Lymphatic absorption of fatty acids and cholesterol in the neonatal rat

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Lymphatic absorption of fatty acids and cholesterol in the neonatal rat. Am J Physiol Gastrointest Liver Physiol 279: G325–G331, 2000.—High-fat diets are essential in suckling animals to ensure adequate calories for postnatal growth, but their lymphatic transport of dietary lipids has not been characterized. We established a lymph fistula model in suckling rats to quantify intestinal uptake and lymphatic transport of dietary lipids and analyzed lipoprotein fractions. Suckling 19-day-old Sprague-Dawley rats had their mesenteric lymph ducts cannulated and gastroduodenal tubes inserted. After overnight recovery, [3H]triolein and [14C]cholesterol were infused for 6 h. Of the total dose, only 38% of triolein and 24% of cholesterol were transported in the lymph of suckling rats. Analyses of residual luminal contents and intestinal mucosal homogenate showed neither reduced absorption nor delayed mucosal processing of ingested lipids to be the cause. Thin-layer chromatographic analysis of radioactive mucosal lipids, however, showed a predominance of free fatty acids (60%) and free cholesterol (67%), implying impaired esterification capacity in these animals. We speculate that this reduced esterification allows for portal transport or direct enteroocyte metabolism of dietary lipids.

suckling rat; fat absorption; chylomicron; intestinal absorption

The ability of the suckling animal to absorb fat efficiently from its diet is important for postnatal growth since fat is the major source of calories in milk. Human studies suggest that babies absorb dietary fat differently from adults. A study on formula-fed babies suggests that term and preterm infants absorb fat less efficiently than do adults (8). This is surprising, because one would expect better fat absorption in babies compared with adults because of their high-fat diet and higher caloric requirement. These findings, however, are based on fecal fat excretion studies and do not provide any information regarding the absorption rate or the contribution of endogenous lipids to the total amount of lipids secreted by the small intestine. Furthermore, any lipid broken down by bacteria is also included in the fraction of lipid absorbed by the gastrointestinal tract. Consequently, the use of fecal fat excretion as a means to determine intestinal lipid absorp-

tion will overestimate the amount of fat absorbed by the small intestine.

Animal studies, which allow more direct measurement of fat absorption and metabolism, have shown more efficient dietary fat absorption and metabolism in suckling rats than in adult rats (7, 10). A comparative study measuring luminal disappearance of [14C]triolein and 14CO2 expiration in suckling and adult rats concluded that the suckling rat absorbs and metabolizes dietary triolein at significantly higher rates than do adult rats (7). The rate of triglyceride secretion by the small intestine into lymph and plasma is also higher than in adult rats (10).

Our knowledge of the ontogeny of the processes involved in lipid absorption is not well characterized. In contrast to adult rats, in which most of the intraluminal hydrolysis of triglycerides occurs in the small intestine by pancreatic lipase, there is considerable triglyceride hydrolysis by lingual lipase in the stomach of the suckling rat (14). Lingual lipase works best with triglycerides rich in medium-chain fatty acids, and milk fat contains significant amounts of medium-chain fatty acids. However, little is known about cellular events that occur after the uptake of lipid digestion products during development, although there are several studies suggesting that intracellular events necessary for lipid absorption are present even in newborn rats (13, 15, 16). The lymphatic transport of dietary lipids in suckling rats is unknown, even though chylomicrons (CMs) and the other lipoprotein fractions have been demonstrated in the lymph of suckling rats (5). Frost et al. (10), using a blocker of lipoprotein clearance from plasma, demonstrated that most of the triglycerides present in plasma of suckling rats arise from intestinal lymphatic secretion rather than hepatic synthesis.

The measurement of intestinal lymph flow and of lipids in lymph is a means to allow direct quantification of intestinal lipid absorption. The mesenteric lymph fistula model has been used extensively by our laboratory as well as others to study intestinal fat absorption in adult rats (21). This model allows one to study lipid absorption in conscious, unanesthetized...
animals under steady-state conditions in which a constant intraduodenal infusion of lipid results in steady lymphatic transport of the lipid. It also allows direct sampling and analysis of CMs and apolipoproteins secreted by the small intestine before they enter the circulation. There has only been one previous study utilizing this model that attempted to examine lymphatic transport of dietary lipids in suckling rats (10). This study, however, only allowed 45 min of recovery after anesthesia, did not quantify the amount of milk present in the stomach before surgery, and only collected lymph for 3 h. This is in contrast to the usual rat mesenteric lymph fistula model in which rats are allowed to recover overnight to ensure that the anesthesia has worn off and are fed exclusively by a gastroduodenal tube to control and quantify the exact amount of lipid administered and in which lymph is collected for 6–8 h to achieve steady-state lymphatic lipid output, during which time the rat remains fully conscious. The only other study to examine lymphatic lipid composition in suckling rats (6) analyzed pooled lymph from several animals collected at a single time point under anesthesia.

The aims of this study, therefore, were 1) to establish a mesenteric lymph fistula model in suckling rats similar to the established model in adult rats to allow direct measurement of intestinal lipid absorption in suckling rats and 2) to test the hypothesis that the suckling rat, consistent with its high-fat diet, is able to transport dietary lipids into lymph more efficiently than the adult animal. We quantified the uptake and lymphatic transport of both triglyceride and cholesterol in the suckling rat and also characterized the lipoprotein fraction and its apolipoprotein composition in the lymph of suckling rats.

**MATERIALS AND METHODS**

**Animal preparation.** Sprague-Dawley rats aged 19 days and weighing 35–45 g were used. The rats were removed from their dams and anesthetized with a mixture of ketamine (40 mg/kg) and xylazine (7.5 mg/kg) intraperitoneally. The main mesenteric lymph duct was cannulated with clear vinyl tubing (0.5-mm OD; Dural Plastics and Engineering, Auburn, Australia) according to the techniques of Bollman et al. (3) in adult rats. Surgery was performed under a dissecting microscope because of the small size of the lymph duct. A polyvinyl chloride tube (0.8-mm OD) was inserted through the fundus of the stomach into the duodenum. A purse-string suture (6-0 silk) and cyanoacrylate glue were used to secure the duodenal infusion tube. The gastroduodenal and lymph duct tubes were externalized through the right flank of the rat to allow easy access for infusion and collection. The skin, abdominal musculature, and peritoneal layers were closed in a single layer (4-0 silk). The rats were kept in restraining cages at an ambient temperature of 30°C and allowed to recover from the anesthesia. Once the animals regained consciousness, 5% glucose in normal saline was infused at 0.3 ml/h during the overnight recovery period to replenish fluid and electrolyte losses due to lymphatic drainage. The infusion rate of 0.3 ml/h was selected because we found that most of the suckling rats had lymph flow rates of ~0.2–0.3 ml/h. Lymph was allowed to drain by gravity into plastic microcentrifuge tubes, and the volume was measured by weighing the tubes before and after lymph collection.

**Experimental procedure.** The next morning, a lipid emulsion containing 6 μmol of [3H]triolein, 0.78 μmol of [14C]cholesterol, 0.87 μmol of egg phosphatidylcholine, and 5.7 μmol of sodium taurocholate (19 mM) in 0.3 ml of phosphate-buffered saline (pH 6.4) was infused at 0.3 ml/h for 6 h. Lymph was collected 1 h before the lipid infusion (fasting) and then hourly for 6 h. This dose of lipid infused was selected because it was comparable in grams per kilogram of body weight of the suckling rat to doses administered to adult rats (1). Although this lipid dose is equivalent to the daily fat intake of an adult rat, it is less than the daily fat intake of suckling rats since milk has a much higher fat content than rat chow. However, we chose to administer this dose to ensure that our experimental conditions were similar to that of adult rat studies thereby allowing for comparison between the two age groups.

**Collection of luminal, mucosal, and liver samples.** The animals were anesthetized with the ketamine-xylazine mixture at the end of the 6 h lipid infusion. The duodenum and terminal ileum were tied off to prevent leakage of luminal contents, and the small intestine was excised in one piece. The excised gut lumen was washed three times with 10 mM sodium taurocholate, and the washings were combined and homogenized. The small intestine was then divided into four equal segments and homogenized in 10 mM sodium taurocholate. The entire liver was removed and homogenized in 10 mM sodium taurocholate. Aliquots of lymph, luminal washings, mucosa, and liver were measured for radioactivity by liquid scintillation spectrometry.

**Thin-layer chromatographic analyses of mucosal lipids.** Lipids from the intestinal mucosal segments were extracted according to the procedure described by Blackenhorn and Ahrens (2). Only the first quarter of the intestine was used for thin-layer chromatographic analyses because most of the residual lipids were in this segment. Samples were plated onto activated silica gel G plates, and the lipids were fractionated using a solvent system of petroleum ether, diethyl ether, and glacial acetic acid (75:15:1.2 vol/vol/vol). Iodine vapor was used to visualize the different lipid classes as well as the comigrating lipid standards. The plates were scraped into scintillation vials, and 1 ml of absolute alcohol was added to help elute the lipids. Radioactivity was then determined after addition of an aqueous miscible scintillant (Opti-Fluor; Packard Instrument, Meriden, CT).

**Radioactivity determination.** Radioactivity was measured in Opti-Fluor. The samples were counted for 10 min in a liquid scintillation spectrometer (Model TR 1900 tri-carb; Packard).

**Preparation of infusate.** Appropriate doses of nonradioactive lipids as well as radioactive lipids were mixed, and the solvent was evaporated under a stream of nitrogen. Sodium taurocholate dissolved in phosphate-buffered saline (19 mM, pH 6.4) was added to make up the appropriate concentration, and the mixture was emulsified with a Branson sonicator (model 250/450 sonifier; Branson Ultrasonics, Danbury, CT). Aliquots were taken from the top, middle, and bottom of the emulsion. Radioactivity was then determined after addition of an aqueous miscible scintillant (Opti-Fluor; Packard).

**Isolation of lipoproteins.** Only fasting lymph and lymph collected during the 4th–6th h of lipid absorption were analyzed. These lymph samples will henceforth be referred to as fasting lymph and fat-infused lymph, respectively. Sequential density ultracentrifugation of lymph samples was accomplished using a Beckman SW 41 Ti rotor at 17°C. The lipopro-
tein classes separated were: CM (d < 1.006 g/ml, 1.06 × 10^6 g · min), very low-density lipoprotein (VLDL; d < 1.006 g/ml, 230 × 10^6 g · min), low-density lipoprotein (LDL; 1.006 g/ml < d < 1.063 g/ml, 268 × 10^6 g · min), and high-density lipoprotein (HDL; 1.063 g/ml < d < 1.21 g/ml, 469 × 10^6 g · min). All lipoprotein fractions were dialyzed at 4°C against 0.01% EDTA, 0.02% azide, 0.0001% chloramphenicol, and 0.00005% gentamicin in deionized water with two changes of dialysate. Albumin is usually present with the CMs and can be removed by repeated washing. The additional washing procedures, however, may have resulted in the loss of other apolipoproteins such as apo A-I and apo A-IV and were therefore not performed in this experiment. Fifty micrograms of each lipoprotein fraction were loaded in each lane before SDS-PAGE. The protein molecular standard was supplied by GIBCO BRL (Grand Island, NY). Bands were stained with 0.1% (wt/vol) Coomassie brilliant blue R-250 in methanol-acetic acid-water (5:1:4 vol/vol/vol) and destained in the same solution.

Materials. Triolein, cholesterol, egg phosphatidylcholine, and sodium taurocholate were purchased from Sigma-Aldrich (St. Louis, MO). The radioactive [9,10-3H(N)]triolein and [4-14C]cholesterol were purchased from New England Nuclear (Boston, MA). All solvents and reagents used were of analytical grade. Solvents were used without redistillation.

RESULTS

Lymph flow. The mean lymph flow rate in 19-day-old suckling rats was relatively constant throughout the experiment at 0.27 ml/h (range 0.22–0.32 ml/h; Fig. 1). The flow rate remained unchanged throughout the lipid infusion.

Recovery of [3H]triolein. [3H]triolein output into lymph increased during the lipid infusion to reach a plateau after 2 h of constant lipid infusion, with a maximum recovery of 50% of the hourly infused dose. Values are means ± SE; n = 7 rats.

We were surprised to recover only 48% of the total infused radioactive triolein. This low total recovery, however, was not due to poor uptake, accumulation of absorbed radioactive lipid in the intestinal mucosa, or reduced transport of triglyceride-rich CMs out of the intestinal epithelial cells. Only 1.2% was present in the liver at the end of the 6-h infusion, which was a small percentage of the total infused radioactive lipid. Simi-
Radioactive $[^{14}\text{C}]$cholesterol recovery. Cholesterol output into lymph increased throughout the lipid infusion to reach a maximum of 39% of the hourly infused dose at 6 h (Fig. 4). The total recovery of $[^{14}\text{C}]$cholesterol in the suckling rat at the end of the experiment was 57% (Fig. 5). Lymph recovery was only 24% of the total infused dose. Luminal contents and mucosal radioactivity were analyzed and found to be 6% and 24% of the total administered dose, respectively. This implies that the low recovery of our label was not due to poor intestinal uptake or lack of CM formation within the enterocyte. Cholesterol was recovered in decreasing amounts from the intestinal segments in a proximal-to-distal gradient (data not shown). Recovery from the liver again represents a small fraction of the total dose (2.2%) and does not explain the discrepancy between infused and recovered radioactivity. Analyses of the stomach, feces, and urine showed negligible amounts of radioactivity and, hence, cholesterol.

Absorptive and transport indices. The absorptive index is defined as the percentage of infused radioactive lipid absorbed by the gastrointestinal tract at the end of the experiment. It is derived by subtracting the percentage of radioactive lipid remaining in the gastrointestinal lumen from 100%. The lymphatic transport index is the percentage of the absorbed lipid that is transported in lymph. This index allows us to assess the ability of the small intestine to transport lipid absorbed by the gastrointestinal tract. The lymph refers to the total $[^{14}\text{C}]$cholesterol present in lymph collected over the 6-h infusion period. Small intestinal luminal contents were washed at the end of the infusion, and the amount of $[^{14}\text{C}]$cholesterol was measured. Homogenates of small intestine (mucosa) and liver harvested at the end of the experiment were used to determine residual $[^{14}\text{C}]$cholesterol. Values are means ± SE; $n = 7$ rats.

Indices show efficient uptake of dietary fat, the marked discrepancy between absorptive and lymph transport indices suggests other means of transport or utilization of dietary fats by suckling rats.

Thin-layer chromatographic analyses of mucosal lipids. Only 8% of the total infused dose of triglyceride was present in the intestinal mucosa at the end of the 6-h infusion. Most of this was in the form of free fatty acids (60 ± 7%) rather than triglycerides (29 ± 7%). Small proportions of diglycerides (4%) and monoglycerides (5%) were also present. When radioactive cholesterol in the small intestinal mucosa was analyzed, most of the cholesterol present in the mucosa was as free cholesterol (83 ± 3%) and the remaining was as cholesterol esters (17 ± 2%).

Lymph apolipoprotein composition. The lipoproteins from fasting lymph samples and lymph collected during fat absorption (4th–6th h) were isolated by density ultracentrifugation and analyzed. The apolipoprotein fractions of lymph lipoproteins in suckling rats are shown in Fig. 6. Apo A-I, apo A-IV, and apo B-48 were present in the CM fraction (Fig. 6A). Fat infusion did not alter apolipoprotein composition of lymph CM. Similar to lymph CM, the major apolipoproteins present in lymph VLDL were apo A-I, apo A-IV, and apo B-48 (Fig. 6B). Small amounts of apo E were also present in CM and VLDL. Fat infusion increased apo A-IV and apo E in lymph VLDL. In contrast to lymph CM and VLDL, lymph LDL contains both apo B-48 and apo B-100 in addition to apo A-I, apo E, and apo A-IV (Fig. 6C). Since apo B-100 is normally not made by the rat small intestine, part of lymph LDL was probably derived by filtration from the circulation. More apo E was present in lymph LDL than in lymph CM and VLDL, again corroborating the speculation that plasma filtration of LDL into lymph occurs because apo E is not synthesized by the intestine. Fat infusion increased the quantity of apo A-IV in lymph LDL. The apolipoprotein fractions of lymph HDL were similar to lymph LDL, except that apo A-I was the predominant

Fig. 5. Total $[^{14}\text{C}]$cholesterol recovery at the end of the 6-h lipid infusion. Lymph refers to the total $[^{14}\text{C}]$cholesterol present in lymph collected over the 6-h infusion period. Small intestinal luminal contents were washed at the end of the infusion, and the amount of $[^{14}\text{C}]$cholesterol was measured. Homogenates of small intestine (mucosa) and liver harvested at the end of the experiment were used to determine residual $[^{14}\text{C}]$cholesterol. Values are means ± SE; $n = 7$ rats.
apolipoprotein (Fig. 6D). The apolipoprotein composition of lymph HDL was unchanged by fat infusion.

**DISCUSSION**

Our data demonstrated several differences in the way the suckling rat handles dietary lipids compared with the adult rat. These differences were not due to experimental techniques because we created a similar model to that of adult rats, attempting to emulate conditions and lipid dosing so that valid comparisons of intestinal lipid absorption and lymphatic transport of dietary fat between suckling and adult rats could be made. Unlike the adult rat, no lymphagogic response (increase in lymph flow during a lipid infusion; Refs. 4 and 17) was noted in the suckling rat during the lipid infusion. This may be a function of the immaturity of the suckling rat. As a comparison, the postprandial inhibitory control of ingestion after lipid feeding in suckling rats was present postnatally at 15 days but not at 12 days (22). It is therefore conceivable that the lymphagogic effect of lipid feeding develops later, perhaps after weaning. Lymph flow plays an important role in the transport of CMs across the lamina propria into the lacteals (20). Tso et al. (19, 20) proposed that the lymphagogic effect of lipid absorption was to facilitate the transport of CMs across the interstitial matrix into the lymphatics. In adult rats, when the lamina propria is well hydrated at a lymph flow rate of 40 μl/min (2.4 ml/h), lymph flow has little influence on the movement of CMs into the lacteals (19, 20). Assuming that the same criteria can be applied to suckling rats, a lymph flow rate of 0.3 ml/h (our suckling rats weigh ~1/8th of the adult rat) is probably sufficient to adequately hydrate the lamina propria to allow unrestricted movement of CMs into lacteals. This would suggest that the lack of lymphagogic response in suckling rats is unlikely to impair CM secretion into lymph. Also, if lymph flow rate were to impede the flow of CMs into lacteals, there would be accumulation of radioactive lipid in the mucosa. Since this was not seen, it again supports the notion that a lack of lymphagogic response in suckling rats does not impair CM secretion into lymph.

Table 1 shows the differences in intestinal lipid absorption between our suckling rats and previously published data from our laboratory on adult rats (1, 18). Our results confirm previous animal studies showing that the suckling rat is very efficient at absorbing dietary lipids (7, 10). Surprisingly however, the suckling rat, despite its high-fat diet, does not transport dietary lipids into lymph as efficiently as the adult rat. Suckling rats had reduced maximum hourly lymph

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**Table 1. Lipid recovery in suckling and adult rats**

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Suckling</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>38</td>
<td>60**</td>
</tr>
<tr>
<td>Lymph</td>
<td>1</td>
<td>6*</td>
</tr>
<tr>
<td>Lumen</td>
<td>8</td>
<td>6−17†</td>
</tr>
<tr>
<td>Mucosa</td>
<td>48</td>
<td>70−80‡</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>24</td>
<td>43†</td>
</tr>
<tr>
<td>Lymph</td>
<td>6</td>
<td>21†</td>
</tr>
<tr>
<td>Lumen</td>
<td>24</td>
<td>22†</td>
</tr>
<tr>
<td>Mucosa</td>
<td>57</td>
<td>86†</td>
</tr>
</tbody>
</table>

Values are means of %recovery of total infused dose of lipid in suckling and adult rats after intraduodenal infusion of lipid under similar conditions. *, Data from Ref. 1; †, data from Ref. 18.
output as well as total lymph recovery of [3H]triolein compared with adult rats under similar conditions (1). Total recovery of [3H]triolein at the end of the experiment was also much lower in suckling rats, with most of the shortfall accounted for by the reduced lymphatic recovery of [3H]triolein in suckling rats. These findings are very clearly demonstrated when comparing absorptive and lymph transport indices between suckling and adult rats. Although little difference is noted in absorptive index, suckling rats have a much lower lymph transport index than adult rats (38.3% vs. 61.4%) (1). These findings suggest either that the sucking rat transports dietary triglycerides through alternative means such as the portal route in addition to the lymphatics or that there is direct metabolism of dietary fat by the enterocytes.

The portal transport of long-chain triglycerides is usually insignificant compared with the lymphatic route, except under certain conditions such as reperfusion following an ischemic injury (11). The sucking rat, however, may utilize this route to allow for more rapid transport of dietary lipids into the circulation to target organs. Intermittent portal blood sampling would need to be taken during the experiment, and information regarding portal blood flow would be necessary to test our speculation, but this would be technically difficult to do and the animals would be less likely to survive due to blood loss and the performance of multiple surgical procedures. Although Fernando-Warnakulasuriya et al. (6) analyzed the lipid components of portal blood in 10-day-old rat pups, no attempt was made to measure portal blood flow, making their data in isolation difficult to interpret.

The other possible explanation for the low recovery of triolein in sucking rats may be because of direct metabolism of dietary triglycerides and fatty acids by enterocytes. The data of Flores et al. (7), which used breath tests, demonstrated that triolein disappearance from intestinal lumen and 14CO₂ excretion (reflecting triglyceride oxidation) was two to three times higher in sucking rats than in adult rats. Their data confirm our speculation, but this would be technically difficult to do and the animals would be less likely to survive due to blood loss and the performance of multiple surgical procedures. Although Fernando-Warnakulasuriya et al. (6) analyzed the lipid components of portal blood in 10-day-old rat pups, no attempt was made to measure portal blood flow, making their data in isolation difficult to interpret.

The third possible explanation for the reduced lymphatic recovery of lipid in neonatal rat is due to reduced lymphatic transport, as evidenced by the significantly reduced transport index (25.4% vs. 41% in adult rat) (18). Unlike adult rats, analyses of residual cholesterol within the intestinal mucosa of sucking rats showed predominance of the free form, suggesting that sucking rats have a problem with reesterification.
The apolipoprotein fractions of lymph CM and VLDL in suckling rats are comparable to those found in adult rats. Similar to the adult rat, only apo B-48 was present in lymph CM and VLDL, implying that apo B-48 editing is intact in baby rats. In contrast to CM and VLDL, lymph LDL contains both forms of apo B, i.e., B-48 and B-100, as well as apo E. Since the intestine only makes apo B-48, the likely explanation for this observation is that lymph LDL is derived partly from the gut and also from plasma through filtration by intestinal capillaries. Our speculation that the gut synthesizes LDL is further supported by the presence of both apo A-I and apo A-IV in addition to the two forms of apo B in lymph LDL. Whether this ability of the gut to synthesize LDL is unique to the suckling rat remains to be demonstrated. Lymph HDL in the suckling rat is an apo A-I-rich particle but also contains apo A-IV and apo B-48. Glickman and co-workers (9, 12) have previously demonstrated that the adult rat intestine secretes HDL. Our data confirms this observation because it shows that the suckling rat intestine is also capable of making HDL. Lipoprotein metabolism in immature animals, however, is an area that is largely unknown, and further studies are indicated.

Our mesenteric lymph fistula suckling rat model has been very useful in contributing to our knowledge of intestinal fat absorption in the immature animal. It has provided information on the efficacy of lymphatic transport of dietary triolein and cholesterol and has demonstrated significant differences in the absorption of dietary lipids compared with adult rats. These differences, which presumably are a result of immaturity of the gastrointestinal system, may be compensated for by the suckling rat’s ability to directly utilize nutrients. Further studies on the esterification capacity of the suckling rat and its metabolism of nutrients obviously are indicated because they will further our understanding of nutrient absorption in infancy.

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