J. Lipid Research, January, 1963
Volume 4, Number 1

The lipoprotein lipase of mammary gland and the correlation of its activity to lactation

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[Manuscript received August 21, 1962; accepted September 21, 1962.]

SUMMARY

The concentration of lipoprotein lipase of guinea pig mammary gland increases markedly just prior to parturition, reaching a maximal level within 2 hr post-partum. This level is maintained until the cessation of suckling, when the enzyme becomes undetectable within 18 hr. Most of the lipoprotein lipase of the mammary gland would appear to be accounted for by that in the retained milk.

It was recently noted in this laboratory that bovine milk contains a lipase with properties characteristic of lipoprotein lipase (1). To gain further insight into the physiological function of this enzyme, studies were initiated on fat metabolism in the lactating mammary gland with particular reference to the role of lipoprotein lipase. The present report is concerned with the correlation of the activity of lipoprotein lipase[1] with mammary gland development and function.

METHODS

Animals. Mammary tissue was obtained from three groups of NIH strain guinea pigs: pregnant, post-partum lactational, and involutional animals. Mid-term pregnancy was defined as the earliest stage at which fetuses could be palpated. The stage of pregnancy of all other animals was estimated from the size of the fetuses and the degree of dilatation of the symphysis pubis. Control observations were made on two groups of nine animals each, estimated to be one and two days ante-partum. Six of the nine animals in the first group delivered within the expected one day and the other three delivered by the third day. In the second group, three of the nine animals delivered on the second day as expected, one delivered the first day, and the other five by the third to fifth days. All animals were allowed a standard diet ad libitum unless otherwise noted. Lactating, post-partum animals were kept with their litters until 1–2 hr before the experiment unless otherwise indicated.

Preparation of Tissue for Assay. Animals were either given light ether anesthesia or were killed by jugular venesection. The mammary glands were removed, quickly weighed, squeezed through a small tissue press, and homogenized in 2–5 vol of cold 0.15 M KCl for 1–2 min in a Potter-Elvehjem glass homogenizer. The homogenates were then either strained through several layers of cheesecloth or centrifuged for 10 min at 800 × *g* to remove the large particulate material. In the centrifuged samples, the fat layer was also discarded. The remaining homogenate was then assayed. In several instances, the activity of whole homogenates and 0.025 M ammonia extracts of mammary gland from lactating and midterm pregnant animals was compared to the activity of the fraction usually assayed. There were no differences in activity among the three types of preparation.

Whenever possible, milk was obtained from post-partum animals by expressing it from the teats just prior to killing the animal. Even when no milk could be obtained in this manner, a small amount of milk would often ooze from excised mammary glands lying on paraffin paper over crushed ice. This milk was collected before weighing and homogenizing the glands. In one experiment, samples of milk obtained in both ways from the same animal were compared, and no differences were observed.

[1] The lipoprotein lipase of mammary gland described in this paper is very similar, or identical, to the lipoprotein lipase of heart and adipose tissue. The tissue enzymes are presumably identical to, and are the source of, the lipase that appears in plasma following the injection of heparin. All of these enzymes have also been called "clearing factor lipase."

TABLE 1. RELATIONSHIP OF PARTURITION TO APPEARANCE OF LIPOPROTEIN LIPASE IN MAMMARY TISSUE AND MILK*

Time Prior to or After Parturition	Size of Mammary Gland	Lipoprotein Lipase Activity	
		Tissue	Milk
	g	*μmoles glycerol/hr/g*	
Midpregnancy	6.9	3.2	...
"	8.2	0	...
−25 days	7.0	6.7	...
−7 days	5.8	8.8	...
−2 days	...	8.5	...
−1 day	18.2	88.0	...
+2 hr	18.4	200	lost
+2 hr	19.6	205	680
+5 hr	11.0	123	490
+2 days	14.8	170	2,100
+5 days	...	232	≫380†
+7 days	28.6	300	1,400
+12 days	18.6	170	1,200
+14 days	29.2	173	500
Involutional 44 days	4.5	0	...

* Homogenates and milk were assayed as described under Methods. Milk could not be obtained from any of the antepartum or involutional animals.

† The smallest aliquot of milk assayed contained too much activity to measure accurately.

TABLE 2. IDENTIFICATION OF LIPOPROTEIN LIPASE IN MAMMARY TISSUE AND MILK

Conditions of Assay*	Relative Lipolytic Activity†		
	Mammary Gland		
	Midterm	Lactational	Milk
Complete system	100	100	100
Plus NaCl, 0.5 M	38	33	28
" NaCl, 1 M	42	0	8
" polylysine·HBr, 200 μg/ml	59	53	50
" polylysine·HBr, 500 μg/ml	41	53	40
Minus serum	58	11	5
" albumin	50	13	20
" substrate + cream, 10 μmoles/ml	18	0	0

* The complete system was as described under Methods, using washed coconut oil plus serum as substrate. When albumin was omitted, 0.5 ml of 0.25 M NH_3-NH_4Cl, pH 8.5, was added. Polylysine·HBr was obtained from Schwartz BioResearch, Inc.

† Activity observed with the complete system was arbitrarily assigned a value of 100.

Substrates. Activated coconut oil refers to the lipoprotein-triglyceride complex formed when Ediol[2] and serum are incubated together. The activated coconut oil was isolated centrifugally and washed repeatedly by centrifuging through 0.15 M NaCl. In some experiments, a 1:12 dilution of Ediol (4% triglyceride) or a washed coconut oil emulsion was used. In these cases, 0.05 ml of normal human serum was added to the incubation mixture. Essentially identical results were obtained with each of the three substrates.

Enzyme assay. Albumin (10%, pH 8.5), $(NH_4)_2SO_4$, (0.05 M), substrate (3–5 μmoles of triglyceride/ml), and varying amounts of the fraction to be assayed were incubated at 38° in a total volume of 1 ml. Duplicate aliquots of 0.05 ml were removed at 0, 30, and 60 min and immediately added to 0.1 ml of 1 N H_2SO_4. Formaldehydeogenic reaction products (glycerol and α-monoglycerides) were determined by a modification of the procedure of Lambert and Neish (2) described previously (3). In all assays, the rate of reaction was constant throughout the period of incubation. Activity is reported as μmoles of glycerol/hr/g tissue. Milk samples were measured volumetrically but results are reported "per gram," assuming 1 ml to be very nearly equivalent to 1 g.

[2] Schenley Laboratories, Lawrenceburg, Indiana. Composition: coconut oil, 50%; sucrose, 12.5%; glyceryl monostearate, 1.5%; polyoxyethylene sorbital monostearate, 2.0%.

RESULTS

Relation of Mammary Lipoprotein Lipase to Lactation. The data in Table 1 demonstrate the relationship between the physiological state of the mammary gland and the activity of lipoprotein lipase in the mammary gland, and in the milk when it could be obtained. Virtually no lipoprotein lipase was present in mammary tissue during most of pregnancy. Enzymic activity appeared in significant quantity not earlier than 1–2 days (and perhaps only a few hours) before delivery and reached its peak within 2 hr after delivery. The activity of lipoprotein lipase then remained relatively constant throughout the entire period of lactation. Lipoprotein lipase was not detectable after cessation of lactation.

Characterization of the Lipase in Mammary Tissue and Milk. The lipase of lactating mammary gland and milk possessed all the characteristic features of lipoprotein lipase (Table 2). It did not catalyze the hydrolysis of simple triglyceride emulsions in the absence of serum, it required a fatty acid acceptor, and it was inhibited by high ionic strength and by polycations. Raw cream was not hydrolyzed by the lipoprotein lipase of milk or mammary tissue in agreement with the findings with the lipoprotein lipase of cow's milk (1). The lipase of

mammary tissue from midpregnant animals exhibited the same characteristics qualitatively but not quantitatively. This might be explained by the requirement for much larger quantities of homogenate in the assay tubes in the latter case, although it might also be due to the presence of some other lipase.

Heparin did not activate the lipoprotein lipase of lactating mammary gland when tested at concentrations of 1–250 μg/ml.[3] The lipoprotein lipases of milk and the mammary gland from midterm pregnant animals were slightly activated by heparin, but never more than 20% in the case of milk nor 35% in the case of midpregnancy mammary tissue. No significant inhibition due to heparin was observed.

Lipoprotein Lipase Content Relative to the Lactose Content of Mammary Tissue. As noted in Tables 1 and 2, the activity of lipoprotein lipase in guinea pig milk was very high. An attempt was made, therefore, to determine whether a significant percentage of the lipoprotein lipase of the mammary gland could be accounted for by the lipolytic activity of the milk retained in the alveoli and ducts.

The amount of "retained fluid" in mammary tissue varies with the stage of pregnancy and lactation and is also dependent on the volume of milk left behind by the suckling litter. As a measure of the retained fluid, lactose was determined by the modified orcinol procedure described by Slater (4). Although orcinol is a relatively nonspecific carbohydrate reagent, essentially all of the carbohydrate in lactating mammary glands is lactose, and, according to Slater (5), the carbohydrate determined in mammary glands at late pregnancy, while not predominantly lactose, is probably a component of the retained fluid. This is supported by our finding that the involutional mammary gland contained only 0.25 mg carbohydrate/g.

The data in Table 3 record the activity of lipoprotein lipase in mammary tissue per milligram of carbohydrate. It is apparent that, with the onset of lactation, there is a greater increase in the lipolytic activity than in the carbohydrate content of the mammary gland. However, in the one case where milk was also analyzed, there was striking agreement between the activity of lipoprotein lipase per milligram of carbohydrate in the milk and in the homogenate of mammary gland. If all of the carbohydrate measured in the mammary gland were contained in the retained fluid, then all of the lipoprotein lipase of the mammary gland would be accounted for by

[3] The absence of appreciable activation by heparin differs from the finding of Robinson (10). This may be due to the fact that Robinson assayed extracts of acetone powders of mammary gland whereas fresh tissue homogenates were used in the present experiments.

TABLE 3. LIPOPROTEIN LIPASE ACTIVITY OF MAMMARY TISSUE AND MILK RELATIVE TO CARBOHYDRATE CONTENT

Expt.	Time After or Before Parturition	Carbohydrate*	Lipoprotein Lipase Activity*	
		mg/g	*μmole glycerol/hr/g*	*μmole glycerol/hr/mg CHO*
1	Midpregnancy	1.31	3.2	2.4
2	−7 days	1.04	8.8	8.5
3	−1 day	3.84	102	26.6
4	+2 hr	3.8	200	52.6
5	+2 hr, mammary tissue	4.84	205	42.4
	+2 hr, milk	15.75	680	43.1

* Assays of carbohydrate and lipoprotein lipase were made on aliquots of the same fraction.

that calculated to be present in the retained fluid. Serum, another possible source of lipolytic activity, was found to contain no significant lipoprotein lipase activity.

Effect of Suckling and Nutritional State on the Lipoprotein Lipase of Mammary Glands. Because of the pronounced nutritional effect on the activity of lipoprotein lipase in rat heart and adipose tissue (6, 7, 8, 9), similar studies were made on the guinea pig mammary gland.

Fasting had no effect (Table 4) on the lipoprotein lipase content of lactating mammary gland. On the other hand, within 18 hr after weaning, lipoprotein lipase was virtually undetectable. This finding was very surprising because one would expect that, even if no additional enzyme were formed after weaning, there would still be appreciable lipoprotein lipase in the retained milk. In subsequent control experiments, however, it was found that when milk was incubated for 3 hr at 38°, 66% of the lipoprotein lipase was inactivated; after 6 hr, only 8% of the original activity remained.

It was interesting to determine how rapidly lipoprotein lipase would return upon resumption of suckling. In one experiment (animal 4, Table 4), a 6-day postpartum guinea pig was removed from its litter and returned to the fasting litter after 20 hr. Samples of milk were obtained immediately before cessation, immediately before resumption of suckling, and at repeated intervals thereafter. Lipoprotein lipase activity had not returned to detectable levels at 9 hr after suckling was resumed, but by 12 hr, minimal activity had appeared. The maximum level, reached by 32 hr, was even slightly higher than the original activity.

TABLE 4. EFFECTS OF SUCKLING AND FASTING ON LIPOPROTEIN LIPASE

Animal No.	Treatment	Lipoprotein Lipase Activity: Milk	Lipoprotein Lipase Activity: Mammary Gland
		μmole glycerol/hr/g	
1	Fasting, suckling	1,950	175
2	Fasting, nonsuckling	<2	4
3	Fed, nonsuckling	<1	5
4	Fed, suckling	1,400	...
4	" nonsuckling	0	...
4	" resuckling 9 hr	0	...
4	" " 12 hr	27.8	...
4	" " 24 hr	950	...
4	" " 32 hr	2,000	...
4	" " 144 hr	2,000	...

Animals were removed from food and/or litters 18 hr prior to the experiment. In one experiment, animal 4, the guinea pig, was returned to the litter and samples of milk obtained at frequent intervals.

DISCUSSION

These experiments, together with those of Robinson[4] reported in the following paper (10), demonstrate a very striking correlation between the activity of lipoprotein lipase in the mammary gland and lactation. The dramatic increase in enzyme concentration occurs over a very short period of time beginning not more than 2 days, and perhaps only a few hours, prior to parturition and reaching maximum levels within 2 hr following delivery. The very rapid decline in activity, to barely detectable levels, that occurs within 18 hr of cessation of suckling is equally striking. Similar changes in other enzymes of the mammary gland have been shown for glutamic dehydrogenase (11), glutamic-aspartic transaminase (11), glucose-6-phosphate dehydrogenase (12), and 6-phosphogluconic dehydrogenase (12).

The effect of lactation on the activity of lipoprotein lipase in the mammary gland is analogous to the nutritional effects on the activities of the same enzyme in heart and adipose tissue. In all cases, the activity of lipoprotein lipase increases under conditions when the uptake of triglycerides by the tissue is also increased. Experiments currently in progress in this laboratory have demonstrated a 20-fold increase in the rate of uptake of chylomicron triglycerides by lactating mammary glands compared to mammary glands of animals at midpregnancy.[5] Thus, these present results may add some strength to the hypotheses (13) that lipoprotein lipase functions in the uptake of lipoprotein triglycerides from the circulation, and that localized changes in the concentration of this enzyme play a regulatory role in fat transport. It must be remembered, however, that data such as these establish only a temporal association and not necessarily a causal relationship. For example, the rates of uptake and esterification of free fatty acids were also found to be much greater in mammary glands of lactating than of nonlactating guinea pigs.[5]

The anatomical site of lipoprotein lipase has been presumed to be at some locus very near the circulation, possibly the capillary walls, and a certain amount of evidence supports this assumption (14). The calculation that most, if not all, of the lipoprotein lipase of mammary glands may be accounted for by that in the retained milk was therefore unexpected. Also, if the function of lipoprotein lipase is in the uptake of triglycerides from the circulation, one might not expect to find the enzyme in the milk. It is possible, however, that its presence in milk is only a reflection of the rupture of cells during milk secretion and not an indication of its functional locus. Furthermore, it is difficult to postulate a role for lipoprotein lipase in milk unless it is in some way related to the secretory process, for the enzyme does not appear to be able to catalyze the hydrolysis of the triglycerides of cream.

[4] We thank Dr. Robinson for permitting us to read his manuscript before publication.

[5] O. W. McBride and E. D. Korn, to be submitted for publication.

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