Cholesterol absorption and transport in thoracic duct lymph lipoproteins of nonhuman primates. Effect of dietary cholesterol level

R. L. Klein and L. L. Rudel
Department of Comparative Medicine and Arteriosclerosis Research Center, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

Abstract
The effect of dietary cholesterol level on cholesterol absorption and its subsequent transport in thoracic duct lymph lipoproteins was studied in two species of nonhuman primates, namely the African green monkey (Cercopithecus aethiops) and the cynomolgus macaque (Macaca fascicularis). Each animal served as its own control because each received sequential, intraduodenal infusions of two fat-rich liquid diets that differed only in the amounts of cholesterol. The percentage of dietary cholesterol absorbed was calculated by dividing the rate of appearance of exogenous cholesterol radioactivity in lymph by the rate of infusion of dietary cholesterol radioactivity at a time when lymph cholesterol specific activity was constant relative to that of diet. The percentage of dietary cholesterol absorbed was similar during both diet infusions in African green monkeys but was significantly decreased during the high cholesterol diet infusion in cynomolgus macaques. Rates of appearance of lymph total cholesterol mass were significantly increased during infusion of high cholesterol diets due to a statistically significant increase in cholesteryl ester transport rates. This increase was due in large part to the preferential esterification of exogenous cholesterol that was incorporated into lymph chylomicrons and VLDL. The rate of appearance of lymph exogenous cholesterol significantly increased during the high cholesterol diet infusion while that of endogenous cholesterol decreased. This decrease or compensation in endogenous cholesterol transport occurred during absorption of increased levels of dietary cholesterol and apparently was due to an approximately 50% decrease in the absorption of luminal cholesterol. Our data demonstrated that there was not a simple one-to-one relationship between the amount of isotopic dietary cholesterol-tracer absorbed from the intestinal lumen and the mass of cholesterol moved into lymph via the intestine. Rather, the amount of sterol transported into the body depends on the degree of the decrease in endogenous cholesterol transport and probably on the efficiency of cholesteryl esterification during absorption.—Klein, R. L., and L. L. Rudel. Cholesterol absorption and transport in thoracic duct lymph lipoproteins of nonhuman primates. Effect of dietary cholesterol level. J. Lipid Res. 1983, 24: 345–356.

Supplementary key words cholesterol esters • chylomicrons • African green monkeys • cynomolgus monkeys • triglycerides

Plasma cholesterol concentration is a widely recognized risk factor in both the development of accelerated atherosclerosis and the high incidence of coronary heart disease (1, 2). As reviewed by McGill (3), there is a significant correlation between the level of dietary cholesterol intake and the level of plasma cholesterol concentration in human beings and we have found this to be true in nonhuman primate models for the study of atherosclerosis (4, 5). Many studies have attempted to quantify the effects of dietary cholesterol on cholesterol absorption by calculating the percentage absorption of the fed sterol (6–8). However, knowledge of this percentage by itself is not adequate for predicting the potential effects of absorbed cholesterol on plasma cholesterol concentration. There are several aspects of the cholesterol absorption and transport pathway that may ultimately be as important as the amount of dietary cholesterol absorption.

The chemical form of absorbed exogenous cholesterol may, in part, determine its fate upon entry into the body. Newly absorbed dietary cholesterol (exogenous cholesterol) is transported into the body via the lymphatics (9–11) and during absorption of cholesterol-rich meals, an increased rate of lymph cholesterol transport has been found (12, 13) in spite of the decreased rate of endogenous cholesterol appearance in lymph (6, 14). This increased transport occurs primarily because of the enhanced incorporation of exogenous cholesterol as cholesteryl ester into the core of the lymph chylomicrons and VLDL (11, 13, 16, 17). In turn, this may result in hepatic cholesteryl ester enrichment due to the rapid uptake and catabolism of chylomicrons, presumably as remnants, by the liver (18, 19). Accumulation of cholesterol in the liver may subsequently

Abbreviations: ACAT, acylcoenzyme A:cholesterol transferase; DTNB, 5,5′dithiobis-2-nitrobenzoic acid; ECSA, ester cholesterol specific activity; EDTA, ethylenediamine tetraacetic acid; FCSA, free cholesterol specific activity; LCAT, lecithin:cholesterol acyltransferase; VLDL, very low density lipoprotein; S, Svedberg flotation rate.

1 Present address: Cornell University, Division of Nutritional Sciences, Ithaca, NY 14853.
result in formation and secretion of cholesteryl ester-enriched lipoproteins (20, 21). Absorption of cholesterol-enriched meals may also result in an increased rate of transport of unesterified (free) cholesterol in lymph (13, 22). This free cholesterol is apparently incorporated into the surface coat of the chylomicrons (23), and appears to be directed into plasma HDL by way of the intravascular metabolism of the redundant chylomicron surface material generated during lipolysis of the core triglyceride (24).

The lipoprotein form in which dietary cholesterol is transported may be another variable that influences the fate of newly absorbed sterol. In some studies, the rabbit has been found to transport a significant proportion of exogenous cholesterol in smaller lymph VLDL instead of larger lymph chylomicrons (15, 25), and when fed cholesterol, the rabbit develops a severe hypercholesterolemia that is characterized by accumulation of cholesteryl ester-rich lipoproteins. In contrast, absorbed dietary cholesterol is transported primarily in chylomicrons in the rat, although some increased cholesteryl ester transport in lymph d > 1.006 g/ml lipoproteins occurred during chronic cholesterol feeding (26), and diet-induced plasma cholesterol increases in this species are more modest.

The present studies were designed to identify the characteristics of the cholesterol absorption and transport pathways in nonhuman primates by using thoracic duct-cannulated animals. Nonhuman primate models were used because of their close phylogenetic relationship to man and because they are frequently used to study dietary cholesterol-induced atherosclerosis. We found that a higher dietary cholesterol level resulted in an increased rate of appearance of cholesteryl esters in lymph chylomicrons and VLDL. Based on the pathways just described, this outcome appeared consistent with the known pattern of hyperlipoproteinemia that has been found to occur in response to cholesterol feeding in nonhuman primates (4, 27), and suggested that increased choles terol absorption does play a role in the hypercholesterolemia of experimental atherosclerosis in these animals.

METHODS AND MATERIALS

Animals and diets

The animals used in these experiments were purchased from Primate Imports, Port Washington, NY. Cynomolgus monkeys (Macaca fascicularis) and African green monkeys (Cercopithecus aethiops) were used. All animals were adult males at the start of the study; they were maintained for at least 3 months prior to the start of the study on a low cholesterol, semipurified diet (75–88B, control diet) whose composition has been published (27). The diet contained 40% of calories as fat, 41% as carbohydrate, and 19% as protein, and the cholesterol level was 0.16 mg/Kcal.

During the constant infusion experiments, each animal received sequential intraduodenal infusions of two cholesterol-containing diets in liquid form, whose compositions have been published (28). The low cholesterol diet contained 0.16 mg of cholesterol/Kcal and the high cholesterol diet contained 0.78 mg of cholesterol/Kcal. The only exception among ingredients was that butter was used as the only source of fat in the present study. Both liquid diets contained 38% of calories as fat, 44% as carbohydrate, and 18% as protein, and were infused at a constant rate using a peristaltic pump. The rate of infusion was set at 90 Kcal/kg per day to approximate the daily caloric requirement of the animal.

Surgical procedures

A thoracotomy was performed on the right side between the ninth and tenth ribs. The thoracic duct was visible through the pleural lining as it coursed between the azygos vein and the aorta. The thoracic duct was exposed and catheterized first against the direction of lymph flow using a 15-in long (0.02 in I.D. × 0.037 in O.D.) piece of silastic tubing (Dow Corning, Midland, MI) which had previously been heparinized with TDMAC-Heparin (Polyscience Inc., Warrington, PA). The tubing was passed through the chest wall and the thoracic duct was then catheterized with the direction of lymph flow. The incision was closed with the silastic tubing positioned beneath the skin. This resulted in a continuous closed-loop of lymph flow.

A midline laparotomy was performed to permit duodenal catheterization. An incision was made in the duodenum approximately 15 cm distal to the pyloric valve. The tubing (42 in, size 8 French Infant Feeding Tube; Cutter Lab, Berkeley, CA) was inserted into the duodenum and the tip was positioned approximately 5 cm distal to the pyloric valve. In some experiments, an additional catheter (Tygon®, formulation S-54-HL, 0.04 in I.D. × 0.07 in O.D.) was positioned in the duodenum approximately 25 cm distal to the tip of the diet infusion catheter.

All animals were permitted to recover in individual animal cages for 10–14 days after surgery before an experiment was begun.

Radioactive diet preparation

Radioabeled cholesterol was purchased from one of three companies (Research Products International, Elk Grove, IL; New England Nuclear, Boston, MA; Amer sham, Arlington Heights, IL). Tritiated cholesterol ([1α,2α(N)-3H]-cholesterol) (300 μCi) was routinely added to the low cholesterol diet and [4-14C]cholesterol
(250 μCi) was added to the high cholesterol diet. The radiolabeled cholesterol was purified before each experiment using thin-layer chromatography on silica gel H plates that were developed using hexane–ethyl ether–glacial acetic acid 70:30:1 (v/v/v).

A clear micellar suspension of radiolabeled cholesterol was prepared for addition to the diet. Ten mg of monooolein (Sigma Chemical Co., St. Louis, MO) was added to a chloroform solution containing the radioactive cholesterol after which the chloroform was evaporated under a stream of nitrogen. Five ml of 20 mM sodium deoxycholate, pH 7.4, was then added, and this mixture was sonicated at 20 watts for 15 sec using a “needle” probe (Branson, Danbury, CT). This micellar solution, containing tracer amounts of cholesterol, was dripped into 800 ml of the preblended diet that was then stirred at 4°C overnight before use. Aliquots of each radioactive diet used were obtained at the beginning and end of each experiment and the cholesterol content and specific activity were determined. One study was performed by dissolving the radiolabeled cholesterol in butter melted to prepare the diet. The diet was then infused in the usual manner. This different manner of addition of radioactive cholesterol to the diet did not alter the outcome of the experiment.

Samples from each lot of [3H]cholesterol were screened for radiochemical reliability after the method of Davidson et al. (29). Briefly, aliquots of each [3H]cholesterol lot were combined with [14C]cholesterol and micelles were prepared as above. A micellar solution of a predetermined 3H/14C ratio was blended with the low cholesterol diet and administered via gastric intubation to several rats and nonhuman primates. Blood samples were taken at selected intervals after the intubation and the plasma 3H/14C ratio determined. The 3H/14C ratio was also determined in the liver of the rats. The 3H/14C ratio in plasma and liver were similar (95.4–100.9% of that in the diet) indicating that the [3H]cholesterol used in these studies had full biological activity.

In one animal, absorption measurements were repeated with the order of radioisotope administration reversed, i.e., [14C]cholesterol was added to the low cholesterol diet and [3H]cholesterol was added to the high cholesterol diet. The order of isotope administration did not appear to bias the data because no differences in the outcome of the two experiments were noted.

Experimental design

To initiate lymph collection, the catheter was exposed and cut and two 1-meter pieces of heparinized silastic tubing (0.025 in I.D. × 0.047 in O.D.) were attached as extensions of the indwelling catheter. The animal was placed in a restraint chair and a drip of Lactated Ringer’s Injection USP (McGaw Labs, Irvine, CA) was begun into the inward-flowing side of the catheter to replace fluid loss while lymph was collected from the outward-flowing side. The flow rate of lymph was 15–30 ml/hr. When lymph turbidity became minimal, about 12–15 hr after the morning meal, an infusion of the liquid low cholesterol diet without radioactivity was begun. After about 6 hr a steady rate of triglyceride appearance in lymph was achieved. The radiolabeled low cholesterol diet was then infused at a constant rate for 36 hr. The infusion of the radioactive high cholesterol diet was then begun and continued for an additional 36 hr. Sequential lymph samples were collected for 2–4-hr intervals during both infusions. After both radioactive diets had been infused, a blood sample was obtained. In all animals for which data are reported, plasma was found to contain less than 1% of the infused radioactivity. This indicated that the collection of intestinal lymph produced throughout the study had essentially been complete and that endogenous cholesterol pools had not become radiolabeled.

Lipoprotein isolation

Thoracic duct lymph was collected at room temperature into tubes containing 0.1% EDTA, 0.02% sodium azide, and 0.04% DTNB, pH 7.4 (final concentration). Lymph was stored at 15°C until processed. Chylomicrons (S, > 400) were floated at 15°C by centrifugation of 10 ml of fresh lymph layered beneath saline (0.9% NaCl, 0.01% EDTA, 0.01% sodium azide, pH 7.4) in a SW 40 rotor in a Beckman L5-50 ultracentrifuge (Beckman Instruments, Inc., Fullerton, CA) for 1 × 106 gav-min. The packed layer of chylomicrons at the top of the tube was separated from the remainder of the sample by tube slicing and was then removed from the centrifuge tube with a Pasteur pipet. The isolated chylomicrons were then completely dispersed in saline by gentle mixing. Ultrapure crystalline sucrose at a final concentration of 10% (w/v) (Schwarz/Mann, Orangeburg, NY) was added to this solution of chylomicrons to raise the density for overlaying with saline. Recentrifugation was then performed for 1 × 107 gav-min. VLDL (S, 20–400) was separated by centrifugation of the chylomicron-free lymph for 2 × 108 gav-min. VLDL was isolated from the centrifuge tube as described for chylomicrons and washed by recentrifugation for 2 × 108 gav-min. All centrifugal isolations of these lipoprotein samples were completed within 4 days of lymph collection. The washed lipoprotein solutions were maintained at 4°C until analyzed.

Analytical procedures

Lipoprotein cholesterol content was determined according to the method of Rudel and Morris (30). Li-
poprotein lipids were extracted in chloroform–methanol 2:1 (v/v). Lipid classes were separated on silica gel H thin-layer chromatography plates using hexane–ethyl ether–glacial acetic acid 75:24:1 (v/v/v) as the solvent system. Areas containing cholesterol and cholesteryl ester were scraped off the plate, the lipid was eluted, and amounts of free and esterified cholesterol were determined as indicated above.

In some animals a catheter was surgically implanted in the duodenum distal to the point of diet infusion through which intestinal lumen contents were aspirated with a syringe at several intervals during each diet infusion. The 0.2–0.3 ml samples were dissolved in 15 ml of chloroform-methanol 2:1 (v/v) and frozen until the specific activity of the cholesterol in the samples could be determined.

Aliquots of the organic solvent extracts of lymph, of lipoprotein solutions, and of free and esterified cholesterol used in cholesterol mass analyses were taken for determination of radioactivity in a Beckman LS-7000 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA). The organic solvents were dried in glass scintillation vials under a stream of nitrogen and a toluene-based counting medium was then added (Liqui-fluor®, New England Nuclear, Boston, MA). Counting efficiency was found to be similar regardless of the type of extract and data were therefore expressed as counts per minute.

Calculations

After 18–24 hr of diet infusion, lymph cholesterol specific activity became constant relative to dietary cholesterol specific activity. Since chemically determined total cholesterol contains both exogenous and endogenous cholesterol, we used radiolabeled cholesterol in the diet so that absorption of both forms could be studied separately. The percentage of total cholesterol derived from dietary (exogenous) sources was calculated from the quotient of the specific activity of cholesterol in lymph divided by the dietary cholesterol specific activity. Exogenous cholesterol mass was calculated as the product of this percentage times the total cholesterol mass chemically determined. Endogenous cholesterol mass was then calculated as the difference between the chemically determined total cholesterol mass and the calculated exogenous cholesterol mass.

Data in the study were analyzed statistically using the paired t-test essentially as described by Snedecor and Cochran (31).

RESULTS

Cholesterol and triglyceride absorption

In these studies, triglyceride absorption was calculated as the rate of appearance of lymph triglyceride divided by the rate of infusion of dietary triglyceride. The percentage of triglyceride absorbed during each infusion was similar in all animals and averaged 75.8 ± 1.9 (mean ± SEM) for the low cholesterol diet and 73.0 ± 3.2 for the high cholesterol diet and ranged from 60.0 to 84.6% for both infusions.

Cholesterol absorption was calculated by three methods and the results are shown in Table 1. The results calculated as in Method 1 indicated that a small difference occurred in the mean percentage of radiolabeled dietary cholesterol recovered in lymph in the first 24 hr of the two diet infusions. Method 2 reflects the percentages of radioactivity recovered in lymph in 36 hr compared to that infused during this time. The percentages calculated using Method 2 were higher than those in Method 1. The magnitude of the difference between the low and high diet percentages in cynomolgus monkeys was greater using Method 2 and was statistically significant (P < 0.003). Using Method 2, no apparent difference was found in African green monkeys.

When the rate of appearance in lymph of exogenous cholesterol radioactivity became constant relative to the rate of infusion of dietary cholesterol radioactivity, cholesterol absorption was determined as in Method 3. The rate of cholesterol radioactivity appearing in lymph of cynomolgus monkeys averaged 65 and 40% of that infused in the low and high cholesterol diets, respectively; this difference was statistically significant (P < 0.001). The value obtained by Method 3 probably represents net dietary cholesterol absorption most accurately, and leads to the conclusion that a statistically significant lesser proportion of dietary cholesterol was absorbed when five times as much cholesterol was present in the diet, at least in cynomolgus monkeys (animals 4–9, Table 1). The percentage of dietary cholesterol absorbed did not significantly decrease during the infusion of the high cholesterol diet in the three African green monkeys (animals 1–3) of this study. These data suggest that a species difference in the response of cholesterol absorption to dietary cholesterol level may occur, although differences between species were not found in any of the other cholesterol transport measurements of this study.

Cholesterol mass transport

The distribution of cholesterol mass transported in lymph lipoproteins was similar during the infusion of the low and high cholesterol diets. In nine animals studied, 55.5 ± 3.0 and 55.2 ± 3.4% (mean ± SEM) of the cholesterol in lymph was transported in chylomicrons during the low and high cholesterol diet infusions, respectively. Less cholesterol appeared in lymph VLDL which transported 28.5 ± 1.6% of the cholesterol in lymph during infusion of the low cholesterol diet and
TABLE 1. Percentage of radiolabeled dietary cholesterol recovered in lymph during infusion of low and high cholesterol level diet

<table>
<thead>
<tr>
<th>Animal</th>
<th>Cholesterol Absorption</th>
<th>1&lt;sup&gt;o&lt;/sup&gt;</th>
<th>2&lt;sup&gt;o&lt;/sup&gt;</th>
<th>3&lt;sup&gt;o&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>C. arthropis</td>
<td>1</td>
<td>39.5 ± 0.3</td>
<td>55.0 ± 0.1</td>
<td>50.2 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>42.2 ± 0.6</td>
<td>55.0 ± 0.2</td>
<td>53.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>45.4 ± 0.7</td>
<td>52.0 ± 0.1</td>
<td>55.4 ± 0.2</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>34.6 ± 0.3</td>
<td>40.0 ± 0.2</td>
<td>45.2 ± 0.1</td>
<td>47.5 ± 0.3</td>
</tr>
</tbody>
</table>

M. fascicularis

|        | 4          | 31.3 ± 0.2  | 30.3 ± 0.3  | 40.7 ± 0.1  | 33.2 ± 0.0  |
|        | 5          | 42.7 ± 0.7  | 30.3 ± 0.1  | 50.8 ± 0.2  | 46.2 ± 0.0  |
|        | 6          | 34.0 ± 0.0  | 25.0 ± 0.1  | 44.3 ± 0.1  | 28.5 ± 0.0  |
|        | 7          | 38.2 ± 0.0  | 30.9 ± 0.1  | 44.6 ± 0.2  | 34.4 ± 0.0  |
|        | 8          | 35.0 ± 0.0  | 33.6 ± 0.1  | 42.9 ± 0.0  | 35.5 ± 0.0  |
|        | 9          | 48.6 ± 0.1  | 31.4 ± 0.1  | 42.1 ± 0.1  | 35.1 ± 0.0  |
| Mean ± SEM | 38.3 ± 0.2  | 32.2 ± 0.2  | 45.9 ± 0.1  | 35.5 ± 0.3  | 65.2 ± 0.1  | 40.2 ± 1.3 |

Significance<sup>d</sup>  

<table>
<thead>
<tr>
<th>Method</th>
<th>1&lt;sup&gt;o&lt;/sup&gt;</th>
<th>2&lt;sup&gt;o&lt;/sup&gt;</th>
<th>3&lt;sup&gt;o&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor 1</td>
<td>&lt;0.05</td>
<td>&lt;0.003</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> Method 1 = (cpm recovered in lymph in 24 hr + CPM infused in 24 hr) × 100.  
<sup>b</sup> Method 2 = (cpm recovered in lymph in 36 hr + CPM infused in 36 hr) × 100.  
<sup>c</sup> Method 3 = [(cpm recovered in lymph/hr) ÷ (CPM infused/hr)] × 100 for the 24–36 hr samples.  
<sup>d</sup> Low vs. high, M. fascicularis.

30.3 ± 2.1% during the high cholesterol diet infusion. The d > 1.006 g/ml lipoprotein fraction transported 16.7 ± 2.7 and 14.2 ± 2.3% of lymph cholesterol during the low and high cholesterol diet infusions, respectively.

A graph of cholesterol transport rates of unfractionated lymph total cholesterol and free and esterified cholesterol of chylomicrons and VLDL from a representative animal is shown in Fig. 1. The data for all nine animals were averaged and the rate of appearance of free cholesterol in the chylomicrons and VLDL increased an average of only 0.3 mg/hr during the high cholesterol meal infusion while the rate of appearance of esterified cholesterol increased an average of 1.49 mg/hr. The increase in the rate of esterified cholesterol appearance in lymph lipoproteins during the infusion of the high cholesterol meal was statistically significant (P < 0.01) and was due to an increase in the rate of esterified cholesterol transport in both chylomicra and VLDL. Thus, during the infusion of the high cholesterol meal, more of the cholesterol in lymph was transported by the chylomicrons and VLDL and most of it was esterified.

**Exogenous cholesterol transport**

The pattern of transport of exogenous cholesterol in lymph lipoproteins is shown for a representative animal in Fig. 2. In each lymph fraction of all animals studied, the rate of appearance of exogenous cholesterol radioactivity steadily increased and reached a plateau after approximately 24 hr during infusion of the low cholesterol diet. The distribution among lipoproteins did not change with dietary cholesterol level. In the nine animals studied, the percentage of exogenous radiolabeled cholesterol transported in chylomicrons averaged 61.9...
UL
I-
Low Cholesterol
High Cholesterol

t
20
10
0
0 6 12 18 24 30 36
Duration of Infusion (hr)

Fig. 2. Rates of appearance of exogenous radiolabeled cholesterol in lymph lipoproteins from animal #6. Each point on the graph represents the midpoint of the collection interval. The (●) symbolize unfractionated lymph, (■) chylomicrons, (■) VLDL, and (□) d > 1.006 g/ml lipoproteins. The distribution of radiolabeled cholesterol in lymph chylomicrons, VLDL, and d > 1.006 g/ml lipoproteins of this animal averaged 65.4%, 31.4%, and 3.2%, respectively, during the low cholesterol infusion, and 66.3%, 28.7%, and 4.9% during the high cholesterol infusion. Dietary cholesterol specific activity was 2140 cpm/μg (¹H) for the low cholesterol level diet and 997 cpm/μg (¹C) for the high cholesterol level diet.

± 1.0 and 60.0 ± 2.1 (mean ± SEM) during the low and high cholesterol diet infusions, respectively. The VLDL transported 28.7 ± 1.4 and 31.3 ± 1.7% of the radioactivity during the low and high diet infusions, respectively, while the d > 1.006 g/ml lipoproteins transported 9.3 ± 1.0 and 8.6 ± 1.7%.

The free cholesterol specific activity (FCSA) and the esterified cholesterol specific activity (ECSA) of both

<table>
<thead>
<tr>
<th>TABLE 2. Normalized lymph lipoprotein cholesterol specific activity during infusion of low and high cholesterol level diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Low Cholesterol Diet</td>
</tr>
<tr>
<td>High Cholesterol Diet</td>
</tr>
<tr>
<td>Significance Low vs. High</td>
</tr>
</tbody>
</table>

Both diets contained radiolabeled cholesterol at a known cholesterol specific activity and were infused at a uniform rate until the rate of appearance and specific activity of lymph cholesterol were constant (Fig. 3). On this plateau, the relative proportions of exogenous and endogenous cholesterol in lymph were determined. An average of 13.3% of the total cholesterol in lymph was of exogenous origin during the low cholesterol infusion, while during the high cholesterol infusion, an average of 46.2% of the cholesterol was of exogenous origin. The latter percentage was more variable among animals but it was always greater than the percentage of cholesterol of exogenous origin during the low cholesterol infusion.

Since the rates of appearance of cholesterol mass in lymph and the percentage of cholesterol from exoge-

origin of lymph lipoprotein cholesterol

Both diets contained radiolabeled cholesterol at a known cholesterol specific activity and were infused at a uniform rate until the rate of appearance and specific activity of lymph cholesterol were constant (Fig. 3). On this plateau, the relative proportions of exogenous and endogenous cholesterol in lymph were determined. An average of 13.3% of the total cholesterol in lymph was of exogenous origin during the low cholesterol infusion, while during the high cholesterol infusion, an average of 46.2% of the cholesterol was of exogenous origin. The latter percentage was more variable among animals but it was always greater than the percentage of cholesterol of exogenous origin during the low cholesterol infusion.

Since the rates of appearance of cholesterol mass in lymph and the percentage of cholesterol from exoge-
cantly greater than that of the chylomicron exogenous free cholesterol during both diet infusions. However, the rate of appearance of exogenous esterified cholesterol in VLDL was higher than that for free cholesterol only during the high cholesterol diet infusion. The average rate of appearance of exogenous esterified cholesterol (3.0 mg/hr) was 1.8 times higher than that for exogenous free cholesterol (1.7 mg/hr) in the d < 1.006 g/ml lymph lipoproteins during the high cholesterol diet infusion, and was higher than the total exogenous cholesterol rate of appearance during the low cholesterol diet infusion.

In contrast to the situation seen for exogenous cholesterol, rates of appearance of endogenous cholesterol were lower in all lymph lipoprotein fractions during the high versus low cholesterol diet infusion. In the chylomicrons, significantly more endogenous cholesterol appeared as free cholesterol than as esterified cholesterol during each infusion. In the VLDL, however, a similar amount of endogenous cholesterol appeared in the free form as in the esterified form during each infusion.

The influence of the level of dietary cholesterol on the origin of lipoprotein cholesterol in lymph may be more easily understood in terms of the difference in the rate of cholesterol appearance between infusions of the low and high cholesterol meals. For individual animals, we subtracted the values obtained during the low cholesterol diet infusion from the values obtained during the high cholesterol diet infusion. A graph of the average differences in the rates of appearance is shown in **Fig. 4**. The data for the chylomicron cholesterol differences are shown in the left panel of the figure. During the high cholesterol diet infusion, more exogenous cholesterol appeared in chylomicron FC, EC, and TC than

---

**TABLE 3.** Influence of the level of dietary cholesterol on the origin of lymph cholesterol mass

<table>
<thead>
<tr>
<th></th>
<th>Exogenous</th>
<th>Endogenous</th>
<th>Significance Low vs. High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>mg/hr</td>
<td>mg/hr</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Unfractionated lymph</td>
<td>1.19 ± 0.13a</td>
<td>5.04 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>Chylomicrons</td>
<td></td>
<td></td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>FC</td>
<td>0.35 ± 0.04</td>
<td>1.08 ± 0.11</td>
<td>2.30 ± 0.16</td>
</tr>
<tr>
<td>EC</td>
<td>0.42 ± 0.05</td>
<td>1.99 ± 0.19</td>
<td>2.05 ± 0.21</td>
</tr>
<tr>
<td>Significance FC vs. EC</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>VLDL</td>
<td></td>
<td></td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>FC</td>
<td>0.17 ± 0.02</td>
<td>0.58 ± 0.06</td>
<td>1.11 ± 0.08</td>
</tr>
<tr>
<td>EC</td>
<td>0.17 ± 0.02</td>
<td>1.02 ± 0.13</td>
<td>1.10 ± 0.14</td>
</tr>
<tr>
<td>Significance FC vs. EC</td>
<td>N.S.</td>
<td>P &lt; 0.001</td>
<td>N.S.</td>
</tr>
<tr>
<td>d &gt; 1.006 g/ml</td>
<td>0.08 ± 0.01</td>
<td>0.37 ± 0.06</td>
<td>1.38 ± 0.27</td>
</tr>
</tbody>
</table>

* Mean ± SEM for nine animals; three to six observations during the 24–36 hr period were averaged for each animal.
* Abbreviations as in Table 2.
during the low cholesterol diet infusion as indicated by the positive deflection of the 'exogenous' bar. Of the increase in exogenous cholesterol appearance rate, significantly more was due to the change in ester cholesterol than to that in free cholesterol ($P < 0.001$). While chylomicron exogenous cholesterol rates of appearance increased during the high cholesterol diet infusion, rates of appearance of chylomicron endogenous cholesterol decreased as indicated by the negative deflection of the 'endogenous' bars. Chylomicron endogenous cholesterol rates of appearance decreased to a similar extent in both free and esterified cholesterol. The increase in exogenous cholesterol rates of appearance during the high cholesterol diet infusion was greater than the concomitant decrease in endogenous cholesterol rates of appearance. This resulted in a significant ($P < 0.01$) net increase in cholesterol appearance during the high cholesterol diet infusion as indicated by the positive deflection of the 'exogenous ± endogenous' bar. The net increase in chylomicron exogenous cholesterol rates of appearance can be seen to be due almost entirely to the increase in ester cholesterol appearance rate.

The graph of the average differences in the rates of appearance of VLDL cholesterol between the low and high cholesterol diet infusions is shown in the right portion of Fig. 4. The conclusions here are the same as for chylomicrons, i.e., that the net increase in exogenous cholesterol rate of appearance was due to the increased ester cholesterol rate of appearance, although free cholesterol also made a significant contribution in VLDL.

**Intestinal lumen cholesterol specific activity**

One series of experiments was carried out to determine the stage of cholesterol absorption responsible for the decreased rate of appearance of endogenous cholesterol. Intestinal lumen contents in three animals were sampled 4–6 times during the infusion of each diet after lymph cholesterol specific activity became constant. Among the samples from a single animal, lumen cholesterol specific activity was highly variable with an average coefficient of variation of 26%. Lumen cholesterol specific activity averaged $31 \pm 4\%$ (mean ± SD) of diet cholesterol specific activity during the low cholesterol diet infusion and $71 \pm 6\%$ of diet specific activity during the high cholesterol diet infusion.

Since the rate of infusion and the percentage dilution of exogenous cholesterol in the intestinal lumen were known, the rate of appearance of endogenous cholesterol in the lumen could be calculated (Table 4). The calculated rates of endogenous cholesterol secretion
into the intestinal lumen were similar during both infusions and were intermediate between the dietary cholesterol infusion rates. Assuming equilibration between endogenous and exogenous cholesterol in the lumen, the appearance rates in lymph of these two sources of lumen cholesterol were then calculated by correcting for the percentage absorption of dietary cholesterol. The contribution of endogenous cholesterol from mucosal cells and lymph was then calculated as the difference between the rate of cholesterol appearance in lymph and the rate of cholesterol flux from the lumen into the mucosa. This source of endogenous cholesterol was equivalent for the two diet infusions, whereas the endogenous cholesterol derived from the intestinal lumen (predominantly from bile) had a decreased appearance rate in lymph during the high cholesterol diet infusion. Based on these calculations, the presence of an increased amount of exogenous cholesterol in the intestinal lumen appears to have resulted in a decreased efficiency of endogenous cholesterol absorption from the lumen resulting in the decreased rate of endogenous cholesterol appearance in lymph.

**Self-feeding studies**

Several studies were carried out in which exogenous cholesterol absorption was measured when low and high cholesterol level diets (75-8 control and test diet, ref. 27) were consumed by the animal of its own volition. This experimental approach was attempted to serve as a physiological control for the constant infusion studies and to approximate the physiological situation so that the influences of hunger, mastication, satiation, stomach emptying, and hormone secretion on cholesterol absorption would be present. The animals usually ate different amounts of diet at each meal, and the amounts of triglyceride and cholesterol appearing in lymph differed between the low and high cholesterol meals and between experiments. Because of this variation, the amount of free and esterified cholesterol appearing in a lymph lipoprotein fraction during a meal was normalized relative to the total amount of triglyceride appearing in that fraction. The results of these calculations are shown in Table 5. The amount of esterified cholesterol transported by both the chylomicrons and VLDL was significantly greater during the high cholesterol meal than during the low, as indicated by the statistically significant decrease in the TG/EC ratio. The differences in the amounts of free cholesterol transported in chylomicrons and VLDL between the two meals were proportionally smaller. The increase in the amount of free cholesterol of chylomicrons was indicated by the statistically significant decrease in the TG/FC ratio during infusion of the high cholesterol meal.

**DISCUSSION**

**Cholesterol absorption**

Cholesterol absorption as measured by Method 3 would seem to be the most accurate measure of the three methods of Table 1. When lymph cholesterol specific activity reaches a plateau and becomes constant relative to dietary cholesterol specific activity, the cholesterol exchange processes that normally occur during cholesterol absorption have reached an equilibrium with respect to isotopic dietary cholesterol. Exogenous cholesterol exchanges 1) with biliary cholesterol in the intestinal lumen, 2) with mucosal cell brush border free

### TABLE 4. Sources of cholesterol in lymph lipoproteins

<table>
<thead>
<tr>
<th>Source of Cholesterol</th>
<th>Infusion or Secretion Rate</th>
<th>Lymph Appearance Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Cholesterol Diet</td>
<td>High Cholesterol Diet</td>
</tr>
<tr>
<td>Diet</td>
<td>1.7 ± 0.2</td>
<td>7.9 ± 0.1</td>
</tr>
<tr>
<td>Intestinal lumen</td>
<td>3.8 ± 0.8</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>Mucosal cell and lymph</td>
<td>6.4 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Total in lymph</td>
<td>10.0 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SEM for three animals; four to six observations for each animal.

1) Calculated as: 1) dietary cholesterol infusion (mg/hr) + (lumen SA/diet SA) = total cholesterol in lumen (mg/hr); 2) total cholesterol in lumen (mg/hr) - dietary cholesterol infusion (mg/hr) = lumenal endogenous cholesterol secretion (mg/hr).

2) Calculated as: infusion or secretion rate (mg/hr) × percentage cholesterol absorption (%) = dietary cholesterol and lumenal cholesterol lymph appearance rate (mg/hr).

3) Calculated as: lumenal endogenous cholesterol secretion (mg/hr) + dietary cholesterol infusion (mg/hr) × percentage cholesterol absorption (%) = lumen cholesterol flux into mucosa (mg/hr); 2) lymph cholesterol appearance (mg/hr) - cholesterol flux into mucosa (mg/hr) = mucosal cholesterol contribution (mg/hr).
TABLE 5. Influence of dietary cholesterol level on cholesterol transport in lymph lipoproteins during self-feeding studies

<table>
<thead>
<tr>
<th>Animal</th>
<th>N</th>
<th>Low</th>
<th>High</th>
<th>Low</th>
<th>High</th>
<th>Low</th>
<th>High</th>
<th>Chylomicrons</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TG/EC</td>
<td></td>
<td>TG/FC</td>
<td></td>
<td>TG/EC</td>
<td></td>
<td>TG/FC</td>
<td></td>
</tr>
<tr>
<td>1398</td>
<td>5</td>
<td>90</td>
<td>51</td>
<td>135</td>
<td>97</td>
<td>38</td>
<td>18</td>
<td>47</td>
<td>30</td>
</tr>
<tr>
<td>66</td>
<td>6</td>
<td>66</td>
<td>52</td>
<td>79</td>
<td>57</td>
<td>18</td>
<td>16</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>144</td>
<td>3</td>
<td>128</td>
<td>94</td>
<td>109</td>
<td>107</td>
<td>46</td>
<td>34</td>
<td>40</td>
<td>48</td>
</tr>
</tbody>
</table>

Mean ± SEM
Significance

<table>
<thead>
<tr>
<th>CHYLOMICRONS</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG/EC</td>
<td></td>
</tr>
<tr>
<td>TG/FC</td>
<td></td>
</tr>
</tbody>
</table>

\[ a \] Total triglyceride appearing in lymph in 24 hr (mg) ÷ total esterified cholesterol appearing in lymph in 24 hr (mg).
\[ b \] Total triglyceride appearing in lymph in 24 hr (mg) ÷ total free cholesterol appearing in lymph in 24 hr (mg).
\[ c \] Low vs. high, paired t-test on 14 observations (3-6 observations on each of three animals).

Cholesterol, 3) with the cholesterol in sloughed mucosal cells, and 4) with cholesterol in transudated plasma lipoproteins after intestinal lipoproteins are secreted into the lymphatics. It is only when exogenous radiolabeled cholesterol exchange has reached an equilibrium with endogenous cholesterol from each of these sources that radiolabeled exogenous cholesterol flux rates approach those of total cholesterol mass; after this has occurred measurements of cholesterol absorption are most accurate. In Method 3 of Table 2 these exchange equilibrium conditions are met.

The esterification of cholesterol during absorption may be an important factor in the regulation of cholesterol absorption. This has been suggested in previous studies in other species (32, 33) and several of the findings in nonhuman primates of the present study emphasize this point. When the dietary cholesterol level was increased, an enhanced rate of esterified cholesterol appearance in lymph occurred that was not matched by the rate of appearance of free cholesterol. This increase in the appearance rate of ester cholesterol was shown to be due to increased exogenous cholesterol transport and occurred together with a real decrease in the rate of endogenous cholesterol transport. The specific activity of the ester cholesterol that appeared in the lymph chylomicrons and VLDL in response to cholesterol feeding was significantly higher than that of the free cholesterol. This indicated that compartmentalization of cholesterol occurred during absorption and that radiolabeled dietary cholesterol did not completely mix and equilibrate with endogenous cholesterol pools before esterification and incorporation into chylomicrons and VLDL. The mechanism by which compartmentalization could occur remains to be demonstrated. However, the data of the present study allow us to speculate on the mechanism.

During absorption, free cholesterol diffuses from the micelle into the membranes of the brush border of the mucosal cell (34). Some of this cholesterol may then become indistinguishably mixed with the endogenous free cholesterol pools in the mucosal cell. However, since dietary cholesterol is preferentially esterified, a significantly greater proportion of the newly absorbed cholesterol apparently diffuses from the brush border membranes along the continuous membranes of the endoplasmic reticulum until it reaches the site where it is esterified. The reduction in the chemical potential of free cholesterol that results from cholesterol esterification may effectively facilitate this diffusion process. Cholesterol in the endoplasmic reticulum is presumably esterified by the ACAT enzyme (35–37) since this enzyme has sufficient activity to account for the observed rates of cholesteryl ester transport in lymph (32, 33).

Although the activity of this enzyme increases with cholesterol feeding (36, 38, 39), it is not known with certainty whether this increase in activity is the result of increased amounts of enzyme or increased substrate availability. The increase in esterifying activity that others have observed with cholesterol feeding could be the result of increased substrate availability. After esterification, the exogenous cholesterol would be effectively separated from free cholesterol inside the cell and maintained in a metabolically and physically distinct pool (40). Cholesteryl ester would then be incorporated into the core of intestinal lipoproteins along with triglyceride while the free cholesterol of the mucosal cell would be incorporated into the lipoproteins, but probably as part of the outer coat. We have suggested that the cholesteryl-esterifying enzyme in our monkeys is ACAT because of the pattern of cholesteryl ester products seen in lymph (41).

In the studies presented here, cholesterol absorption significantly decreased during the high cholesterol diet infusion in cynomolgus monkeys but not in African...
green monkeys (Table 1). The cholesterol absorption ranged from 53–60% during both infusions in the African green monkeys. These results are in agreement with those of St. Clair, Wood, and Clarkson (8) where cholesterol absorption in African green monkeys was determined by a modification of the method first described by Borgström (7) and later modified by Crouse and Grundy (42). St. Clair et al. (8) fed radiolabeled cholesterol for 2 days before determining cholesterol absorption. Exogenous and endogenous cholesterol exchange had presumably approached equilibrium in their experimental design, as they had in our constant infusion studies. Cholesterol absorption in Macaca fascicularis ranged from 58 to 73% during the low cholesterol diet infusion and decreased to 34–45% during the high cholesterol diet infusion. Our results are not consistent with those previously reported for this species (43). The composition of the diets and methods of measurement of cholesterol absorption differed considerably between the two studies.

Endogenous cholesterol compensation

The results of the present studies indicate that absorption measurements of the dose of radioactive cholesterol administered do not accurately predict the changes in rates of cholesterol transport to the blood vascular compartment in response to a given amount of dietary cholesterol. Cholesterol in the low cholesterol diet was infused at an average rate of 2 mg/hr. A total lymph cholesterol rate of appearance of 9.11 mg/hr (Table 3) occurred during this infusion. Cholesterol in the high cholesterol diet was infused at 10 mg/hr and the total lymph cholesterol rate of appearance increased to only 11 mg/hr. Exogenous cholesterol transport rates increased an average of 3.89 mg/hr during the high cholesterol diet infusion in these studies while endogenous cholesterol transport decreased 1.96 mg/hr during the infusion and the result was an observed net increase in cholesterol transport of only 1.89 mg/hr. This phenomenon, which we have termed endogenous cholesterol compensation, has been reported previously. Sylvén and Borgström (6) reported an average 0.3 mg/hr decrease in endogenous cholesterol rate of appearance in lymph when comparing rats fed a bolus of 38.7 mg versus 19 mg of cholesterol. In a later study, Green (14) observed a 0.2 mg/hr decrease in the rate of appearance of endogenous cholesterol in lymph when rats infused with an artificial emulsion containing cholesterol (11.6 mg/hr) were compared to those presented a cholesterol-free infusion.

The mechanism by which endogenous cholesterol compensation occurs has not been identified, but we have carried out calculations in an attempt to identify possibilities (Table 4). Sources of endogenous cholesterol in lymph include cholesterol arising from sloughed mucosal cells, cholesterol synthesized and exchanged in the intestinal mucosal cell, and biliary cholesterol. The calculated values presented in Table 4 suggested that the endogenous cholesterol contribution from the mucosal cell was similar regardless of the level of dietary cholesterol. Rather, it appeared that the decrease in endogenous cholesterol transported in lymph during the high cholesterol diet infusion was due primarily to an approximately 50% decrease in the absorption of endogenous luminal cholesterol. It has been assumed that, prior to absorption, dietary cholesterol and biliary cholesterol mix indistinguishably in the intestinal lumen (44, 45) and this assumption was made in order to do the calculation of Table 4. If this assumption is correct, cholesterol from both sources would be absorbed similarly since cholesterol absorption has the characteristics of passive diffusion (6). During the high cholesterol versus low cholesterol diet infusion, dietary cholesterol could more effectively compete with endogenous cholesterol in the intestinal lumen for incorporation into micelles, thereby reducing the probability of absorption of endogenous cholesterol molecules. In summary, a more effective dilution of endogenous cholesterol in the intestinal lumen may have occurred during the high cholesterol diet infusion resulting in the observed decrease in endogenous cholesterol transport in lymph.

Preferential esterification of dietary cholesterol

During the high cholesterol diet infusion, ECSA exceeded FSCA and thus dietary cholesterol was preferentially esterified. These results are consistent with those previously reported in the rabbit (15), the rat (14), and in man (11). It has been suggested by some (14, 18) that preferential esterification does not occur; rather, unlabeled endogenous free cholesterol in lymph exchanges with exogenous radiolabeled free cholesterol, lowering the FCSA and resulting in a higher ECSA than FCSA. Based on the experimental design of these studies, it is unlikely that this would be the answer for the differences we have observed. We infused radioactive cholesterol at a constant specific activity and allowed time (24 hr) for the specific activity of the lymph free cholesterol to become constant relative to dietary specific activity. During this time period, the numerous exchange processes between free cholesterol pools of the enterocyte and between those of lymph chylomicrons and the lymphoid cells should have brought their FCSAs into equilibrium. If this equilibrium had not been reached, we assume that chylomicron FCSA would continue to increase as equilibration took place, but it was found to be constant after 24 hr of diet infusion (data summarized in Table 2). However, a source of unlabeled free cholesterol that continuously appears in
lymph is that of the d > 1.006 g/ml lipoproteins filtered into lymph from plasma. This cholesterol remains unlabeled because we continue to collect lymph quantitatively and prevent endogenous cholesterol from becoming labeled. The rate of appearance in lymph of d > 1.006 g/ml lipoprotein cholesterol averaged 1.57 mg/hr during the high cholesterol diet infusion (Table 3). A maximum of 35% of the cholesterol in the d > 1.006 g/ml lymph lipoprotein fraction was found to be present as free cholesterol in these animals (41). Therefore, the rate of appearance of free cholesterol in this lipoprotein fraction would have been 0.55 mg/hr. Free cholesterol transport in d < 1.006 g/ml lipoproteins averaged 4.22 mg/hr during this infusion (Table 3). Free cholesterol specific activity could, therefore, be reduced by a maximum of 0.55/(4.22 ± 0.55) or 12%. As can be seen in Table 2, chylomicron and VLDL FCSA and ECSA differed by more than 60% during the high cholesterol diet infusion, not 12%. Therefore, lymph chylomicron and VLDL FCSA could not have been lowered sufficiently to account for the lower FCSA than ECSA because there is not enough free cholesterol mass in the d > 1.006 g/ml lipoproteins.

In addition we have identified cholesteryl ester transfer activity in the lymph from several of the nonhuman primates used in these studies. Others have identified activity of the enzyme lecithin:cholesterol acyltransferase in rat mesenteric lymph (46, 47) that could potentially synthesize cholesteryl esters from endogenous free cholesterol during the period of time required for lymphatic transport of the chylomicrons and VLDL. These two factors would act to decrease the esterified cholesterol specific activity, and evidence for a decrease in the cholesteryl linoleate specific activity of the chylomicrons and VLDL in lymph of the monkeys of these studies has been obtained (41). Thus, the processes occurring in lymph could decrease ECSA in addition to FCSA. Taken in sum, our data provide strong evidence that preferential esterification of dietary cholesterol does occur. This implies that compartmentalization of exogenous cholesterol occurs during absorption and it seems likely that this is accomplished by esterification as discussed above.

Experimental design

Since these studies were carried out in an experimental design where the high cholesterol meal was administered immediately after the low cholesterol meal, two control experiments were carried out to determine the effect of this sequence of events on the outcome. In one cynomolgus monkey, the low cholesterol meal was administered and lymph was collected and analyzed as usual for 36 hr, then lymph flow back into the animal was re-established. Ten days later, the same animal was administered the high cholesterol meal for 36 hr and the second portion of the study was carried out. All of the conclusions regarding cholesterol absorption and endogenous cholesterol compensation were the same as presented for the other animals. Subsequent to this high cholesterol meal period, a low cholesterol meal infusion was begun and continued for 36 hr. The rate of cholesterol appearance in lymph was reduced during this second low cholesterol meal infusion from 10.6 mg/hr during the first low cholesterol meal to 5.8 mg/hr during the second low cholesterol meal infusion. This decrease was primarily due to a decrease in the rate of endogenous esterified cholesterol appearance (reduced to 1.85 mg/hr). During infusion of the second low cholesterol meal the rate of exogenous cholesterol appearance, 0.58 mg/hr, was not markedly reduced when compared to the rate, 0.65 mg/hr, during infusion of the first low cholesterol meal. Therefore, the sequence of diet administration may have had an effect on the outcome of the study. Administration of a high cholesterol meal immediately prior to the low cholesterol meal markedly reduced the rate of ester cholesterol appearance during the low meal. The mechanism of this effect will have to be determined in future studies.

The work was supported in part by a grant from the North Carolina Heart Association, an R. J. Reynolds Industries, Inc. Special Fellowship (RLK) and NIH-NHLBI Grants HL-14164 and HL-24736. The authors wish to acknowledge the technical assistance of Mr. Ramesh Shah and Mr. Larry West and the surgical expertise of Mr. Al Shircliffe, and to thank Ms. Shirley Pegram and Ms. Linda Odham for their help in manuscript preparation. Portions of this work were presented at the American Heart Association meeting in Anaheim, CA in November, 1979. An abstract of this presentation has appeared: R. Klein and L. Rudel, Intestinal regulation of cholesterol transport by lymph lipoproteins during absorption. Circulation. 65-II: 32 (1979).

REFERENCES


