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Review

The influence of positional distribution of fatty acids in native, interesterified and structure-specific lipids on lipoprotein metabolism and atherogenesis

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The arrangement of fatty acids (FA) on the glycerol backbone of triacylglycerols (TAG) among natural fats has been indicated to be responsible for specific effects on lipoprotein metabolism and atherogenesis which may not be predicted from the unsaturated index alone. Typically, hypercholesterolemic animal fats such as bovine milk fat and lard contain mainly saturated FA on the sn-*2 position. In contrast, in most vegetable oils such as cocoa butter unsaturated FA occupy the* sn-*2 position and saturated FA are usually located in the* sn-*1 and* sn-*3 positions. Human and animal feeding studies are discussed in which the TAG stereospecific composition of the fed fats has been altered via chemical or enzymatic interesterification. These processes either rearrange FA in each TAG position to one third of its total content within a fat, or generate novel synthetic fats from the interchange of FA between discrete TAG molecules from different fat sources. Such studies have shown effects of interesterification of milk fat, lard, and peanut oil on fat absorption, plasma cholesterol, and TAG concentrations and atherogenesis. Kinetic rat studies using radiolabeled FA and cholesterol have indicated a slowed chylomicron metabolism in structure-specific TAG containing a saturated FA at the* sn-*2 position. On the other hand, negligible effects of TAG structure on chylomicron and lipoprotein metabolism have been observed in recent humans and animal studies from the feeding of interesterified forms of palm oil in which palmitic acid is enriched at the* sn-*2 position. Metabolic parameters not measured in these investigations, however, such as alterations in platelet aggregation activity and in free cholesterol concentrations at the level of serum and liver have been noted from other interesterified palm oil feeding studies pointing to the need for further research. The role of TAG structure in natural, interesterified, and structure-specific fats on FA and cholesterol absorption, chylomicron clearance, and lipoprotein metabolism is discussed as well as the confounding aspects that need consideration in terms of these studies.* © Elsevier Science Inc. 1996 (J. Nutr. Biochem. 7:530–541, 1996.)

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Introduction

Numerous clinical and animal investigations have shown that saturated fats increase circulating concentrations of serum cholesterol and triacylglycerols (TAG) and that their replacement by liquid polyunsaturated or monounsaturated oils is hypocholesterolemic.[1,2] Individual fatty acids (FA) or specific dietary fats appear to affect cholesterol metabolism via changes in the absorption, synthesis, distribution, and excretion of cholesterol.[3] However, the understanding of the nature of dietary FA or fats in influencing cholesterol metabolism has undergone constant evolution and the field continues to develop. The study of diet-lipid interactions is

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complex as different combinations of dietary cholesterol and type of fat can produce identical concentrations of total serum cholesterol with varying proportions of the concentrations of very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) cholesterol.[4] Keys et al.[5] developed a predictive equation for calculating the relative change in serum cholesterol concentrations that considered the percentage of energy derived from saturated and unsaturated FA as well as the dietary cholesterol intake. This equation indicated that saturated FA increase serum cholesterol concentrations on a gram for gram basis about twice as much as polyunsaturated FA (PUFA) lower them. Studies of Keys et al.[5] and Hegsted et al.[6] also showed that saturated FA seemed to influence plasma cholesterol concentrations differently, depending on chain length. Thus, stearic acid (18:0) has been indicated to be neutral with respect to serum cholesterol as opposed to the hypercholesterolemic properties of lauric (12:0), myristic (14:0), and palmitic (16:0) acids.[5,7] Controversy still exists, however, regarding the relative importance of the individual saturated FA. Dietary studies in nonhuman primates have suggested that lauric and myristic acids are the primary FA responsible for elevating serum cholesterol,[8] whereas others have implicated palmitic acid in humans.[3] Monounsaturated FA, primarily oleic acid (18:1 *n*-9), have now been indicated to be hypocholesterolemic,[9,10] whereas these FA were once thought to be neutral with respect to serum cholesterol. Another aspect that could impact the hyperlipidemic effect of a natural fat is the *trans* versus the *cis* configuration of the double bonds as recent work has indicated that *trans* FA may be even more potent than saturated FA in elevating LDL cholesterol concentrations.[11]

Although most dietary FA are in the form of TAG relatively little is known about the importance of the stereospecific composition of TAG in the biological activity of dietary FA. The glycerol backbone of TAG is esterified in distinct patterns with FA (*Figure 1*), usually palmitate, stearate, oleate, and linoleate (18:2 *n*-6) and large differences are seen in the positional distribution of naturally occurring TAG among common dietary oils and fats that are unique to each fat (*Tables 1* and *2*).[12] Typically, animal fats such as

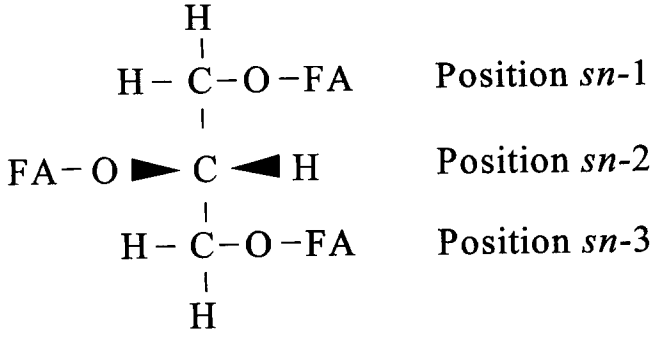

Figure 1 The stereochemical configuration of a TAG molecule showing the *sn* numbering of the carbon atoms of glycerol. When the FA on the middle carbon is situated on the left hand side (in the plane of the page) then the top carbon is numbered *sn*-1 and the bottom carbon is numbered *sn*-3 (behind the plane of the page).

bovine milk fat and lard (pork fat) contain mainly saturated FA on the *sn*-2 position whereas in most vegetable oils unsaturated FA occupy the *sn*-2 position and saturated FA are usually located in the *sn*-1 and *sn*-3 positions (*Table 1*).[12–14] Such differences in the positional distribution of FA among natural fats have been indicated to have specific effects on the profile, structure, and composition of lipoproteins that may not be predicted from the unsaturated index alone. Evidence for a role of TAG structure in the absorption and metabolism of natural fats has primarily come from studies that suggest that these qualities can be altered by rearrangement of the positions of the FA on the glycerol backbone of the TAG. A common approach to assess the effects of TAG structure has been to use the process of randomization, a process that is commonly used in the edible oil industry to modify the physical properties of fats and oils. In contrast to naturally occurring fats that exhibit a specific FA distribution, the FA in randomized fats are rearranged so that every FA is present in each TAG position to one third of its total content. In addition, chemical or enzymatic interesterification can be employed to produce unique TAG structures via a process whereby FA are exchanged between discrete TAG molecules from different fat sources. In this manner, fats and oils with similar FA composition can be studied in terms of their metabolic effects depending on the positional distribution of the FA.

Lipid absorption

Modulation of saturated FA absorption by the positional distribution of dietary FA has been suggested to be a key factor in the influence of dietary TAG structure on subsequent lipid and cholesterol metabolism. The majority of digestion and absorption of TAG does not occur until food has reached the small intestine where dietary fats are exposed to bile and pancreatic juice. As most TAG cannot be absorbed into cell walls, transport requires further metabolism of the TAG in the gut. The pancreatic lipase complex preferentially hydrolyses FA in the *sn*-1 and *sn*-3 position of TAG leaving a 2-monoacylglycerol (2-MAG)[15] that, due to its polar (glycerol) and nonpolar (FA) moieties, functions as an excellent emulsifying agent.[16] The lipolysis products including FA, MAG and diacylglycerols (DAG) are solubilized together with phospholipids and cholesterol by lysophospholipids and by bile salts into micelles and thus absorbed.[12,17,18] Because the rate of hydrolysis at the *sn*-2 position of the glyceride is very slow, the FA in the *sn*-2 position remain intact as 2-MAG during digestion and absorption.

Because the enzymes involved in the TAG digestion are specific for both the *sn*-1 and *sn*-3 positions,[12,17] a variety of FA, MAG, and DAG intermediates are formed whose nature is determined by the positional distribution of the FA in the original TAG molecules. Many of these molecules have specific melting points above body temperature, which may influence subsequent digestion, absorption, and metabolism. MAG readily form mixed micelles[18] and are subsequently absorbed, but free FA have variable incorporation into mixed micelles.[19] Digestion and absorption of long-chain saturated FA occurs less readily than for shorter chain

Table 1 Positional distribution of fatty acids in TAG of natural oils and fats*

		Fatty Acid (mol%)														
Source	Position	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	24:0
Cow's milk	1	5	3	1	3	3	11	36	15	21	1					
	2	3	5	2	6	6	20	33	6	14	3					
	3	43	11	2	4	3	7	10	4	15	<1					
Pig (outer back)	1						1	10	30	51	6					
	2						4	72	2	13	3					
	3						—	—	7	73	8					
Beef (depot)	1						4	41	17	20	4	1				
	2						9	17	9	41	5	1				
	3						1	22	24	37	5	1				
Cocoa butter	1							34	50	12	1					
	2							2	2	87	9					
	3							37	53	9	—					
Peanut	1							14	5	59	18		1	1	—	1
	2							1	<1	58	39		—	—	—	<1
	3							11	5	57	10		4	4	6	3
Corn	1							18	3	27	50	1				
	2							2	<1	26	70	<1				
	3							13	3	31	51	1				
Soybean	1							14	6	23	48	9				
	2							1	<1	21	70	7				
	3							13	6	28	45	8				
Olive	1							13	3	72	10	<1				
	2							1	—	83	14	1				
	3							7	4	74	5	1				

*Brockerhoff and Yurkowski[13] and Nawar.[79]

or more highly unsaturated FA as unsaturated FA and 2-MAG require lower concentrations of bile salts to achieve emulsification into micellar form.[18,20] Impaired absorption of long chain saturated FA in the free state may also be due to their high individual melting points above body temperature, which does not allow adequate incorporation in the liquid phase[12,21] and the tendency of unesterified FA to form hydrated acid-calcium soaps that are insoluble in aqueous media at the pH of the intestine.[12,21]

Many studies have indicated that the content of long-chain saturated FA and the arrangement of those saturated FA are the major factors that determine digestibility.[12–14] In particular, the positional specificity of pancreatic lipase has been indicated to be an advantage for the absorption of saturated FA located at *sn*-2 position due to the better absorption of saturated FA as the 2-MAG rather than as free FA.[1,22,23] The effect of chemical randomization on fat absorption has been studied in human infants and animals to determine the importance of the arrangement of saturated FA in dietary TAG on digestibility.

Newborn infants were given a formula consisting either of lard that has an abundance of palmitic acid in the *sn*-2 position (*Tables 1* and *2*), or lard that had been previously randomized in which the palmitic acid was equally distributed in all three positions of the glyceride.[24] Infants fed native lard were shown to absorb 95% of all FA, whereas infants fed randomized lard resulted in absorption of only 72% of all FA. Also, infants were found to absorb 88% of the stearic acid when fed lard but only 40% when fed randomized lard. The greater absorption of stearic acid from lard was thought to be due to the greater extent micellization in presence of larger amounts of 2-monopalmitin during digestion. Fat absorption has been linearly related to the amount of palmitic acid in the *sn*-2 position. Lien et al.[25] showed that chemical interesterification of coconut oil and palm olein increased the amount of palmitic acid in the *sn*-2 position and rendered the fat more absorbable in rats than a simple mixture of the two fats mixtures. Studies in rats have demonstrated that the positional distribution of saturated FA predominantly located in the 1,3-positions also influence the absorption of cholesterol, energy, and FA.[23,26] In a rat study of digestibility of a number of fats and fat mixtures, Tomarelli et al.[26] concluded that total fat absorption was influenced adversely by the total stearic acid content of the fat and the amount of palmitic acid in the 1 and 3 positions. Cocoa butter, despite containing more than 50% saturated FA, possesses a high unsaturated character of the 2-position

Table 2 Primary TAG species of natural oils and fats*

Source	Major TAG species[a]		
Butterfat	PPB	PPC	POP
Lard	SPO	OPL	OPO
Beef tallow	POO	POP	OPO
Cocoa butter	POS	SOS	POP
Palm oil	POP	POO	POL
Peanut oil	OOL	POL	OLL
Corn oil	LLL	LOL	LLP
Soybean oil	LLL	LLO	LLP
Olive oil	OOO	OOP	OLO

*Small.[12]
[a]Abbreviations used to describe acyl chains of TAG: B = butyric (14:0), C = capric (10:0), P = palmitic (16:0), S = stearic (18:0), O = oleic (18:1 *n*-9), L = linoleic (18:2 *n*-6).

of cocoa butter (*Table 1*) (mainly oleic and linoleic, 87% and 9%, respectively).[14] A reduced bioavailability of stearic acid due to its distribution on the 1- and 3-positions has been suggested as a possible mechanism for the observed neutral effect of cocoa butter on serum cholesterol compared with other highly saturated fats containing FA with saturated FA primarily positioned on the *sn*-2 position of the glycerol backbone.[27] Saturated FA in cocoa butter triacylglycerols are less absorbed than those of its randomized counterpart having saturated FA predominantly in the *sn*-2 position.[12] Fecal lipid elimination was found to be significantly increased in rats fed cocoa butter, with palmitic and stearic acids being the major FA excreted.[28] Mattson et al.[26] demonstrated that in rats fed a series of TAG isomers, the stearic acid esterified at either the *sn*-1 or *sn*-3 positions in cocoa butter was released as a free acid and poorly absorbed in the presence of calcium and magnesium. Animals fed cocoa butter had an increased food intake without any subsequent increase in body weight. Chylomicron FA composition has revealed that as a result of this poor absorption, palmitic and stearic acids are less available for resynthesis into chylomicron TAG.[29]

There is also evidence that the structure of dietary TAG could affect absorption of dietary and endogenous cholesterol as cholesterol transport is affected by the micellar solubilization ability of hydrolyzed products of TAG. In particular, unhydrolyzed dietary fat has been shown to inhibit cholesterol absorption when compared with hydrolyzable TAG.[30] In a study comparing digestion and absorption of cocoa butter, palm kernel oil and corn oil in rats, in which thoracic duct lymph lipids were analyzed after duodenal infusion of each fat, a reduced intestinal absorption of cholesterol into the thoracic duct lymph was associated with a poor absorption of cocoa butter.[31] Decreases in body weight gain, plasma cholesterol, and liver cholesterol concentrations and increases in fecal weight and fecal neutral steroids in rats fed a hydrogenated soybean oil-rich diet have been attributed to the high concentration of tristearoylglycerols (up to 57%) in this oil.[32] There is evidence that the poor absorptivity of tristearoylglycerol might impair intestinal cholesterol absorption by influencing cholesterol transport in a similar manner to water-soluble dietary fiber.[32] Some of the properties of tristearoylglycerol that could impair cholesterol absorption include an impairment of micelle formation, a suppression of intraintestinal mobility of cholesterol and a reduction of absorptivity by the sequestration of cholesterol. The suggestion that has arisen from these studies is that a consequence of the reduced absorption of saturated FA and cholesterol in fats such as cocoa butter and randomized lard would be a decreased tendency to raise plasma lipid concentrations.

Although animal and human infant data support the role of molecular structure of TAG influencing saturated FA absorption, there are questions in the human adult regarding the relevance of positional distribution of saturated FA on their absorption. There is suggestion that the human adult can absorb efficiently most dietary FA whether in non-esterified or monoacyl form[33] despite the apparent limited capacity of newborn infants to absorb saturated FA in their non-esterified form. Controversy still exists, however, regarding the efficiency of absorbability of free FA in the human adult. Jones et al.[34] studied whole body oxidation and fat malabsorption in men consuming diets of normal foods containing labeled stearic, oleic and linoleic acids as measured by $^{13}CO_2$ produced in the breath. Both dietary and labeled stearic acid were observed to be less well absorbed than either oleic or linoleic acids when given in the diet as free FA.[34] On the other hand, another study indicated that in humans the intestinal absorbability of stearic acid was similar to that of palmitic acid with both saturated FA being absorbed almost as well as oleic acid.[7] In the latter study, however, direct measurement of fat malabsorption was not made but rather compositional analysis of chylomicrons were performed at time intervals after every test meal and the assumption was made that relative proportions of FA in chylomicron lipids reflected their relative absorption rates. The degree that free FA are efficiently absorbed in the human adult and the role of positional distribution in this regard are issues that still need further investigation.

Chylomicron metabolism

An increase in plasma TAG concentrations are observed after ingestion of a meal as a result of the appearance of chylomicrons and their remnants. The plasma concentration of TAG depends on both the amount and type of fat in the diet as the duration of the postprandial rise in TAG is longer after an acute dietary load with saturated fat than with polyunsaturated fat.[35] In addition, the type and amount of fat consumed on a chronic basis also has an effect on the residence time and concentration of TAG.[35] Delayed chylomicron remnant clearance has been strongly associated with the atherosclerotic lesions induced by intake of high saturated, high cholesterol diets in laboratory animals and in persons at risk for CHD.[36,37] The recognition of the importance of postprandial lipoproteins in the etiology of atherosclerosis has led to interest in determining the characteristics of dietary TAG, which increase the length and level of post-prandial lipemia.

There has been indication that stereospecificity in natural oils and fats could play a role in terms of the postprandial TAG response. The FA in the 2-position of the MAG formed during digestion and the FA released from the 1- and 3-position are absorbed in mucosal cells and resynthesized into TAG by the monoglyceride pathway. In the human adult, most of the FA at the 2-position of glycerol remain intact as the 2-MAG during digestion and absorption of dietary TAG.[12,38] Because MAG provide the basic structure for the re-synthesis of chylomicron TAG in the enterocyte, *sn*-2-located saturated acyl chains present in ingested dietary TAG are conserved and incorporated into circulating chylomicron TAG.[22] Studies on human subjects showed that the feeding of lard, which is high in palmitic acid in the 2-position, resulted in increased proportions of palmitate in the 2-position of VLDL and chylomicron TAG.[39] Myher et al., however, did not measure the effect of lard feeding on chylomicron concentrations.[39] More recently, Nestel showed that positional distribution was maintained in TAG chylomicrons after feeding a interesterified palm oil blend to hypercholesterolemic men.[38] The intramolecular structure of dietary TAG has been suggested to influence the metabolism of chylomicrons to chylomicron remnants be-

cause lipoprotein lipase, like pancreatic lipase, shows positional specificity for the 1,3 positions of TAG.[40,41] Hence, the primary products of lipoprotein lipase-mediated lipolysis are FA and 2-MAG, which may have different metabolic fates.[42] There is evidence, for example, that the 2-MAG released upon lipoprotein lipase activity may partition to extrahepatic tissues differently than the released free acids (*Figure 2*).[42]

Several kinetic studies in rats have suggested that the presence of saturated FA at the *sn*-2 position in dietary TAG administered either orally or intravenously, slows down the metabolism of chylomicrons via both lipoprotein lipase hydrolysis of TAG and chylomicron remnant removal by the liver.[43–46] In experiments by Redgrave et al.[46] chylomicrons were prepared in donor rats by feeding positional isomers 1,2-dioleyl-3-stearoyl glycerol (OOS) and 1,3-dioleyl-2-stearoyl glycerol (OSO) to donor rats. As approximately 75% of the *sn*-2 position FA are conserved throughout the

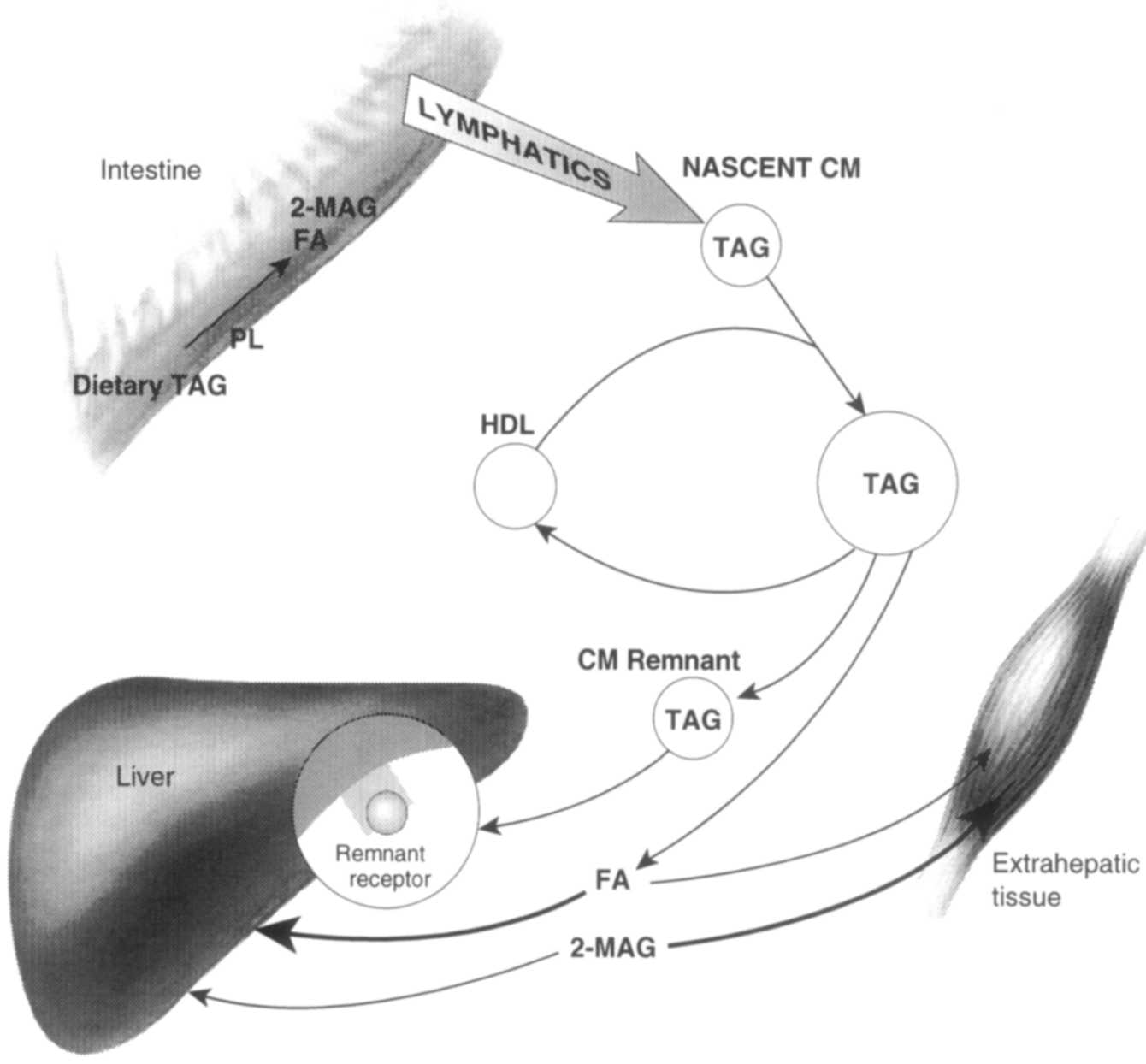

Figure 2 A representation of the dynamics of chylomicron metabolism. Action of pancreatic lipase (PL) on dietary TAG produce 2-MAG and free FA, which are emulsified by bile salts and absorbed into enterocytes. After absorption, TAG are resynthesized in a process whereby the FA character of the *sn*-2 position is predominantly maintained.[15] After secretion from enterocytes, chylomicrons (CM) acquire surface components (apoprotein CII, CIII, and E) from HDL, along with free and esterified cholesterol and phospholipids.[98] Under the activation of apo CII, pancreatic lipase (LPL) hydrolyzes TAG to yield 2-MAG and free FA and as the TAG core is depleted surface components (apo C and apo A) are transferred to HDL.[98,99] The loss of apo CII along with increasing inaccessibility of the TAG core to LPL action ceases further TAG removal producing chylomicron remnants.[98,99] The apoprotein E in chylomicron remnants serves as a ligand to enable clearance via a specific hepatic receptor.[98] A substantial fraction of chylomicron FA released by LPL hydrolysis are recirculated in the plasma free FA pool to reach the liver,[100] whereas MAG recirculate to a much lesser extent and tend to preferentially partition toward the tissue where lipolysis takes place.[42]

process of digestion and absorption,[22] collection of lymph chylomicrons resulted in chylomicrons in which stearic and oleic acids were largely retained in the *sn*-2 position of OSO and OOS chylomicrons, respectively.[46] Upon intravenous reinjection of these harvested chylomicrons into conscious recipient rats, the fractional clearances of both TAG and cholesteryl ester, monitored by radioactive tracers, were significantly slower for chylomicrons prepared by OSO feeding than those obtained from OOS feeding.[46] Similar results were observed when model emulsions were injected containing mixtures of phosphatidylcholine, free cholesterol, and cholesteryl oleate with the TAG being OSO or triolein (OOO), respectively. Hence, the presence of stearic acid at the 2-position of the TAG in chylomicrons or model emulsions slowed lipolysis and remnant removal. Explanations of altered rates of TAG hydrolysis and chylomicron remnant uptake in these studies have been based upon the physical properties of the 2-MAG of the surface of the chylomicron. Lipoprotein lipase acting on these chylomicrons would generate 2-monostearin from OSO and 2-monolein from OOS. The melting point of 2-monostearin is well above body temperature (74.5°C), whereas the melting point of 2-monolein is well below body temperature.[12] The suggestion was made that as hydrolysis of OSO chylomicrons proceeded, accumulation of 2-MAG containing saturated FA such as 2-monostearin at the surface of remnant particles could act to increase the surface rigidity of the particles. A more compact chylomicron surface could exclude apolipoproteins such as apo CII (involved in lipoprotein lipase activation) and lipoprotein lipase itself from the interface of chylomicrons. Further, the increased surface rigidity could inhibit binding of apo E to the surface of the chylomicron particles or alter the conformation of the apo E protein so that it could no longer be recognized by hepatic remnant receptors. In support of this concept, only chylomicron models with fluid surfaces have been shown to undergo lipolysis, and subsequent remnant uptake by the liver was demonstrated to be blocked by a solid surface.[47,48] To investigate the effect of 2-MAG at the interface of chylomicrons, Mortimer et al.[44] prepared chylomicron-like emulsions with small amounts of saturated or polyunsaturated 2-MAG and injected them into rats. They found that addition of 1% of 2-MAG containing a saturated acyl carbon chain from 12 to 20 carbons to triolein chylomicron-like emulsions slowed plasma clearance and decreased liver uptake of the remnants relative to the addition of monolein. In contrast, MAG with unsaturated chains were inconsistent in their effects on remnant clearance. The demonstration that saturated MAG added exogenously to emulsions delayed remnant clearance further supported the concept that perturbations caused by 2-MAG could account for the metabolic differences associated with altered positional distribution of saturated FA in ingested TAG. Other studies have indicated a role of the intramolecular structure of *n*-3 PUFA on chylomicron metabolism. Mesenteric lymph chylomicrons were prepared following intragastric injection after dissolving radiolabeled ^{3}H-cholesterol and ^{14}C-palmitic acid into two marine oils rich in *n*-3 PUFA but that differed whereby *n*-3 FA were present predominantly either at the *sn*-2 position (fish oil) or the *sn*-1,3 positions (seal oil).[49] The disappearance of ^{3}H-cholesterol radiolabel was faster from the plasma when seal oil chylomicrons were injected indicating a faster clearance of chylomicrons containing *n*-3 PUFA primarily at the *sn*-1,3 positions. In contrast, a higher rate of hepatic uptake of ^{14}C-palmitic acid was observed after injection of fish oil chylomicrons as compared with chylomicrons prepared from seal oil. This was suggested to be due greater incorporation of the free radiolabeled palmitate in the *sn*-1,3 positions in fish oil chylomicrons due to the conservation of the *n*-3 polyunsaturated occupied *sn*-2 position during absorption. The greater partitioning of palmitate at the *sn*-1,3 positions toward hepatic tissue was indicated to result in the increased hepatic accretion of the palmitate radiolabel.

The extrapolation of the animal kinetic studies using structure specific lipids to humans would suggest that eating natural dietary fats containing a predominance of *sn*-2-saturated TAG might delay chylomicron metabolism resulting in elevated plasma remnant concentrations. Experiments have been recently performed in human adults to test this hypothesis using Betapol (tradename), an enzymatically generated synthetic fat.[50] Betapol is obtained by *sn*-1,3 enzymic interesterification of a palm fraction with sunflower oil and canola FA, followed by solvent fractionation of the resultant TAG to produce a fat predominantly occupied by palmitic acid in the 2-position of the TAG. Although the Betapol fat is composed of a variety of TAG molecules, this fat contains 1,3-dioleoyl-2-palmitoylglycerol as the principal TAG (54.1%) followed by 1-oleoyl-2-palmitoyl-3-linoleoylglycerol (22.8%).[51] Sixteen healthy men were fed a liquid test meals containing either Betapol or a control oil blend that was characterized by a relatively high proportion of the symmetrical TAG, palmitic-oleic-palmitic structure.[50] They reported no differences between the control oil blend and the modified-fat meal on postprandial lipemia. The study did not rule out, however, possible differences in rates of chylomicron metabolism that could occur over longer post-prandial time intervals in a chronic-feeding situation. In a recent rat feeding study only a transient effect of Betapol was observed in comparison with a control blend on chylomicron metabolism as seen by a greater accumulation of ^{14}C-palmitate in the chylomicrons and liver in the Betapol-fed rats that disappeared 24 hr after the meal.[52] On the other hand, radiolabeled studies in the rat have shown that fats such as randomized cocoa butter that contain a high proportion of TAG in a solid state at body temperature showed significantly reduced rates of TAG hydrolysis and chylomicron removal in comparison with fed fats containing TAG liquid at body temperature.[53] As Betapol and control blends have been indicated to be essentially liquid at body temperature (37°C),[54] the influence of TAG structures with a different melting point characteristics on chylomicron metabolism was not made in the Betapol studies. Because randomization of TAG can increase the percentage of TAG remaining in a solid state at body temperature,[55] further study is required on postprandial chylomicron metabolism that compared dietary fats containing a preponderance of TAG positional isomers with melting point characteristics either above or below body temperature.

Cholesterol metabolism and atherogenesis

A number of human and animal dietary trials using interesterified fats have implicated the TAG structure, in addition to FA composition, as a determining factor in the cholesterolemic and atherogenic potency of a fat. Indeed, as both Kritchevsky[56] and Hayes et al.[57] have recently pointed out that some of the variabilities observed in humans studies in the effect of individual FA on serum cholesterol concentrations could be accounted by differences in positional distribution involving interesterified fats and whether the FA is given in the unbound form as opposed to bound to TAG in a specific configuration. Zock et al.[58] used interesterified fats enriched in specific saturated FA to compare their effects on serum lipid and lipoprotein concentrations of men and women. Serum cholesterol concentrations from the feeding a myristate-enriched TAG modified by interesterification were demonstrated to be only 1.5 times more cholesterolemic as palmitate as opposed to a 4 fold difference observed by Hegsted[59] in humans fed natural butterfat or coconut oil. McGandy et al.[60] tested in humans a number of synthesized fats with an excess of specific saturated FA in an effort to characterize the cholesterol-raising potential of each saturated FA. The prepared fats were achieved by transesterifying natural fats with either trilaurin, trimyristin, tripalmitin, or almost a fully hydrogenated soybean oil (85 percent stearic acid) in a ratio of 3:1. The results obtained with the interesterified fats (in which the tested saturated FA was randomly distributed over each of the three positions) conflicted with studies using naturally occurring fat sources. McGandy[60] found that the stearic acid interesterified fat showed a similar hypercholesterolemic effect in comparison with the palmitic or myristic acid fats. This contrasted with previous work by Hegsted et al.[59] who showed that stearic acid when fed as cocoa butter (in which it is predominantly esterified to the 1 and 3 positions) was neutral with respect to blood cholesterol-raising potential. McGandy et al.[60] concluded that the difference in the cholesterolemic action of stearic acid may have been due to the structure of the TAG being fed. This contrasted with work by Grande et al.[61] who noted no changes in plasma cholesterol concentrations in human adults fed native cocoa butter versus interesterified fats with a similar FA composition. The effect of specific TAG species of stearic acid on cholesterol metabolism remains to be established as interesterification can produce a variety of stearic acid-containing TAG species.

Further suggestion of role of TAG structure in cholesterol metabolism has been indicated in animal studies that indicated that the cholesterol-lowering properties of PUFA could be in part dependent on their positional distribution on the glycerol molecule.[62,63] Fat blends were prepared containing TAG with approximately equal amounts of essential FA but differing in their positional distribution. Studies in which rats were fed these fats showed that the cholesterol-lowering effects of dietary polyunsaturated FA were significantly diminished if the PUFA were not esterified at the 2-position of the dietary TAG.[62]

Native butterfat-feeding has been consistently shown to elevate serum total cholesterol levels, in particular LDL-cholesterol when fed to humans[59,64] and also to lead to the development of atherosclerotic lesions when fed to rats.[65] Mechanisms proposed to explain how saturated fats, such as butterfat, result in these physiological consequences include the observation that saturated FA cause modifications in the composition, structure, and relative viscosity of LDL culminating in decreased binding, internalization, and degradation of LDL.[66,67] In addition, the feeding of the saturated FA lauric, myristic, and palmitic acids has been shown to enrich hepatic lipid pools with saturated FA and suppress cholesterol ester formation[68] as these saturates are poor substrates of acyl-CoA:cholesterol acyltransferase (ACAT).[69] As a consequence of the decrease in cholesterol ester formation, more sterol is diverted into the free cholesterol pool, which is believed to regulate LDL-receptor transcription. Hence, as hepatic tissue concentrations of free cholesterol increase, a greater inhibition of LDL-receptor endocytosis is observed resulting in higher concentrations of plasma LDL-cholesterol.[68] On the other hand, an enrichment of hepatic tissue with unsaturated FA such as oleic acid, the preferred substrate of ACAT, cholesterol is moved out of the regulatory free sterol pool into the biologically inert pool of cholesterol ester. As a result, LDL-receptor transcription is increased, leading to increased uptake of LDL-cholesterol from the circulation.[70]

Butterfat contains predominantly hyperlipidemic saturated FA (mainly palmitic and myristic, approximately 32% and 18%, respectively) at the *sn*-2 position of the TAG molecule (*Table 1*).[71] We have characterized butterfat TAG that contain hypercholesterolemic FA with carbon chain lengths of C12 or greater and have demonstrated these fractions to account for up to 30% of the total butterfat TAG.[72] These TAG also showed the predominant presence of hypercholesterolemic FA in the *sn*-2 position. Because milk fat also contains appreciable quantities of oleic acid (26%), which is randomly distributed (21%, 14%, and 15% oleic acid at positions 1, 2, and 3, respectively),[71] interchange of oleic acid with saturated FA at the 2-position as would happen through randomization could be indicated to suppress the cholesterol-raising potential of butter fat. Christophe et al.[73] and Verdonk and Christophe[74] presented work suggesting that the hyperlipidemic effect of butterfat could be alleviated by the process of chemical randomization. Christophe et al.[73] reported that substitution of randomized butter for natural butter in the diet of healthy young men for a period of 25 days reduced serum TAG concentrations by approximately 35% and serum cholesterol by 12%. Randomization also increased approximately 10 fold the rate at which pancreatic lipase was able to hydrolyze the butterfat TAG in vitro.[74] This work indicated that in addition to the total composition of FA in dietary fat the discrete positional distribution of these FA on the glycerol backbone of TAG molecules may influence their subsequent metabolism in terms of serum lipid concentrations. Lower concentrations of liver cholesterol esters have been observed after a 5-week period in rats fed native butterfat compared with rats fed either randomized butterfat or a canola oil diet at 50% of energy in the diet; however, serum lipid concentrations were not altered.[53] In a 4-week feeding study, we fed Syrian hamsters cholesterol (0.1%)-containing diets with 9.5% (w/w) of synthesized lipids that differed in the proportions

of acylglycerol components with saturated (palmitate or myristate) versus unsaturated (oleate) FA at the *sn*-2 position, but that contained a similar FA composition.[75] Animals fed the acylglycerol mixture with the higher degree of *sn*-2 saturation showed higher plasma TAG concentrations and a significant rise in plasma apolipoprotein B concentrations.[75] Miniature pigs fed diets containing either native butterfat and either chemically or enzymatically interesterified milk fat showed variable effects on plasma cholesterol.[76] Pigs fed enzymatically interesterified milk fat showed an increase in total plasma cholesterol, whereas chemically randomized milk fat had reduced LDL cholesterol concentrations by 10.8%. Differences in the specific molecular TAG species produced via chemical and enzymatic interesterification and interspecies differences in terms of digestibility and the degree of endogenous randomization of TAG after absorption may partially account for differences between the studies. More human and animal investigations are needed into the potential effects of specific butterfat TAG structures on blood cholesterol metabolism.

Although the beef tallow and lard diets have similar levels of saturated and unsaturated FA, feeding studies have indicated that each dietary fat produce different plasma VLDL, intermediate density lipoprotein, and HDL lipoprotein concentrations.[77] Such differential effects could be due to differences in the position of specific FA on the glycerol molecule or to a 10 fold difference in linoleic acid content.[78] As opposed to lard in which the *sn*-2 position is occupied by 72% palmitic acid, beef tallow contains only 17% palmitic acid in the *sn*-2 position (*Table 1*).[79] The relatively higher percentage of palmitic acid in the *sn*-2 position in lard could result in an increased uptake of palmitate relative to the feeding of beef tallow. Beef tallow has been indicated to be less hypercholesterolemic than originally thought, as rats showed similar plasma cholesterol concentrations when fed nonpurified diets containing either beef tallow or soybean oil.[80] Moreover, beef tallow intake showed a decreased level of atherogenicity in rabbits in comparison with lard feeding.[81] Randomization of the two fats, on the other hand, was indicated to produce an equivalent degree of atherogenicity.[81] As a reduced absorption of saturated FA has been observed from the feeding of randomized lard[24,82] this may have an effect on its atherogenic action as well as the tendency of lard to raise plasma lipid concentrations. Rat fed native or randomized lard showed that interesterification of lard resulted in significantly lower plasma concentrations of TAG in comparison to the feeding of native lard.[82] Randomized lard has also been associated with a reduction in atherogenicity in rabbits fed 2% cholesterol.[83]

Peanut oil is unexpectedly atherogenic despite its relatively high iodine value for rats,[84] rabbits,[85] and Vervet monkeys.[86] The atherogenic effect of peanut oil was reduced significantly after randomization suggesting that the TAG structure of peanut oil might be responsible for its physiological effects.[86] The mechanism by which randomization of peanut oil leads to reduced atherogenicity is not clear, although a simple effect on cholesterol absorption would appear to be excluded.[87] Peanut oil contains 4 to 7% long-chain saturated FA including arachidic (20:0), behenic (22:0), and lignoceric (24:0) acids, almost all of which are present in the *sn*-3 position (*Table 1*).[88] In comparison with the randomized form, native peanut oil has more TAG with linoleic acid in the *sn*-2 position and saturated FA in the *sn*-1 and -3 positions.[88] It has been suggested that the presence of C20–24 saturates in the *sn*-3 position of peanut oil might render linoleic acid in the *sn*-2 position relatively unavailable, which could promote the atherogenicity of the oil.[89] However, the introduction of the long-chain FA into a synthetic fat with an identical FA composition to peanut oil produced a fat with considerably lower atherogenic effect.[89] Alternatively, the reduction of the unusual substances such as lections found in peanut oil after randomization has been suggested to contribute to the reduction of atherogenicity.[90] The atherogenesis in animals fed native peanut oil does not seem to be mediated by elevated plasma cholesterol concentrations as randomization appear to have variable effects on plasma cholesterol concentrations,[80,91] which do not appear to correlate with atheroma formation.[92]

Recent work in humans and animals has focused on the comparison of *sn*-2-saturated TAG in the form of Betapol versus a fat blend enriched with *sn*-1,3 16:0 rich fractionated palm oil. In piglet studies, Innis et al.[93] showed higher fasting plasma total and HDL-cholesterol concentrations in newborn piglets fed for 17 days formulas containing Betapol than a palm oil formula high in *sn*-2-oleate glycerides. On the other hand, Zock et al.[54] showed negligible effects on plasma lipoprotein concentrations in healthy males and females after a 4-week feeding of Betapol versus a control blend with a equivalent FA composition but a high proportion of TAG with the palmitic-oleic-palmitic structure. The authors suggested that the differences observed between their results and that of Innis et al.[93] could have been due to the limited capacity of newborn animals to absorb efficiently saturated FA located at *sn*-1 or -3 positions in comparison of the high absorptive efficiency in the adult.[33] The lack of a hyperlipidemic effect of Betapol in this study was consistent with previous findings of de Fouw et al.[51] who demonstrated that Betapol exerted no significant effects in adult rats on either cholesterol or TAG metabolism after a 6-week feeding regime. Another recent 28-day feeding study in hamsters has confirmed the lack of an effect of Betapol on fasting serum lipid concentrations in relation to a control palm oil blend.[52] An interesting observation from the authors of the latter study, however, was that another strain of hamsters susceptible to hypertriglyceridemia unexpectedly showed reduced plasma TAG concentrations upon feeding Betapol. Hence, strain and age differences can result in marked differences in lipid metabolism from the feeding of interesterified fat. A recent double-blind crossover study has showed no effect of positional distribution on plasma lipoprotein and TAG concentrations in hypercholesterolemic men fed a high palm oil margarine versus an interesterified blend containing higher concentrations of *sn*-2 palmitate.[38] As human studies have thus far centered on the interchange of palmitic with oleic and linoleic acids, further studies comparing different FA mixtures are needed to examine the effect of interesterification on serum lipids in human adults.

Although palm oil, rich in palmitic acid, is typically considered as a hypercholesterolemic saturated fat, the 2-position is predominantly occupied by oleic and linoleic acids

(*Table 1*).[94] Palm oil is characterized by a relatively high proportion of the symmetrical TAG, palmitic-oleic-palmitic structure (30 to 36%)[94] and this structure has been suggested to affect the cholesterolemic nature of palmitic acid. In studies by Hegsted et al.[59] when natural fats were fed, myristic acid accounted for 67% of the total variance in the cholesterol levels of their subjects, whereas palmitic acid has a much lesser effect. The differential effects of myristic and palmitic acids were not observed, however, when randomly reesterified fats were fed. In accordance with these observations, palm oil has been shown to be less hypercholesterolemic than predicted from its high palmitic acid content. Feeding experiments with Rhesus monkeys[95] have indicated that the saturated FA of palm olein did not cause the hypercholesterolemic effect predicted by the equations of Keys et al.[5] and Hegsted et al.[59] In the Keys et al.[5] and Hegsted et al.[59] studies, however, the major source of palmitic acid was butterfat, where it is primarily esterified at the *sn*-2 position. The role of dietary fat structure in natural fats such as palm oil is, however, complicated by the presence other components within the fat (i.e., linoleic acid and phytosterol content, *n*-3/*n*-6 PUFA ratios), which can make comparison difficult between natural fats and override effects of positional distribution on lipid metabolism. The comparison of palm oil and randomized palm oil showed no difference in lipid absorption or on total serum cholesterol concentrations of rats, although hepatic cholesterol or plasma-free cholesterol concentrations were not measured. On the other hand, chemical interesterification of palm oil resulted in increased platelet aggregation induced by ADP[82] suggesting that the molecular structure of palm oil is a reason why palm oil is not as thrombogenic in rats as might be indicated by its high saturated FA composition.[96] The type of interesterification performed, however, can affect the lipidemic characteristics of palm oil. Chemically interesterified palm oil contains palmitic acid in almost equal proportions in the 1-, 2- and 3- positions, while palmitic acid predominantly resides at the 1- and 3- positions of TAG in lipozyme-interesterified palm oil. Similar to other human and animal findings seen in the studies with Betapol, the total plasma cholesterol and TAG concentrations were unaffected when rats were fed the two types of interesterified palm oil.[97] Unexpectedly, despite the higher saturated *sn*-2 content in the chemically interesterified fat, free cholesterol plasma concentrations were lower in the rats fed this fat as opposed to rats fed enzymatically randomized palm oil. Moreover, hepatic cholesterol ester concentrations were indicated to be lower in the chemically interesterified palm oil group. No differences between the two groups were observed in terms growth response, food efficiency ratio, or the coefficient of digestibility indicating that differences in fat absorption could not account for the observed findings. The relationship between these findings and other human and animal studies is not clear as the effects on serum free cholesterol concentrations or hepatic cholesterol concentrations were not measured from the feeding studies using Betapol or interesterified palm oil margarines.

The contrasting findings in terms of positional distribution may be partially reconciled on the basis of possible differential effects of TAG structure on saturated FA absorption and further metabolism. Depending on factors such as the species and age of the animal and the type of fat (i.e., lard with a high saturated *sn*-2 content) and other dietary components (i.e., calcium and magnesium content), diminished absorption of saturated FA may occur after randomization resulting in decreased uptake at the gut and hepatic levels. In our study, for example, a lower feed efficiency was observed in hamsters fed the acylglycerol diet containing a lower proportion of saturated *sn*-2 acylglycerols indicating a decreased fat absorption. A lowered saturated fat uptake could, in turn, be responsible for a plasma lipid-lowering action. On the other hand, there is evidence that after lipoprotein lipase-mediated lipolysis 2-MAG recirculate to a lesser degree than free FA[42,100,101] indicating that 2-MAG may partition more toward extrahepatic tissues where lipolysis takes place. Hence, in circumstances whereby saturated FA absorption is not greatly affected, the presence of a greater proportion of saturated FA at the *sn*-2 position could decrease hepatic uptake of saturated FA, whereas a relative increase in oleic and linoleic acids at the *sn*-1,3 positions may increase their hepatic uptake. As oleic and linoleic acids are the preferred substrates for ACAT this could account for the greater hepatic cholesterol ester concentrations observed in rats fed the chemically randomized palm oil enriched in *sn*-2 saturated TAG. More studies are needed to resolve these and other findings including the decreased plasma lipid concentrations observed following randomization of butterfat and lard.

Conclusion

The above studies point out the complexities regarding the study of molecular structure of TAG with respect to lipid metabolism. Controversy still exists with respect the role of positional distribution of FA on lipid absorption, metabolism, and atherogenesis. Differences can be observed among studies using interesterified fats depending on the type of fat studied, species, strain, and age of animals as well as the methodology used to interesterify the fed fat. Additional human and animal studies are required to investigate interesterification of lard and butterfat, which have shown effects on plasma lipid concentrations and atherogenesis. For the most part, the study of Betapol and interesterified palm oil in animals and humans has suggested a negligible effect on plasma lipid concentrations caused by the redistribution of palmitate on the *sn*-2 position. Some animal studies, however, have suggested that changes in molecular structure of palm oil can affect the platelet aggregation response to ADP as well as concentrations of plasma-free cholesterol and hepatic cholesterol. The biological importance of these observations needs to be clarified due to the use of randomization in the edible oil and fat industry to increase the level of saturates in the *sn*-2 position of TAG and thereby increase the solidity of fat in products such as margarines. In general, more experiments with diverse native and randomized fats are needed as there is still much to be learned with respect to the relationship of TAG structure to the cholesterolemic and atherogenic properties of fats.

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