octadecatrienoic acid) have

several health benefits to combat cardiovascular disease, immune disorders and inflammation, renal disorders, allergies (70,71), diabetes (72), and cancer (73). Eskimos in Greenland have shown lower serum cholesterol and triacylglycerol levels and lower incidence of cardiovascular disease (74). Dyerberg et al. (75) suggested that the relatively high EPA content of their diet is related to the lower incidence of cardiovascular disease. Studies with nonhuman primates and human newborns suggest that DHA is essential for the normal functioning of the retina and brain, particularly in premature infants (76). Other studies have shown that n-3 fatty acids can decrease the number and size of tumors and increase the time elapsed before the appearance of tumors (77).

Fish oil is a rich source of EPA, although the amount depends on the species of fish. Cod liver, menhaden, sardine, and anchovy contain >22% of EPA and DHA. Because of their high unsaturation, they are easily susceptible to oxidation. Tocopherols, especially γ-tocopherols, are the most effective in preventing their oxidation (70). Fish oil has been known to reduce the synthesis and secretion of TAG and the very low density lipoprotein (VLDL) from liver (66,78). This may be due to the inhibition of de novo fatty acid synthesis (79,80), increase in hepatic fatty acid oxidation (81), or increase in lipoprotein lipase (LPL) activity to remove circulating TAG (82).

Metabolically, EPA is an antagonist of the arachidonic acid cascade and competes with arachidonic acid as substrates for cyclooxygenase and lipoxygenase to produce eicosanoids (Fig. 5). EPA is used for the synthesis of eicosanoids such as series-3 prostaglandin which ameliorate immunodysfunction. Arachidonic acid may form the series-2 prostaglandin which may impair the immune function (64,66). n-3 PUFA are essential for growth and development throughout the life cycle of humans and therefore should be included in the diet. Two to 5% of n-3 PUFA in SL is considered the optimum level for clinical nutrition (83).

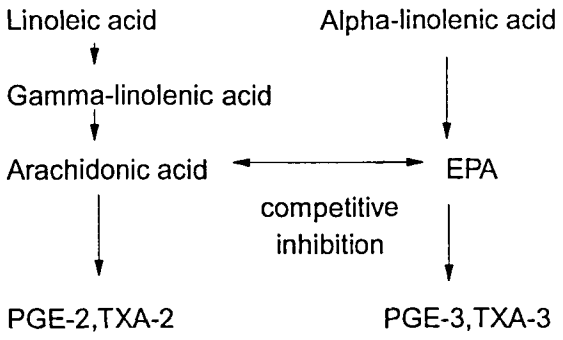


Figure 5. Different n-6 vs n-3 fatty acid metabolic pathways. Where EPA = eicosapentaenoic acid; PGE-2 = prostaglandin-2; TXA-2 = thromboxane-2; PGE-3 = prostaglandin-3; TXA-3 = thromboxane-3.

Lipid modulation of cell-mediated cytotoxic activity has been demonstrated by several investigators. Fritsche and Johnston (84) reported that feeding mice a diet rich in α-linolenic acid (18:3, n-3) can significantly enhance cell-mediated cytotoxicity when compared with diets rich in n-6. A group of female mice (BALB/c) fed a diet containing 10% (by weight) of oil (2:1 mixture of n-6:n-3 fatty acid) showed reduced levels of PGE2, increased cell yields from spleen, and increased cell-mediated cytotoxicity after viral infection. Arachidonic acids (20:4, n-6), γ-linolenic acid (18:3, n-6), and EPA (20:5, n-3) were studied for effects on murine IL2 dependent T-cell growth in vitro (85). The study demonstrated that suppression of IL2 dependent murine T-cell proliferation occurred in a dose-dependent manner, particularly with γ-linolenic acid. Arachidonic acid showed slight inhibition and EPA had no effect on suppression.

Diets containing high levels of n-6 fatty acids may increase the production of PGE2, decrease IL2 production, alter T cell response to IL2, inhibit macrophage collagenase synthesis, and enhance platelet aggregation (65,86,87). Feeding high levels of n-3 PUFA will lead to substitution of some arachidonic acid with EPA. The PGE3 formed from EPA has less inflammatory effect than PGE2. IL1 production is also lowered by n-3 PUFA while IL2 is increased (65,66).

OTHER FATTY ACIDS FOR STRUCTURED LIPIDS

Short-chain fatty acids (SCFA) range from C2 to C6. A rich dietary source is bovine milk, which has a TAG mixture containing approximately 5–10% butyric acid and 3–5% caproic acid (88,89). SCFA are more rapidly absorbed in the stomach and provide fewer calories than LCFA (acetic acid, 3.5 kcal; propionic acid, 5.0 kcal; butyric acid, 6.0 kcal; caproic acid, 7.5 kcal). Linoleic acid cannot be synthesized by humans and mammals but are essential for the production of arachidonic acid, which is the precursor of prostaglandin (PGE2), thromboxane (TXA2), and leukotrienes (LTB4). Approximately 3–4% n-6 fatty acids is needed to meet the EFA requirement (83). Oleic acid (monounsaturate; 18:1, n-9) can be biosynthesized and can reduce plasma cholesterol levels in the body (90).

On the other hand, the influence of saturated fatty acids on cholesterol levels are different depending on their chain length and the positional distribution of fatty acids in TAG molecule (11). It has been generally known that palmitic acid is more hypercholesterolemic and hyperlipidemic than lauric and myristic acid, while stearic acid seem to be neutral (11,91).

NUTRITIONAL STUDIES WITH STRUCTURED LIPIDS CONTAINING FISH OIL AND MCFA

Wall et al. (92) compared the dietary effects of MCT + soybean oil (8:0 + 10:0, 37%; 18:1, 11%; 18:2, 24%), MCT + fish oil (8:0 + 10:0, 39%; 18:1, 11%; 18:2,

22%; EPA + DHA, 0.7%) and sow milk (18:1, 38%, 16:0+18:0, 35%) on plasma and organ lipid contents in piglets and found that feeding sow milk resulted in higher concentration of plasma total cholesterol. Even though sow milk has a relatively high content of 18:1, known as hypocholesterolemic fatty acid, its cholesterol lowering effect might be off-set by 16:0, known as hypercholesterolemic fatty acid. On the other hand, higher content of hepatic TAG in MCT + soybean oil than MCT + fish oil group suggest that fish oil may inhibit the synthesis of TAG in the liver (92).

In the study by Teo et al. (93), the effects of enteral feeding with SL composed of MCT and fish oil (8:0, 36%; 10:0, 18.5%; EPA and DHA, 9.9%) and safflower oil (18:2, 80.5%) on energy metabolism were compared in burned rats. The SL was made by random transesterification of a mixture of 60% MCT and 40% menhaden oil. A decrease in total energy expenditure (7%) and improved nitrogen balance were obtained in the SL group, suggesting that SL reduced the net protein catabolic effects of burn injury. When SL (emulsion of MCT + fish oil composed of 50% MCT, 40% fish oil, and 10% canola oil) and soybean oil were provided to rats enterally, TAG and cholesterol levels in liver were lowered in the SL group (94).

SL containing caprylic and n-3 polyunsaturated fatty acids was synthesized and this enzymatically produced SL vs soybean oil (20% of diet weight) were fed to female mice for 21 d. The result showed that the concentration of total cholesterol (–49%), LDL cholesterol (–35.4%), and triacylglycerol (–53.2%) were significantly decreased in SL-fed group (Lee and Akoh, 1997, unpublished data).

CONCLUSIONS

Bioconversion of lipids or fats with lipase has become an important process to change their physical/chemical characteristics in the oil processing industry and to provide some nutritional and/or pharmaceutical benefits to the public. To lower production cost, it is preferrable that the lipases be immobilized with suitable methods and supports to ensure process stability. Screening for novel lipases from organisms or production of a thermostable or *sn*-2 specific lipase that is rare in nature through protein engineering or molecular biology are desirable for industrial application. Metabolic pathways and clinical benefits of individual fatty acids that are components of SL should be studied and accurately evaluated to design novel lipid molecules for clinical or nutritional purposes. The need to provide essential fatty acids in SL emulsion or at least in part from the diet components should also be considered. Optimization of enzymatic processes and understanding of kinetics and mechanisms of reactions are needed. Collaboration between industry and academia is vital to the successful commercialization of the enzymatic process for structured lipid production.

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