Intestinal absorption of specific structured triacylglycerols

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Abstract To clarify the intestinal absorption pathway of medium-chain fatty acids from MLM-type structured triacylglycerols containing both medium- and long-chain fatty acids, we studied the lymphatic transport of 1,3-dioctanoyl-2-linoleoyl-sn-glycerol (8:0/18:2/8:0), 1,3-didecanoyl-2-linoleoyl-sn-glycerol (10:0/18:2/10:0), and 1,3-didodecanoyl-2-linoleoyl-sn-glycerol (12:0/18:2/12:0) in a rat model. Safflower oil was used in the absorption study in order to compare the absorption of medium-chain fatty acids and long-chain fatty acids. The triacylglycerol species of lymph lipids were separated on a reversed-phase high performance liquid chromatograph (RP-HPLC) and identified by atmospheric pressure chemical ionization mass spectrometry. The composition of triacylglycerols was quantified by RP-HPLC with evaporative light scattering detection. The intact MLM-type triacylglycerols were detected in the lymph lipids after administration of the specific structured triacylglycerols (STAG). The recoveries of 8:0/18:2/8:0, 10:0/18:2/10:0, and 12:0/18:2/12:0 were 0.6%, 12%, and 5%, respectively. Several new triacylglycerol species were detected in the lymph lipids, including MLL-, LLL-, and MMM-type triacylglycerols. From the present study we conclude that the medium-chain fatty acids from STAG, in addition to absorption into the portal blood as free fatty acids, are absorbed by the same pathway as the conventional long-chain triacylglycerols, that is, they are hydrolyzed into free fatty acids, absorbed and activated into CoA, and reacylated into triacylglycerols in the enterocyte. The hydrolysis of MLM-type STAG is predominantly partial hydrolysis, whereas part of the STAG can also be hydrolyzed to free glycerol and free fatty acids.—Mu, H., and C-E. Høy. Intestinal absorption of specific structured triacylglycerols. J. Lipid Res. 2001. 42: 792–798.

Supplementary key words identification • hydrolysis • long-chain fatty acids • lymph • medium-chain fatty acids • rat • reacylation

In addition to the overall fatty acid profile of dietary fat, the triacylglycerol structure and the species composition are factors of nutritional importance. This has been demonstrated convincingly for triacylglycerols containing long-chain fatty acids, in particular in infant nutrition, that is, palmitic acid can be better absorbed when it is located at the sn-2 position than at the primary positions (1, 2). Most of the palmitic acid molecules in human milk fat are actually located at the sn-2 position (3–5). Therefore an infant formula enriched with palmitic acid at the sn-2 position is more similar to human milk, provides more energy, and results in lower fecal losses of fat and calcium (1, 6, 7).

Also for structured triacylglycerols (STAG) containing medium-chain fatty acids and polyunsaturated fatty acids, the triacylglycerol structure is important (8–14). These fats were initially manufactured in randomized form and provided significant metabolic benefits in injured animals, such as a protein-sparing effect and improved nitrogen balance (15). The distribution of medium-chain fatty acids in STAG also affects the absorption of long-chain fatty acids (16, 17), because the medium-chain fatty acids can be hydrolyzed faster than long-chain fatty acids (18). Therefore MLM (M, medium-chain fatty acid; L, long-chain fatty acid)-type STAG containing essential fatty acids have been suggested to improve the clinical nutrition of patients suffering from malabsorption because they supply both energy and essential fatty acids (16, 19).

In studies of absorption of MLM-type STAG, medium-chain fatty acids have also been detected in lymph lipids (16, 20–22). The level of medium-chain fatty acids detected in lymph increased with the increase in chain length of medium-chain fatty acids (16, 21). Even though several studies of the absorption of structured lipids have been reported (16, 18, 19, 23, 24), the absorption pathway is still unclear and the intestinal absorption of medium-chain fatty acids from MLM-type STAG is not well understood.

The most frequently used method to monitor lipid digestion and absorption is gas-liquid chromatography, which is used to analyze the fatty acid profiles (16, 19, 25), whereas the individual triacylglycerol molecules have not been examined because of the experimental complexity. In the present study, we studied in a normal rat model the lymphatic transport of MLM-type STAG containing various medium-chain fatty acids. The triacylglycerol species of lymph lipids were separated by reversed-phase high performance liquid chromatography; STAG, structured triacylglycerol.

Abbreviations: 8:0/18:2/8:0, 1,3-dioctanoyl-2-linoleoyl-sn-glycerol; 10:0/18:2/10:0, 1,3-didecanoyl-2-linoleoyl-sn-glycerol; 12:0/18:2/12:0, 1,3-didodecanoyl-2-linoleoyl-sn-glycerol; APCI MS, atmospheric pressure chemical ionization mass spectrometry; ELSD, evaporative light scattering detector; RP-HPLC, reversed-phase high performance liquid chromatography; STAG, structured triacylglycerol.

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performance liquid chromatography (RP-HPLC) and unknown triacylglycerols were identified by HPLC with atmospheric pressure chemical ionization-mass spectrometry (APCI MS). The intact MLM-type triacylglycerols and MLL-, LLL-, and MMM-type triacylglycerols were detected in the lymph lipids after administration of MLM-type STAG.

EXPERIMENTAL PROCEDURES

Solvents and reagents
Chloroform and methanol were from Rathburn Chemicals (Walkerburn, Scotland), and acetonitrile, hexane, and isopropanol were from BDH Laboratory Supplies (Poole, England). Taurocholate (98%), choline (99%), trinonanoin (99%), triptenadecanoin (99%), and ammonium acetate (ultra min 98%) were from Sigma (St. Louis, MO). EDTA (Titriplex III GR) was obtained from Merck (Darmstadt, Germany).

Structured triacylglycerols
MLM-type STAG were produced by lipase-catalyzed acidolysis of safflower oil (Róco, Copenhagen, Denmark) and medium-chain fatty acids, that is, caprylic acid, capric acid, and lauric acid (Sigma) in a packed-bed reactor (26) and purified by preparative HPLC (21). Purified STAG were examined by gas-liquid chromatography and Grignard degradation (21) to verify the fatty acid profile and the locations of the medium-chain fatty acids. Table 1 lists the fatty acid compositions of the purified 1,3-dioctanoyl-2-linoleoyl-sn-glycerol (8:0/18:2/8:0), 1,3-didodecanoyl-2-linoleoyl-sn-glycerol (10:0/18:2/10:0), and 1,3-didodecanoyl-2-linoleoyl-sn-glycerol (12:0/18:2/12:0).

Animal experiments
The animal experiments were approved by the Danish Animal Experiments Inspectorate. Male albino Wistar rats (about 250 g) were used. The mesenteric lymph duct of the rats was cannulated with clear vinyl tubing; meanwhile, a silicon tube was inserted into the stomach. More details about the animal experiments have been described previously (21). The rats were kept fasted overnight after surgery, but had free access to tap water and were kept hydrated by infusion of physiological saline (0.9% NaCl, 2 ml/h) through the gastrostomy feeding tube. The lymph collection was initiated 1 h before the administration of lipids to obtain a baseline level of intestinal fat. Four groups of rats (five or six rats in each group) were administered standard to establish the calibration curve of ELSD, whereas tripentadecanoin was used as the external standard.

An HP 1100 series LC/MSD system (Hewlett-Packard, Waldbronn, Germany) fitted with an APCI source was used to identify lymph triacylglycerols. The APCI was operated in the positive mode. The vaporizer temperature was 400°C, the nebulizer gas pressure was 60 psi, the drying gas temperature was 325°C, and the flow rate was 4.0 l/min. A Supelcosil LC-C18 column (length, 25 cm; inner diameter, 4.6 mm; particle size, 5 μm; Supelco, Bellefonte, PA) with a binary solvent system of acetonitrile (solvent A) and isopropanol–hexane 2:1 (v/v) (solvent B) (28). The content of solvent B in the mobile phase was increased from 25% to 40% over 15 min and then further increased to 45% over another 25 min. Ten to 30 μl of sample solution was injected for HPLC analysis. A calibration curve was established to quantify the amount of triacylglycerol in the samples; tripentadecanoin was used as the external standard.

The HPLC results were calculated with both internal standard and external standard. Trinonanoin was used as the internal standard to measure the recovery of lymph lipids after lipid extraction, whereas tripentadecanoin was used as the external standard to establish the calibration curve of ELSD, assuming that the triacylglycerols in the lymph samples have a similar response factor in ELSD.

Statistical methods
The statistic program InStat (GraphPad Software, San Diego, CA) was used in the statistical evaluation. One-way ANOVA was applied in the analysis of differences among groups for each hour of lymph collection. The Tukey-Kramer multiple comparisons test was used to evaluate the statistical significance.

RESULTS

Identification of acylglycerols in lymph lipids
The triacylglycerol species in lymph lipids after administration of various STAG and safflower oil were identified by APCI LC-MS in the positive mode. Figure 1 shows the total ion current chromatogram of lymph triacylglycerols 2 h after administration of STAG 8:0/18:2/8:0, 10:0/...
18:2/10:0, and 12:0/18:2/12:0, and of safflower oil. The triacylglycerol species were identified on the basis of their ammonium adduct molecular ions and diacylglycerol fragment ions, and by the relative intensity of the fragment ions (22, 29). The identified triacylglycerols in lymph samples 2 h after administration of different STAG and the oil are listed in Table 2.

Diacylglycerols (DG) were also detected in lymph samples. They were identified with the pseudo-molecular ion [M-17]/H and the monoacylglycerol fragment ions (29). For instance, DG 10:0/18:2 was identified in the lymph lipids after administration of 10:0/18:2/10:0; DG 12:0/18:2 was also detected in the lymph samples after administration of 12:0/18:2/12:0.

**Lymph triacylglycerols after administration of 8:0/18:2/8:0**

After extraction of total lymph lipids, the triacylglycerol molecular species were analyzed by RP-HPLC. Several triacylglycerols containing C8:0 were detected in the lymph lipids (Fig. 1A). Triacylglycerol 8:0/18:2/8:0 was detected in the lymph lipids and a maximal level of 8:0/18:2/8:0 was observed 1 h after administration of the STAG (Fig. 2). There was no more 8:0/18:2/8:0 in the lymph lipids 4 h after administration of the STAG (Fig. 2). The recovery of STAG 8:0/18:2/8:0 was only 0.6%.

Triacylglycerol 8:0/18:2/18:2 was also detected in the lymph lipids and a maximal level of 8:0/18:2/18:2 was observed 2 h after administering STAG 8:0/18:2/8:0. The level of 8:0/18:2/18:2 was significantly higher than that of 8:0/18:2/8:0.

The major triacylglycerol detected in lymph lipids after administration of STAG 8:0/18:2/8:0 was trilinolein (TG 18:2) (Fig. 2). A maximal level of trilinolein was observed 2 h after administration of the STAG and the level was significantly higher than that of 8:0/18:2/8:0 and 8:0/18:2/18:2. Significant levels of other long-chain triacylglycerols such as 18:1/18:2/18:2 and 16:0/18:2/18:1 were also observed in the lymph triacylglycerols after administration of STAG 8:0/18:2/8:0, reflecting endogenous contribution of fatty acids.

**Lymph triacylglycerols after administration of 10:0/18:2/10:0**

After administration of STAG 10:0/18:2/10:0, several triacylglycerols containing C10:0 were detected; the major triacylglycerols were 10:0/18:2/10:0, 10:0/18:2/18:2, and TG 18:2 (Fig. 1B). A maximal level of 10:0/18:2/10:0 and 10:0/18:2/18:2 was observed 2 h after administration of STAG 10:0/18:2/10:0, and it was still possible to detect those triacylglycerols in lymph lipids 8 h after administration of the STAG (Fig. 3). The recovery of STAG 10:0/18:2/10:0 in lymph lipids was 12%. The accumulated level
of 10:0/18:2/18:2 was significantly higher than that of 10:0/18:2/10:0. Medium-chain triacylglycerol tricaprin (TG 10:0) was also detected in lymph lipids.

Trilinolein was also detected in lymph lipids after administration of STAG 10:0/18:2/10:0; the maximal level of TG 18:2 was similar to that of 10:0/18:2/10:0 but lower than that of 10:0/18:2/18:2. Other long-chain triacylglycerols were also detected in lymph lipids after administration of STAG 10:0/18:2/10:0, but the contents of the long-chain triacylglycerols were lower than was found after administration of STAG 8:0/18:2/8:0.

**Lymph triacylglycerols after administration of 12:0/18:2/12:0**

The major triacylglycerols in lymph lipids after administration of STAG 12:0/18:2/12:0 were similar to those in lymph lipids after administration of STAG 10:0/18:2/10:0; they are 12:0/18:2/12:0, 12:0/18:2/18:2, and TG 18:2 (Fig. 1C). A maximal level of 12:0/18:2/12:0 and 12:0/18:2/18:2 was observed 2 h after administration of STAG 12:0/18:2/12:0, and it was still possible to detect those triacylglycerols in lymph lipids 8 h after administration of the STAG (Fig. 4). The recovery of STAG 12:0/18:2/12:0 in lymph lipids was 5%. The maximal level of 12:0/18:2/18:2 was significantly higher than that of 12:0/18:2/12:0.

Medium-chain triacylglycerol trilaurin (TG 12:0) was also detected in lymph lipids, and the level of TG 12:0 was higher than that of TG 10:0, which was detected after administration of STAG 10:0/18:2/10:0. The level of TG 18:2 was higher than that of 12:0/18:2/12:0, but it was significantly lower than that of 12:0/18:2/18:2.

### Table 2: Concentration of various triacylglycerols in lymph lipids 2 h after administration of STAG or safflower oil

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**Abbreviations:** ECN, equivalent carbon number; TG, triacylglycerol; i.s., internal standard; DG, diacylglycerol.
Lymph triacylglycerols after administration of safflower oil

In contrast to the lymph lipids after administration of the STAG, the triacylglycerols detected in lymph lipids after administration of safflower oil reflected the triacylglycerols of the safflower oil. The major triacylglycerols were TG 18:2, 18:2/18:2/18:1, and 18:2/18:2/16:0 (Fig. 1D). A maximal level of those long-chain triacylglycerols was observed 2 h after administration of the safflower oil (Fig. 5).

DISCUSSION

The triacylglycerols detected in lymph lipids after administration of safflower oil reflected the triacylglycerols in the safflower oil; the major species were TG 18:2, 18:2/18:2/18:1, and 18:2/18:2/16:0 (Fig. 1D). The results are in agreement with previous reports that lymph fatty acids reflected the fatty acids administered (25, 30). The hydrolysis and resynthesis of the major safflower oil triacylglycerols still resulted in similar triacylglycerol species in lymph. This is quite different from the absorption of a single structured triacylglycerol. After administration of MLM-type STAG 8:0/18:2/8:0, 10:0/18:2/10:0, and 12:0/18:2/12:0, many new triacylglycerol species were detected in lymph lipids, and some of the triacylglycerols contained medium-chain fatty acids. Because MLM-type triacylglycerols were the major triacylglycerols administered, the new triacylglycerol species containing medium-chain fatty acids must be the resynthesized product in the enterocyte, which indicates that the specific STAG were hydrolyzed, and part of the medium-chain fatty acids in the form of free fatty acids were adsorbed and resynthesized to triacylglycerols in the enterocyte.

The presence of MLM-type triacylglycerols in the lymph lipids may indicate that they could be absorbed in the intact form. However, because the absorption level of 10:0/18:2/10:0 was much higher than that of 8:0/18:2/8:0, it is unlikely that MLM-type triacylglycerols were absorbed in the intact form, considering the size of the molecules.

The low recovery of 8:0/18:2/8:0 in lymph lipids may be due to both the fast hydrolysis of caprylic acid from the STAG, followed by fast transport via the portal vein com-

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Fig. 2. The lymphatic transport of the major MLM-, MLL-, and LLL-type (M, medium-chain fatty acid; L, long-chain fatty acid) triacylglycerols after administration of STAG 8:0/18:2/8:0. TG 18:2, Trilinolein.

Fig. 3. The lymphatic transport of the major MLM-, MLL-, and LLL-type triacylglycerols after administration of STAG 10:0/18:2/10:0.

Fig. 4. The lymphatic transport of the major MLM-, MLL-, and LLL-type triacylglycerols after administration of STAG 12:0/18:2/12:0.

Fig. 5. The lymphatic transport of the major long-chain triacylglycerols after administration of safflower oil.
bined with low binding ability to FABP and low affinity toward the acyl transferase, and therefore only a small part of the C8:0 from the STAG was used in the resynthesis of triacylglycerols in the enterocyte and resulted in low recovery of 8:0/18:2/8:0. Triacylglycerol 8:0/18:2/8:0 in lymph lipids reached the maximal level 1 h after administration of the STAG, further suggesting that C8:0 was rapidly hydrolyzed from the STAG in the intestine and transported via the portal vein.

A significantly higher level of 10:0/18:2/10:0 was detected in the lymph, which reached the maximal level 2 h after administration of the STAG, indicating that C10:0 was hydrolyzed slower from the STAG than C8:0. Relatively slower transport of C10:0 via the portal vein allowed more C10:0 to be utilized for resynthesis into triacylglycerols in the enterocyte by the monoacylglycerol pathway. The observation is in agreement with the order of the affinity of fatty acids for acyl transferase and the chyloportal partition of the medium-chain fatty acids (31), that is, lower substrate concentration of C8:0 in the enterocyte in comparison with C10:0. The difference between the lymphatic transports of different MLM-type triacylglycerols may thus reflect the overall difference in the rates of hydrolysis in the intestine, transport rate via the portal vein, affinities toward FABP, activation to CoA esters, and the affinity for acyl transferases to form the chylomicron triacylglycerols.

After administration of MLM-type STAG more MLL-type triacylglycerols were formed than MLM-type triacylglycerols in the enterocyte, suggesting that long-chain fatty acids are required in the resynthesis of triacylglycerols. A comparison of lymph triacylglycerols after administration of STAG 8:0/18:2/8:0, 10:0/18:2/10:0, and 12:0/18:2/12:0, STAG with C8:0 which resulted in major portal transport also resulted in more long-chain triacylglycerols in lymph (Fig. 1). That is in agreement with the previous observation by Tso et al. (20) that long-chain fatty acids of exogenous or endogenous origin are necessary to promote absorption of STAG.

Medium-chain triacylglycerols were also detected after administration of 10:0/18:2/10:0 and 12:0/18:2/12:0, but not after administration of 8:0/18:2/8:0. Because there were no medium-chain triacylglycerols in the administered STAG, these medium-chain triacylglycerols detected in lymph lipids may represent newly synthesized triacylglycerols from medium-chain fatty acids and glycerol phosphate in the enterocyte (32). Because the level of medium-chain triacylglycerols was much lower than that of the other triacylglycerols containing medium-chain fatty acids, it is most likely that the hydrolysis of the specific STAG predominantly is a partial hydrolysis, that is, they were hydrolyzed into diacylglycerols or monoacylglycerols and free fatty acids. The formation of medium-chain triacylglycerols might also result from acyl migration of diacylglycerols, that is, the medium-chain fatty acids migrated to the sn-2 position. A further hydrolysis resulted in the formation of sn-2 monoacylglycerols containing medium-chain fatty acids, which were used for resynthesis of triacylglycerols and resulted in the formation of medium-chain triacylglycerols. The higher level of TG 12:0 than TG 10:0 in lymph lipids is also in accordance with the chyloportal partition as discussed above.

In the present study we found that the maximal level of TG 18:2 was similar after administration of STAG 8:0/18:2/8:0 and 10:0/18:2/10:0; however, the level of triolein was lower after administration of STAG 12:0/18:2/12:0. This difference may be caused by the different endogenous contribution of long-chain fatty acids, that is, there was more endogenous contribution of long-chain fatty acids when most of the C8:0 and C10:0 was transported via the portal vein, whereas there was less endogenous contribution for STAG 12:0/18:2/12:0 because most of the lauric acid was transported via the lymphatic system. The formation of TG 18:2 may also occur by complete hydrolysis of some MLM-type STAG to glycerol and free fatty acids followed by use of the linoleic acid released from the sn-2 position for the resynthesis of TG 18:2 by the monoacylglycerol pathway.

Complete hydrolysis of MLM-type STAG and resynthesis of TG 18:2 occurred at a slower rate and resulted in a delay of the maximal level of TG 18:2 in comparison with MLM-type triacylglycerols. The slower resynthesis rate from lauric acid resulted in the lower level of 12:0/18:2/12:0, but higher level of 12:0/18:2/12:0, indicating that the resynthesis of triacylglycerols in the enterocyte has selectivity for the fatty acids.

Diacylglycerols were also detected in lymph lipids; for instance, DG 10:0/18:2 was detected after administration of 10:0/18:2/10:0. After administration of STAG 12:0/18:2/12:0, we found two diacylglycerol 12:0/18:2, which may be the 1,2-diacylglycerol and 1,3-diacylglycerol, because they can be separated on an RP-column (28). The 1,2-diacylglycerol 12:0/18:2 could be the direct hydrolysis product of the structured triacylglycerol 12:0/18:2/12:0, whereas the 1,3-diacylglycerol could be a migration product of the 1,2-diacylglycerol or the randomized resynthesized product after total hydrolysis into free fatty acids and glycerol. Alternatively, they were the hydrolysis product from 12:0/12:0/18:2, which also existed in the administered STAG, because acyl migration was higher for this product.

In summary, this study provides evidence that the absorption pathway of MLM-type STAG occurs by hydrolysis into free fatty acids and monoacylglycerols, but some total hydrolysis into free fatty acids and glycerol occurs in parallel. Some of the medium-chain fatty acids are resynthesized to triacylglycerols and transported via the lymphatic system. Caprylic acid can be hydrolyzed from MLM-type STAG and transported via the portal vein much faster than capric acid and lauric acid.

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