

# Studies on fat digestion, absorption, and transport in the suckling rat. I. Fatty acid composition and concentrations of major lipid components

Germain J. P. Fernando-Warnakulasuriya, Joan E. Stagers, Susan C. Frost, and Michael A. Wells<sup>1</sup>

Department of Biochemistry, College of Medicine, University of Arizona, Tucson, AZ 85724

**Abstract** The lipids of rat milk, the contents of 9–10-day-old rat stomach and intestine, lymph, plasma, and liver were quantitated and their fatty acids were analyzed. Rat milk consists of 97% triacylglycerols, of which 35% of the fatty acids are of medium chain length (C<sub>8</sub>–C<sub>12</sub>). However, stomach triacylglycerols show a 25% reduction in medium chain fatty acids, which indicates preferential hydrolysis of medium chain fatty acids in the stomach. The intestinal lumen free fatty acid composition shows decreased medium chain fatty acids compared to long chain fatty acids, indicating preferential absorption of the former. Lymph was shown to contain a significant amount (~22%) of medium chain fatty acids. The decreased content of medium chain fatty acids in vena cava blood compared to portal blood, and also the lower concentration of medium chain fatty acids in liver compared to blood, indicates preferential use of these fatty acids by the liver. — **Fernando-Warnakulasuriya, G. J. P., J. E. Stagers, S. C. Frost, and M. A. Wells.** Studies on fat digestion, absorption, and transport in the suckling rat. I. Fatty acid composition and concentrations of major lipid components. *J. Lipid Res.* 1981. **22:** 668–674.

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Although lipid digestion and absorption have been well studied in the adult rat (1, 2), the processes remain to be fully characterized in the suckling rat. During the neonatal period, about 70% of energy is derived from milk lipids, primarily triacylglycerols (TG) (3). The suckling rat utilizes milk lipids efficiently and gains weight rapidly. Due to the lack of knowledge about enzymatic and absorptive mechanisms in the suckling rat, the processes by which the pups utilize the exogenous (milk) lipids remain obscure.

Considerable hydrolysis of milk TG occurs in the stomach of the suckling rat (4, 5). This activity can be largely attributed to lingual lipase, an enzyme released from the serous glands of the tongue, which is active within gastric contents of the suckling and adult rat, as

well as in man and other species possessing this enzyme (6). Gastric lipolysis of long-chain TG is nearly abolished in adult rats when saliva is diverted from the stomach (4) or lipid is provided via an esophageal fistula (7). Homogenates of lingual tissue from suckling (4, 8) or adult (4) rats produce in vitro lipolysis products very similar to the in vivo products of gastric lipolysis. In the adult rat, various pancreatic lipolytic enzymes acting within the intestinal lumen are thought to be the principal enzymes responsible for lipid digestion (1). Only the classic pancreatic lipase (EC 3.1.1.3) has been studied in the suckling rat. The results indicate that neonates have a low activity of this enzyme (3), or utilize a different form of the enzyme than the adult (9).

In order to study lipid metabolism in suckling rats, it is necessary to know the fatty acid composition and concentrations of lipid components in milk, stomach, intestinal contents, lymph, plasma, and liver. The fatty acid composition of rat milk has been published (5, 10, 11), but a complete analysis, particularly of the major polyunsaturated fatty acids, has not been reported. These data, as well as ours, demonstrate a high medium chain (C<sub>8</sub>–C<sub>12</sub>) fatty acid content in rat milk TG. The milk lipid fatty acid composition was shown to undergo minor changes when dams were fed diets of different fatty acid compositions (12, 13). The fatty acid composition and concentrations of stomach acylglycerols and free fatty acids in the suckling rat have been reported (5). The total lipid fatty acid composition of stomach contents also has been analyzed (12, 14) and quantitated (5). Similar studies on the intestinal contents

Abbreviations: ANS, 8-anilino-1-naphthalene sulfonic acid; TG, triacylglycerol; DG, diacylglycerol; MG, monoacylglycerol; FFA, free fatty acids; PL, phospholipids; CE, cholesteryl esters; CH, cholesterol.

<sup>1</sup> To whom correspondence should be directed.

have not been reported. The concentration of plasma fatty acids, triacylglycerols, and total cholesterol of suckling rats has been published (15–17), but the fatty acid composition of each of the classes has not been reported. Liver lipids of neonatal rats have been measured and their fatty acid composition has been published (14).

The present study was undertaken to provide data concerning the quantitative analysis and the fatty acid composition of tri-, di-, and monoacylglycerols, free fatty acids, phospholipids, and cholesteryl esters in milk, stomach, intestine, lymph, plasma, and liver of suckling rats. In addition, analyses of the acylglycerols and free fatty acids are reported for intestinal mucosal cells.

## METHODS

Sprague-Dawley rats were obtained from a breeding colony maintained by the Division of Animal Resources of the College of Medicine. At 2 days after birth the litters were reduced to ten pups. Pups 9–10 days of age, weighing 23–30 g, were used in all experiments. Animals were killed within 30 min after removal from the mother. All surgical procedures were carried out under pentobarbital anesthesia (50  $\mu\text{g/g}$  body weight, i.p.)

Intestinal lymph was collected from the cisterna chyli via a 50- $\mu\text{l}$  glass micropipet whose tip had been drawn out to a diameter of approximately 0.2 mm. Five to ten  $\mu\text{l}$  of lymph could be collected from each animal. The lymph from ten animals was pooled, diluted with an equal volume of physiological saline, and centrifuged in a Brinkmann Eppendorf 3200 Centrifuge for 2 min to remove any fibrin clots and blood cells. The floating layer and supernatant were carefully removed and resuspended in saline. Lipids were extracted with 20 volumes of  $\text{CHCl}_3$ – $\text{CH}_3\text{OH}$  2:1 (v/v), and precipitated protein was removed by centrifugation (18).

An attempt was made to assess the role of the liver in altering the fatty acid composition of blood lipids by comparing the lipids obtained from portal and vena cava blood. A suture was used to tie off the portal vein just below the liver. The animal was then exsanguinated through a cut in the vein which was made between the suture and the gastrointestinal tract. For the vena cava sample, the suture was placed between the liver and the heart, and the animal was exsanguinated through a cut in the vena cava between the liver and the suture. Blood from individual animals was centrifuged in plastic tubes for 4 min to remove red cells. The plasma was removed and

stored on ice until all samples were collected. Plasma lipids were extracted by the method of Wells and Dittmer (19), omitting the acidified chloroform–methanol extraction. Livers were freeze-clamped immediately after removal, and the powdered frozen tissue was extracted by the above method. For the analysis of FFA in plasma, lymph, and liver, the lipid extracts were not washed, but analyzed directly. By measuring the recovery of added fatty acid standards, it was determined that this procedure resulted in loss of less than 5% of  $\text{C}_8$  and  $\text{C}_{10}$  and less than 1% of longer chain fatty acids. Washing of gastrointestinal lipid extracts with saline was shown not to alter the fatty acid composition.

Mothers were milked under ether or pentobarbital anesthesia after intraperitoneal injection of 200 munits of oxytocin. Milk was extracted as described for plasma.

Intact gastrointestinal tracts (esophageal sphincter to cecum) were surgically excised and placed in ice-cold 0.9% saline. Each tract was sectioned by cutting at the pyloric sphincter. The intestines were blotted dry and weighed. The stomach was cut open and the contents removed and homogenized. Weighed samples of stomach contents (approximately 0.5 g) were extracted as for plasma. Intestines were lavaged with ice-cold 0.9% saline (3–4 ml) to remove their contents. Intestinal content weight was determined as the difference between full and empty tract weight. Total lipid extractions were performed on intestinal contents and empty intestines as described for plasma.

### Separation of lipid classes

Lipids from milk, lymph, plasma, and liver were separated on silica gel G or H plates using hexane–ether–formic acid 80:20:2 (20). Lipids from the gastrointestinal tract were separated using either heptane–ether–methanol–acetic acid 85:15:3:2 (21) for TG, DG, and FFA, or heptane–ether–methanol–acetic acid 65:30:5:2 for MG and PL. Lipids were detected either with 0.1% aqueous ANS (22) or iodine vapor. Each band was scraped from the plate and extracted by a modification of the method of Arvidson (23) in which ammonium hydroxide replaced acetic acid and the extracts were washed with saline. In the case of the free fatty acid band, the original method was used except that the extracts were washed with saline.

### Quantitative analysis of lipids

Phospholipids were determined by measuring the amount of phosphorous in the lipid extract by the method of Bartlett (24) after digestion with 70% perchloric acid. Cholesterol was measured by the

method of Bowman and Wolf (25), reducing the total volume of the assay to 1 ml.

Acylglycerols were quantitated by measurement of the glycerol content of each fraction. Acylglycerol samples (25–100 nmol) were subjected to alkaline hydrolysis in 0.25 ml of 0.7 M KOH in 90% ethanol (26). After addition of 0.8 ml of water, the free fatty acids were removed by adsorption onto 0.2 g of anion exchange resin (Dowex AG 1 – ×8). Samples were centrifuged and the free glycerol concentration of the supernatant was assayed according to the enzymatic method of Weiland (27).

Free fatty acids from gastrointestinal samples were methylated as described below and quantitated by addition of an internal standard (0.1 to 0.25  $\mu$ mol of tridecanoic acid; a modification of the method of

Christie, Noble, and Moore (20)). Certain quantitative determinations of glycerolipids were also done by this method.

Methyl esters were prepared by transmethylating the lipids according to a modification of the method of Doss and Oette (28). Lipids (0.01 to 2 mg) were dissolved in 100  $\mu$ l of dichloromethane and 100  $\mu$ l of 2 M sodium methoxide in anhydrous methanol was added; the mixture was kept at room temperature under nitrogen in the dark for 5 min (for cholesteryl esters the incubation time was prolonged to 25 min). The reaction was stopped with 100  $\mu$ l of acetic acid, followed by 1.5 ml of water, and the methyl esters were extracted with 5 ml of hexane. Free fatty acids were methylated with diazomethane, according to the method of Schlenk and Gellerman (29).

TABLE 1. Fatty acid composition of gastrointestinal acylglycerols and free fatty acids from 9–10-day-old suckling rats

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2n-6	18:3n-3 + 20:0	20:4n-6	22:5n-3	22:6n-3
	<i>mole %<sup>a</sup></i>												
<b>Triacylglycerols</b>													
Milk	5.8 (0.6)	16.5 (1.7)	13.4 (1.2)	12.4 (1.0)	20.9 (2.3)	1.7 (0.5)	2.2 (0.2)	12.3 (1.7)	12.7 (1.6)	0.9 (0.1)	0.7 (0.1)	0.2 (0.1)	0.4 (0.1)
Stomach	3.4 (0.6)	11.7 (0.4)	11.1 (1.0)	12.1 (1.7)	23.4 (1.0)	1.8 (0.1)	2.6 (0.4)	15.0 (1.8)	15.9 (1.7)	1.2 (0.1)	0.8 (0.1)	0.4 (0.1)	0.5 (0.1)
Lumen	1.1 (0.1)	7.3 (0.9)	12.4 (1.0)	13.9 (1.5)	27.0 (2.1)	1.9 (0.4)	3.1 (0.3)	14.9 (1.5)	16.9 (1.4)	1.1 (0.2)	0.8 (0.2)	0.3 (0.1)	0.5 (0.2)
Mucosa	0.6 (0.1)	4.9 (0.2)	12.7 (0.7)	14.0 (1.2)	28.0 (1.6)	1.8 (0.1)	2.8 (0.1)	14.7 (0.7)	16.5 (1.4)	1.1 (0.0)	1.2 (0.2)	0.4 (0.0)	0.7 (0.1)
<b>Diacylglycerols</b>													
Stomach	2.9 (0.5)	10.2 (0.8)	11.4 (0.6)	15.9 (1.3)	27.8 (1.8)	1.7 (0.1)	2.6 (0.2)	11.5 (1.2)	13.6 (1.0)	1.0 (0.1)	0.6 (0.0)	0.3 (0.1)	0.5 (0.1)
Lumen	0.9 (0.4)	5.7 (1.0)	11.2 (0.5)	16.4 (1.5)	34.9 (3.0)	1.9 (0.5)	3.8 (0.4)	10.9 (1.1)	11.7 (2.3)	0.9 (0.3)	0.8 (0.3)	0.3 (0.1)	0.7 (0.2)
Mucosa	0.7 (0.4)	4.2 (0.3)	10.2 (0.2)	16.7 (0.5)	32.0 (0.2)	1.6 (0.1)	5.1 (0.1)	9.4 (0.4)	11.4 (0.3)	1.0 (0.2)	1.5 (0.2)	0.4 (0.1)	1.0 (0.1)
<b>Monoacylglycerols</b>													
Stomach	3.4 (0.6)	12.5 (1.0)	13.5 (0.4)	20.1 (1.4)	29.5 (0.6)	2.1 (0.4)	1.2 (0.2)	7.1 (0.7)	8.7 (0.6)	0.9 (0.2)	0.4 (0.0)	0.4 (0.0)	0.3 (0.1)
Lumen	2.8 (0.2)	5.3 (0.9)	11.4 (0.8)	18.6 (0.6)	37.6 (0.7)	2.5 (0.6)	1.9 (0.5)	7.3 (0.9)	9.4 (0.9)	0.8 (0.0)	1.0 (0.2)	0.3 (0.1)	1.1 (0.1)
Mucosa	0.7 (0.1)	4.2 (0.1)	10.5 (0.1)	19.0 (0.3)	38.2 (0.3)	2.0 (0.2)	2.9 (0.1)	7.4 (0.1)	10.8 (0.2)	1.1 (0.2)	2.1 (0.5)	0.3 (0.1)	0.8 (0.2)
<b>Free fatty acids</b>													
Stomach	10.9 (0.6)	40.1 (0.3)	23.2 (1.4)	5.7 (0.6)	5.4 (0.4)	0.6 (0.1)	0.9 (0.1)	5.3 (0.7)	7.0 (1.0)	0.6 (0.1)	0.6 (0.1)	0.2 (0.1)	0.1 (0.0)
Lumen	0.3 (0.1)	5.6 (0.2)	12.5 (0.7)	12.0 (1.2)	26.3 (0.5)	1.6 (0.2)	5.4 (0.6)	15.3 (1.0)	15.0 (0.6)	1.0 (0.2)	3.7 (0.9)	0.3 (0.1)	1.0 (0.3)
Mucosa	0.4 (0.1)	2.8 (0.2)	7.2 (0.1)	9.0 (0.4)	20.6 (2.5)	2.0 (0.2)	6.7 (0.3)	23.5 (0.8)	20.7 (1.5)	1.1 (0.2)	4.6 (0.2)	0.6 (0.2)	0.8 (0.2)

<sup>a</sup> Mean value for at least three different samples of ten pups each. The standard deviation is in parentheses.

TABLE 2. Fatty acid composition of triacylglycerols from 9–10-day-old suckling rats

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2n-6	18:3n-3 + 20:0	20:4n-6	22:5n-3	22:6n-3
	<i>mole %<sup>a</sup></i>												
Lymph	0.9 (0.2)	6.9 (0.9)	14.3 (1.7)	14.1 (2.8)	25.3 (3.1)	2.5 (0.6)	2.3 (0.2)	16.1 (3.4)	14.8 (1.7)	1.2 (0.2)	0.8 (0.2)	0.2 (0.1)	0.6 (0.2)
Portal blood	0.7 (0.4)	4.6 (0.6)	10.8 (1.0)	12.2 (1.3)	26.2 (1.9)	2.2 (0.2)	2.4 (0.2)	15.8 (0.8)	16.9 (1.4)	1.0 (0.1)	2.7 (0.9)	1.1 (0.5)	3.6 (1.2)
Liver	0.8 (0.3)	3.8 (1.5)	4.7 (1.7)	5.8 (1.5)	25.0 (0.8)	1.8 (0.2)	3.2 (0.3)	20.2 (1.8)	23.0 (2.4)	0.8 (0.1)	3.6 (0.5)	1.7 (0.1)	5.6 (1.2)
Vena cava blood	0.7 (0.2)	4.8 (0.9)	9.3 (2.3)	9.5 (2.7)	24.1 (1.9)	2.4 (0.5)	2.6 (0.4)	17.6 (3.5)	18.3 (1.2)	0.9 (0.1)	3.8 (1.8)	1.1 (0.5)	4.9 (2.6)

<sup>a</sup> Mean for at least three different samples of ten pups each. The standard deviation is in parentheses.

In order to avoid loss of methyl esters of C<sub>8</sub> and C<sub>10</sub>, samples were never taken completely to dryness and were kept at room temperature or below in tightly closed containers.

Gas-liquid chromatography was performed on a Shimadzu GC mini 1 gas chromatograph equipped with a Shimadzu C-RIA recording data processor. Columns (glass 1.8 m, i.d. 2.6 mm) were filled with 10% Silar 10C on 100/120 Gas-Chrom Q packing (Applied Science Labs, State College, PA). A linear temperature program from 130 to 230°C at a rate of 4°C/min was used.

All solvents and chemicals used were reagent grade except hexane, dichloromethane, chloroform, and methanol used in GLC analysis which were nanograde (Mallinckrodt) or omnisolve grade (MCB). Lipid standards were obtained from Nu-Chek-Prep Inc. (Elysian, MN).

## RESULTS AND DISCUSSION

The 9–10-day-old rat was chosen for these studies because it is in the middle of the suckling period,

and especially because at this age the only source of food is mother's milk. The concentrations and fatty acid composition of the major lipid components involved in digestion, absorption, and transport are shown in **Tables 1–7**.<sup>2</sup> In milk, TG constitutes 97% of the lipids, while DG, FFA, PL, CH, and CE account for the remainder (Table 6); no MG was detected in these samples. Our results differ from those reported by Helander and Olivecrona (5) especially with regard to TG content and the presence of MG. This variation may be due to the diet and/or methods used for obtaining milk. However fatty acid composition of the milk TG agrees well with other reported values (5, 10, 11, 13).

It should be noted that ~35% of the milk TG consists of medium chain fatty acids (C<sub>8</sub> to C<sub>12</sub>). It is interesting to note that the concentration of medium chain fatty acids is reduced to ~25% in the TG of the stomach. This change is most notable for

<sup>2</sup> It should be noted that in all samples, especially milk, there were odd chain length fatty acids and also fatty acids besides those reported in the C<sub>20</sub>–C<sub>22</sub> range. Since their concentrations were low, they are not reported.

TABLE 3. Fatty acid composition of phospholipids from 9–10-day-old suckling rats

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2n-6	18:3n-3 + 20:0	20:4n-6	22:5n-3	22:6n-3
	<i>mole %<sup>a</sup></i>												
Lymph	0.1 (0.2)	0.3 (0.2)	0.5 (0.3)	1.5 (0.4)	24.3 (2.0)	1.2 (0.4)	19.5 (1.9)	5.5 (0.8)	30.5 (0.7)	0.3 (0.1)	12.6 (0.5)	0.7 (0.3)	3.2 (0.2)
Portal blood	0.0	0.1 (0.2)	0.2 (0.1)	1.4 (0.2)	28.1 (0.8)	tr	16.8 (0.3)	4.7 (0.5)	24.9 (1.6)	tr	15.6 (1.6)	1.0 (0.7)	7.1 (0.4)
Liver	0.0	tr	0.1 (0.1)	0.7 (0.3)	26.4 (1.5)	0.2 (0.2)	18.1 (0.9)	4.8 (0.3)	11.2 (0.5)	0.1 (0.1)	22.4 (0.6)	0.8 (0.4)	14.5 (1.0)
Vena cava blood	0.0	0.1 (0.1)	0.2 (0.1)	1.2 (0.3)	27.8 (2.6)	0.1 (0.1)	16.7 (0.6)	5.0 (0.7)	24.0 (0.6)	0.1 (0.1)	16.6 (2.0)	0.9 (0.4)	7.4 (1.4)

<sup>a</sup> Mean for at least three different samples of ten pups each. Standard deviation in parentheses.

TABLE 4. Fatty acid composition of cholesteryl esters from 9–10-day-old suckling rats

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2n-6	18:3n-3 + 20:0	20:4n-6	22:5n-3	22:6n-3
	mole % <sup>a</sup>												
Lymph	tr	1.7 (0.1)	2.1 (0.3)	3.3 (0.2)	17.9 (2.8)	3.7 (0.2)	4.4 (2.3)	27.9 (4.1)	19.9 (2.1)	0.9 (0.2)	17.1 (6.4)	0.6 (0.1)	0.5 (0.8)
Portal blood	0.0	tr	0.6 (0.1)	2.7 (0.4)	16.8 (1.6)	2.8 (0.3)	1.4 (0.3)	9.7 (1.8)	24.9 (2.0)	0.9 (0.6)	38.0 (3.6)	tr	2.3 (0.5)
Liver	tr	0.6 (0.4)	0.9 (0.2)	2.7 (0.7)	22.7 (3.6)	3.2 (0.2)	4.3 (0.5)	28.5 (2.8)	18.8 (2.0)	0.9 (0.3)	14.9 (0.3)	0.3 (0.3)	2.2 (0.7)
Vena cava blood	0.0	0.1 (0.1)	0.3 (0.1)	2.5 (0.4)	15.8 (1.3)	3.0 (0.3)	1.4 (0.1)	11.6 (1.5)	24.2 (2.4)	0.4 (0.1)	38.7 (2.9)	0.1 (0.1)	2.2 (0.3)

<sup>a</sup> Mean for at least three different samples of ten pups each. Standard deviation in parentheses.

C<sub>8</sub>, which is reduced by ~55%, and is due to the preferential hydrolysis of medium chain fatty acids from TG in the stomach (5, 8). This preferential hydrolysis of medium chain fatty acids from TG in the stomach is also shown by the composition of FFA. Hydrolysis of TG in the stomach produced DG, MG, and FFA, as shown by the data in Table 7. The relative amounts of DG and especially of MG and FFA are high in the intestinal lumen; this suggests that further hydrolysis occurs in the lumen. The lumen FFA composition shows a low concentration of medium chain fatty acids, indicating their preferential absorption. Our results do not preclude the possibility of direct absorption of medium chain fatty acids from the stomach as suggested by Aw and Grigor (30). Medium chain fatty acids are low in both TG and FFA fractions of intestinal mucosa. The fatty acid composition of mucosal acylglycerols shows a marked similarity to that of intestinal lumen contents (Table 1).

The majority of lipid present in lymph is TG

(85%) which carries a significant amount of C<sub>8</sub> and C<sub>10</sub> (~8%) fatty acids (Tables 2 and 6). In addition a large amount of C<sub>12</sub> is present. Phospholipid comprises about 9% of lymph lipid and is notable for its high content of arachidonic acid (20:4n-6) as is also seen in the small amount of cholesteryl ester present (Table 3). The high content of free fatty acids (~3%) in lymph has not been reported with normal adult rats (31), although free fatty acids are found in lymph after biliary diversion (32). The presence of free fatty acid in the lymph of the suckling rat may be a result of the large amount of fat being digested. The composition of the lymphatic free fatty acids reflects that in the intestinal lumen (Table 5).

The comparison of lipids present in portal and vena cava plasma do not show significant differences except for the FFA fraction (Tables 5 and 6). In portal blood the FFA fraction has an increased content of medium chain fatty acids (C<sub>8</sub>, C<sub>10</sub>, C<sub>12</sub>) as compared to vena cava blood. These data support

TABLE 5. Composition of free fatty acids from 9–10-day-old suckling rats

	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2n-6	18:3n-3 + 20:0	20:4n-6	22:5n-3	22:6n-3
	mole % <sup>a</sup>												
Lymph	0.5 (0.4)	3.9 (2.2)	11.2 (6.3)	8.1 (2.3)	25.5 (11.5)	1.6 (1.3)	8.0 (3.4)	21.4 (2.6)	11.9 (3.0)	2.3 (1.6)	4.1 (2.9)	2.4 (3.3)	0.3 (0.5)
Liver	1.2 (1.3)	3.9 (0.7)	5.6 (0.6)	5.3 (0.7)	28.3 (6.6)	1.9 (0.2)	10.5 (1.3)	14.8 (5.1)	9.0 (1.3)	0.6 (0.2)	8.1 (2.3)	3.1 (1.6)	7.7 (1.2)
Portal blood	3.7 (1.3)	14.9 (1.6)	14.7 (2.5)	8.8 (0.5)	18.3 (1.1)	2.0 (0.4)	5.0 (0.6)	11.7 (2.1)	10.4 (0.9)	1.1 (0.3)	3.8 (0.4)	1.0 (0.5)	4.6 (0.6)
Vena cava blood	2.4 <sup>b</sup> (0.5)	6.1 <sup>c</sup> (3.9)	9.6 <sup>b</sup> (4.6)	8.5 <sup>d</sup> (1.4)	21.5 <sup>e</sup> (0.9)	2.6 <sup>d</sup> (0.7)	8.7 <sup>c</sup> (1.9)	15.4 <sup>d</sup> (4.7)	9.7 <sup>d</sup> (1.6)	1.8 <sup>d</sup> (1.2)	6.2 <sup>c</sup> (1.7)	1.1 <sup>d</sup> (0.4)	1.7 <sup>b</sup> (2.1)

<sup>a</sup> Mean for at least three different samples of ten pups each. The standard deviation is in parentheses.

<sup>b</sup>  $P < 0.1$ .

<sup>c</sup>  $P < 0.05$ .

<sup>d</sup> Not significantly different, for portal-vena caval samples.



TABLE 6. Quantitative analysis of lipid components of 9–10-day-old suckling rats<sup>a</sup>

	Milk	Lymph	Portal Plasma	Vena Cava Plasma	Liver
	<i>mmol/liter</i>	<i>mmol/kg</i>	<i>mmol/liter</i>	<i>mmol/liter</i>	<i>mmol/kg wet weight</i>
TG	140.1 ± 69.5	85.3 ± 14.2	1.1 ± 0.2	1.0 ± 0.4	8.9 ± 2.3
PL	0.6 ± 0.2	9.3 ± 4.1	2.8 ± 0.3	2.5 ± 0.2	23.4 ± 5.0
FFA	<0.3	2.7 ± 0.7	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.1
CE	0.2 ± 0.1	2.0 ± 0.9	1.6 ± 0.6	1.6 ± 0.4	1.2 ± 0.2
CH	1.2 ± 0.7	1.7 ± 0.9	1.1 ± 0.1	1.2 ± 0.1	6.1 ± 0.6

<sup>a</sup> The results presented are means ± S.D. for at least three determinations.

the notion of direct absorption of these fatty acids from the intestinal lumen and suggest rapid removal by the liver. Liver TG shows a lower C<sub>10</sub> to C<sub>14</sub> fatty acid content compared to plasma, which indicates that the liver may be using these preferentially for energy production.<sup>3</sup> The amounts of 18:0, 18:1, 18:2, and 22:6 are somewhat higher in liver TG than in plasma. In the PL fraction the major differences between liver and plasma are in the content of 18:2, 20:4, and 22:6 (Table 3). The concentrations of 16:0, 18:0, and 18:1 in cholesteryl esters are higher in liver compared to plasma, whereas the concentrations of 18:2 and 20:4 are lower (Table 4).

The data presented in this paper reiterate the importance of hydrolysis of milk TG in the stomach contents of suckling rats (6, 8). This hydrolysis is of particular importance in releasing medium chain fatty acids. As indicated in the introduction, this lipolytic activity is most likely due to lingual lipase. Further support for the importance of lingual lipase in milk TG hydrolysis is found in the accompanying paper (8). The relatively high content of FFA in intestinal contents suggests further lipolysis, although the nature of this lipolytic activity is unknown. As far as can be determined by these studies, long chain fatty acids are absorbed and transported by processes similar to those operative in adults.

Caution should be exercised in equating gastric metabolism of milk TG, which on the average con-

tains only one medium chain fatty acid (8), with that of medium chain TG (MCT). Although adult gastric mucosa contains a lipase specific for MCT (33, 34), this activity has not been investigated in suckling rats. Further, the activity of gastric lipase on milk TG is unknown. Therefore we suggest that milk TG be considered different from MCT in gastric digestion. Studies to elaborate this point further are in progress. ■

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<sup>3</sup> Frost, S. C., and M. A. Wells. Unpublished data.

TABLE 7. Quantitative analysis of neutral lipids in the gastrointestinal tract of 9–10-day-old suckling rats<sup>a</sup>

	Milk	Stomach	Intestinal Lumen
	<i>mmol/liter</i>	<i>mmol/kg</i>	<i>mmol/kg</i>
TG	140.1 ± 69.5	99.3 ± 21.9	13.5 ± 2.3
DG	1.6 ± 0.7	84.7 ± 17.8	9.3 ± 5.0
MG		19.3 ± 6.7	8.0 ± 4.3
FFA	<0.3	67.5 ± 8.1	28.6 ± 3.4

<sup>a</sup> The results presented are means ± S.D. for at least three determinations.

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