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The Contribution of Chylomicron Cholesterol to Milk Cholesterol in the Rat[1] (41012)

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Abstract. The contribution of chylomicron cholesterol to total milk cholesterol was studied in the lactating rat. Pregnant rats were fed a high-fat, high-cholesterol diet or standard rat diet. On the 13th day of lactation, dams were intubated with 25 μCi [^{3}H]cholesterol (25 μCi/μmole). Milk was collected at 5 and 10 hr following intubation; plasma was collected at 5, 8, and 10 hr following intubation. There was a lower specific activity of chylomicron cholesterol in dams fed the high-fat, high-cholesterol diet compared with control animals at 5, 8, and 10 hr after intubation ($P < 0.05$) with radiolabeled cholesterol. There was no difference in the specific activity of milk cholesterol between both groups at 5 hr. These data indicate that chylomicron cholesterol does not significantly contribute to milk cholesterol levels in the lactating rat. At 8 and 10 hr following intubation with radiolabeled cholesterol, the specific activity of plasma cholesterol from dams fed the high-fat, high-cholesterol diet was lower than that from control animals ($P < 0.05$). The specific activity of milk cholesterol at 10 hr was also lower in experimental animals ($P < 0.05$). Taken together, these data suggest that plasma lipoproteins other than chylomicrons contribute significantly to milk cholesterol levels.

Atherosclerosis is still the leading cause of death and debilitation in the Western world (1). While the major clinical event does not usually occur until midlife, it has been suggested that the precursors of atherosclerosis are established in childhood (2–4). Many factors contribute to the development of coronary heart disease (5). Of these, diet and plasma lipids, have been implicated as predominate risk factors in the pathogenesis of coronary heart disease (6). Considerable controversy exists among investigators with regard to restricting and/or modifying fat and cholesterol intake in early life (7). It is not clear whether infants on diets high in saturated fat and cholesterol have an increased predisposition for developing premature atherosclerosis in adulthood (7).

The Committee on Nutrition of the American Academy of Pediatrics recommends human milk or commercially prepared iron-fortified formula exclusively for the first 6 months of life (8). Breast-fed infants will ingest approximately 200 mg of cholesterol daily, compared with an intake of approximately 20 mg daily by the formula-fed infant (7). Although the fatty acid pattern of human milk may change with maternal dietary changes, cholesterol content does not appear to be influenced by diet even with moderate alterations in plasma cholesterol (9). However, with marked maternal hypercholestrolemia (9, 10) levels of cholesterol in breast milk are elevated (10). These findings suggest: (a) that endogenous cholesterol has a greater effect on milk cholesterol levels than dietary cholesterol in the human, and (b) that the concentration of milk cholesterol is homeostatically controlled. Indeed, the contribution of dietary cholesterol to milk cholesterol appears to be relatively modest. However, further examination of the actual contribution of chylomicron cholesterol to milk cholesterol is especially germane in view of the animal work which has demonstrated a significant contribution of dietary cholesterol to milk cholesterol levels.

Studies conducted with rats and guinea

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pigs have demonstrated that milk cholesterol is derived from three sources: diet, the liver–plasma pool, and *de novo* synthesis in the mammary tissue (11, 12). Approximately 11 to 20% of milk cholesterol is ultimately of dietary origin (1, 2). These values have been derived from the ratio of the specific activity of milk cholesterol to dietary cholesterol after isotopic equilibrium had been achieved following [^{14}C]cholesterol feeding. This method is based upon the assumption that at isotopic equilibrium all plasma lipoprotein constituents are uniform with regard to the sp act of the cholesterol moiety. However, the validity of this method has been questioned (11). In particular, it has been suggested that the ^{14}C-labeled dietary cholesterol associated with chylomicra has a considerably higher sp act than that of other plasma lipoprotein fractions which contain cholesterol (11). Therefore, the possibility exists that chylomicron cholesterol may contribute significantly to cholesterol levels in milk and be considerably underestimated by inappropriately using techniques where the contribution of plasma cholesterol to milk cholesterol is assessed by comparing the sp act of total plasma cholesterol (including chylomicron cholesterol) with sp act of milk cholesterol.

The uptake of cholesterol from circulating plasma chylomicron by mammary tissue has been previously quantified (11, 13). Approximately 12–30% of ^{14}C-labeled cholesterol associated with the chylomicron fraction has been found in rat mammary tissue after the intravenous injection of labeled chylomicra (11, 13) or after perfusion of mammary tissue with chylomicra containing radiolabeled cholesterol (14, 15).

We investigated the contribution of chylomicron cholesterol to milk cholesterol in the lactating rat by producing a very high chylomicron cholesterol sp act and comparing the sp act of milk cholesterol in these rats with the sp act of milk cholesterol in lactating dams with a considerably lower chylomicron cholesterol sp act. Results reported herein indicate that chylomicron cholesterol does not contribute significantly to milk cholesterol in the rat.

Materials and methods. At 14 days gestation, Holtzman rats (Holtzman Co., Madison, Wisc.) were allocated to one of two experimental dietary groups: Group 1 was fed a purified diet (Table I) containing 15.5% butter, 2% cholesterol, and 0.78% sodium cholate (high-fat, high-cholesterol diet): and Group 2 was fed a standard rat diet (Purina Rat Chow, Ralston Purina, St. Louis, Mo.). Five days postpartum, litters were adjusted to six pups. On the 13th day of lactation, dams in the fed state were intubated with 25 μCi [^{3}H]cholesterol (25 μCi/μmole, New England Nuclear, Boston, Mass.) in 1.0 ml of corn oil. Polyethylene tubing (i.d. 0.023 in. × o.d. 0.038 in., Clay Adams, Parsippany, N.J.) was directed down the esophagus and into the stomach while dams were under light ether anesthesia. A syringe containing the corn oil and labeled cholesterol mixture was used to administer a single dose to each dam. Dams were then separated from their litters and allowed free access to their respective diet. Milk was collected at 5 and 10 hr following intubation and blood was collected at 5, 8, and 10 hr following intubation (Day 1). Milk was collected while dams were under light anesthesia 10 min after a 0.2-ml intraperitoneal injection of Pitocin (10 units/ml, Parke Davis and Co., Detroit, Mich.). Each dam was milked until no more milk could be collected. Blood samples were collected (about 2 ml) from the tail into tubes containing EDTA. Forty-eight hours after intu-

TABLE I. COMPOSITION OF HIGH-FAT, HIGH-CHOLESTEROL PURIFIED DIET

	Grams per 100 grams of diet
Cholesterol[a]	1.94
Butter (salt free)	15.51
Casein[b]	31.03
Sucrose	32.12
Sodium cholate[b]	0.78
Choline chloride[b]	1.55
Celluflour[b]	7.76
Salt mix W[b]	6.21
Vitamin diet fortification mixture[b]	3.10

[a] USP grade. Sigma Chemical Co., St. Louis, MO
[b] ICN Pharmaceuticals, Inc., Cleveland, OH

bation, each dam was again intubated, but with 1.0 ml of corn oil only (Day 3). Milk and blood samples were collected at the same intervals as 2 days prior. In addition, milk was collected on Days 13, 14, and 18 from rats which had not been intubated with radiolabeled cholesterol to assess the effect of a high-cholesterol diet on milk cholesterol.

Chylomicra were separated from plasma in a Beckman L2-65B ultracentrifuge (Beckman Instruments, Inc., Lincolnwood, Ill.) (16). Chylomicron-free plasma and chylomicron cholesterol were determined by the Autoanalyzer II method (17). Milk cholesterol was determined by a method developed by Morin and Elms (18) utilizing a Packard gas chromatograph 837 (Packard Instrument Co., Inc. Downers Grove, Ill.). Milk cholesterol was analyzed utilizing a gas chromatograph because of limitations in sample size, especially at the 10-hr period. Previous reports from our lab have shown that these two methods yield comparable results (19).

An aliquot of the plasma, chylomicron, and milk lipid extracts was evaporated to dryness. Ten milliliters of PPO–POPOP scintillation fluid containing 0.5% diphenyloxazole and 0.5% *p*-bisphenylorazolylbenzene in toluene was added to each sample. All samples were counted in an Ansitron liquid scintillation counter, II 1300 (Picker Nuclear, New Haven, Conn.). Corrections for quenching were made.

Differences between two treatments were determined using a simple *F* test (20).

Results. There was a lower ($P < 0.05$) sp act of the chylomicron cholesterol at 5, 8, and 10 hr after intubation with labeled cholesterol in dams fed the high-fat, high-cholesterol diet compared with those fed standard rat diet (Table II). There was no difference in the sp act of milk cholesterol

TABLE II. SPECIFIC ACTIVITY[a] OF CHYLOMICRON, PLASMA, AND MILK CHOLESTEROL ON DAY 1

		Chylomicra		
	n	5 hr	8 hr	10 hr
High-fat, high-cholesterol diet	7	$1.8 \pm 0.3 \times 10^4$ *	$1.5 \pm 0.1 \times 10^4$ *	$1.2 \pm 0.1 \times 10^4$ *
Standard rat diet	7	$1.6 \pm 0.3 \times 10^5$	$6.4 \pm 0.5 \times 10^4$	$1.6 \pm 0.2 \times 10^5$
		Chylomicron-free plasma		
	n	5 hr	8 hr	10 hr
High-fat, high-cholesterol diet	7	$3.5 \pm 0.5 \times 10^4$ N.S.[b]	$2.5 \pm 0.3 \times 10^4$ *	$2.3 \pm 0.3 \times 10^4$ *
Standard rat diet	7	$5.8 \pm 1.3 \times 10^4$	$4.8 \pm 0.5 \times 10^4$	$5.8 \pm 0.6 \times 10^4$
		Milk		
	n	5 hr		10 hr
High-fat, high-cholesterol diet	7	$5.5 \pm 1.4 \times 10^3$ N.S.		$2.5 \pm 0.3 \times 10^4$ *
Standard rat diet	7	$9.6 \pm 2.1 \times 10^3$		$4.9 \pm 0.7 \times 10^4$

Note. Values are means and standard errors. All comparisons were made between high-fat, high-cholesterol diet and standard rat diet.

[a] Specific Activity = cpm/mg cholesterol.

[b] Not significant.

* $P < 0.05$.

between both groups of dams at 5 hr following intubation with labeled cholesterol.

The sp act of milk cholesterol at 10 hr was lower in dams fed the high-fat, high-cholesterol diet ($P < 0.05$). There were no differences in the sp act of plasma cholesterol at 5 hr between both groups of dams. At 8 and 10 hr, however, the sp act of plasma cholesterol from dams fed a purified high-fat, high-cholesterol diet was lower than that from dams fed standard rat diet ($P < 0.05$).

On Day 3, the sp act of the plasma and milk cholesterol of dams fed the high-fat, high-cholesterol diet was lower than that of dams fed standard rat diet at all times ($P < 0.005$); (Table III).

The serum cholesterol of dams fed the high-fat, high-cholesterol diet was higher than the serum cholesterol of dams fed standard rat diet (397 ± 40 versus 50 ± 3 mg/100 ml; $P < 0.005$). These results agree with previous reports from our laboratory (19). The chylomicron cholesterol of dams fed the high-fat, high-cholesterol diet was higher than the chylomicron cholesterol of dams fed standard rat diet (Day 1 at 5 hr: 289 ± 62 vs 1.5 ± 0.67 mg/100 ml, $P < 0.005$: at 8 hr: 256 ± 51 vs 2.2 ± 2.0 mg/100 ml; $P < 0.005$; and at 10 hr: 337 ± 56 vs 1.0 ± 0.8 mg/100 ml, $P < 0.005$). On the 13th day of lactation, the milk cholesterol of dams fed the high-fat, high-cholesterol diet was higher than the milk cholesterol of dams fed standard rat diet (58 ± 5 vs 38 ± 2 mg/100 ml, respectively; ($P < 0.01$) (Fig. 1). In addition, the milk cholesterol continued to increase throughout the course of lactation in dams fed the high-fat, high-cholesterol diet and was higher than the milk cholesterol from dams fed the standard rat diet at all times ($P < 0.01$) (Fig. 1).

Discussion. The data presented in Table II demonstrate no significant contribution of chylomicron cholesterol to milk cholesterol; the sp act of chylomicron cholesterol was significantly different between dams consuming a high-fat, high-cholesterol diet and standard rat diet, but there was no difference in the sp act of milk cholesterol at the 5-hr period. Therefore, chylomicron cholesterol does not appear to be a signifi-

TABLE III. SPECIFIC ACTIVITY[a] OF PLASMA AND MILK CHOLESTEROL ON DAY 3

	n	Chylomicron-free plasma			Milk		
		5 hr	8 hr	10 hr	5 hr	10 hr
High-fat, high-cholesterol diet	7	$1.8 \pm 0.35 \times 10^4$ *	$2.0 \pm 0.4 \times 10^4$ *	$1.7 \pm 0.3 \times 10^4$ *	$1.6 \pm 0.2 \times 10^4$ *	$1.8 \pm 0.2 \times 10^4$ *
Standard rat diet	7	$5.0 \pm 0.6 \times 10^4$	$4.8 \pm 0.4 \times 10^4$	$4.2 \pm 0.4 \times 10^4$	$3.8 \pm 0.3 \times 10^4$	$5.2 \pm 0.4 \times 10^4$

Note. Values are means and standard errors of the mean. All comparisons were made between high-fat, high-cholesterol diet and standard rat diet.

[a] Specific activity = cpm/mg cholesterol.

* $P < 0.005$

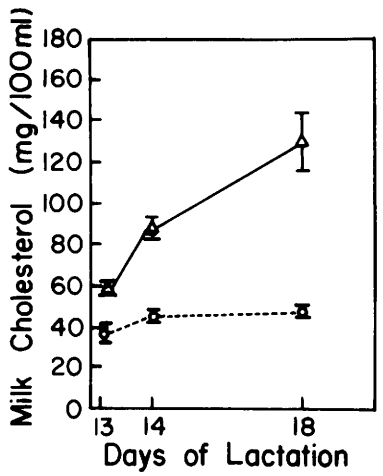

FIG. 1. Average milk cholesterol of dams on standard rat diet (○——○) and high-fat, high-cholesterol diet (△——△). Bars indicate standard error of the mean. Milk cholesterol from dams fed the high-fat, high-cholesterol diet was significantly higher than milk cholesterol from dams fed standard rat diet on Days 13, 14, and 18. ($P < 0.01$)

cant source of milk cholesterol under our experimental conditions.

It is worth noting, however, that at other time points, i.e., especially before the 5-hr period, differences in the sp act of the milk cholesterol might, in fact, exist between treatments. An increase in the size of the total body cholesterol pool with cholesterol feeding could affect the rate at which the exogenous dose of labeled cholesterol was utilized *in vivo*. Additional data for more time points would be helpful in clarifying this matter.

Very recently, Fielding *et al.* (21) have demonstrated high-affinity binding of chylomicra to vascular endothelial cells in culture. Subsequent to binding, the chylomicron cholesterol ester and triglyceride moieties are internalized and hydrolyzed to free cholesterol and unesterified fatty acids by a lysosome-dependent pathway. It may be that this process occurs in mammary tissues as well. In addition, from the studies of Scow and cohorts (22), who demonstrated a partial uptake of whole chylomicra by mammary tissue, it is apparent that exogenous cholesterol, transported by chylomicra, is transferred/taken up by mammary tissue. However, our results demonstrate that chylomicron cholesterol does not contribute significantly to milk cholesterol. Previous reports from our laboratory have demonstrated a decrease in *de novo* cholesterol synthesis in mammary tissue following cholesterol feeding in the lactating rat concomitant with an increase in mammary tissue cholesterol (23). The possibility exists, as well, that after uptake of cholesterol by mammary tissue, efflux of cholesterol into the circulation with subsequent uptake by plasma lipoproteins could occur. It appears that while chylomicron cholesterol enters mammary tissue and subsequently inhibits *de novo* cholesterol synthesis, it does not contribute appreciably to cholesterol levels in milk.

It is probable that the majority of radioactivity in the milk at the 5- and 10-hr interval is derived from plasma lipoproteins of endogenous origin. Radiolabeled chylomicron cholesterol could readily exchange with nonisotopic cholesterol in circulating endogenous lipoproteins which in turn could contribute to the radioactivity found in milk within 5 hr after dams had been intubated with radiolabeled cholesterol. These results are consistent with the finding of Raphael and associates (24), who have shown that the plasma lipoproteins of endogenous origin may act as a fairly homogenous source of milk cholesterol. While there is no difference in the sp act of plasma cholesterol and milk cholesterol between treatments at the 5-hr interval, there is a significant difference in the sp act of plasma and milk cholesterol between treatments at the 10-hr interval. These data suggest that plasma cholesterol transported via lipoproteins, contributes significantly to milk cholesterol. Accordingly, these results agree with the earlier reports of Connor and Lin (12), who demonstrated that approximately 60% of milk cholesterol emanated from plasma cholesterol. Since the rats in our investigation were not in isotopic equilibrium, the precise contribution of plasma cholesterol to milk cholesterol cannot be ascertained accurately. However, the sp act of milk cholesterol is approximately 80% of the plasma cholesterol sp act on Day

3. These data suggest that it is plasma cholesterol, i.e., endogenous lipoprotein cholesterol, which contributes significantly to milk cholesterol, rather than chylomicron cholesterol.

Clarenburg and Chaikoff (11) suggested that plasma cholesterol exclusively in the form of chylomicron lipoproteins, could be a significant source of milk cholesterol. While they suggested this was a very tentative possibility, they nevertheless cautioned against the exclusion of this hypothesis. Our data demonstrate otherwise, i.e., that the contribution of chylomicron cholesterol to total milk cholesterol does not appear to be of quantitative significance in the lactating rat under the experimental conditions reported herein.

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