Uptake of free fatty acids and chylomicron glycerides by guinea pig mammary gland in pregnancy and lactation

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SUMMARY Mammary tissue of lactating guinea pigs was shown to take up both free fatty acids and chylomicron glycerides from plasma approximately twenty times more rapidly than mammary glands from midpregnant animals. The rates of removal of both forms of lipid by lactating mammary gland are comparable with those of liver. Free fatty acids cross the placental membrane and are rapidly taken up by fetuses at midpregnancy. There is no transfer of chylomicron glyceride across the placental membrane.

MAMMARY TISSUE of guinea pigs contains a lipoprotein lipase, the activity of which is greater in lactating than in nonlactating animals (1, 2). Although the function of lipoprotein lipase in any tissue in which it occurs is not certain, it is thought that this enzyme may function in the uptake of lipoprotein triglycerides from the circulation (3). This report is concerned with the correlation between the uptake of free fatty acids and glycerides by the mammary gland and the physiological state of this tissue.

MATERIALS AND METHODS

Two groups of N.I.H. strain guinea pigs were studied. One group was at the midterm of pregnancy and the other group was approximately 1 week post partum lactational. Six suckling piglets were placed with each of the lactating post partum animals for 72 hr prior to the experiments in order to stimulate maximal metabolic activity in the mammary glands. The suckling litters were kept with the lactating animals until 1–2 hr before the experiments. All animals were allowed a standard diet ad lib.

Palmitic acid-1-C^{14} (29.7 μc/μmole) was obtained from the Nuclear-Chicago Corporation, Chicago, Ill. It was purified from nonacidic contaminants and short-chain fatty acids as described in an accompanying paper (4). Analysis of the palmitic acid-C^{14} by gas–liquid chromatography after methylation demonstrated that it was more than 99% pure; traces of radioactivity corresponding to methyl myristate were found.

Albumin-I^{131} (Abbott Laboratories, Chicago, Ill.) was dialyzed extensively against 0.15 M NaCl immediately prior to use to remove any free I^{131} that may have been present.

Palmitate-C^{14}–Serum Mixture

Palmitic acid-C^{14} (0.2 μmole, 6 μc) was dissolved in 0.5 ml of ethanol and dilute aqueous NaOH was added to a final pH of approximately 8. The ethanol was evaporated by heating to 80°, 0.1 ml of water was added, and the solution was quickly mixed with 3 ml of normal guinea pig serum. Dialyzed albumin-I^{131} (12 μc) was mixed with this material prior to injection.

Chylomicrons Labeled with Esterified Palmitate-C^{14}

Palmitic acid-C^{14} (50 μc) was dissolved in 1.5 ml of olive oil and administered to rats with cannulated cisternae chyli. The chyle was collected under ice for 24 hr, and the chylomicrons were then isolated by centrifugation of the chyle at 100,000 × g for more than 30 min. The tubes were sliced just below the packed layer of chylomicrons. The chylomicrons were reemulsified by repeated aspiration through a 19-gauge needle and were mixed with a solution of 5% bovine serum albumin, pH 7.5, in an effort to decrease any contamination

TABLE 1 UPTAKE OF PALMITATE-1-C^{14} (FREE FATTY ACID) BY TISSUES OF MIDPREGNANT AND LACTATING GUINEA PIG

Physiological State	% of Injected Dose in				Uptake per Gram	
	Serum	Mammary Glands	Liver	Fetuses	Mammary Glands	Liver
					% dose/g	
Midpregnancy	2.30	0.76	42.3	9.6	0.09	1.51
Midpregnancy	2.53	0.69	44.3	16.9	0.06	1.43
Lactating	2.11	15.1	39.6	—	0.52	1.17
Lactating	4.85	13.6	42.4	—	0.56	1.27

of the chylomicrons with unesterified palmitate-C^{14}. The chylomicrons were washed twice by layering under saline and reisolating them ultracentrifugally. The chylomicrons were then emulsified in 0.15 M NaCl and mixed with guinea pig serum and dialyzed albumin-I^{131} to give a final triglyceride concentration of approximately 30 μmoles/ml.

Procedure

Animals were laparotomized under light ether anesthesia and 1 ml of the palmitate-C^{14}–albumin-I^{131}–serum mixture, or chylomicron-C^{14}–albumin-I^{131}–serum mixture was injected into the inferior vena cava. The abdomen was kept moist with saline. Five minutes after injection, approximately 5 ml of blood was removed by cardiac puncture and allowed to clot in a centrifuge tube at 0°. The thoracic aorta was also transected at that time. Both mammary glands, the liver, and one fetus in the case of pregnant animals were removed in toto, in that order, quickly weighed, and homogenized in 20 volumes of chloroform–methanol 2:1 (v/v) in Waring blendors. All tissues were homogenized within about 8 min of the time of injection. The serum and an aliquot of the injected material were also extracted in chloroform–methanol. After extraction for several hours the protein precipitates were collected by centrifugation, and the lipid extracts were partitioned and washed to remove nonlipid material (5). The lipid extracts were evaporated to dryness on a rotary evaporator at 40° under reduced pressure and redissolved in known volumes of chloroform. Aliquots of the whole lipid extracts were separated into neutral lipids and phospholipids by silicic acid chromatography (6). Free fatty acids were separated from the neutral lipids by partitioning in heptane–isopropanol–water–1 N NaOH 40:40:30:1. The free fatty acids were recovered by acidifying the aqueous isopropanol phase and extracting with heptane. Aliquots of the total lipid extracts, neutral lipids, phospholipids, and free fatty acids were evaporated to dryness under air in scintillation vials. The samples were dissolved in 0.4% diphenyloxazole in toluene and assayed for radioactivity in a liquid scintillation spectrometer. Counting efficiency was approximately 80%. Corrections for quenching were made in all cases by the addition of internal standards.

The protein precipitates were hydrolyzed in 4 volumes of 25% KOH and aliquots of these digests were assayed for radioactivity in a thallium-activated sodium iodide scintillation counter. From the C^{14}/I^{131} ratio in the blood and the I^{131} content of the tissues, uptakes of palmitate-C^{14} were corrected for the amount of C^{14} remaining in blood in the tissues. These corrections were usually negligible in the case of the experiments with free fatty acids, but frequently were significant in the experiments with chylomicrons. Aliquots of the lipid extracts were also assayed for I^{131}; there was never any significant contamination.

Lipid Assays

Free fatty acids were titrated by the method of Dole (7). Triglycerides were determined as glycerol following saponification (8). Esters were determined by the hydroxamate procedure (9).

RESULTS

UPTAKE OF FREE FATTY ACIDS

Comparison of Tissues

There were many similarities in the experiments with lactating and pregnant animals (Table 1). In each case almost all the palmitate-C^{14} had been removed from the plasma within the 5 min of the experiment, and 40–45% of the injected radioactivity was found in the liver. However, the activity of the mammary glands in the two physiological states was quite different. Mammary glands of pregnant guinea pigs removed less than 1% of the injected palmitate-C^{14}, whereas the lactating mammary glands removed about 15% of the dose. Even when compared on a weight basis, the lactating mammary glands were five to ten times as active as the nonlactating glands.

TABLE 2 DISTRIBUTION OF RADIOACTIVITY AMONG THE LIPIDS OF THE TISSUES AFTER UPTAKE OF PALMITATE-1-C^{14} (FREE FATTY ACID)

Tissue	% of Radioactivity in		
	FFA	Neutral Lipids	Phosphatides
Injected Fatty Acid	99.3	0.7	0.1
Midpregnant			
Liver	1.2	72.5	22.4
Mammary	33.4	31.4	40.7
Fetuses	4.9	75.9	20.3
Serum	96.1	3.9	0
Lactating			
Liver	1.9	69.1	28.1
Mammary	1.6	83.9	11.5
Serum	94.3	2.6	0.2

Free fatty acids are apparently able to cross the placenta and are taken up readily by the fetuses.

The tissues studied accounted for approximately 60% of the injected palmitate-C^{14}. The majority of the remainder was probably removed by skeletal muscle and other tissues, and some may have been lost by oxidation.

Distribution of Radioactivity among the Lipids of Each Tissue

The data for the two experiments described in Table 1 were essentially identical and are averaged in Table 2. Virtually all the palmitate-C^{14} taken up by both the liver and the lactating mammary glands had been esterified within the 5-min period of the experiment. In contrast to this, 33% of the radioactivity taken up by the nonlactating mammary glands was still present as free fatty acid. This difference in rates of esterification is particularly striking when it is remembered that the nonlactating glands took up only 5–10% as much palmitate as the lactating glands. Also, a much higher percentage of the esterified palmitate-C^{14} was incorporated into phospholipids in the nonlactating glands than in the lactating mammary glands. It is also apparent from Table 2 that the residual radioactivity in the serum is still in free fatty acid. This confirms the finding of Laurell (10) that conversion of free fatty acids to glycerides and phospholipids and their reappearance in the plasma in esterified form occur only after 5–10 min.

Uptake of Chylomicron C^{14}-Glycerides

Comparison of Tissues

In these studies three different preparations of chylomicrons were studied at two dose levels. The data from Experiment 1 (Table 3) show clearly that the mammary glands of lactating guinea pigs are much more active in the uptake of chylomicrons than are the mammary glands of nonlactating animals. The difference in relative activity is even greater than was found with free fatty acids. In contrast to what was found with free fatty acids, the fetuses were unable to take up chylomicrons.

Total uptake, as judged by the amount of radioactivity left in the serum, was greater by the lactating animals in Experiment 1 than by those in Experiments 2 and 3. The differences were accounted for by the greater uptake by liver in Experiment 1, the uptakes by the mammary gland being very similar in the three experiments. One possible explanation for this discrepancy is that the chylomicrons used in the first experiment may have been partially denatured, leading to greater uptake by the reticuloendothelial system. The percentages of injected radioactivity remaining in the plasma in Experiments 2 and 3 are in reasonable agreement with the results of others in different species (11, 12).

In Experiments 2 and 3 (Table 3) the uptake of chylomicrons per gram of tissue was generally greater for lactating mammary gland than for liver. The relative uptake of the two tissues appears to be independent of the size of the dose. It should be pointed out that no important part of the uptake by either liver or lactating mammary gland can be accounted for by the uptake of free fatty acids. The three batches of chylomicrons used contained only 1, 0.6, and 0.9%, respectively, of the radioactivity as free fatty acid, and, at the end of the experiments no more than 3% of the injected radioactivity was in the plasma as free fatty acid (Tables 3 and 4).

Distribution of Radioactivity among the Lipids of the Tissues

In the chylomicrons that were injected, over 95% of the radioactivity was in the glycerides (Table 4), and very little in the phospholipids and free fatty acids. At the end of the experiment the radioactivity remaining in the plasma was still largely in the glycerides, but approximately 10% of this residual radioactivity was in free fatty acids. As mentioned above, this represented less than 3% of the injected radioactivity.

TABLE 3 Uptake of Palmitate-1-C^{14}-Labeled Chylomicrons by Midpregnant and Lactating Guinea Pigs

Experiment*	Physiological State	Injected Dose	% of Radioactivity in				Uptake per Gram	
			Serum	Mammary Glands	Liver	Fetuses	Mammary Glands	Liver
		μmole of triglyceride					*% dose/g*	
1	Midpregnancy	6.4	15.0	0.44	35.6	0.10	0.03	1.0
	"	30.0	13.0	0.30	47.8	0.02	0.02	1.1
	Lactating	9.0	6.0	8.6	61.0	—	0.34	1.7
	"	30.0	11.9	20.0	39.0	—	0.69	1.3
2	Lactating	30.0	65.7	10.2	7.2	—	0.45	0.19
3	Lactating	10.7	29.1	14.5	9.4	—	0.78	0.30
	"	30.0	52.5	9.9	15.0	—	0.34	0.44

*A different preparation of chloymicrons was used in each experiment.

The distribution of radioactivity among the lipids of the liver was very similar to that of the injected material in all the experiments. But the phospholipids of the mammary glands, both lactating and nonlactating, contained a significantly greater percentage of the radioactivity than did the phospholipids of the chylomicrons. Only in the case of the nonlactating mammary glands was any appreciable radioactivity found in the free fatty acids. This is similar to what was found in the experiments on the uptake of free fatty acids (Table 2).

Distribution of Radioactivity among the Fatty Acids of Mammary Gland

In one experiment in which C^{14}-palmitate was injected, and in one experiment with C^{14}-chylomicrons, the distribution of radioactivity among the fatty acids of the lactating mammary glands was determined. An aliquot of the total lipids was methanolyzed in 0.5 N methanolic NaOH (13), and the methyl esters were analyzed by gas–liquid chromatography. Another aliquot was chromatographed on a preparative column; the emerging fractions were collected on anthracene and their radioactivity was measured directly in a scintillation spectrometer (14). The fatty acid analyses were essentially identical in the two experiments and the mean values are shown in Table 5. The distribution of radioactivity was also very similar in the two experiments. In both cases, most of the radioactivity was still in palmitate, with 10–20% in oleate and some in stearate (Table 5).

TABLE 4 DISTRIBUTION OF RADIOACTIVITY AMONG THE LIPIDS OF THE TISSUES AFTER UPTAKE OF PALMITATE-1-C^{14}-LABELED CHYLOMICRONS

Experiment	Tissue	% of Radioactivity in FFA	Neutral Lipids	Phospholipids
1	Injected Chylomicrons	1.0	98	1
	*Midpregnant**			
	Serum	9	79	2
	Mammary Glands	11	77	13
	Liver	1	98	3
	*Lactating**			
	Serum	15	74	5
	Mammary Glands	1	87	9
	Liver	1	100	1
2 and 3	Injected Chylomicrons	0.5	97	2
	Lactating†			
	Serum	7	94	4
	Mammary Glands	2	80	15
	Liver	0.3	93	8

* Average of two experiments.
† Average of three experiments.

DISCUSSION

The source of milk lipids has been a subject of considerable interest and some controversy, as discussed in several reviews (15–17). It is clear that the fatty acids of mammary tissue are in part synthesized in the gland from nonlipid precursors (18) and in part derived from the circulation, but estimates of the relative contributions from each source vary widely. Some of the difficulties may lie in species differences and in the influence of the nutritional state of the animal (19).

Although it is generally agreed that mammary tissue is capable of removing lipid from the circulating blood, the nature of the lipid removed has not been established with certainty. Many previous studies were concerned with the appearance in milk of orally administered lipids. The experiments lasted for hours or days and obviously gave little information concerning the immediate circulating precursor. Under more carefully controlled conditions, Lauryssens et al. (20) have demonstrated that the isolated perfused cow udder takes up stearate-1-C^{14} (free fatty acid). More than 50% of the fatty acid was esterified and about 50% of the total had been converted to oleate. The quantitative significance of plasma free fatty acids as the source of milk lipids remained in some doubt, however, since the lipid content of the perfusing solution was not significantly altered during passage through the gland.

Robinson et al. (21) were unable to find any arteriovenous difference in the concentration of free fatty acids across the mammary gland of lactating goats. The composition of the free fatty acids was significantly altered,[1] however, and it is possible that uptake of free fatty acids by mammary tissue was obscured by simultaneous release of free fatty acids by adipose tissue.

In the present study, the rate of uptake of palmitate-C^{14} by lactating mammary gland was 50% as great as the uptake by liver. This uptake was undoubtedly due to mammary tissue rather than to adipose tissue both because the uptake was greater by lactating than by nonlactating glands, and because Bragdon and Gordon (22) have shown that adipose tissue takes up very little free fatty acid. The fact that virtually all the palmitate-C^{14} removed by the mammary gland was esterified shows that the process was not just one of simple exchange of plasma and tissue free fatty acids.

If one assumes that the removal of radioactivity is a measure of net uptake, the contribution of plasma free fatty acids to mammary gland lipids can be calculated. The plasma volume of the guinea pigs was found to be approximately 30 ml (from the concentration of albumin-I^{131}) and the plasma free fatty acid concentration determined as 0.5–0.7 μeq/ml. From the data in Table 1, the *maximal* half-life of plasma free fatty acids was approximately 1.5 min, and about 15% of the fatty acids

[1] Personal communication, D. S. Robinson.

removed went to mammary gland. Using these data, the rate of uptake of free fatty acids by lactating guinea pig mammary glands can be calculated to be approximately 400 mg/24 hr.

There is general agreement on the ability of the lactating mammary gland to take up triglycerides. Robinson (21) found a pronounced arteriovenous difference across the goat udder in the concentration of the triglycerides of chylomicrons plus $d < 1.019$ lipoproteins. Similar results have been obtained by direct measurement while perfusing isolated udders (23), and by the use of radioactive substrates (24).

The data reported here confirm the earlier results and extend them to another species. They also emphasize the magnitude of the uptake by mammary gland, which was at least as active as liver on a weight basis, and show the great difference between lactating and nonlactating glands. The fact that the percentage of the chylomicron C^{14}-glycerides removed by liver did not appear to be influenced by the size of the load injected does not support the hypothesis (25) that other tissues remove chylomicrons from the circulation only when the capability of the liver to do so is exceeded. Lactating mammary gland, at least, removes chylomicron glycerides even when they are present in plasma at very low concentrations.

It might be pointed out that correlation between the uptake of chylomicrons by mammary gland and the physiological activity of the tissue agrees well with what was previously observed in regard to the lipoprotein lipase content of mammary gland (1, 2). One cannot conclude from this, however, that lipoprotein lipase is involved in the uptake of chylomicrons. For example, the uptake of free fatty acids, in which lipoprotein lipase is certainly not involved, is also much higher in lactating mammary glands than in nonlactating glands.

The uptake of lipid by the fetuses was studied both for its intrinsic interest and also because any large uptake by the fetuses might have reduced the plasma lipid concentration enough to influence the comparison of uptakes by other tissues. In the midpregnant animals studied, chylomicron glycerides were not taken up by the fetuses, but the free fatty acids were rapidly removed. With the same assumptions discussed above, it can be calculated that the three fetuses present in each pregnant animal would remove approximately 250–400 mg of free fatty acid per day from the maternal circulation. These data support earlier reports that free fatty acids can pass from the maternal circulations to the fetus[2] (26), although most fetal fat appears to be synthesized *in situ* (27). The relative contributions from the maternal circulation and from fetal synthesis may vary with the stage of gestation; there is some evidence that lipogenesis is very rapid in human fetuses during the last few weeks of pregnancy (28).

[2] Personal communication, K. Ono and D. Fredrickson.

TABLE 5 DISTRIBUTION OF RADIOACTIVITY AMONG THE FATTY ACIDS OF MAMMARY GLAND AFTER UPTAKE OF PALMITATE-1-C^{14} OR PALMITATE-1-C^{14}-LABELED CHYLOMICRONS

Fatty Acid	Composition	Distribution of Radioactivity derived from: Free Fatty Acid	Distribution of Radioactivity derived from: Chylomicrons
	Weight %	*cpm*	*cpm*
14:0	0.1	0	0
16:0	40.0	1385	438
16:1	1.8	23	8
18:0	4.3	69	17
18:1	35.1	251	43
18:2	15.5	0	0
18:3	3.3	0	0

Fatty acid analyses were made by gas–liquid chromatography of the methyl esters on ethylene glycol succinate polyester at 185° and 14 psi argon. Areas preceding, between, and after peaks gave 0 cpm. Fatty acids designated by chain length and number of double bonds.

We acknowledge the assistance of Dr. A. Karmen in the gas–liquid chromatography, and of Mr. Carlos Schultz in the preparation of the chylomicrons.

Manuscript received December 20, 1963; accepted February 14, 1964.

REFERENCES

1. Robinson, D. S. *J. Lipid Res.* **4:** 21, 1963.
2. McBride, O. W., and E. D. Korn. *J. Lipid Res.* **4:** 17, 1963.
3. Robinson, D. S., P. M. Harris, and C. R. Ricketts. *Biochem. J.* **71:** 286, 1959.
4. McBride, O. W., and E. D. Korn. *J. Lipid Res.* **5:** 448, 1964.
5. Folch, J., M. Lees, and G. H. Sloane Stanley. *J. Biol. Chem.* **226:** 497, 1957.
6. Borgström, B. *Acta Physiol. Scand.* **25:** 101, 1952.
7. Dole, V. P. *J. Clin. Invest.* **35:** 150, 1956.
8. Van Handel, E., and D. B. Zilversmit. *J. Lab. Clin. Med.* **50:** 152, 1957.
9. Rapport, M. M., and N. Alonzo. *J. Biol. Chem.* **217:** 193, 1955.
10. Laurell, S. *Acta Physiol. Scand.* **47:** 218, 1959.
11. Havel, R. J., and D. S. Fredrickson. *J. Clin. Invest.* **35:** 1025, 1956.
12. Bragdon, J. H., R. J. Havel, and R. S. Gordon, Jr. *Am. J. Physiol.* **189:** 63, 1957.
13. Morgan, T. E., D. J. Hanahan, and J. Ekholm. *Federation Proc.* **22:** 414, 1963.
14. Karmen, A., L. Giuffrida, and R. L. Bowman. *J. Lipid Res.* **3:** 44, 1962.
15. Folley, S. J., and M. L. McNaught. In *Milk: The Mammary Gland and Its Secretion*, edited by S. K. Kon and A. T. Cowie. Academic Press, Inc., New York, 1961, Vol. 1, pp. 481–82.
16. Glascock, R. F. *Proc. Roy. Soc. (London), Ser. B* **149:** 402, 1958.

17. Garton, G. A. *J. Lipid Res.* **4:** 237, 1963.
18. Dils, R., and G. Popják. *Biochem. J.* **83:** 41, 1962.
19. Insull, W., Jr., J. Hirsch, A. T. James, and E. H. Ahrens, Jr. *J. Clin. Invest.* **38:** 443, 1959.
20. Lauryssens, M., R. Verbeke, and G. Peeters. *J. Lipid Res.* **2:** 383, 1961.
21. Robinson, D. S., J. M. Barry, W. Bartley, and J. L. Linzell. *Biochem. J.* **87:** 23P, 1963.
22. Bragdon, J. H., and R. S. Gordon, Jr. *J. Clin. Invest.* **37:** 574, 1958.
23. Lough, A. K., W. E. H. Duncan, G. A. Garton, and G. Peeters. Proc. 4th Intern. Congr. Biochem., 1958. Vol. 18, *Biochemistry of Lipids*, edited by G. Popják. Pergamon Press, New York, 1960, p. 64.
24. Riis, P. M., J. R. Luick, and M. Kleiber. *Am. J. Physiol.* **198:** 45, 1960.
25. Borgström, B., and P. Jordan. *Acta Soc. Med. Upsalien.* **64:** 185, 1959.
26. Goldwater, W. H., and D. Stetten, Jr. *J. Biol. Chem.* **169:** 723, 1947.
27. Chaikoff, I. L., and A. Robinson. *J. Biol. Chem.* **100:** 13, 1933.
28. Hirsch, J., J. W. Farquhar, E. H. Ahrens, Jr., M. L. Peterson, and W. Stoffel. *Am. J. Clin. Nutr.* **8:** 499, 1960.