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THE EFFECTS OF GLYCERIDE STRUCTURE ON ABSORPTION AND METABOLISM

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CONTENTS

Introduction, Scope, and Nomenclature

This review attempts to highlight an area that has been generally neglected in studies of the absorption and metabolism of dietary fats. In short, how does the stereospecific structure of dietary triacylglycerols affect their absorption from the gut, their metabolism in enterocytes, their incorporation into lipoproteins and subsequent metabolism, and their distribution into tissues? Two brief reviews alluded to some of these questions recently (58, 103), but the authors found scant data to provide answers. A major reason for the lack of a large body of creditable data concerning the absorption and metabolism of specific acyl glycerols is that the chemical determination of acyl glycerol structures is not a trivial exercise. Dietary fat in a single meal often comes

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from a variety of sources (dairy, meat, vegetable). These mix in the stomach and small intestine to form an extremely complex succus entericus, which then undergoes digestion and absorption (17, 86). Surprisingly, absorption from the lumen is generally quite efficient, and only about 4% of the ingested fat escapes into the feces (17). However, absorption of specific, highly saturated fats may become a problem.

Each source of fat (vegetable oil, margarine, meat fat, butter, etc) is composed of hundreds of different complex triacylglycerols, and the majority of these have not been analyzed completely. The general structure of a triacylglycerol is shown in Figure 1. The three R groups stand for different acyl groups. Using the IUPAC-IUB nomenclature (49) with the carbon in the mid or secondary position in the plane of the page and the primary or end carbons behind the plane of the page, if the OH is drawn to the left then the top carbon becomes *sn*-1, the mid carbon becomes *sn*-2, and the bottom carbon becomes *sn*-3. If the groups at the 1 and 3 position are different (e.g. as shown for the different acyl groups in Figure 1), then the molecule has an asymmetric carbon at the 2 position, and the optical isomers 1R',2R'',3R'''-*sn*-glycerol and 1R''',2R'',3R'-*sn*-glycerol are possible.

Most of the data given for the triacylglycerol composition of oils, fats, lipoproteins, etc, simply report the total overall fatty acid composition of the triacylglycerol mixture. This information is easily obtained and is valuable because it tells us which major fatty acids are esterified to the glycerol; however, it does not tell us their position or how many specific triacylglycerol species are present in a given sample. If the sample is treated with a lipase

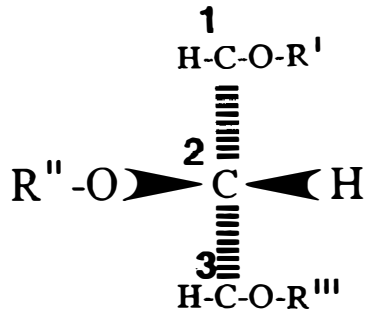

Figure 1 The IUPAC-IUB Commission on Biochemical Nomenclature (1978) (Ref. 49) has recommended the following nomenclature for substituted glycerides, e.g. the phosphoglycerides. With the carbon in the 2-position in the plane of the page and the 1- and 3-carbons behind the plane of the page, if the –OH on the 2-position of the glycerol is drawn to the left, then the top carbon becomes 1 and the bottom becomes 3. A triglyceride with myristic acid on the 1-position, oleic acid on the 2-position, and palmitic acid on the 3-position would be described as *sn*-glycerol-1-myristate-2-oleate-3-palmitate, or simply as *sn*-MOP.

such as pancreatic lipase (13) to cleave the fatty acids in the primary 1 and 3 positions to produce 2 mol of fatty acids and 1 mol of 2-monoglyceride and if these in turn are analyzed, then we learn globally what fatty acids are in the 2 positions and in the 1 and 3 positions. This procedure does not distinguish between the 1 and 3 positions or reveal which primary fatty acids are linked to which monoglyceride. In theory the number of triacylglycerols (N) that could be present in a sample having n fatty acids is $N \simeq n^3$ (34). This includes positional isomers and enantiomers, that is optical isomers in which a specific fatty acid is either at the *sn*-1 or *sn*-3 position. If one simply considers racemic mixtures where the 1 and 3 positions can be interchanged, then $N = (n^3 + n^2)/2$. If one considers only the fatty acid combinations on the 3 positions and ignores positional isomers, then $N = (n^3 + n^2 + 2n)/6$. Thus, if a specific sample contains only three different fatty acids (e.g. R', R'', R'''), then the total number of isomers including positional isomers and enantiomers would be 27. If we exclude optical isomers, there would be 18, and if we exclude positional isomers, there would be 10. Considering that many dietary fats and oils often have 10 or more major fatty acids, the number of potential individual triacylglycerols becomes enormous. In fact, butterfat has both short and long chain fatty acids as well as many unsaturated ones (14) and therefore probably consists of thousands of individual stereospecific triacylglycerols. For this reason even the major stereospecific triacylglycerols of milk have not been completely analyzed (75).

Dietary Triglycerides: Methods of Analysis

The techniques for stereospecific analysis of single, mixed chain triacylglycerols were first worked out by Brockerhoff (15, 16) and by Myher & Kuksis (73). As generally outlined by Kuksis (58a) and diagrammed in Figure 2, the unknown triacylglycerol is hydrolyzed by pancreatic lipase to release the fatty acids in the 1 and 3 positions. The fatty acid in the 2 position persists as a monoglyceride that is separated from the free fatty acids and analyzed to give the fatty acid in the 2 position. Myher & Kuksis then used the Grignard reagent to make diacylglycerols. The 1,2- and 2,3-diacyl-*sn*-glycerols were separated from the 1,3-diacylglycerol and derivatized, for instance, to phosphatidylcholines or phosphotidylphenols, which were then reacted with phospholipase C. The 1,2-diacyl-*sn*-glycerol derivatives are hydrolyzed rapidly in approximately 2 min, whereas the 2,3 derivatives require approximately 4 hr. Thus, the 1,2-diacyl-*sn*-glycerols can be separated from the 2,3 derivatives. Pancreatic lipase hydrolysis of the 1,2-diacyl-*sn*-glycerol yields the fatty acid in the 1 position and confirms the fatty acid in the 2 position on the monoglyceride. Chemical analysis of the fatty acids on the 2,3 derivative reveals the fatty acid in the 3 position, since that in the 2 position is known. An alter-

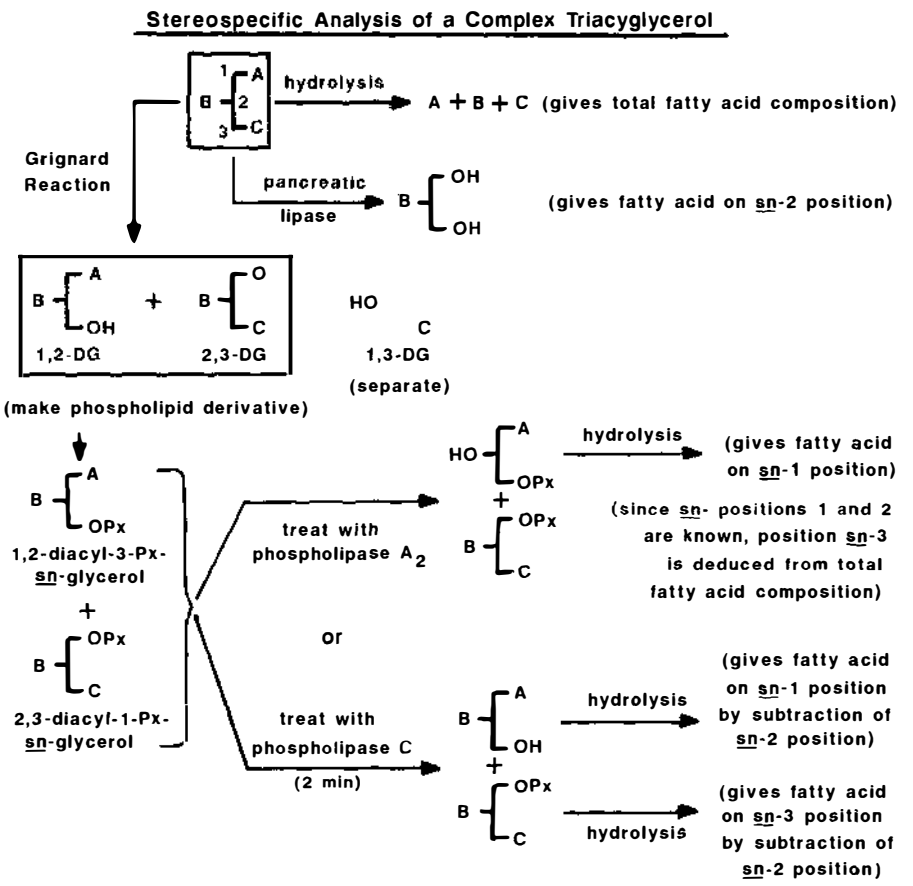

Figure 2 Scheme for the stereospecific chemical analysis of the complex triacylglycerol, 1A,2B,3C-triacyl-*sn*-glycerol. A, B, and C stand for different acyl groups. 1,2-diacyl-3-Px-*sn*-glycerol and 2,3-diacyl-2-Px-*sn*-glycerol are enantiomeric phospholipid derivatives (such as phosphotidylcholines or phosphotidylphenols), which are distinguished from each other by the phospholipases. For further explanation see text.

nate method of reacting the 1,2 and 2,3 derivatives with phospholipase A_2 provides a corroboration of the structure.

By a combination of chromatographic separations to isolate individual or racemic mixtures followed by chemical and enzymatic steps (15, 16, 58a, 73), the stereospecific structure of individual triacylglycerols in a mixture can be estimated. In the last 10 to 15 years a number of new techniques by which triacylglycerol classes can be easily separated have been developed. These include HPLC (21), gas chromatography (22), and the use of silver nitrate

Table 1 Composition and melting of some natural fats and oils[a]

Fat or oil	m.p (°C)[b]	Major TAG's			References
Butterfat	37–38	PPB[c]	PPC[c]	POP[c]	14, 38, 75
Horse fat		OOO	POO	LOO	101
Lard	46–49	SPO[c]	OPL[c]	OPO[c]	73
Tallow (beef)	40	P$\underline{O}$O[d]	P$\underline{O}$P	P$\underline{O}$S	63
Cocoa butter	28–36	P$\underline{O}$S[d]	S$\underline{O}$S	P$\underline{O}$P	38, 52, 83, 88, 91
Coconut oil	24–27	DDD	C$\underline{D}$D	C$\underline{D}$M	96
Palm kernel oil	24–29	DDD	MOD	ODO	96
Almond oil		OOO	OLO	OLL	38
Corn oil	–14	LLL	LOL	LLP	29, 32, 34, 60
Cottonseed oil	5–11	PLL	POL	LLL	5
Egg triglycerides		POO	PLO	POS	23
Grapeseed oil	8	LLL	OLL	POL	34
Hazelnut oil		OOO	OLO	POO	38
Olive oil	–7	OOO	OOP	OLO	29, 32, 83, 87, 101
Palm oil	30–36	POP	POO	POL	21, 22, 29, 32, 88
Peanut oil	–8–12	OOL	POL	OLL	92
Rice bran oil		PLO	OOL	POO	105
Safflower oil	–15	LLL	LLO	LLP	90
Soybean oil	–14	LLL	LLO	LLP	32, 82, 83
Sunflower oil	–17	LLL	OLL	LOO	34, 90
Walnut oil		LLL	OLL	PLL	38
Rapeseed oil (low Er)	5	OOO	LOO	OOLn	34, 88
Linseed oil	–17	LnLnLn	LnLnL	LnLnO	90, 101
Rapeseed oil (high Er)		ErOEr	ErLEr	ErLnEr	20
Mustard seed oil[e]		ErOEr[c]	ErLEr[c]	OOEr[c]	76

[a] Abbreviations used for acyl chains in the triacylglycerols: B = C-4 : 0 (butyric), C = C-10 : 0 (capric), D = C-12 : 0 (dodecanoic), M = C-14 : 0 (myristic), P = C-16 : 0 (palmitic), S = C-18 : 0 (stearic), O = C-18 : 1 *(cis)* (oleic), E = C-18 : 1 *(trans)* (elaidic), L = C-18 : 2 (linoleic), Ln = C-18 : 3 (linolenic), G = C-20 : 1 (gogoleic), Er = C-22 : 1 (erucic).

[b] The melting points or ranges of melting of these fats and oils are taken from References 4, 47, 96.

[c] Specific TAG's estimated from stereospecific fatty acid analyses, as in 1S,2P,3O-*sn*-glycerol.

[d] Underline denotes fatty acid in the *sn*-2 position; otherwise the stereospecific composition is uncertain.

[e] The stereospecific composition of the eight most prevalent triacylglycerols of mustard seed oil (76), which comprise 40% of the total, is ErOEr = 8.2%, ErLEr = 6.8%, OOEr = 5.9%, ErLnEr = 5.3%, OLEr = 4.9%, OLnEr = 3.8%, GOEr = 3.3%, GLEr = 2.7%.

chromatography either on plates or on columns (22). HPLC and gas chromatography separate the triglycerides by the total number of carbons in the acyl chains, whereas argentation chromatography separates on the basis of the number of double bonds. Using a combination of these separation techniques followed by the chemical and enzymatic techniques (15, 16, 58a, 73, 93) or mass spectrometry (12, 61, 65, 73) requires a great deal of work, but can lead to the stereospecific configuration of individual triacylglycerols in a complex mixture. If one is not interested in the optical isomers, each peak corresponding to a triacylglycerol may be treated with pancreatic lipase to determine the fatty acid in the 2 position and in the 1 and 3 positions.

However, the stereospecific nature of the triacylglycerols may be important in their absorption, metabolism, and possibly in atherogenesis. It has been pointed out that randomization of fats such as lard or peanut oil alters their absorption and atherogenicity in the cholesterol-fed rabbit model (58).

In Table 1 are listed estimates of the major triacylglycerols found in a variety of natural fats and oils. Most of these have not been fully analyzed and the specific stereoisomers are generally not reported. In some examples positional isomers are indicated and in a few rare cases (76) the optical isomers are given. The melting point (or range of melting) of the fat is also given.

Digestion, Absorption, Transport, and Tissue Accumulation of Fat

The metabolism of the triacylglycerols (Figure 3) begins when several enzymes digest fats and oils in the intestinal lumen to form fatty acids and 2-monoacylglycerols. Fatty acids and 2-monoacylglycerols are absorbed into intestinal mucosal cells and resynthesized into triacylglycerols, which are assembled into chylomicrons and secreted into lymph. The fundamental early studies of Mattson & Volpenhein (66, 67) on racemic positional isomers and labeled mono-, di-, and tri-glycerols in rats laid the foundations for our present understanding of the rearrangements of acyl glycerols that occur during the process of digestion, absorption, and chylomicron formation. From the thoracic duct, chylomicrons pass into plasma where certain physical-chemical changes take place in the chylomicrons (69) and lipolysis of triacylglycerols in the capillary bed occurs (81). Finally, fatty acids and partial glycerides are taken up by adipose and muscle cells and are reesterified to triacylglycerols to complete the cycle. The intestinal absorption of fat has been reviewed from the point of view of enzymes (13, 59), physical behavior of lipids (17, 86), movement of fatty acids to the intestinal microvillus (102), and reesterification of triacylglycerols in the intestine (11, 50, 59, 64). A summary of the general pathway is given in Figure 3.

Consider a single specific triacylglycerol with three separate chains R',R'',R''' as it passes through the cycle from dietary fat to tissue fat. In the upper intestinal tract (tongue to stomach), lingual or gastric lipase begins the process by hydrolyzing some of the fatty acids from the *sn*-3 position to generate a 1,2-diacyl-*sn*-glycerol and fatty acid. The short chains at the *sn*-3 position in cow milk (see butterfat, Table 1) are efficiently hydrolyzed by gastric lipases (100, 104). A mixture of triacylglycerol, diacylglycerol, and some fatty acid proceeds into the duodenum and small intestine where it is acted on by two other enzymes. The first, pancreatic lipase and its colipase (13), hydrolyze the fatty acid from the 1 position of the 1,2-diacyl-*sn*-glycerol and generate 2-monoacylglycerol and fatty acid. Pancreatic lipase also attacks

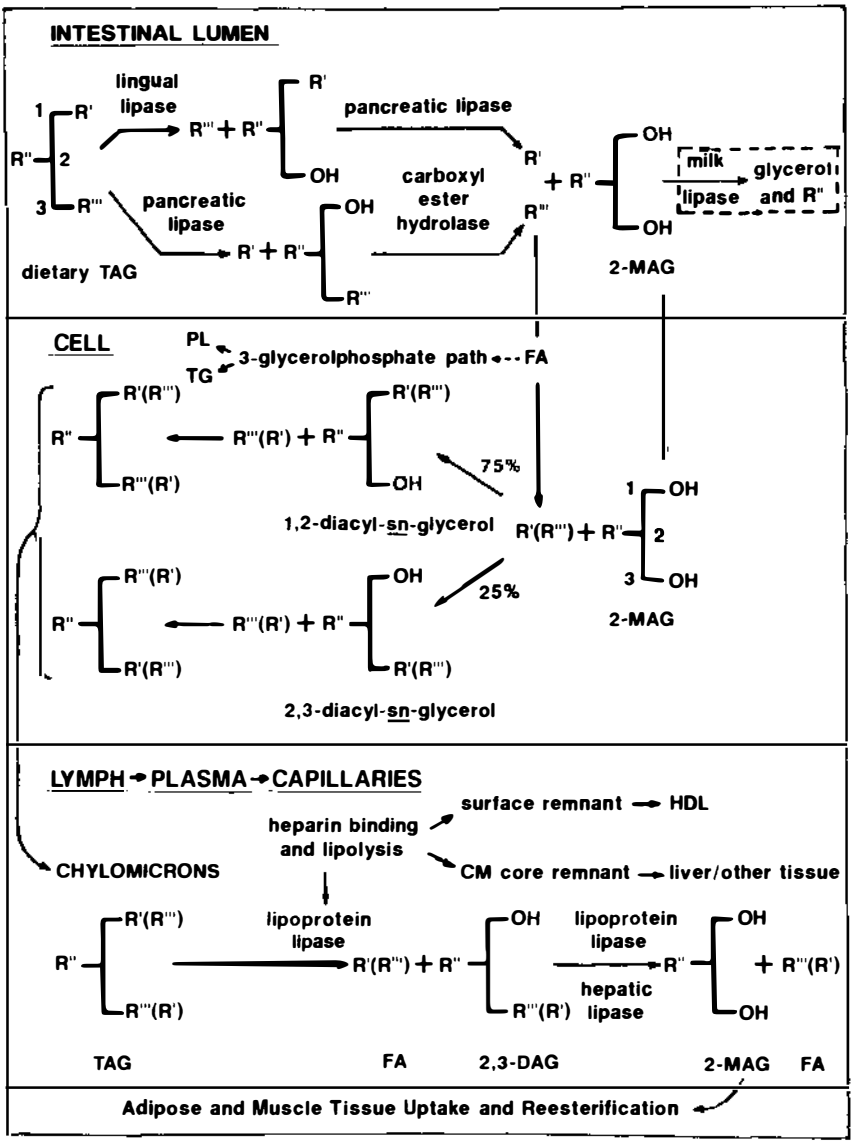

Figure 3 General scheme for the movement of stereospecific triacylglycerols from dietary fat to depot fat.

the triacylglycerol, which escaped the gastric lipase, with a slight preference for the *sn*-1 position to create a 2,3-diacyl-*sn*-glycerol and fatty acid. The 2,3-diacyl-*sn*-glycerol is then attacked by carboxyl ester hydrolase or by pancreatic lipase to generate the 2-monoacylglycerol and another mole of fatty acid (104). A very small fraction of the 1,2- or 2,3-diacyl-*sn*-glycerols are thought to undergo acyl migration to the 1,3-diacylglycerol, which is probably cleaved rapidly by the lipases or, if absorbed, cleaved in the mucosa to glycerol. The importance of carboxyl ester hydrolase is highlighted when the diacylglycerol contains long-chain polyunsaturated fatty acids, especially those found in fish oil (eicosapentaenoic acid) or arachidonic acid. These diacylglycerols are poor substrates for pancreatic lipase, but carboxy ester hydrolase hydrolyzes them efficiently (18, 19). The net result is that 2 mol of fatty acid and 1 mol of 2-monoacylglycerol are produced (66, 67). In the adult most of the 2-monoacylglycerol is absorbed without further hydrolysis and enters the mucosal pool. Some small fraction, about one quarter in the rat (67), isomerizes to the 1 or 3 position to produce 1(3)-monoacylglycerol, about three quarters of this is hydrolyzed to glycerol and fatty acid (67), and the remaining fraction is absorbed. In newborn infants a fourth lipase appears to be important (10); the bile salt activated milk lipase, which is different from milk lipoprotein lipase (104). This lipase is probably responsible for the hydrolysis of 2-monoacylglycerol to free glycerol and fatty acid, which occurs in the intestine of neonates fed breast milk. This procedure apparently leads to an efficient absorption of fat even in neonates with very low bile salt concentrations (10). The absorption of fatty acids is generally efficient, but under specific conditions some fatty acids, especially saturated (25) or trans-unsaturated (9), are less well absorbed.

After the fatty acids and 2-monoacylglycerol are absorbed into the enterocyte, the 2-monoacylglycerol is reacylated by one enzyme, monoacylglycerol transferase (50), to both 1,2-diacyl-*sn*-glycerols and 2,3-diacyl-*sn*-glycerols (11). The preference appears to be for the 1 position which produces about 3 times as much 1,2- as 2,3-diacyl-*sn*-glycerol (80). The activated fatty acids (fatty acyl-CoAs) (2, 11) used for the acylation seem to be similar for either the 1 position or the 3 position. Thus, apparently equivalent utilization of fatty acids in the diacylglycerols leads to the formation of optical isomers. The activated fatty acid pool used in the reacylation can come from the newly synthesized pool of fatty acids in the cell and from fatty acids absorbed from the lumen as a result of bile phospholipid and fat digestion. The newly formed 1,2- and 2,3-diacyl-*sn*-glycerols are acylated by diglyceride acyl transferase to produce triacylglycerols. Since acyl migration is probably slow at 37°C (55), the newly synthesized mucosal triacylglycerols largely retain the original fatty acid in the 2 position. However, the fatty acids in the 1 and 3 positions tend towards a random selection of activated fatty acids in the intestinal mucosa. While it is generally accepted that fatty acids less than 12

carbons are transported in the free form into the portal vein, if the fatty acid in the 2 position was originally a shorter chain fatty acid such as capric (C-10:0), it is at least partly carried through the reacylation process and secreted into the lymph lipids as a triglyceride with a medium or short chain in the 2 position (11).

The reacylation of 2-monoacylglycerols appears to be the predominant pathway utilized during active fat absorption. The utilization of free glycerol through the 3-glycerolphosphate path is important when the flux of monoacylglycerols through the gut is minimal. Generally the activated fatty acid pool used to acylate 3-glycerolphosphate to form the 1,2-diacyl-*sn*-glycerol phosphate tends to put unsaturated fatty acids at the 2 position, especially linoleate (18:2) and arachidonate (20:4), and a saturated fatty acid at the 1 position. This is the predominant path for synthesizing chylomicron phospholipids. De-phosphorylation and reacylation at the 3 position produce triacylglycerols. Triacylglycerols generated by this mechanism generally do not maintain the original 2 position as it was in the diet. However, during fat absorption the monoglyceride path predominates in the formation of triacylglycerols (11).

Newly synthesized mucosal triacylglycerols, which are the major pool of enterocyte triglyceride (64), are then packaged into chylomicrons and intestinal very low density lipoprotein (VLDL) and secreted into the lymph. As they pass from the lymph into the plasma they pick up some apoproteins from the HDL fraction, lose some phospholipid, and gradually increase free cholesterol (3, 69). They then circulate to various capillary beds where they bind to the capillary surface, probably through heparin-binding sites on the apolipoproteins (81). Lipoprotein lipase acting in conjunction with apoC-2 (81) and perhaps apoA-IV (40) attacks both the 1 and 3 positions (79). The substrate is most probably surface-located triacylglycerol (68, 69) in which the 1 and 3 positions are equally exposed to the aqueous interface (26, 41, 44, 45). Consistent with the surface location of the substrate is the fact that when cholesterol increases to 1:1 mole ratio to phospholipid (C/PL = 1) the triacylglycerol is forced out of the interface (99) and hydrolysis stops (69). Lipoprotein lipase prefers the 1 position (1, 70, 84), and fatty acids in this position are hydrolyzed more rapidly to produce the 2,3-diacyl-*sn*-glycerol. Studies using enantiomeric diacylglycerols as substrates also show that the 1 position is preferred by lipoprotein lipase (71). If the 3 position is occupied by a 16–18 carbon fatty acid, then it is also ultimately hydrolyzed to produce a 2-monoacylglycerol with the same configuration as the 2-monoacylglycerol produced in the intestine by pancreatic lipase. If, however, arachidonic acid or eicosapentaenoic acid are esterified at the 1 or 3 positions, then they are not well hydrolyzed by lipoprotein lipase, and diacylglycerols containing the long-chain unsaturated fatty acids are produced (31). These diacylglycerols appear to be a good substrate for hepatic lipase (78). In the absence of

significant plasma activity of hepatic lipase, apparently some diacylglycerols containing arachidonic or eicosapentaenoic acid are retained in the chylomicron remnant and may be returned to the liver (31) where they are removed by hepatic lipase or by remnant uptake. Thus, during lipoprotein lipase action on chylomicrons, fatty acids that are about half ionized (97, 98), monoacylglycerols, and perhaps some diacylglycerols are transiently present in the remnant particle. Moreover, in the capillary bed where active lipolysis takes place the concentration of albumin may not be adequate to remove all of the fatty acid and monoglyceride produced. Consequently, a particle with significant amounts of these molecules on the surface could be produced and could affect the further metabolism of the remnant particle. Fatty acids at pH 7.4 partition favorably into albumin, but if the albumin:FA falls, the fatty acid will partition into lipoproteins (97) and membranes (43, 98). Some of the fatty acid and monoglyceride are probably returned to the liver through transport on albumin where they are utilized for energy or lipid synthesis. The rest of the fatty acid and monoglyceride that leave the chylomicron enter the capillary bed and its surrounding tissues to be reesterified to triacylglycerols or phospholipids. If the monoacylglycerol pathway is active in adipose tissue or other tissues where triacylglycerols are stored, the original *sn*-2 position should be retained. However, it is more likely that monoacylglycerols are hydrolyzed and a different distribution of fatty acids occurs in adipose tissue triacylglycerols. Thus animals such as pigs that eat a diet with a high polyunsaturated fatty acid in the 2 position of the diet triacylglycerol make depot fat with palmitate in the 2 position and linoleate in the 3 position (see Table 1).

Although a relatively random distribution of fatty acids on the 1 and 3 positions would be expected from this scheme, some specificity of fatty acid incorporation into the 1 or 3 positions has been noted. Mustard seed oil (76) (see footnote e, Table 1) contains erucic acid (22:1 Ω 9) largely in the 3 position. Virtually none was found in the 2 position. When mustard seed oil (60, 77) was fed to rats and lymph lipoproteins (77) and tissue (76) were examined, the 2 position contained oleic and linoleic acids expected from the oil composition, but in the chylomicrons erucic acid was still enriched in the 3 position although not to the same extent as in the original mustard seed oil (60). Even in the heart adipose tissue the erucic acid was enriched in the 3 position (76). Therefore, some specificity for certain fatty acids may be incorporated into the 1 or 3 positions, but the mechanisms for this have not been elucidated.

Physical Properties of Triglycerides, Diglycerides, Monoglycerides, and Fatty Acids

Tables 2 to 4 give the melting and crystallization temperatures for a variety of triacylglycerols, diacylglycerols, and monoacylglycerols that could be pres-

ent during fat metabolism. These tables illustrate the fact that the melting points of many of these molecules are above body temperature. It is well known that tristearin is not digested or absorbed, presumably because the melting temperature is too high. Once hydrolysis begins, fatty acids are released. Long-chain saturated fatty acids also have quite high melting points; for instance, the melting points for myristic, palmitic, and stearic acids are 54, 63, and 70°C respectively. However, at the pH of the intestine (5.5–7.5) the fatty acid would be rapidly converted into hydrated acid-soaps (24). Like protonated fatty acids, these compounds are insoluble in aqueous media, and the melting temperatures for acid-soaps are only slightly less than the melting temperatures for the acids themselves. For instance, the melting temperatures for hydrated potassium acid-soaps are 43°C (myristic), 51°C (palmitic), and 61°C (stearic) (24). Thus if either the acid or the hydrated acid-soap were to precipitate from the intestinal contents, it would not be absorbed readily. Furthermore, if sufficient calcium is present in the intestinal lumen, calcium soaps with very high melting points (94) can be formed and pass out in the feces (17). Saturated diacylglycerols (Table 3) also have high melting points (56), which are not affected appreciably by water. These molecules partition into the surface of lipoproteins and membranes (42) and as surface constituents of chylomicrons could influence the types of apoprotein bound. Monoacylglycerols (Table 4) in the hydrated state have considerably lower melting temperatures (62) than in the dry state (about 25°C lower). However, even in water the longer chain monacylglycerols are crystalline above body temperature and could potentially segregate into partially crystalline structures on the surfaces of fat globules in the intestine or plasma lipoproteins if present in high enough quantities.

The Relation Between the Degree of Fatty Acid Saturation and Physical Properties of Triglyceride-Rich Lipoprotein

Bennett-Clark et al (6) were able to feed rats a diet rich in palmitate and produce chylomicrons in which palmitate constituted about 58–72% of the fatty acids and stearate 6–16%. The mean triglyceride fatty acids were 74 ± 1% saturated (6). These chylomicrons circulated as metastable, undercooled particles at 37°C in the rat. However, if these particles were cooled to below 30°C, they crystallized the saturated fatty acid triacylglycerols, which distorted the shape of the particles and increased their density (6). These particles had crystalline triglycerides that melted over a fairly broad range between about 30° and 60°C with a peak at about 54°C (6). Compared to particles with much less saturated triacylglycerols, these saturated particles bound more apoC's and less apoA-I and apoA-IV (35). Monkeys fed butter fat also had highly saturated triacylglycerides in their chylomicrons and intestinal VLDL. These particles had melting points above body temperature (46, 85), but were secreted from the intestine into lymph with the triglycerides in an un-

Table 2 Melting (T_m) and crystallization (T_c) temperatures of triacylglycerols[a]

	T_m (°C)	T_c (°C)	Reference
All saturated acyl chain			
CCC	31.5	−15	94
DDD	46.5	15	94
MMM	57.0	33	94
PPP	66.4	44.7	94
SSS	73.1	54.9	94
sn - PPB[b]	44.0	21.5	53
sn - PPM	58.8	39.5	53
sn - DMD	50.2		39
sn - MDM	50.0		39
sn - DPP	54		39
sn - SPP	62.7	47.4	4
sn - PSP	68.6	46.5	4
sn - PSS	65.2	50.6	4
sn - SPS	68.5	51.0	4
One unsaturated acyl chain			
sn - DOD	16.5	−7.1	4
sn - MOM	26.3	2.1	4
sn - POP	35.2	12.0	4
sn - SOS	41.6	22.3	4
sn - PEP	55.0	33.0	4
sn - SES	61.0	40.0	4
sn - DDO	16.0		39
sn - PPO	34.5		39
sn - SSO	41.6		39
sn - PPL	36–38		39
sn - SSL	36–38		39
racemic POS	37.5–38		39
racemic OPS	40.5–41		39
racemic OSP	41–41.5		39
Two unsaturated acyl chains			
sn - OPO	22.0	−11.0	54
sn - OSO	25.2	−7.0	54
sn - OOD	6.5		54
sn - OOM	12.5	−10.6	54
sn - OOP	18.2	4.0	54
sn - OOS	24.0	−1.0	54
All unsaturated acyl chains			
OOO	5.5	−32	94
EEE	42	15.5	94
LLL	−13.1	−43	94
LnLnLn	−24.2	−44.6	94
ErErEr	30	6	94

[a] Abbreviations as in Table 1.

[b] The prefix *sn*- means that the compounds listed after are stereospecific isomers; for instance, *sn*-PPB is 1,2-dipalmitoyl, 3-buteryl *sn*-glycerol.

Table 3 Melting (T_m) and crystallization (T_c) temperatures of diacylglycerols[a]

	T_m (°C)	T_c (°C)	References
1,2-Diacyl-*sn*-glycerols			
DD	46.9	14.2	56
MM	59	37.5	4
PP	70.1	47.3	56
SS	77.2	56.8	56
1,3-Diacyl-*sn*-glycerols			
di-C	44.5	37	4
di-D	56.5	49.5	4
di-M	65.5	60	4
di-P	74.9	65.5	56
di-S	78.4	69.1	56
di-O	25	18	4
di-E	55	49	4
di-Er	46.5	41	4
Mixed chain racemic diacylglycerols			
CM	48	39	4
DP	59.5	51	4
CS	59.5		4
DS	61.5		4
MS	67	56	4
PS	71.5		4
DO	32		4
MO	41		4
PO	46		4
SO	54		4

[a] Abbreviations as in Table 1.

dercooled, metastable, liquid state. Thus, it is possible to force animals to make highly saturated triacylglycerols that are packaged and secreted as metastable, undercooled triglycerides in chylomicrons and intestinal VLDL. A fairly good correlation ($r = 0.79$) was found between the combined percentage of 16:0 and 18:0 of chylomicron triacylglycerols and the temperature of crystallization, T_c (69). The equation relating T_c in degrees C to saturation was: $T_c = 5 + (\%\ 16{:}0 + \%\ 18{:}0)$. We do not yet know if such high melting particles are metabolized differently from more unsaturated particles with melting points below body temperature.

The Metabolism of Single Stereospecific Triacylglycerols

To simplify the problems involved in feeding a diet with an uncontrollable variety of triacylglycerols, we synthesized individual stereospecific triacyl-

Table 4 Melting (T_m) and crystallization (T_c) temperatures of monoacylglycerols[a]

	T_m(°C)	T_c(°C)	References
2-Monoacyl-*sn*-glycerols			
C	40.4		94
D	51.0		94
M	61.2		94
P	68.5		94
S	74.5		94
3-Monoacyl-*sn*-glycerols			
C	56.0	29.2	57
D	62.0	42.0	57
M	64.0	55.0	57
P	70.5	63.8	57
S	77.0	72.0	57

[a] Abbreviations as in Table 1.

glycerols, studied them physically, then fed them to rats, harvested the chylomicrons, and reinjected them into recipient rats to study chylomicron hydrolysis and remnant uptake. We started with 1,2-dioleyl-3-acyl-*sn*-glycerols (OOX), a set of triacylglycerols with oleic acid in the 1 and 2 position and saturated fatty acids from C14 to C24 in the 3 position (33). The longer chain fatty acids C20, C22, and C24 model to some extent the composition of some of the triacylglycerols in peanut oil (92). About 15% of the fatty acids in the 3 position are C-20 to C-24 in peanut oil. The fatty acid composition of the chylomicron triglyceride (89) largely reflected the composition of the fed triacylglycerol except for these long chain fatty acids. Oleic acid was the major fatty acid and represented about two thirds of the fatty acids recovered in the chylomicron. Small amounts of 16:0 (4% of total), 18:2 (~ 3% of total), and 20:4 (~ 1%) were found, indicating the incorporation of some fatty acids from bile and mucosa. When OOM, OOP, and OOS were fed, 28–32 mole% of these fatty acids were recovered in the chylomicron—a recovery rate of ~ 90%. However, in the longer series, 1,2-dioleyl-3-arachidyl-*sn*-glycerol (OOA), 1,2-dioleyl-3-behenyl-*sn*-glycerol, and 1,2-dioleyl-3-lignoceryl-*sn*-glycerol, only 20, 8, and 2 mol%, were recovered in the harvested chylomicron triacylglycerols. Thus, arachidic (C20), behenic (C22), and lignoceric (C24) acids were incoporated into chylomicron triacylglycerols at progressively lower rates. The fact that decreased amounts of long chain saturated acids were accompanied by increases in oleic acid suggests that the oleic acids were absorbed but the long chain fatty acids were not. Since the melting point of the acid soaps of these long chain fatty acids is very high (> 65°C), they may precipitate in the intestine

and be lost in the feces. The uptake of remnants of OOP, OOS, and OOA chylomicrons was slower than that of OOO chylomicrons. Unfortunately, no stereospecific analysis of these chylomicron triacylglycerols was made.

When the positional isomers OSO and OOS were fed to donor rats, the composition of the collected chylomicrons was quite similar although there was considerably more stearic acid in the OSO than in the OOS chylomicrons (89). One explanation is that in the 2 position stearate is retained whereas in the 3 position it is partially lost in the gut. When these harvested chylomicrons were injected into recipient rats, both lipolysis and especially the uptake of remnants were slower in the chylomicrons obtained by OSO feeding than in those obtained by OOS feeding. The scheme in Figure 3 suggests that the stearic and oleic acids are retained in the 2 position in the OSO and OOS chylomicrons respectively. When lipoprotein lipase acts on the triacylglycerols in these particles it generates 2-monostearin from OSO and 2-monolein from OOS. The melting point of 2-monostearin is considerably above body temperature, whereas 2 monolein is well below (Table 4). Possibly, lipolysis might produce a more compact surface from which apoproteins like apoC and enzymes like lipoprotein lipase might be excluded. Furthermore, apoE might be dissociated or its conformation changed so that the OSO chylomicron remnant did not bind well to its hepatic receptor. This might account for its slow uptake and longer plasma residence time. We know that the fluidity of the surface, as modified by cholesterol content, governs apoA-I and apoE-3 absorption (28). In egg yolk-triolein-cholesterol emulsions the binding constant, K_d, for apoA-I and apoE-3 is unchanged but the surface concentration is greatly diminished as the surface cholesterol is increased from 1/5 C/PC to 1/1 C/PC (28). Small amounts of partly ionized fatty acid that partition strongly to the surface (30) enhance apoprotein binding (27). Furthermore, modulating the surface fluidity between fluid and solid by using different saturated chain phosphatidylcholines and cholesterol greatly changes the mass and the nature of the absorbed proteins (95). When chylomicron models with different surfaces (37) are injected into rats and lipolysis and remnant uptake is monitored, only chylomicron models with fluid surfaces undergo lipolysis, and remnant uptake by the liver is virtually blocked by a mainly solid surface (7, 8). These surface changes, however, are brought about by phospholipids and/or cholesterol and not by monoglycerides.

To attempt to model these chylomicrons (69), low cholesterol emulsions were made with egg phosphatidylcholine and either OSO or OOS and were injected into rats (89). If a saturated fatty acid was presented in the 2 position, lipolysis was slowed and uptake by the liver retarded. Emulsions were also made with OEO and OVO, which had the *trans* fatty acids, elaidic or vaccinic, substituted in the 2 position (54). Uptake of the remnant particle derived from these emulsions was also slowed, although hydrolysis was not

affected (89). To look at the effect of monoacylglycerols in the interface, Mortimer et al prepared emulsions with small amounts of monoglycerols and injected them into rats (72). These authors found that 1.8–2.5% of either 1 or 2 monostearyl glycerol (but not monooleyl glycerol) retarded emulsion uptake by the liver but did not influence lipolysis. It should be noted that monoacylglycerols added to an emulsion may not form the same quality of surface as monoacylglycerols added by hydrolysis of triacylglycerols. In the formation of emulsions the surface pressure of egg PC emulsions varies from about 18 dynes per cm in low cholesterol emulsions to 25 dynes per cm in high cholesterol emulsions (D. M. Small, unpublished observations). Lipoprotein remnants might be at a considerably higher surface pressure due to rapid formation of surface active molecules (partial glycerols and fatty acids) generated during lipolysis. Thus, high melting monoacylglycerols on the surface slow remnant removal by the liver and may, if generated by lipolysis, act to impede further hydrolysis. Careful analysis of the apoproteins bound to such artificial remnants should help us understand the mechanism for slowed removal.

A few studies correlate the stereospecificity of diet fat with that of chylomicrons in humans. Kayden et al (51) infused positionally specific radiolabeled glycerides and/or glycerol into patients with thoracic duct fistula. Chyle was collected during and after the infusion, and lipids were analyzed. A maximum of 4% of the radiolabeled free glycerol was recovered in triacylglycerols. Doubly labeled 2-monolein was highly conserved in chylomicron triacylglycerols in the first few hours of the perfusion, and only later, presumably after some acyl migration and/or hydrolysis of 2-monolein in the intestine occurred, did the labels separate.

To study the effect of stereospecificity on the absorption of fatty acids, Filer et al (36) gave newborn infants a formula made either of lard that has an abundance of 16:0 in the 2 position (Table 1) or of randomized lard in which 16:0 is distributed to all positions, and they measured fat absorption by balance. Whereas 95% of all fatty acids were absorbed from lard, only 72% were absorbed from randomized lard. In fact, from randomized lard unsaturated fatty acids were absorbed to greater than 90% while palmitic and stearic acids were malabsorbed at 58 and 40% respectively. Although the mechanisms of action are not understood, positional specificity certainly seems to play a role in the luminal phase of fat absorption. Animal studies using cocoa butter and randomized cocoa butter also show marked differences in absorption (58).

Studies of the stereospecific positions of triacylglycerol fatty acids in chylomicrons isolated from plasma after more complicated fat meals have been even more difficult to interpret. However, the brave attempts by Myher et al (74) to solve this problem should be mentioned. These authors gave a 74-g lard breakfast to subjects and collected blood 4 hr later. A chylomicron

fraction was isolated and stereospecific analysis of triacylglycerols made. While VLDL fractions on control mixed diets contained very little palmitic acid (<10%) in the 2 position, 33-42% of the 2 position of the lard-fed chylomicrons was palmitate (see Table 5). This finding indicates fair retention of the original 2 position palmitate present in the fed fat. Furthermore, stearic acid is almost exclusively present in the 1 position in lard (73), and predominance of stearate in the *sn*-1 position was maintained in the chylomicron fraction. If the findings concerning the metabolism of chylomicrons with saturated fatty acids in the 2 position of triacylglycerols in rats (89) can be extrapolated to humans, then eating lard or lard-like triacylglycerols might slow both hydrolysis of chylomicron triacylglycerols and liver uptake of chylomicron remnants. This could lead to increased plasma remnant concentration and possibly to increased arterial bed uptake of remnants.

Summary

The subtle effects of the stereochemistry of acyl glycerols are apparent from the cited studies. It is not adequate to simply measure the fatty acid composition of dietary lipids or chylomicrons generated from them. To understand the importance of stereospecific acyl glycerols, simplification of the systems is necessary because of the incredible diversity found among dietary triacylglycerols. By feeding simple analogues corresponding to major triacylglycerols found in oils and fats it should be possible to determine the absorption, uptake into the mucosa, resynthesis into chylomicrons, and the stereospecificity that remains. Hydrolysis and uptake of these defined chylomicron triacylglycerols into adipose tissue or muscle could be followed and stereospecificity again determined in depot fats. Finally, the composition of the remnants, particularly the core and surface (69), and of the bound apoproteins needs to be related to their plasma residence time, hepatic removal, and deposition in nonhepatic

Table 5 Comparison of stereospecific analyses of lard in the diet and resultant chylomicrons

Fatty acids	Diet lard (73)			Human chylomicron fraction (74)				
				Subject 1			Subject 2	
	1-*sn*	2-*sn*	3-*sn*	1-*sn*	2-*sn*	3-*sn*	1 + 3-*sn*	2-*sn*
14:0	1	3	1	2	2	1	1	2
16:0	10	74	3	41	33	10	19	42
16:1	2	4	2	3	4	3	3	5
18:0	29	2	5	17	5	9	15	5
18:1	49	13	69	30	41	60	48	34
18:2	7	4	19	6	13	18	12	15

tissues such as spleen, bone marrow (48), and arterial intima. Since increased serum cholesterol and atherogenicity in animals have been related to different dietary fats (58), some of the effects noted here, that is the retention of certain kinds of remnants in plasma, may lead to increased serum cholesterol and atherogenesis. We must study the metabolism of a few key stereospecific triacylglycerols in more detail to increase our understanding of their effects on hypercholesterolemia and atherogenesis.

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Literature Cited

1. Akesson, B., Gronowitz, S., Herslof, B. 1976. Stereospecificity of hepatic lipases. *FEBS Lett.* 71(2):241–44
2. Akesson, B., Gronowitz, S., Herslof, B., Ohlson, R. 1978. Absorption of synthetic, stereochemically defined acylglycerols in the rat. *Lipids* 13:338
3. Atkinson, D., Small, D. M. 1986. Recombinant lipoproteins. Implications for structure and assembly of native lipoproteins. *Annu. Rev. Biophys. Biophys. Chem.* 15:403–56
4. Bailey, A. E. 1950. Melting and solidification of pure compounds. In *Melting and Solidification of Fats*, pp. 117–237. New York: Interscience
5. Balesdent, D., Kapseu, C., Kayem, G. J. 1989. Characterization of triglycerides of cottonseed oil by high performance liquid chromatography (HPLC) in one step. *Rev. Fr. Corps Gras* 36:27–33
6. Bennett-Clark, S., Atkinson, D., Hamilton, J. A., Forte, T., Russell, B., et al. 1982. Physical studies of d 1.006 g/ml lymph lipoproteins from rats fed palmitate-rich diets. *J. Lipid Res.* 23:28–41
7. Bennett-Clark, S., Derksen, A. 1987. Phosphatidylcholine composition of emulsions influences triacylglycerol lipolysis and clearance from plasma. *Biochim. Biophys. Acta* 920:37–46
8. Bennett-Clark, S., Derksen, A., Small, D. M. 1991. Plasma clearance of emulsified triolein in conscious rats: Effects of phosphatidylcholine species, cholesterol content and emulsion surface physical state. *Q. J. Exp. Physiol.* 76: 39–52
9. Bernard, A., Echinard, B., Carlier, H. 1987. Differential intestinal absorption of two fatty acid isomers: elaidic and oleic acids. *Am. J. Physiol.* 253(6): G751–59
10. Bernback, S., Blackberg, L., Hernell, O. 1990. The complete digestion of human milk triacylglycerol in vitro requires gastric lipase, pancreatic colipase-dependent lipase, and bile salt-stimulated lipase. *J. Clin. Invest.* 85 (4):1221–26
11. Bezard, J., Bugaut, M. 1986. Absorption of glycerides containing short, medium, and long chain fatty acids. See Ref. 59, pp. 119–58
12. Bhati, A. 1986. Gas chromatography–mass spectrometry of triglycerides and related compounds. In *Analysis of Oils and Fats*, ed. R. J. Hamilton, J. B. Rossell, pp. 207–41. New York: Elsevier
13. Borgstrom, B. 1984. Pancreatic lipase. In *Lipase*, ed. B. Borgstrom, H. L. Brockman. Amsterdam: Elsevier
14. Breckenridge, W. C. 1978. Stereospecific analysis of triacylglycerols. In *Handbook of Lipid Research, Fatty Acids and Glycerides*, ed. A. Kuksis, 1:197–232. New York: Plenum
15. Brockerhoff, H. 1965. A stereospecific analysis of triglycerides. *J. Lipid Res.* 6:10
16. Brockerhoff, H. 1965. A stereospecific analysis of triglycerides: an alternative method. *J. Lipid Res.* 8:167
17. Carey, M. C., Small, D. M., Bliss, C. M. 1983. Lipid digestion and absorption. *Annu. Rev. Physiol.* 45:651–77
18. Chen, Q., Sternby, B., Akesson, B., Nilsson, A. 1990. Effects of human pancreatic lipase-colipase and carboxyl ester lipase on eicosapentaenoic and arachidonic acid ester bonds of triacylglyc-

erols rich in fish oil fatty acids. *Biochim. Biophys. Acta* 1044(1):111–17
19. Chen, Q., Sternby, B., Nilsson, A. 1989. Hydrolysis of triacylglycerol arachidonic and linoleic acid ester bonds by human pancreatic lipase and carboxyl ester lipase. *Biochim. Biophys. Acta* 1004(3):372–85
20. Christie, W. W. 1986. The positional distributions of fatty acids in triglycerides. See Ref. 12, pp. 313–39
21. Christie, W. W. 1987. *HPLC and Lipids: A Practical Guide*. New York: Pergamon
22. Christie, W. W. 1989. *Gas Chromatography and Lipids A Practical Guide*. Ayr, Scotland: Oily
23. Christie, W. W., Moore, J. H. 1970. The structure of egg yolk triglycerides. *Biochim. Biophys. Acta* 218:83–88
24. Cistola, D. P., Atkinson, D., Hamilton, J. A., Small, D. M. 1986. Phase behavior and bilayer properties of fatty acids: Hydrated 1:1 acid soaps. *Biochemistry* 25:2804–12
25. Constantin, M. J., Savary, P. 1965. On the incorporation of stearic acid in the triglycerides of rat-lymph chylomicrons. *Biochim. Biophys. Acta* 106:248–66
26. Deckelbaum, R. J., Hamilton, J. A., Bengtsson-Olivecrona, G., Butbul, E., Carpentier, Y. A., et al. 1990. Medium-chain vs long-chain triacylglycerol emulsion hydrolysis by lipoprotein lipase and hepatic lipase: Implications for the mechanisms of lipase action. *Biochemistry*. In press
27. Derksen, A., Ekman, S., Small, D. M. 1989. Oleic acid allows more apoprotein A-I to bind with higher affinity to large emulsion particles saturated with cholesterol. *J. Biol. Chem.* 264(12): 6935–40
28. Derksen, A., Small, D. M. 1989. Interaction of apoA-I and apoE-3 with triglyceride-phospholipid emulsion containing increasing cholesterol concentrations. Model of triglyceride-rich nascent and remnant lipoproteins. *Biochemistry* 28:900–5
29. Dong, M. W., DeCesare, J. L. 1983. Improved separation of natural oil triglycerides by liquid chromatography using columns packed with 3-μm particles. *J. Am. Oil Chem. Soc.* 60:788–91
30. Ekman, S., Derksen, A., Small, D. M. 1988. The partitioning of fatty acid and cholesterol between core and surfaces of phosphatidylcholine-triolein emulsions at pH 7.4. *Biochim. Biophys. Acta* 959(3):343–48
31. Ekstrom, B., Nilsson, A., Akesson, B. 1989. Lipolysis of polyenoic fatty acid esters of human chylomicrons by lipoprotein lipase. *Eur. J. Clin. Invest.* 19:259–64
32. El-Hamdy, A. H., Perkins, E. G. 1981. High performance reversed phase chromatography of normal triglyceride mixtures: critical pair separation. *J. Am. Oil Chem. Soc.* 58:867–72
33. Fahey, D. A., Small, D. M., Atkinson, D., Kodali, D. R., Redgrave, T. G. 1985. Structure and polymorphism of 1,2-dioleoyl-3-acyl-*sn*-glycerols three and six layered structures. *Biochemistry* 24:3757–64
34. Farines, M., Soulier, R., Soulier, J. 1988. Analysis of the triglycerides of some vegetable oils. *J. Chem. Ed.* 65(5):464–66
35. Feldman, E. B., Russell, B. S., Chen, R., Johnson, J., Forte, T., Bennett Clark, S. 1983. Dietary saturated fatty acid content affects lymph lipoproteins. Studies in the rat. *J. Lipid Res.* 24:967–76
36. Filer, L. J. Jr., Mattson, F. H., Fomon, S. J. 1969. Triglyceride configuration and fat absorption by the human infant. *J. Nutr.* 99:293–98
37. Gantz, D., Bennett Clark, S., Derksen, A., Small, D. M. 1990. Size and shape determination of fixed chylomicrons and emulsions with fluid or solid surfaces by three-dimensional analysis of shadows. *J. Lipid Res.* 31(1):163–71
38. Geeraet, E., Sandra, P. 1987. Capillary GC of triglycerides in fats and oils using a high temperature phenylmethylsilicone stationary phase. Part II. The analysis of chocolate fats. *J. Am. Oil Chem. Soc.* 64:100–5
39. Golborn, P. 1969. Triglyceride composition of lard by differential thermal analysis. *J. Am. Oil Chem. Soc.* 46:385–86
40. Goldberg, I. J., Scheraldi, C. A., Yacoub, L. K., Saxena, U., Bisgaier, C. L. 1990. Lipoprotein ApoC-II activation of lipoprotein lipase. Modulation by apolipoprotein A-IV. *J. Biol. Chem.* 265(8):4266–72
41. Hamilton, J. A. 1989. Interactions of triglycerides with phospholipids: Incorporation into the bilayer structure and formation of emulsions. *Biochemistry* 28:2514–20
42. Hamilton, J. A., Bhamidipati, S., Kodali, D. R., Small, D. M. 1991. The interfacial conformation and transbilayer movement of diacylglycerols in phospholipid bilayers. *J. Biol. Chem.* 266:1177–86
43. Hamilton, J. A., Cistola, D. P. 1986. Transfer of oleic acid between albumin

and phospholipid. *Proc. Natl. Acad. Sci. USA* 83:82–86
44. Hamilton, J. A., Miller, K. W., Small, D. M. 1983. Solubilization of triolein and cholesteryl oleate in egg phosphatidylcholine vesicles. *J. Biol. Chem.* 258:12821–26
45. Hamilton, J. A., Small, D. M. 1981. Solubilization and localization of triolein in phosphatidylcholine bilayers: A ^{13}C NMR study. *Proc. Natl. Acad. Sci. USA* 78(11):6878–82
46. Hamilton, J. A., Small, D. M., Parks, J. 1983. ^{1}H NMR studies of lymph chylomicra and very low density lipoproteins from nonhuman primates. *J. Biol. Chem.* 258:1172–79
47. Hannewijk, J., Haighton, A. J. 1958. Differential thermal analysis. III. Melting curves of oils and fats. *J. Am. Oil Chem. Soc.* 35:457–61
48. Hussain, M. M., Mahley, R. W., Boyles, J. K., Fainaru, M., Brecht, W. J., Lindquist, P. A. 1989. Chylomicron-chylomicron remnant clearance by liver and bone marrow in rabbits. Factors that modify tissue-specific uptake. *J. Biol. Chem.* 264(16):9571–82
49. IUPAC-IUB Commission on Biochemical Nomenclature (CBN). 1968. The nomenclature of lipids. *Chem. Phys. Lipids* 2:156
50. Johnston, J. M. 1978. Esterification reactions in the intestinal mucosa and lipid absorption. In *Disturbances in Lipid and Lipoprotein Metabolism,* ed. J. M. Dietschy, A. M. Gotto Jr., J. A. Ontko, pp. 57–58. Bethcsda: Am. Physiol. Soc.
51. Kayden, H. J., Senior, J. R., Mattson, F. H. 1967. The monoglyceride pathway of fat absorption in man. *J. Clin. Invest.* 46:1695–1703
52. Kimmey, R. L., Perkins, E. G. 1984. Confectionary fat analysis with high performance lipid chromatography. *J. Am. Oil Chem. Soc.* 61:1209–11
53. Kodali, D. R., Atkinson, D., Redgrave, T. G., Small, D. M. 1984. Synthesis and polymorphism of 1,2-dipalmitoyl-3-acyl-*sn* glycerols. *J. Am. Oil Chem. Soc.* 61(6):1078–84
54. Kodali, D. R., Atkinson, D., Redgrave, T. G., Small, D. M. 1987. Structure and polymorphism of 18-carbon fatty acyl triacylglycerols: effect of unsaturation and substitution in the 2-position. *J. Lipid Res.* 28:403–13
55. Kodali, D. R., Atkinson, D., Small, D. M. 1990. Acyl migration in 1,2-dipalmitoyl-*sn*-glycerol. *Chem. Phys. Lipids* 52:163–70
56. Kodali, D. R., Fahey, D. A., Small, D. M. 1990. Structure and polymorphism of saturated monoacid 1,2-diacyl-*sn*-glycerols. *Biochemistry* 29:10771–79
57. Kodali, D. R., Redgrave, T., Small, D. M., Atkinson, D. 1985. Synthesis and polymorphism of 3-acyl-*sn*-glycerols. *Biochemistry* 24:519–25
58. Kritchevsky, D. 1988. Effects of triglyceride structure on lipid metabolism. *Nutr. Rev.* 46:177–81
58a. Kuksis, A. 1984. Quantitative and positional analysis of fatty acids. In *Lipid Research Methodology,* ed. J. A. Story, 10:78–131. New York: Liss
59. Kuksis, A., ed. 1986. *Fat Absorption,* Vol. 1. Boca Raton, Fla: CRC Press
60. Kuksis, A. 1987. Effect of dietary fat on formation and secretion of chylomicrons and other lymph lipoproteins. In *Fat Absorption,* ed. A. Kuksis, 2:135–66. Boca Raton, Fla: CRC Press
61. Kuksis, A., Myher, J. J., Marai, L. 1984. Lipid methodology-chromatography and beyond. Part I. GC/MS and LC/MS of glycerolipids. *J. Am. Oil Chem. Soc.* 61:1582–89
62. Larsson, K. 1968. The structure of mesomorphic phases and micelles in aqueous glyceride systems. *J. Phys. Chem.* 56:173
63. Luddy, F. E., Hampson, J. W., Herb, S. F., Rothblat, H. L. 1973. Development of edible tallow fractions for specialty fat uses. *J. Am. Oil Chem. Soc.* 50:240–44
64. Mansbach, C. M. II, Parthasarathy, S. 1982. A re-examination of the fate of glyceride-glycerol in neutral lipid absorption and transport. *J. Lipid Res.* 23:1009–19
65. Marai, L., Myher, J. J., Kuksis, A. 1983. Analysis of triacylglycerols by reversed-phase high pressure liquid chromatography with direct liquid inlet mass spectrometry. *Can. J. Biochem.* 61:840–49
66. Mattson, F. H., Volpenhein, R. A. 1962. Rearrangement of glyceride fatty acids during digestion and absorption. *J. Biol. Chem.* 237:53–55
67. Mattson, F. H., Volpenhein, R. A. 1964. The digestion and absorption of triglycerides. *J. Biol. Chem.* 239:2772–77
68. Miller, K. W., Small, D. M. 1982. The phase behavior of triolein, cholesterol, and lecithin emulsions. *J. Colloid Interface Sci.* 89(2):466–78
69. Miller, K. W., Small, D. M. 1987. Structure of triglyceride-rich lipoproteins: An analysis of core and surface phases. In *New Comprehensive Biochemistry. Plasma Lipoproteins,* ed. A.

M. Gotto Jr., 14:1–75. New York: Elsevier
70. Morley, N., Kuksis, A. 1972. Positional specificity of lipoprotein lipase. *J. Biol. Chem.* 247(20):6389–93
71. Morley, N. H., Kuksis, A., Buchnea, D., Myher, J. J. 1975. Hydrolysis of diacylglycerols by lipoprotein lipase. *J. Biol. Chem.* 250(9):3414–18
72. Mortimer, B. C., Simmonds, W. J., Joll, C. A., Stick, R. V., Redgrave, T. G. 1989. The effect of added monoacylglycerols on the removal from plasma of chylomicron-like emulsions injected intravenously in rats. *Biochim. Biophys. Acta* 1002:359–64
73. Myher, J. J., Kuksis, A. 1979. Stereospecific analysis of triacylglycerols via racemic phosphatidylcholines and phospholipase C. *Can. J. Biochem.* 57:117–24
74. Myher, J. J., Kuksis, A., Breckenridge, W. C., McGuire, V., Little, J. A. 1985. Comparative studies of triacylglycerol structure of very low density lipoproteins and chylomicrons of normolipemic subjects and patients with Type II hyperlipoproteinemia. *Lipids* 20(2):90–101.
75. Myher, J. J., Kuksis, A., Marai, L. 1988. Identification of the more complex triacylglycerols in bovine milk fat by gas chromatography–mass spectrometry using polar capillary columns. *J. Chromatogr.* 452:93–118
76. Myher, J. J., Kuksis, A., Vasdev, S. C., Kako, K. J. 1979. Acylglycerol structure of mustard seed oil and of cardiac lipids of rats during dietary lipidosis. *Can. J. Biochem.* 57:1315–27
77. Myher, J. J., Kuksis, A., Yang, L. Y., Marai, L. 1987. Stereochemical course of intestinal absorption and transport of mustard-seed oil triacylglycerols in the rat. *Biochem. Cell Biol.* 65(9):811–21
78. Nilsson, A., Landin, B., Schotz, M. C. 1987. Hydrolysis of chylomicron arachidonate and linoleate ester bonds by lipoprotein lipase and hepatic lipase. *J. Lipid. Res.* 28(5):510–17
79. Nilsson-Ehle, P., Egelrud, T., Belfrage, P., Olivecrona, T., Borgstrom, B. 1973. Positional specificity of purified milk lipoprotein lipase. *J. Biol. Chem.* 248(19):6734–37
80. O'Doherty, P. J. A., Kuksis, A., Buchnea, D. 1972. Enantiometric diglycerides as stereospecific probes in triglyceride synthesis in vitro. *Can. J. Biochem.* 50:881
81. Olivecrona T., Bengtsson-Olivecrona, G. 1987. Lipoprotein lipase from milk—the model enzyme in lipoprotein lipase research. In *Lipoprotein Lipase,* ed. J. Borensztajn, pp. 15–58. Chicago: Evener
82. Ottenstein, D. M., Wittig, L. A., Silvius, P. H., Hometchko, D. J., Pelick, N. 1984. Column types for the chromatographic analysis of oleochemicals. *J. Am. Oil Chem. Soc.* 61:390–94
83. Palmer, A. J., Palmer, F. J. 1989. Rapid analysis of triacylglycerols using high-performance liquid chromatography with light scattering detection. *J. Chromatogr.* 465:369–77
84. Paltauf, F., Esfandi, F., Holasek, A. 1974. Stereospecificity of lipases. Enzymic hydrolysis of enantiometric alkyl diacylglycerols by lipoprotein lipase, lingual lipase and pancreatic lipase. *FEBS Lett.* 40:119–23
85. Parks, J. S., Atkinson, D., Small, D. M., Rudel, L. R. 1981. Physical characterization of lymph chylomicra and very low density lipoproteins from nonhuman primates fed saturated dietary fat. *J. Biol. Chem.* 256:12992–99
86. Patton, J. S. 1981. Gastrointestinal lipid digestion. In *Physiology of the Gastrointestinal Tract,* ed. L. R. Johnson, pp. 1123–46. New York: Raven
87. Pauls, R. E. 1983. A time normalization study of the separation of olive oil triglycerides. *J. Am. Oil Chem. Soc.* 60: 819–22
88. Petersson, B., Podhala, O., Töregård, B. 1981. HPLC separation of natural oil triglycerides into fractions with the same carbon number and numbers of double bonds. *J. Am. Oil Chem. Soc.* 58:1005–9
89. Redgrave, T. G., Kodali, D. R., Small, D. M. 1988. The effect of triacyl-*sn*-glycerol structure on the metabolism of chylomicrons and triacylglycerol-rich emulsions in the rat. *J. Biol. Chem.* 263(11):5118–23
90. Rhodes, J. H., Netting. A. G. 1988. Normal-phase high-performance liquid chromatography of triacylglycerols. *J. Chromatogr.* 448:135–43
91. Schlichter-Aronhime, J., Garti, N. 1988. Solidification and polymorphism in cocoa butter and the blooming problems. In *Crystallization and Polymorphism of Fats and Fatty Acids,* ed. N. Garti, K. Sato. 31:363–93. New York: Dekker
92. Sempore, G., Bezard, J. 1986. Qualitative and quantitative analysis of peanut oil triacylglycerols by reversed-phase liquid chromatography. *J. Chromatogr.* 366:261–82
93. Shukla, V. K. S. 1989. Application of modern analytical techniques for evalua-

tion of lipid structures. *J. Disp. Sci. Technol.* 10:581–616
94. Small, D. M. 1986. *Handbook of Lipid Research*, Vol. 4, ed. D. Hanahan, New York: Plenum
95. Small, D. M., Bennett Clark, S., Tercyak, A., Steiner, J., Gantz, D., Derksen, A. 1991. The lipid surface of triglyceride-rich particles can modulate (apo)protein binding and tissue uptake. *Proc. 5th Int. Colloq. Atherosclerosis, Brussels, Belgium. Hypertriglyceridemia, Hypercholesterolemia, Hypocholesterolemia, and In Vivo Kinetics*, ed. C. L. Malmendier. New York: Plenum
96. Sonntag, N. O. V. 1979. Composition and characteristics of individual fats and oils. In *Bailey's Industrial Oil and Fat Products*, ed. D. Swern, 1:289–478. New York: Wiley. 4th 3d.
97. Spooner, P. J. R., Bennett Clark, S., Gantz, D. L., Hamilton, J. A., Small, D. M. 1988. The ionization and distribution behavior of oleic acid in chylomicrons and chylomicron-like emulsion particles and the influence of serum albumin. *J. Biol. Chem.* 263(3):1444–53
98. Spooner, P. J. R., Gantz, D. L., Hamilton, J. A., Small, D. M. 1990. The distribution of oleic acid between chylomicron-like emulsions, phospholipid bilayers and serum albumin. A model for fatty acid distribution between lipoproteins, membranes, and albumin. *J. Biol. Chem.* 265(21):12650–55
99. Spooner, P. J. R., Small, D. M. 1987. Effect of free cholesterol on incorporation of triolein in phospholipid bilayers. *Biochemistry* 26:5820–25
100. Staggers, J. E., Fernando-Warnakulasuriya, G. J. P., Wells, M. A. 1981. Studies on fat digestion, absorption, and transport in the suckling rat. II. Triacylglycerols: molecular species, stereospecific analysis, and specificity of hydrolysis by lingual lipase. *J. Lipid Res.* 22:675–79
101. Stolyhwo, A., Colin, H., Guiochon, G. 1985. Analysis of triglycerides in oils and fats by liquid chromatography with the laser light scattering detector. *Anal. Chem.* 57:1342–54
102. Thomson, A. B. R., Dietschy, J. M. 1981. Intestinal lipid absorption: major extracellular and intracellular events. See Ref. 86, pp. 1147–1220
103. Triacylglycerols: influence of structure on metabolism in the rat. Editorial. *Nutr. Rev.* 47:
104. Wang, C-S. 1986. Hydrolysis of dietary glycerides and phosphoglycerides: fatty acid and positional specificity of lipases and phospholipases. See Ref. 59, pp. 83–117
105. Wong, R., Hwang, L. S. 1989. Capillary GC analysis of triglyceride composition of fats and oils. *Shih P'inK'o Hsueh, Taipei* 16:1–13