ASBMB JOURNAL OF LIPID RESEARCH

Studies on fat digestion, absorption, and transport in the suckling rat. II. Triacylglycerols: molecular species, stereospecific analysis, and specificity of hydrolysis by lingual lipase

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Abstract Triacylglycerols (TG) of rat milk supply about two-thirds of the energy consumed by suckling rat pups. The present studies were undertaken to determine stereospecific fatty acid composition and molecular species distribution of milk TG and TG produced during digestion, transported in lymph and blood, and present in the liver of 9–10-day-old pups. Results support non-random stereochemical fatty acid and molecular species distribution for all TG's analyzed. Stereospecific compositional results show loss of medium chain fatty acids (MCFA) during digestion, producing a shift to a larger average molecular weight TG than present in milk. These MCFA are esterified primarily at the *sn*-3 position of milk TG and appear to be hydrolyzed by the action of lingual lipase in the stomach. In vitro incubation of milk with tongue homogenate yields free fatty acids and glyceride products that resemble those found in suckling stomach contents. Further TG metabolism appears to involve redistribution of the long chain fatty acids that remain esterified in TG following gastric lipolysis and release of MCFA.—**Staggers, J. E., G. J. P. Fernando-Warnakulasuriya, and M. A. Wells.** 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.* 1981. **22:** 675–679.

Supplementary key words lingual lipase · neonatal rat · stereospecific triacylglycerol analysis · gastric lipolysis

The distribution of fatty acids in rat milk triacylglycerols (TG) has been shown to be non-random by gas–liquid chromatographic analysis of intact TG molecular species (1) and by stereochemical analysis of the esterified fatty acids (2). The rat, like certain other species, produces milk TG with a large proportion of medium chain fatty acids (1, 3–6).

We have recently reported (7) the fatty acid composition of TG in rat milk and stomach contents, lymph, liver, and plasma of 9–10-day-old suckling rats. The present paper reports the molecular species and stereospecific analysis of these TG.

In 1973 Hamosh and Scow (8) reported a lingual lipase in the serous secretions of the suckling and adult rat tongue. Lingual lipase produces partial glycerides and free fatty acids in the stomach (8–10) and has been suggested to contribute significantly to efficient utilization of the high dietary fat levels consumed during the neonatal period (11–13). We now report an in vitro incubation of rat milk with suckling rat tongue homogenate that closely reproduces the lipid composition of stomach contents in the suckling rat.

MATERIALS AND METHODS

Except as noted below these are described in the previous paper (7). Pancreatic lipase (EC 3.1.1.3, porcine type VI) was purchased from Sigma Chemical Co., St. Louis, MO. Phospholipase A_2 (EC 3.1.1.4) was purified from *Crotalus adamanteus* venom by the method of Wells (14).

Stereospecific TG analysis was performed by the method of Brockerhoff (15), as modified by Christie and Moore (16). The *sn*-2-monoacylglycerols were generated by the method of Mattson and Volpenhein (17) and the *rac*-1,2-diacylglycerols were generated from TG by the method of Myher and Kuksis (18). Phosphatidylphenols were prepared by the procedure of Brockerhoff (15), as described by Breckenridge (19), and these were stereospecifically hydrolyzed by phospholipase A_2, according to the method of Christie and Moore (16). The fatty ester composition of the *sn*-3 position was calculated from the composition of the hydrolysis products.

Abbreviations: ANS, 8-anilino 1-naphthalene sulfonic acid; TG, triacylglycerol; MCFA, medium chain fatty acids.

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The TG molecular species were analyzed on a Hewlett-Packard model 402 gas chromatograph equipped with a Hewlett-Packard model 3380A integrator. Columns (glass, 20 in., i.d. 1/8 in.) were filled with 3% JXR on 100/120 Gas-Chrom Q (Applied Science Laboratories, State College, PA). A linear temperature program from 250 to 350°C at 4°C/min was used.

Tongues were surgically excised from anesthetized rat pups and the tissue immediately surrounding the circumvallate papilla (~10–15 mg/pup) was dissected out under a microscope and homogenized in 20 volumes of cold Krebs-Ringer phosphate-bicarbonate buffer. The homogenate was spun at 1,000 rpm for 10 min in a clinical centrifuge to pellet tissue fragments and the supernatant (henceforth referred to as tongue homogenate) was retained. Tongue homogenate not used immediately was stored in 1-ml aliquots at −20°C and it retained enzymatic activity for at least 3 weeks.

Hydrolysis of milk TG by tongue homogenate was

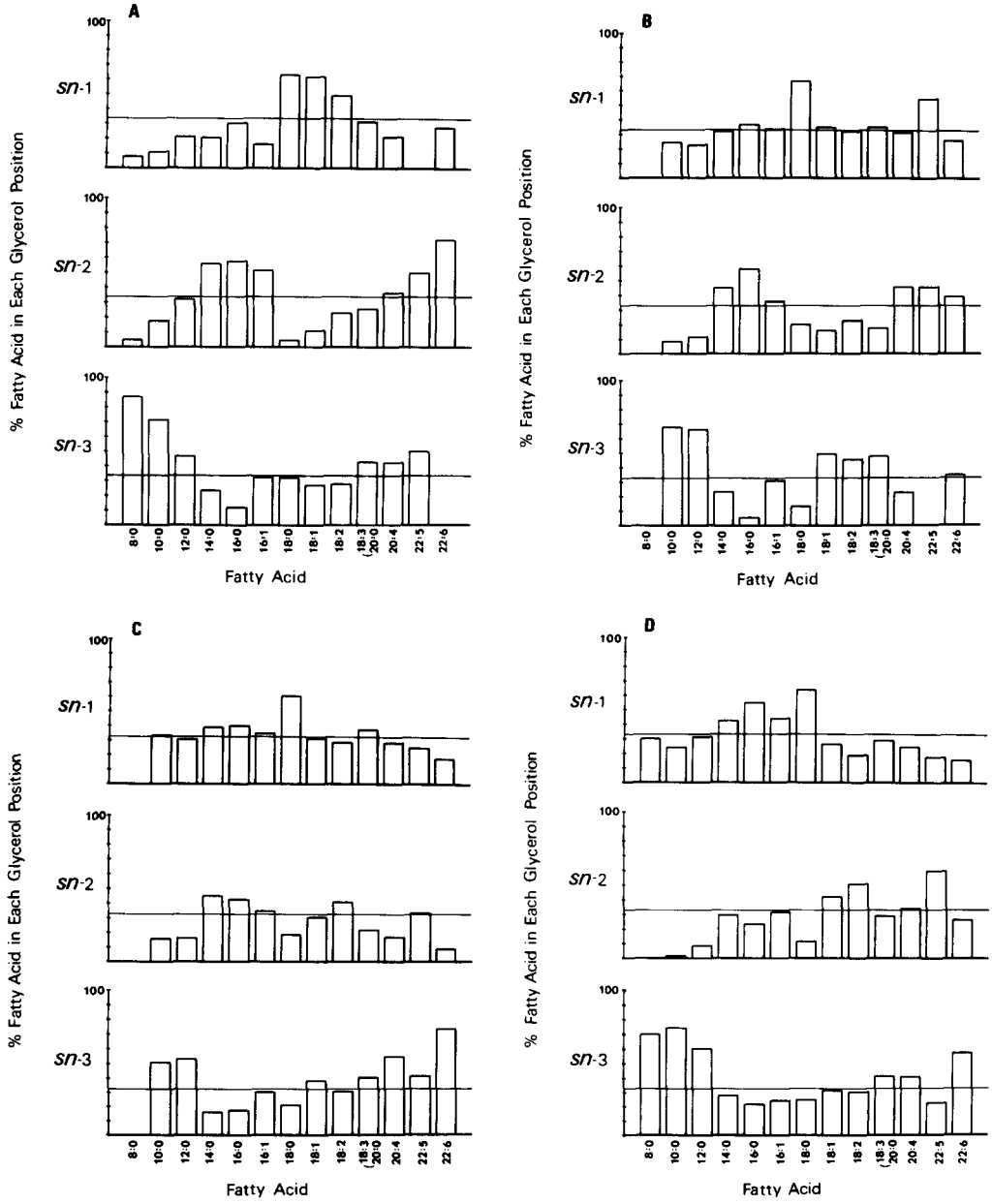

Fig. 1. Stereospecific composition of triacylglycerols. A, milk; B, lymph; C, plasma; and D, liver. Vertical bars represent the percent of each fatty acid at each glycerol position (*sn*-1, *sn*-2, or *sn*-3). The horizontal line in each graph indicates 33 1/3 percent, the proportion of each fatty acid expected on the basis of a random positional distribution.

performed by modification of a method reported by Hamosh (9). The reaction mixture, containing 20 μl of rat milk, 200 μl of tongue homogenate, and 300 μl of 0.1 M citrate-Na_2HPO_4 buffer (pH 5.4) containing 5% bovine serum albumin, was incubated at 37°C in a shaking water bath for 0–120 min. The reaction was stopped by addition of 30 μl of concentrated HCl and lipids were extracted from the mixture by the method of Bligh and Dyer (20). Separation and quantitative analysis of lipids in the reaction products were performed as described previously for gastrointestinal lipids (7).

RESULTS AND DISCUSSION

Stereospecific TG analysis

Fig. 1 shows the positional distribution of the major fatty acids in TG from milk and lymph, plasma, and liver in 9–10-day-old rats. In each graph the horizontal line represents purely random distribution. The results show that none of these TG has a random positional distribution of fatty acids.

Fig. 1A shows the strongly non-random stereospecific composition of rat milk TG. The *sn*-1 position is enriched in 18-carbon fatty acids. The 18:0, 18:1, and 18:2 acids together account for over one-half of *sn*-1 acids. The 14- and 16-carbon saturated acids and 16:1 are enriched at *sn*-2 and together represent 61 mol% of acids at that position. The 22:5 and 22:6 polyunsaturated fatty acids together represent less than 1 mol % of all fatty acids in these milk TG, but show strongly non-random distribution (*sn*-2). Arachidonic acid is slightly enriched at *sn*-3. Examination of the *sn*-3 position reveals a markedly enriched content of medium chain (C_8–C_{12}) fatty acids (87, 72, and 47%, respectively, of total C_8, C_{10}, and C_{12}). Altogether these three MCFA account for one-third of the acids in the milk TG (7) and over one-half of *sn*-3 acids. The MCFA are relatively minor constituents at *sn*-1 and *sn*-2. These data on milk TG agree closely with those reported by Lin, Smith, and Abraham (2). The conclusions drawn by these authors (2) and by Cooper and Grigor (6) support the positioning of long-chain fatty acids at *sn*-1 and *sn*-2, particularly with saturated acids at *sn*-2, and enrichment of MCFA at *sn*-3.

The assimilation of large amounts of milk TG by the gastrointestinal tract of the suckling rat produces a high concentration of TG in intestinal lymphatic fluid (7). The stereospecific fatty acid distribution of lymph TG (Fig. 1B) is also non-random and differs from that of milk TG. Although the *sn*-3 of lymph TG shows enrichment of C_{10} and C_{12}, it is noteworthy that C_8 is present only in trace amounts. The major differences between lymph and milk TG reflect a redistribution of long chain acids into the *sn*-3 position to replace MCFA lost during digestion (7).

TABLE 1. Triacylglycerol molecular species distribution (weight %) in lymph, plasma, and liver of 9–10-day-old rats[a]

Acyl Carbon Number	Lymph	Portal Plasma	Liver	Vena Cava Plasma
26	—	—	—	—
28	tr	—	—	tr
30	0.2 ± 0.1	0.1 ± 0.0	—	0.1 ± 0.0
32	0.3 ± 0.1	0.1 ± 0.0	—	0.1 ± 0.1
34	0.8 ± 0.3	0.4 ± 0.1	—	0.2 ± 0.1
36	1.3 ± 0.4	1.0 ± 0.1	—	0.5 ± 0.2
38	2.5 ± 0.4	1.8 ± 0.1	—	0.8 ± 0.2
40	4.1 ± 0.4	3.3 ± 0.0	0.4 ± 0.0	1.5 ± 0.2
42	6.1 ± 0.5	5.3 ± 0.0	1.1 ± 0.4	2.4 ± 0.2
44	8.9 ± 0.7	8.2 ± 0.1	2.2 ± 0.3	3.7 ± 0.5
46	12.6 ± 0.7	11.9 ± 0.1	4.3 ± 0.5	5.9 ± 0.6
48	15.1 ± 1.7	15.3 ± 1.1	5.9 ± 0.8	9.2 ± 0.3
50	15.9 ± 0.8	20.3 ± 0.5	15.1 ± 1.4	11.7 ± 0.3
52	16.8 ± 0.9	19.3 ± 0.6	30.6 ± 1.6	16.5 ± 0.6
54	12.8 ± 2.4	8.2 ± 0.5	21.4 ± 1.0	21.3 ± 1.8
56	2.5 ± 0.9	4.6 ± 0.5	13.3 ± 1.7	14.4 ± 1.4
58	0.1 ± 0.0	0.2 ± 0.1	5.1 ± 0.8	10.9 ± 2.5
60	—	—	0.6 ± 0.0	0.8 ± 0.4
$\bar{N}$[b]	47.9 ± 0.3[c]	48.5 ± 0.3	52.4 ± 0.7[d]	51.5 ± 1.2[e]

[a] Mean ± S.D. for at least three determinations.
[b] Mean acyl carbon number.
[c] Significantly different from milk ($P < 0.01$) and portal plasma ($P < 0.10$).
[d] Significantly different from portal plasma and milk ($P < 0.01$).
[e] Significantly different from portal plasma ($P < 0.05$).

TABLE 2. Triacylglycerol molecular species distribution (weight %) in milk, stomach contents, and in vitro incubation[a]

Acyl Carbon Number	Milk	Stomach	Milk + Lingual Lipase
26	0.1 ± 0.0	—	tr
28	0.4 ± 0.1	tr	tr
30	1.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
32	2.3 ± 0.4	0.7 ± 0.3	0.8 ± 0.2
34	3.9 ± 0.5	1.3 ± 0.4	1.4 ± 0.3
36	6.1 ± 0.7	2.9 ± 0.2	2.5 ± 0.9
38	8.1 ± 1.1	5.2 ± 0.2	5.3 ± 0.8
40	10.5 ± 1.1	7.9 ± 0.4	6.7 ± 0.7
42	12.3 ± 1.5	10.9 ± 1.0	9.5 ± 1.0
44	13.8 ± 1.3	13.6 ± 1.5	12.4 ± 1.2
46	10.6 ± 0.6	12.7 ± 1.3	12.8 ± 1.4
48	6.8 ± 1.5	10.3 ± 0.8	10.4 ± 2.2
50	5.8 ± 1.3	10.1 ± 0.2	10.7 ± 2.3
52	9.5 ± 1.1	14.2 ± 2.0	15.6 ± 2.7
54	7.2 ± 0.9	8.0 ± 2.2	10.4 ± 1.6
56	1.1 ± 0.5	1.6 ± 0.7	1.1 ± 0.4
58	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
60	—	—	—
$\bar{N}$[b]	43.5 ± 1.2[c]	46.1 ± 0.5	46.1 ± 2.3

[a] Mean ± S.D. for at least three determinations.
[b] Mean acyl carbon number.
[c] $P < 0.05$.

Fig. 1C shows the positional distribution of acids in plasma TG. The plasma TG closely resembles lymphatic TG except for an increased content of polyunsaturated fatty acids (20:4, 22:5, 22:6) (7) which are enriched at *sn*-3. In liver TG (Fig. 1D), C_8 is present in measurable amounts. The liver TG is enriched with MCFA at the *sn*-3 position, as observed with milk TG. The other acids in liver TG are non-randomly distributed, although the stereospecificity is less striking than in milk TG. The total fraction contributed by polyunsaturated fatty acids in liver TG (10.9 mol %) is several-fold that (1.3 mol %) found in milk TG (7). Conversely the liver TG contain less total MCFA than milk TG, 9.3 and 34.7 mol %, respectively (7).

TG molecular species

The distribution of TG species is shown in **Tables 1 and 2.** The TG molecular size classes are represented by total acyl carbon numbers. For each TG source the mean acyl carbon number ($\bar{N}$) is shown. In each case the molecular species show a non-random size distribution. These results agree with those of Smith, Watts, and Dils (1) for milk TG.

A comparison of milk TG and intestinal lymphatic TG (Tables 1 and 2) shows that the value of $\bar{N}$ increases from 43.5 to 47.9 ($P < 0.01$). This shift to larger average TG reflects the loss of MCFA. As shown in Table 1, the average portal plasma TG is significantly (3.0 carbons) smaller ($P < 0.05$) than the average TG in vena cava plasma. The average liver TG is significantly (3.9 carbons) larger than that in portal plasma ($P < 0.01$) and 8.9 carbons larger than the average TG present in milk ($P < 0.01$). This may suggest preferential utilization of MCFA by liver.

The molecular species and stereospecific analyses on milk, lymph, and plasma TG show a loss of MCFA and a positional rearrangement of long chain fatty acids. These data are in accord with our observations about the composition of acids released during TG digestion (7). In order to characterize these changes further, we have investigated the TG molecular species and the acylglycerol class composition of stomach contents, as well as the effects of lingual homogenate on milk TG.

TABLE 3. Acylglycerol class distribution (mol %) in milk, suckling stomach contents, and produced in vitro by milk + lingual homogenate[a]

	Triacylglycerol	Diacylglycerol	Monoacylglycerol
Milk[b]	98.8 ± 0.2	1.4 ± 0.1	tr
Stomach[c]	61.0 ± 5.5	34.9 ± 5.4	4.0 ± 1.3
In vitro[c]	65.3 ± 4.7	29.8 ± 5.1	4.2 ± 0.9

[a] Mean ± S.D. for at least three determinations.
[b] This distribution is significantly different from the others ($P < 0.01$).
[c] These distributions are not significantly different ($P > 0.1$).

TABLE 4. A comparison of the composition of free fatty acids in stomach contents, those released from milk TG by lingual lipase, and those at the *sn*-3 position of milk TG (mol %)[a]

Fatty Acid	Stomach	Tongue Homogenate + Milk In Vitro	Milk TG *sn*-3
8:0	10.9 ± 0.6	9.9 ± 2.1	11.9 ± 0.9
10:0	40.1 ± 0.3	34.3 ± 3.0	28.7 ± 2.2
12:0	23.3 ± 1.4	21.1 ± 3.6	15.3 ± 1.2
14:0	5.5 ± 0.6	6.9 ± 0.7	7.3 ± 0.4
16:0	5.2 ± 0.4	8.1 ± 1.2	7.5 ± 0.1
16:1	0.6 ± 0.0	0.9 ± 0.1	1.8 ± 0.1
18:0	0.9 ± 0.1	1.2 ± 0.3	2.4 ± 0.5
18:1	5.3 ± 0.7	7.8 ± 2.1	12.2 ± 2.7
18:2	7.0 ± 1.0	8.1 ± 3.5	10.8 ± 1.8
18:3 + 20:0	0.6 ± 0.1	0.9 ± 0.1	1.1 ± 0.0
20:4	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.1
22:5	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.0
22:6	0.1 ± 0.0	0.1 ± 0.1	tr

[a] Mean ± S.D. Fatty acid distributions of the three groups are different ($P < 0.05$) by one-way analysis of variance.

Tongue homogenate activity

Table 3 compares the acylglycerol class distribution produced from milk incubated in vitro with tongue homogenate (≥30 min) and that found in stomach contents. The distributions are not significantly different. **Table 4** presents the molar composition of free fatty acids produced by the in vitro reaction and by in vivo gastric lipolysis. These data show that a large proportion of MCFA are present in the acids released from milk TG under both conditions. In stomach contents

MCFA represent 74 mol % of free fatty acid and in vitro 65 mol % of free fatty acid (Table 4). The molecular species distributions of milk TG remaining after incubation of milk with tongue homogenate (lingual lipase activity) and TG in stomach contents are shown in Table 2. The average size ($\bar{N}$) of TG remaining both in stomach and in vitro is 46.1, significantly larger than the average TG species in milk ($\bar{N}$ = 43.5) ($P < 0.05$).

Stereospecific analysis of milk TG showed enrichment of MCFA at *sn*-3 (Fig. 1A). Data have also been presented suggesting that lingual lipase attacks TG at *sn*-3 at twice the rate of *sn*-1 (21). Although the substrates used were long chain acyl-alkyl analogs and actually may not be comparable to milk TG as substrates for lingual lipase, these results (21) would predict ~45 mol % MCFA released from these milk TG, whereas strict *sn*-3 specificity predicts ~56 mol % MCFA. In fact, MCFA comprise ~70 mol % of released fatty acid (Table 4). These data therefore suggest that lingual lipase shows a preference for hydrolysis of MCFA, in addition to possible *sn*-3 positional specificity.

The importance of lingual lipase during the suckling period is supported by our results. In the stomach of the suckling rat, lingual lipase acts on milk TG to produce partial glycerides, largely diacylglycerol, and free fatty acid, largely MCFA. The partial glyceride products may be important as substrates and/or emulsification factors (13) for further gastrointestinal lipid utilization. Our studies show that the MCFA content in intestinal and lymphatic lipids is greatly reduced. The released MCFA are available for preferential absorption (7, 22).

Although there is preferential release of MCFA during digestion of milk TG, it is clear that not all MCFA is transported as free fatty acid in portal blood, since lymph and plasma TG contain substantial amounts (7). The mechanism whereby the MCFA are incorporated into these TG is unknown.

Further studies in our laboratory are being carried out to examine the metabolic fate(s) of the MCFA.

This research was supported by a grant (HD 10954) from the National Institutes of Health.

Manuscript received 14 July 1980, in revised form 20 November 1980, and in re-revised form 30 January 1981.

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