Eur J Nutr (2006) 45:363–368
DOI 10.1007/s00394-006-0607-x

ORIGINAL CONTRIBUTION

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The recovery of ^{13}C-labeled oleic acid in rat lymph after administration of long chain triacylglycerols or specific structured triacylglycerols

Received: 11 January 2006
Accepted: 21 May 2006
Published online: 29 June 2006

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Abstract *Background* Consumption of specific structured triacylglycerols, MLM (M = medium chain fatty acid, L = long chain fatty acid), delivers fast energy and long chain fatty acids to the organism. *Aim of the study* The purpose of the present study was to compare lymphatic absorption of ^{13}C-labeled MLM and ^{13}C-labeled LLL in rats. Stable isotope labeling enables the separation of the endogenous and exogenous fatty acids. *Methods* Lymph was collected during 24 h following administration of MLM or LLL. Lymph fatty acid composition and ^{13}C-enrichment were determined and quantified by gas chromatography combustion isotope ratio mass spectrometry. *Results* The recovery of 18:1n-9 was higher after administration of LLL compared with MLM (58.1% ± 7.4% and 29.1% ± 3.9%, respectively, $P < 0.001$). This may be due to a higher chylomicron formation stimulated by a higher amount of long chain fatty acids in the intestine after LLL compared with MLM administration. This was confirmed by the tendencies of higher lymphatic transport of endogenous fatty acids. *Conclusion* The study revealed a higher lymphatic recovery of the administered long chain fatty acids after LLL compared with MLM consumption.

Key words oleic acid – caprylic acid – stable isotopes – absorption – lymphatic transport

Introduction

Specific structured triacylglycerol (MLM) is composed of medium chain fatty acids (M) in the *sn*-1,3 positions and a long chain fatty acid (L) in the *sn*-2 position. The medium chain fatty acids deliver fast energy to the organism due to rapid hydrolysis and absorption followed by oxidation in the liver [1–5]. The long chain fatty acids are absorbed as *sn*-2 monoacylglycerols in the enterocytes, reesterified to triacylglycerols, incorporated into chylomicrons and distributed by the lymph for oxidation or deposition in the tissue [6].

Lymphatic transport of specific structured triacylglycerols compared with randomized oils (the same fatty acid composition, but no specific structure at the glycerol backbone) and long chain triacylglycerols (LLL) in rats has been examined in several studies. Christensen et al. [7] observed a faster mesenteric lymphatic transport of eicosapentanoic acid (20:5n-3) and docosahexanoic acid (22:6n-3) the first 6 h after administration of MLM (L = 20:5n-3 and 22:6n-3, M = caprylic acid, 10:0) compared with a randomized oil. After 24 h, however, similar total transport of 20:5n-3 and 22:6n-3 was observed in the experimental groups. Straarup and Høy [8] observed a higher

mesenteric lymphatic transport of oleic acid (18:1n-9) and linolenic acid (18:3n-3) after administration of MLM (L = rapeseed oil fatty acids, M = 10:0) compared with a randomized oil, a physical mixture of medium and long chain triacylglycerols and rapeseed oil. Ikeda et al. [9] observed a higher transport of 18:2n-6 during 24 h of thoracic lymph collection after administration of MLM (L = 18:2n-6, M = caprylic acid, 8:0) compared with a physical mixture of medium and long chain triacylglycerols. However, in contrast to these studies and with another study design, the lymphatic recoveries of the exogenous long chain fatty acids after continuous infusion of ^{14}C-labeled LLL were significantly higher compared with infusion of two structured triacylglycerols, ^{14}C-labeled MLM and ^{14}C-labeled MML (L = 18:2n-6, M = 8:0) [10].

The use of isotope labeled dietary triacylglycerols enables distinguishing exogenous and endogenous fatty acids in lymph, but this technique was only utilized in one of the above-mentioned studies. In the other studies the endogenous and exogenous fatty acids are mixed and termed "lymphatic transport." It follows, that these studies cannot conclude on the exact uptake and lymphatic content of the dietary fatty acids, because the amount and composition of the endogenous lymph fatty acids remains unknown.

The ^{13}C-labeled fatty acids analysis with gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) gives a very sensitive and precise determination of the ^{13}C-enrichment of individual fatty acid species allowing us to trace the administered fatty acids also if they are converted into other fatty acid species.

The present study evaluated the lymphatic recovery of ^{13}C-labeled MLM (L = 18:1n-9, M = 8:0) in order to estimate differences in digestion and absorption of the exogenous fatty acids due to the triacylglycerol structure of the experimental oils.

Material and methods

Feeding and housing of rats

Male rats (130 g, Wistar, Møllegården Breeding and Research Centre, Lille Skensved, Denmark) were kept for 10 days in the animal housing before the experiment. They were fed chow pellets (Altromin no. 1324, Chr. Petersen A/S, Ringsted, Denmark) and kept at 21 ± 1°C, a humidity of 50 ± 5% and a light period from 6 a.m. until 18 p.m. They had free access to tap water. Rats weighed 221 ± 19 g (SD) at the time of surgery.

Production of specific structured triacylglycerols

The specific triacylglycerols were produced by enzyme catalyzed interesterification as described previously [11]. The LLL oil was purchased from Sigma Aldrich, Germany. The ^{13}C-labeled specific structured triacylglycerols were produced by batch interesterification of caprylic acid (M = 8:0, Sigma Aldrich) and L*L*L* (L = 18:1n-9, * = ^{13}C-labeled fatty acid, Cambridge Isotope Laboratories, MA, USA). The two components were mixed at a substrate ratio of 8:1 (mol:mol), added immobilized *sn*-1,3 specific lipase (10 wt% of the solution, Lipozyme IM, Novozymes A/S, Denmark) and incubated at 50°C for 5 h with constant stirring. After incubation the solution was filtered to remove the lipase. ML*M was purified by preparative-HPLC [12].

The experimental oils

At the beginning of the experiment (see below) rats received a bolus of 0.3 ml MLM or LLL and 0.3 ml taurocholate solution (20 mM taurocholate and 10 mg/ml choline). The solution was mixed 2 times for 10 s at a Whirly mixer and sonicated 10 s (M.S.E. 150 watt Ultrasonic Disintegrator; M.S.E. Inc. Crawley, England). The LLL bolus was added 5 µl L*L*L* and the MLM bolus was added 5 µl ML*M.

Analysis of oils

The LLL and MLM oils were transmetylated by KOH in methanol [13] for determination of the triacylglycerol fatty acid composition. The triacylglycerol structure illustrated by the fatty acid composition of the *sn*-2 position was determined by Grignard degradation [14] followed by thin layer chromatography (Silica gel 60, Merck, Darmstadt, Germany) separation, recovery of the *sn*-2 monoacylglycerol and KOH methylation. The fatty acid methyl esters were analyzed by a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard GmbH, Walbronn, Germany) on a fused silica capillary column (SP-2380, 60 m, ID 0.25 mm, 0.20 µm film, Supelco Inc., Bellefonte, PA, USA). The injector was 260°C and it was used in the split mode with a split ratio of 1:20. The fatty acids were separated by an initial temperature of 70°C, which was kept for 0.5 min, then raised to 160°C at 15°C/min, at 1.5°C/min to 200°C, which was held for 15 min and finally raised at 30°C/min to 225°C. This temperature was maintained for 10 min. The temperature of the detector (flame ionization) was 300°C. The fatty acid compositions of the triacylglycerols and of the *sn*-2 position are listed in Table 1. The relatively low mol% of 8:0 indicates that the specific structured oil con-

Table 1 Fatty acid composition (mol%) of the triacylglycerols (TG) and *sn*-2 monoacylglycerols (2-MG) of the experimental oils

Fatty acids	MLM		LLL	
	TG	2-MG	TG	2-MG
8:0	23.2	1.8	n.d.	n.d.
16:0	2.7	1.8	3.7	0.5
18:0	3.4	2.4	3.2	n.d.
18:1n-9	65.0	87.7	87.1	93.4
18:2n-6	4.8	6.3	5.9	5.4

Data represent the average of 3 determinations.
MLM (L = 18:1n-9, M = 8:0)
LLL (L = 18:1n-9)
n.d. = not detected

sisted of MLM and MLL/LLM triacylglycerol species, but the oil will be referred to as MLM.

Animal experiment

The experiment was approved by the Danish Committee for Animal Experiments. At the day of surgery, rats were anesthetized with 0.055 ml/100 g body weight Zoletil-mixture (consisting of Zolazepam (125 mg/10.5 ml), Tiletamin (125 mg/10.5 ml), Narcoxyl Vet (10 ml of 20 mg/ml), Veterinaria AG, Schweiz, and Torbugesic (0.5 ml of 10 mg/ml) Fort Dodge Laboratories, Fort Dodge, IA, USA). A clear vinyl tube (OD 0.8 mm, ID 0.5 mm, Critchley Electrical Products Pty. Ltd, NSW, Australia) was inserted into the mesenteric lymph vessel [15]. The tube was subcutaneously led to the abdomen of the rat, through the skin and fastened with tape [16]. The lymph was collected in a 5 ml plastic bottle taped to the trunk of the rat. To prevent the rats from eating the catheter a plastic collar was placed around the neck of the rat. After surgery, rats were administered 0.05 ml antidote (Antisedan, Farmos, Finland) intramusculary and 5 ml saline water (9 g/l NaCl) subcutaneously to prevent dehydration. Rats were placed in individual plastic cages with free access to tap water and a glucose solution (55 g/l glucose, Sigma Aldrich, Steinheim, Germany). Then, 2–4 h after surgery, rats received 0.2 ml Torbugesic (diluted 1:10 with sterile water).

The next day, a 5-ml bottle with 0.1 mL EDTA (10% (w/vol) Na_2-EDTA-2H_2O, Merck, Darmstedt, Germany) was fastened to the abdomen of the rat and lymph was collected for 1 h ("0-sample"). Afterwards rats were fed by gavage, a bolus of LLL or MLM. Lymph was collected in 1 h fractions for the next 8 h, a night fraction of 15 h and an 1 h fraction the next morning beginning at 23 h. After 24 h of lymph collection, rats were killed by an overdose of sodium pentobarbital. Until analysis lymph samples were kept at −20°C.

Comparisons of this non-restrained lymph collection model with the conventional restrained model of lymph collection [17] revealed that the data obtained from the two models were comparable (data not shown).

Lymph analysis

An internal triacylglycerol standard (TG-15:0, Sigma Aldrich, Steinheim, Germany) was added to the lymph samples followed by extraction [18]. The extract was methylated with KOH-catalyzed method and analyzed by GC-C-IRMS (Delta^{PLUS}, Thermo-Finnigan, Bremen, Germany) with separation and quantification of fatty acids and determination of the $^{13}C/^{12}C$ ratio of the fatty acid carbon atoms. The samples were injected at 250°C with a split ratio of 1:15 and separated on a fused silica capillary column (same as above) with the following temperature program: The initial temperature (70°C) was maintained for 3 min and then raised to 150°C at 15°C/min, to 169°C at 1.5°C/min, to 173°C at 0.5°C/min, to 188°C at 3°C/min and finally raised to 200°C at 20°C/min and maintained for 14 min. The stability of the measurements was checked every day by standard on/off test (18 injections of CO_2) and B-scan (control of all mass values, especially water, nitrogen, and argon). CO_2 injections in the beginning and end of each run ensured correct determinations of sample values. The individual fatty acids were identified by comparing retention times with retention times of authentic standards (Nu-Chek Prep Inc., Elysian, MN, USA).

Calculations

The atom percent was used for quantitative description of the ^{13}C-enrichment of a sample. The atom percent of a sample (AP_S) was calculated using the Eq. 1 from the ratio of $^{13}C/^{12}C$ of the sample (δ_S) and a reference δ of a specific limestone, Pee Dee Belemnite (δ = 0.0112372) [19].

$$AP_S = \frac{100 \times 0.0112372 \times (0.001 \times \delta_S + 1)}{1 + 0.0112372 \times (0.001 \times \delta_S + 1)} \quad (1)$$

[20].

The lymphatic transport of ^{13}C-labeled 18:1n-9 is calculated by multiplying AP_S with the total lymphatic transport of 18:1n-9 in the sample. The recovery of 18:1n-9 is then found by dividing this value with the amount of administered ^{13}C-labeled 18:1n-9 at the beginning of the experiment.

Statistics

Amounts and ratios are stated as average ± SE. Lymphatic recovery of ^{13}C-enriched 18:1n-9 after LLL and MLM administration and accumulated lymphatic

transport of fatty acids was compared with student's *t*-test. All statistics were performed with SigmaStat (ver. 2.03, Jandel Corporation, Erkrath, Germany). The level of significance was $P < 0.05$.

Results

Lymph flow

The lymph flows in the 2 two experimental groups did not differ. They were 0.58 ± 0.03 vs. 0.66 ± 0.08 ml/h for LLL and MLM, respectively ($P = 0.40$).

Lymphatic recovery of ^{13}C-enriched 18:1n-9

Figure 1 illustrates that the lymphatic recovery of ^{13}C-enriched 18:1n-9 was significantly higher after LLL compared with MLM administration (58.1% ± 7.4% and 29.1% ± 3.9%, respectively, $P < 0.001$).

The only ^{13}C-enriched fatty acid detected in the lymph samples was ^{13}C-labeled 18:1n-9, which indicates that neither elongation nor chain shortening of the fatty acid had taken place.

Absorption of medium chain fatty acids

Caprylic acid was detected during the first 8 h of lymph collection in rats receiving the MLM bolus (Fig. 2). The lymphatic transport of 8:0 peaked 2 h after oil administration with a maximum transport of 438 ± 107µg/h. The accumulated transport 8 h after MLM administration constituted approximately 3% of the administered dose.

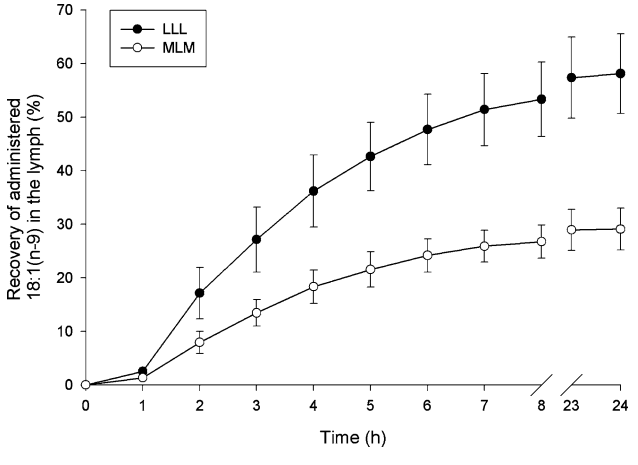

Fig. 1 Recovery (in %) of ingested 18:1n-9 in rat lymph after feeding a bolus of LLL or MLM, $n = 11–14$. Average ± SE

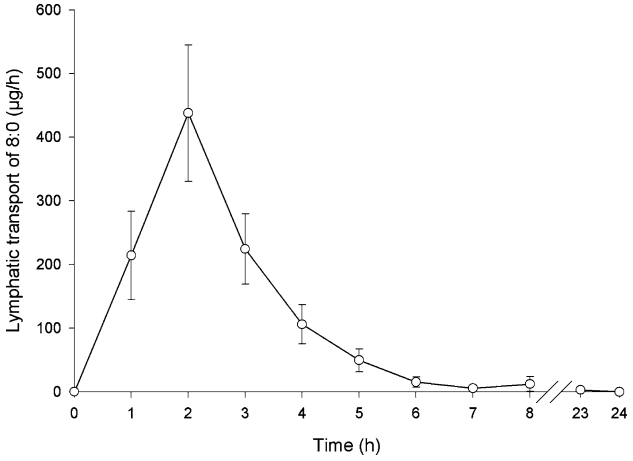

Fig. 2 Lymphatic transport of 8:0 (µg/h) after administration of MLM ($n = 14$). Average ± SE

Lymphatic transport of long chain fatty acids

The accumulated lymphatic transport of 18:1n-9 tended to be higher after LLL compared with MLM administration ($P = 0.14$, Fig. 3A). The accumulated lymphatic transport of 18:1n-9 24 h after LLL and MLM administration were 66.2 ± 6.5mg and 50.7 ± 7.3 mg, respectively. Furthermore, there were tendencies of higher lymphatic transport of 16:0, 18:0, and 18:2n-6 following LLL compared with MLM administration ($P = 0.06$, $P = 0.15$, and $P = 0.11$, respectively, Fig. 3B–D).

Discussion

The long chain fatty acids delivered within the MLM structure (together with medium chain fatty acids) was thought to be absorbed faster than those in triacylglycerols consisting of three long chain fatty acids, since medium chain fatty acids were hydrolyzed faster. However, the maximum lymphatic recovery of orally administered ^{13}C-enriched 18:1n-9 was higher when the fatty acid was incorporated in LLL compared with MLM. Similar results were obtained by Tso and colleagues [10], who observed a significantly higher lymphatic recovery of ^{14}C-labeled fatty acids when administered as LLL compared with MLM. They observed that the lipolysis of MLM and LLL did not differ. However, the uptake of long chain fatty acids into the enterocyte was slower, when ingested as MLM compared with LLL. Therefore, Tso et al. suggested that low concentrations of long chain fatty acids available for triacylglycerol resynthesis in the enterocyte might explain the lower lymphatic long chain fatty acid recovery after administration of MLM. This might also be the explanation of the results of the

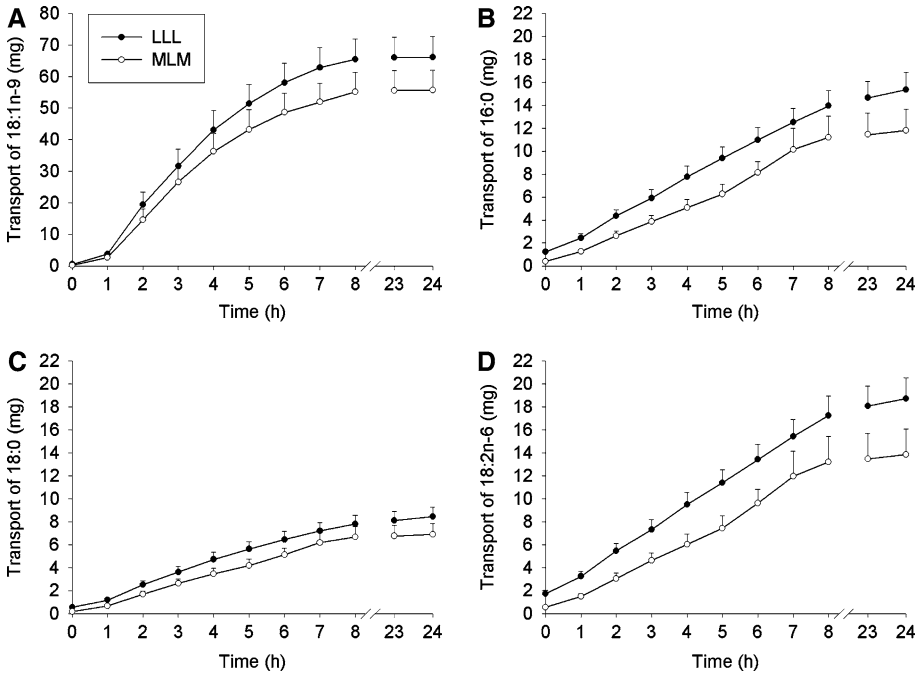

Fig. 3 Accumulated lymphatic transport in mg of 18:1n-9 (**A**), 16:0 (**B**), 18:0 (**C**) and 18:2n-6 (**D**) following LLL or MLM administration, $n = 11–14$. Average ± SE. Please notice, that the maximum value of the *y*-axis in panel **A** is higher than in panel **B**–**D**

present study. This is further supported by the lymphatic transport of long chain fatty acids (16:0. 18:0, and 18:2n-6), which in this experiment solely originates from endogenous sources (bile, old intestinal cells, and enterocyte) and which all tend to be higher after LLL administration. This indicates that the level of triacylglycerol synthesis (based on both endogenous and exogenous fatty acids) is related to the amount of consumed long chain fatty acids. This is in line with the observations of Kalogeris [21], who demonstrated that dietary long chain triacylglycerols in contrast to short and medium chain triacylglycerols stimulated chylomicron formation. Thus, a higher amount of long chain fatty acids in the enterocyte might increase chylomicron formation with subsequently increased transport of endogenous fatty acids.

The present results do not agree with other lymph studies of MLM administration [7–9], where it was found that the total lymphatic transport of several long chain fatty acids was increased after administration of MLM compared with LLL or randomized oil. However, this might be due to different experimental designs. The present study compared MLM with LLL, while Christensen et al. [7] compared MLM with the randomized oil (same fatty acid composition as MLM, but different TAG structure). This could have influenced the results because LLL supplied the enterocyte with plenty of long chain fatty acids compared with the randomized oil, which had the similar (low) content of long chain fatty acids as MLM. Furthermore, the present study used ^{13}C-labeled fatty acids, and was therefore, able to separate the absorption of the exogenous fatty acids from the transport of endogenous fatty acids and to provide more evidences for understanding lipid absorptions.

The recovery of the oleic acid in this study is calculated as the proportion between the ^{13}C-labeled enrichment of the administered fatty acid and the ^{13}C-enrichment of the lymphatic fatty acid. This could be termed as the "apparent" recovery, because of obvious reasons we could not take into account the endogenous fatty acid pool (ready for triacylglycerol synthesis in the enterocyte). However, as we assume that the ^{13}C-labeled fatty acids are hydrolyzed and absorbed at the same rate as the unlabeled exogenous fatty acids, our determinations of the recovery of the ^{13}C-labeled fatty acids in the lymph give a fairly good indication of the relative absorption of the exogenous fatty acids.

No other ^{13}C-labeled fatty acids were observed in the lymph, which revealed that 18:1n-9 was not transformed into other fatty acids. The expected fate of the non-essential fatty acid 18:1n-9 is oxidation in organs and peripheral tissue, which was demonstrated by Watkins et al. [22], who observed a higher oxidation of ^{13}C-labeled trioleylglycerol (TG-18:1n-9) (11%) compared with ^{13}C-labeled 16:0 administered as free fatty acid (7%) after simultaneous administration of the triacylglycerols.

Up to 3% of the administered 8:0 was found in the lymph, which was comparable to the findings of Ikeda et al. [9] and Mu and Høy [12]. The majority of the ingested 8:0 was not esterified into TAGs in the enterocyte, but transported through the cell and via the portal vein to the liver [3, 23].

The lymph flow observed in the present study was low compared with other lymph cannulation studies. This was mainly due to our experimental setup, where rats could move freely and drank ad libitum from a water bottle in the cage, but did not receive 1–2 saline ml/h through a stomach tube as in the restrained model.

In summary, the present study demonstrates that the lymphatic recovery of 18:1n-9 is higher when administered as triacylglycerols consisting only of long chain fatty acids (LLL) compared with MLM (long and medium fatty acids). The composition of the dietary fat influences fat absorption after a single fat meal.

One of the implications of the study is that administration of MLM should be supplemented with other sources of long chain fatty acid to ensure maximal absorption and lymphatic transport of long chain fatty acids. On the other hand, the present results also indicate that MLM containing 18:1n-9 could be used for treatment of obesity. It might have a weight-reducing effect, because a comparable dietary amount of MLM compared with LLL resulted in a lower lymphatic output of long chain fatty acids and thereby a potential lower energy uptake.

Acknowledgments We thank Xuebing Xu for preparing the unlabeled structured triacylglycerols and Zahra Roudaki, Karen Jensen, Lillian Vile, and Egon Christensen for technical assistance. The present address of Bodil Vistisen is: The Division of Pathophysiology, The Department of Medical Physiology, the PANUM Institute, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N.

References

1. Clark S, Brause B, Holt P (1969) Lipolysis and absorption of fat in the rat stomach. Gastroenterology 56(2):214–222
2. Greenberger N, Rodgers J, Isselbacher K (1966) Absorption of medium and long chain triglycerides: factors influencing their hydrolysis and transport. J Clin Invest 45(2):217–227
3. Hashim S, Bergen S, Krell K, Van Itallie T (1964) Intestinal absorption and mode of transport in portal vein of medium chain fatty acids. J Clin Invest 43(11):1238
4. Bennett S (1964) Intestinal absorptive capacity and site of absorption of fat under steady state conditions in the unanaesthetized rat. Quat J Exp Physiol Cog 49(1):210–218
5. Tantibhedyangkul P, Hashim S (1971) Clinical and physiological aspects of medium-chain triglycerides: alleviation of steatorrhea in premature infants. Bull N Y Acad Sci 47(1):17–33
6. Phan C, Tso P (2001) Intestinal lipid absorption and transport. Front Biosci 6:d299–d319
7. Christensen M, Høy C, Becker C, Redgrave T (1995) Intestinal absorption and lymphatic transport of eicosapentaenoic (EPA), docosahexaenoic (DHA), and decanoic acids: dependence on intramolecular triacylglycerol structure. Am J Clin Nutr 61:56–61
8. Straarup E, Høy C-E (2000) Structured lipids improve fat absorption in normal and malabsorbing rats. J Nutr 130:2802–2808
9. Ikeda I, Tomari Y, Sugano M, Watanabe S, Nagata J (1991) Lymphatic absorption of structures glycerolipids containing medium-chain fatty acids and linoleic acid, and their effect on cholesterol absorption in rats. Lipids 26:369–373
10. Tso P, Karlstad M, Bistrian B, DeMichele S (1995) Intestinal digestion, absorption, and transport of structured triglycerides and cholesterol in rats. Am J Physiol (G L P) 268(31):G568–G577
11. Xu X, Mu H, Høy C-E, Adler-Nissen J (1999) Production of specifically structured lipids by enzymatic interesterification in a pilot packed bed reactor: optimization by response surface methodology. Fett/Lipid 101:207–214
12. Mu H, Høy C-E (2000) Effects of different medium-chain fatty acids on intestinal absorption of structured triacylglycerols. Lipids 35:83–89
13. Christopherson S, Glass R (1969) Preparation of milk fat methyl esters by alcoholysis in an essentially nonalcoholic solution. J Dairy Sci 52:1289–1290
14. Becker C, Rosenquist A, Hølmer G (1993) Regiospecific analysis of triacylglycerols using allyl magnesium bromide. Lipids 28:147–149
15. Bollman J, Cain J, Grindlay J (1948) Techniques for the collection of lymph from the liver, small intestine, or thoracic duct of the rat. J Lab Clin Med 33(11):1349–1352
16. Hanberg A, Trossvik C (1995) In situ collection of intestinal lymph in the non-restrained rat. Scand J Lab Anim Sci 22(4):329–334
17. Bollman J (1948) A cage which limits the activity of rats. J Lab Clin Med 33(11):1348
18. Folch J, Lees M, Sloane Stanley G (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
19. Craig H (1957) Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim Cosmochim Acta 12:133–149
20. Brossard N, Pachiaudi C, Croset M, et al. (1994) Stable isotope tracer and gas-chromatography combustion isotope ratio mass spectrometry to study the in vivo compartmental metabolism of docosahexanoic acid. Anal Biochem 220:192–199
21. Kalogeris T, Monroe F, DeMichele S, Tso P (1996) Intestinal synthesis and lymphatic secretion of apolipoprotein A-IV vary with chain length of intestinally infused fatty acids in rats. J Nutr 126:2720–2729
22. Watkins J, Klein P, Schoeller D, Kirschner B, Park R, Perman J (1982) Diagnosis and differentiation of fat malabsorption in children using 13-C-labeled lipids: trioctanoin, triolein, and palmitic acid breath tests. Gastroenterology 82:911–917
23. Playoust M, Isselbacher K (1964) Studies on the intestinal absorption and intramucosal lipolysis of a medium chain triglyceride. J Clin Invest 43(5): 878–885