Intestinal Lipid Absorption in the Nephrotic Rat

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ABSTRACT
There is limited data on intestinal lipid absorption in the nephrotic syndrome. This study investigated whether the efficiency of intestinal lipid absorption is altered in nephrotic lymph-fistula rats. The nephrotic syndrome was induced in nine Sprague-Dawley rats by an iv injection of puromycin aminonucleoside in saline; seven control rats received saline only. At 10 to 14 days after injection, the main intestinal lymph duct was cannulated for collection of lymph. The duodenum was also cannulated and a fasting saline-glucose solution was infused overnight at 3 mL/h. The next day, the infusate was changed to a lipid emulsion that contained (14C)cholesterol and (3H)triglyceride (triolein) that was infused at 3 mL/h for 8 h. During the last hour of fasting and during the lipid infusion, lymph flow in the Nephrotic group averaged 0.6 mL/h higher than the Control group (P = 0.02). No significant differences were found between the two groups in recovery of infused radioactive cholesterol (P = 0.37) or triglyceride (P = 0.38) from the gastrointestinal lumen, small intestinal mucosa, or lymph. Lymphatic output of chemically measured cholesterol was also similar (P = 0.96). These results suggest that mucosal uptake and lymphatic output of cholesterol and triglyceride are not altered in the nephrotic syndrome.

Key Words: Hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, small intestine, puromycin aminonucleoside

The mechanisms of hyperlipidemia in the nephrotic syndrome involve widespread changes in lipid metabolism, including increased hepatic synthesis and decreased catabolism of lipoproteins (1,2).

Despite much study, however, the underlying reasons for these changes remain unknown (2).

An enhanced efficiency of intestinal cholesterol absorption in the nephrotic syndrome would contribute to the hyperlipidemia. In healthy rats and humans, the efficiency of cholesterol absorption is relatively poor, ranging from 25 to 75%, depending on experimental conditions (3–8). The hyperresponsive rabbit, which absorbs cholesterol with approximately 90% efficiency, demonstrates the effect of enhanced cholesterol absorption (9). During cholesterol feeding, these rabbits have a high flux of cholesterol into the liver, causing increased synthesis of very low-density lipoprotein (VLDL). Increased hepatic secretion of VLDL then causes increased production of low-density lipoprotein (LDL) cholesterol.

It is also plausible that lipid absorption might be reduced in the nephrotic syndrome. Intestinal absorption of diuretics is delayed in edematous states, possibly because of reduced gastrointestinal motility or bowel-wall edema (10–12). Rats that have been rendered nephrotic by puromycin aminonucleoside reportedly have reduced jejunal transport of amino acids and monosaccharides (13). There is currently little data on lipid absorption in the nephrotic syndrome. In nephrotic rats and humans, restriction of fat intake reduces lipoprotein levels (14,15). Thus, lipid metabolism is sensitive to dietary fat intake, but whether this involves an altered efficiency of cholesterol absorption is unknown. Lymphatic triglyceride output is reportedly increased two-fold in nephrotic rats (16), but this contradicts other studies that show triglyceride absorption is approximately 90% efficient in the normal rat, leaving little room for improvement (8,17–20). In this study, we used the conscious lymph-fistula rat model (21) to determine whether mucosal uptake and lymphatic output of cholesterol and triglyceride are altered in rats with puromycin aminonucleoside-induced nephrotic syndrome.

METHODS

Animals

This study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (weighing 300 to 350 g) were randomly assigned to Nephrotic or Control groups. The nephrotic syndrome was induced in nine rats by an iv injection of puromycin aminonucleoside (7.5 mg/kg) as a 2.5% solution in 0.15 M saline; seven Control rats received saline only. Both groups underwent a timed urine collection for protein measurement in metabolic cages for 1 to 4 days before and 8 to 12 days after injection. On the days that urine collections were completed, fasting plasma samples were obtained for cholesterol and triglyceride measurement. The rats were fed Harlan Teklad 22/5 Rodent Diet (Madison, WI), a standard rodent diet that contains 5.4% fat. Because

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2 This study was presented at the 26th Annual Meeting of the American Society of Nephrology, November 1993, and published in abstract form (J Am Soc Nephrol 1993;4:780).
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preliminary experiments showed that food intake decreased for only 1 to 2 days after the puromycin aminonucleoside injection, the rats were allowed to eat and drink water ad libitum.

Surgery

Nine to 13 days after injection, the rats fasted overnight. The next morning, they were anesthetized with halothane, and the main intestinal lymph duct was cannulated with vinyl tubing (0.8 mm o.d.) by the method of Bollman et al. (22). The cannula was secured with a drop of cyanoacrylate glue. A silicone tube (2.2 mm o.d.) was passed through the fundus of the stomach into the duodenum for 1 to 2 cm, and secured with a purse-string suture and a drop of cyanoacrylate glue. After surgery, the rats were infused intraduodenally with a saline-glucose solution (145 mM NaCl, 4 mM KCl, 0.28 M glucose) at 3 ml/h. The rats recovered from surgery overnight in restraint cages that were kept in a chamber maintained at 30°C.

Experimental procedure

The next morning, the saline-glucose infusion was replaced with a lipid emulsion that contained 40 µmol triglyceride (triolein labelled with glycerol tri(9,10(n)-3H)oleate, 1.0 µCi), 8.6 µmol cholesterol (labeled with [4-14C]cholesterol, 0.1 µCi), 8.7 µmol phosphatidylcholine, and 57 µmol sodium taurocholate, in 3 ml of phosphate-buffered saline (pH 6.4). The emulsion was prepared as described previously (23), and infused at 3 ml/h for 8 h. The sodium taurocholate was necessary to ensure that the emulsion was stable throughout the 8-h infusion. Lymph was collected during the last hour of the saline-glucose infusion (referred to as the fasting period), and 0 to 2, 2 to 4, 4 to 5, 5 to 6, 6 to 7, and 7 to 8 h after starting the lipid infusion. Aliquots of lymph were taken for the measurement of radioactivity and for the measurement of cholesterol content by chemical assay.

At the end of the lipid infusion, the rats were anesthetized with halothane and exsanguinated. The entire small intestine was removed, placed on ice, and divided into four equal segments with ligatures. The luminal contents of each segment were eluted three times with 3-ml aliquots of 5 mM sodium taurocholate in 0.15 M NaCl. The stomach and colon were also removed and their contents were eluted with the same solution. The volumes collected from the six luminal segments were measured separately, and the samples were homogenized. Aliquots from these samples were taken for the measurement of luminal radioactivity. Luminal radioactivity indicated infused lipid that was not absorbed by small intestinal mucosa.

The four segments of small intestine were then opened longitudinally, placed in separate Erlenmeyer flasks that contained chloroform-methanol (2:1, vol/vol), and then minced with scissors. Mucosal lipid was extracted by the method of Folch et al. (24), and aliquots were taken for the measurement of mucosal radioactivity. Mucosal radioactivity indicated lipid that was absorbed by small intestinal mucosa, but not yet transported into lymph.

Assays and Materials

Radioactivity was determined in an aqueous miscible scintillant (Poly-Fluor; Packard, Downers Grove, IL). Samples were counted in a liquid-scintillation spectrometer, and corrected for quenching by reference to a series of 3H and 14C standards that were progressively quenched. Urine protein was determined by Bradford’s procedure (Bio-Rad Laboratories, Richmond, CA). Plasma cholesterol and triglyceride were determined enzymatically (Sigma Chemical Co., St. Louis, MO). Lipid was extracted from lymph by the method of Blankenhorn and Ahrens (25), and lymphatic cholesterol content was determined chemically by the method of Rudel and Morris (26). Puromycin aminonucleoside and sodium taurocholate were obtained from Sigma Chemical. Glycerol tri(9,10(n)-3H)oleate and [4-14C]cholesterol were obtained from New England Nuclear Research Products (Boston, MA).

Statistical Analysis

Data are reported as mean ± SE. Comparisons were made by t test, two-factor analysis of variance, or two-factor analysis of variance with repeated measures on one factor (time) (27). All analysis of variance P values are from the overall comparison of the Nephrotic group with the Control group; no multiple comparisons were made. P values of less than 0.05 were considered significant.

RESULTS

In the Control group, daily urinary protein excretion and plasma cholesterol and triglyceride levels changed minimally after the saline injection (Table 1). In the Nephrotic group, however, all three measurements increased markedly after the puromycin aminonucleoside injection.

Lymph Flow

During the 1-h fasting period, intestinal lymph flow in the Control group was 2.9 ± 0.2 ml/h (mean ± SE) (Figure 1), which is similar to flows previously reported in normal rats (17-20). Fasting lymph flow in the Nephrotic group was 3.6 ± 0.3 ml/h and averaged 0.6 ml/h higher than the Control group during the combined 1-h fasting and 8-h lipid infusion period.

<table>
<thead>
<tr>
<th>TABLE 1. Daily urinary protein excretion, and fasting plasma cholesterol and triglyceride levels</th>
<th>Control Group (N = 7)</th>
<th>Nephrotic Group (N = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Saline</td>
<td>Post-Saline</td>
</tr>
<tr>
<td></td>
<td>Pre-PAN</td>
<td>Post-PAN</td>
</tr>
<tr>
<td>Protein (mg/day)</td>
<td>12.6 ± 1.4</td>
<td>11.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>15.5 ± 3.0</td>
<td>341.1 ± 35.0c</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>2.25 ± 0.12</td>
<td>2.05 ± 0.15b</td>
</tr>
<tr>
<td></td>
<td>2.14 ± 0.10</td>
<td>9.16 ± 1.22c</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.57 ± 0.05</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>0.64 ± 0.12</td>
<td>1.34 ± 0.29d</td>
</tr>
</tbody>
</table>

a Samples were obtained before and after injection of saline or puromycin aminonucleoside (PAN). Data are mean ± SE.
b P < 0.05 versus before injection.
c P < 0.001 versus before injection.
Triglyceride Output in Lymph

Lymphatic output of radioactive triglyceride (expressed as a percentage of hourly infused dose) increased similarly in both groups during the 8-h lipid infusion (Figure 2). By 7 to 8 h, output was 69.1 ± 7.3% in the Control group and 82.5 ± 7.8% in the Nephrotic group.

Recovery of Triglyceride from Lumen, Mucosa, and Lymph

After completing the lipid infusion, the recovery of radioactive triglyceride (expressed as a percentage of total infused dose) in the gastrointestinal lumen, small intestinal mucosa, and lymph was similar in both groups (Figure 3). Mucosal uptake of triglyceride was nearly complete because the percentage of infused radioactivity recovered in the lumen was 9.4 ± 1.9% in the Control group and 10.0 ± 2.8% in the Nephrotic group. The luminal and mucosal distributions of triglyceride were similar in both groups (69% of recovered luminal radioactivity was in the proximal one half of small intestine in the Control group, and 65% in the Nephrotic group; 90% of recovered mucosal radioactivity was in the proximal one half of small intestine in the Control group, and 98% in the Nephrotic group).

Cholesterol Output in Lymph

Lymphatic output of radioactive cholesterol (expressed as a percentage of hourly infused dose) increased similarly in both groups during the lipid infusion (Figure 4). By 7 to 8 h, output was 60.3 ± 6.8% in the Control group and 65.6 ± 4.9% in the Nephrotic group. Cholesterol in intestinal lymph is derived not only from diet, but from endogenous sources as well. To determine total lymphatic cholesterol output, cholesterol was measured chemically. There were no significant differences between the two groups during the 9-h experiment (Figure 5). At the end of the infusion,
output was 5.95 ± 0.35 μmol/h in the Control group
and 6.02 ± 0.14 μmol/h in the Nephrotic group.

Recovery of Cholesterol from Lumen, Mucosa, and Lymph

Recovery of radioactive cholesterol (expressed as a percentage of total infused dose) in the lumen, mucosa, and lymph demonstrated similar incomplete absorption in both groups (Figure 6). The percentage of infused radioactivity that remained in the lumen or mucosa was 48.0 ± 2.6% in the Control group and 52.0 ± 2.7% in the Nephrotic group. The luminal and mucosal distributions of cholesterol also demonstrated incomplete absorption (74% of recovered luminal radioactivity was in the distal three quarters of small intestine in the Control group, and 73% in the Nephrotic group; 67% of recovered mucosal radioactivity was in the proximal one half of small intestine in the Control group, and 73% in the Nephrotic group). In comparison, more triglyceride was in the proximal mucosa and less in the distal lumen because of more complete proximal absorption.

DISCUSSION

This study was designed to determine whether intestinal lipid absorption is altered in nephrotic rats. We found no significant differences between the Control and Nephrotic groups in the recovery of infused radioactive cholesterol or triglyceride from the gastrointestinal lumen, small intestinal mucosa, or lymph. Cholesterol absorption remained incomplete since approximately 50% of the infused cholesterol was found in the lumen or mucosa of both groups. In contrast, only approximately 28% of the infused triglyceride was found in the lumen or mucosa of both groups.

Approximately 50% of luminal cholesterol is endogenous and is derived primarily from biliary secretion and sloughed intestinal epithelial cells (28). To determine total lymphatic cholesterol output, we measured cholesterol chemically and found no significant differences between the two groups. This suggests that biliary secretion of cholesterol was not increased in the Nephrotic rats. Because of inefficient intestinal absorption, increased biliary secretion would help compensate for increased hepatic synthesis by removing cholesterol from the body. Whether biliary secretion of cholesterol is altered in nephrotic humans is unknown.

This study shows that Nephrotic and Control rats absorb cholesterol similarly when fed a standard rodent diet. The efficiency of cholesterol absorption often decreases as dietary cholesterol increases (29,30). This adaptive mechanism is useful because it reduces the effect of diet on plasma cholesterol levels. Because we did not study absorption during a high-cholesterol diet, we cannot be certain this adaptation is unchanged in the nephrotic syndrome.

Intestinal triglyceride absorption was more complete than cholesterol in this study because only 9 to 10% of infused triglyceride was found in the lumen and 19% in the mucosa of both groups. Most of the mucosal triglyceride was destined to be exported into lymph. This result is consistent with previous studies of triglyceride absorption in normal rats (8,17–20). Thus, triglyceride absorption is normally so efficient that the only significant improvement possible in the nephrotic syndrome was an increase in the initial rate of lymphatic output, which was not observed.

In contrast, Levy et al. have reported lymphatic triglyceride output is twofold increased in nephrotic lymph-fistula rats (16). If this is true, it would be important because the effect of enhanced triglyceride absorption might be similar to an increase in dietary triglyceride (as saturated fat), which causes increased VLDL synthesis and decreased LDL catabolism (31). However, triglyceride absorption was inefficient in their Control group because less than 35% of infused triglyceride was recovered from lymph. The reason for the discrepancy between their study and ours is un-
clear, but might be the result of a low fasting lymph flow in their rats.

The time required for chylomicrons to appear in intestinal lymph after starting a lipid infusion is influenced by three factors: mucosal uptake of luminal fat, intracellular formation and secretion of chylomicrons, and interstitial chylomicron movement from the intercellular space to the central lacteal. The rate of chylomicron movement increases with lymph flow provided that flow is less than approximately 2.4 mL/h (32,33). The flow dependence of chylomicron movement is probably a result of enhanced interstitial matrix hydration during feeding. Enhanced hydration increases the equivalent pore radius of the matrix, which reduces frictional interaction with chylomicrons (34).

In this study, lymph flow in the Nephrotic group averaged 0.6 mL/h higher than the Control group. The higher flow was probably a result of enhanced matrix hydration caused by the edema of the nephrotic syndrome. The fact that flow rates of all rats were usually at least 2.4 mL/h may explain why the lymphatic output of lipids was unaffected by differences in flow. It has been hypothesized that delayed absorption of diuretics in edematous states may be caused by decreased gastrointestinal motility or bowel-wall edema (10–12). In this study, the distributions of infused lipid in the gastrointestinal lumen and small intestinal mucosa allowed an indirect comparison of motility. The similar lumenal and mucosal distributions of cholesterol and triglyceride in the two groups suggest that motility was not reduced in the Nephrotic group. Also, bowel-wall edema does not appear to have adversely affected lipid absorption in the Nephrotic group.

To summarize, intestinal lymph flow was higher in the Nephrotic group than the Control group, probably because of increased interstitial hydration. Mucosal uptake and lymphatic output of cholesterol and triglyceride, however, were similar in both groups. Intestinal motility does not appear to have been reduced and bowel-wall edema does not appear to have adversely affected lipid absorption in the Nephrotic group. These results suggest that intestinal lipid absorption is not altered in the nephrotic syndrome.

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REFERENCES

316-460.

"The latest challenge to the versatility of the kidney is surgical transplantation, during which it is permanently deprived of its nerve supply, partially asphyxiated and placed in an immunologically hostile environment. Here, it is expected to respond to physiologic stimuli of a new host, and begin at once to regulate and defend the alien milieu."