Lymphatic Transport of Dietary Cholesterol Oxidation Products, Cholesterol and Triacylglycerols in Rats

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Rats were fed on a diet containing 0.5% cholesterol oxidation products (oxysterols) or 0.5% cholesterol for 30 min, and their lymph was collected for 7 h. The amount of each of the individual oxysterols absorbed in the lymph depended on the ingested amounts, but the recovery was the highest for 5α,6α-epoxycholesterol (10.5%), this being followed by 7-ketocholesterol (5.8%), cholestanetriol (5.2%), 7β-hydroxycholesterol (4.8%), 7α-hydroxycholesterol (3.4%), 5β,6β-epoxycholesterol (2.2%), and 25-hydroxycholesterol (1.8%).

A diet enriched with oxysterol, but not cholesterol, resulted in increased transport of triacylglycerols in the lymph. These results suggest that the absorption rate of oxysterols depends on the type, and indicate that the effect of dietary oxysterols on the lymphatic transport of triacylglycerols differs from that of dietary cholesterol. It therefore remains to be determined which oxysterol was responsible for the triacylglycerol transport.

Key words: cholesterol; triacylglycerols; lymph; oxysterols

Studies on humans1) and animals2,3) have shown that dietary cholesterol oxidation products (oxysterols) can be absorbed and are transported into the lymph as chylomicrons. Vine et al.3) have reported that the presence of oxysterols in an infusate enhanced the chylomicron triacylglycerol secretion rate into the lymph 2-4 h post-presentation of the lipid load. In contrast, Osada et al.2) have shown that the presence of oxysterols in an infusate delayed the lymphatic absorption of oleic acid as triacylglycerols. Furthermore, the apparent recovery of the individual oxysterols in the lymph markedly differed between both studies. Vine et al.3) were unable to detect 5β,6β-epoxycholesterol in chylomicrons whereas it was absorbed at the highest rate according to Osada et al.2) These discrepancies can be ascribed to the dose, model and vehicle used to administer the oxysterols.4)

The lymphatic transport of dietary fats in rats has often been measured by infusing a fat-emulsion into the stomach or duodenum.5,6) This method has the advantage of making the interpretation of data easier, since it does not need to take into account the interaction of the lipid emulsion with other dietary components. Conversely, there is a drawback to this method, since the interaction of dietary components, particularly proteins, carbohydrate and phospholipids, which influence the emulsification of dietary fats, from the mouth to intestine is not taken into consideration.7) In addition, the rats used for this purpose have not always recovered from surgery-related stress.8) To address these problems, a method of permanent cannulation of the thoracic duct has been adopted in rats,8,9) but the application of this method has been limited; Carvajal et al.10) used this method to characterize the lymphatic transport of dietary lipids during active dietary fat absorption.

In the present study, rats were fed on a diet containing oxysterol or cholesterol, and the lymphatic transport of oxysterols, triacylglycerols and cholesterol was determined in the rats by the permanent lymph duct cannulation method.

Materials and Methods

Materials. 5α-cholestan-3β-ol, cholest-5-en-3β-ol-7-one (7-ketocholesterol), cholestan-3β,5α,6β-triol (cholestanetriol), 7α-hydroxycholesterol and cholest-5-en-3β,25-diol (25-hydroxycholesterol) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Cholest-5-en-3β,7α-diol (7α-hydroxycholesterol), cholest-5-en-3β,6β-diol (7β-hydroxycholesterol), cholest-5-en-3β,7β-diol; 7β-epoxycholesterol, cholest-5-en-3β,6β-epoxy-3β-ol; 27-hydroxycholesterol, 25R-cholest-5-en-3β-26-diol

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Abbreviations: HMDS, 1,1,1,3,3,3-hexamethyldisilazane; TMCS, trimethylchlorosilane; cholesterol, Cholest-5-en-3β-ol; oxysterols, cholesterol oxidation products; 7-ketocholesterol, cholest-5-en-3β-ol-7-one; cholestanetriol, cholest-5α,6β-triol; 7α-epoxycholesterol, cholestan-5α,6α-epoxy-3β-ol; 25-hydroxycholesterol, cholest-5-en-3β,25-diol; 7α-hydroxycholesterol, Cholest-5-en-3β,7α-diol; 7β-hydroxycholesterol, cholest-5-en-3β,7β-diol; 7β-epoxycholesterol, cholest-5β,6β-epoxy-3β-ol; 27-hydroxycholesterol, 25R-cholest-5-en-3β-26-diol
en-3β,7β-diol (7β-hydroxycholesterol), cholestan-5β,6β-epoxy-3β-ol (β-epoxycholesterol), and 25R-cholest-5-en-3β,26-diol (27-hydroxycholesterol) were obtained from Steraloids, Inc. (Wilton, NH, U.S.A.). Cholest-5-en-3β-ol (cholesterol) was purchased from Daichii kagaku (Tokyo, Japan) and pyridine, trimethylchlorosilane (TMCS), and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) were products from Nacalai Tesque (Kyoto, Japan). All organic solvents were of guaranteed reagent grade. Oxidized cholesterol was prepared by heating cholesterol at 150°C for 12 h.11) The heated products were applied to a silica acid column (Silica Gel 60, 70–230 mesh, Nacalai Tesque, 3 cm2×30 cm) and fractionated by successive elution with 100 ml of n-hexane, 100 ml of 5% diethyl ether in hexane, and finally 200 ml of methanol.13) The polar fraction rich in cholesterol oxidation products was eluted by methanol. This operation was conducted three times. The oxidized cholesterol mixture was analyzed by GC-MS in the SCAN mode (Shimadzu, Kyoto, Japan). The oxysterol mixtures contained 4.2% cholesterol and large amounts of unidentified polar compounds (41.2%) (Table 1). The most abundant oxysterol was 7-ketocholesterol, this being followed by β-epoxycholesterol, α-epoxycholesterol, 7β-hydroxycholesterol, 25-hydroxycholesterol, 7α-hydroxycholesterol and cholestanetriol.

Diets. All diets were based on the AIN-93G formulation12) as described previously.10) Palm oil (10 g/100 g diet) was used in the basal diet, to which cholesterol or the oxysterol preparation was supplemented at the level of 0.5 g/100 g diet.

Animals and permanent lymph duct cannulation. Male Sprague-Dawley rats, 8-wk-old, obtained from Seiwa Experimental Animals (Fukuoka, Japan) were maintained in a temperature-controlled room. The rats were trained to consume the basal diet twice a day from 10 a.m.–11 a.m. and 4 p.m.–5 p.m., respectively, for one week. Deionized water was freely available throughout the feeding periods. All the rats were anesthetized with Nembutal prior to permanent lymph duct cannulation as described previously.10) Briefly, a cannula (SH silicon tube, 0.5 mm i.d. and 1.0 mm o.d., Kaneka Medics, Osaka, Japan) filled with heparinized saline was inserted into the thoracic duct and secured within the abdominal cavity. The rats were returned to their cages and provided with the basal diet twice a day, as just described. On the third day, the rats were attached to a long PE cannula (0.58 mm i.d. and 0.97 mm o.d., Becton Dickinson and Company, MD, U.S.A.) to collect the lymph. The end of the cannula was 5–10 cm below the bottom of the cage in order to provide sufficient underpressure to allow the lymph to drain into the cannula. The lymph was collected for 15 min, and the rats were then given free access to the basal diet containing cholesterol or oxysterols for 30 min, before the lymph was again collected every hour for 7 h. The rats had free access to deionized water during the collection of the lymph. After removing fibrin, ethylenediaminetetraacetic acid at a final concentration of 0.01% was added to the lymph solutions. Each lymph solution was flushed with argon (99.9% purity, Hakata Kyoudou Sannso Co., Fukuoka, Japan) and stored at −30°C for up to 12 h.

These experiments were carried out under the Guidelines for Animal Experiments of the Faculty of Agriculture and the Graduate Course at Kyushu University (Fukuoka, Japan) and the Law (No. 105) and Notification (No. 6) of the Government of Japan.

Determination of oxysterols in the lymph. To 200 μl of the lymph, 50 μg of 5α-cholestanol (100 μg/ml in ethanol) as an internal standard was added with a transfer pipette. Lipids were extracted with 20 volumes of chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene according to the method of Folch et al.13) A screw-capped tube was flushed with argon, and 4 ml of a freshly prepared 1 M ethanolic potassium hydroxide solution was added. Each sample was allowed to saponify at room temperature overnight in the dark. To each saponified sample, 4 ml of H2O was added, and the unsaponified lipids were extracted (shaken for 5 min, and the upper hexane layer recovered) three times with 4 ml of hexane. A part of the residue was used to determine cholesterol by GC,10) the rest being dried under N2 and the residue dissolved in 1 ml of toluene. After removing the solvent under N2, the dried residue was converted to trimethylsilyl ethers in a mixture of TMCS, HMDS, and dried pyridine (1:3:9, v/v/v) for 30 min at room temperature. Each sample was dried under N2 and dissolved in hexane (50 μl) for a GC-MS analysis.

GC-MS was performed with a Shimadzu GC-17A ver. 3 gas chromatograph coupled to an SPB-1 fused silica capillary column (60 m×0.25 mm, 0.25 μm)

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>4.2</td>
</tr>
<tr>
<td>Oxysterols</td>
<td>95.7</td>
</tr>
<tr>
<td>7α-Hydroxycholesterol</td>
<td>2.0</td>
</tr>
<tr>
<td>7β-Hydroxycholesterol</td>
<td>5.1</td>
</tr>
<tr>
<td>β-Epoxycholesterol</td>
<td>13.0</td>
</tr>
<tr>
<td>α-Epoxycholesterol</td>
<td>9.3</td>
</tr>
<tr>
<td>Cholestanetriol</td>
<td>0.5</td>
</tr>
<tr>
<td>7-Ketocholesterol</td>
<td>22.5</td>
</tr>
<tr>
<td>25-Hydroxycholesterol</td>
<td>2.1</td>
</tr>
<tr>
<td>Unknowns</td>
<td>41.2</td>
</tr>
</tbody>
</table>

* Each value is the mean of triplicate experiments.
phase thickness, Supelco, Inc., Bellefonte, PA, U.S.A.) and connected to a Shimadzu QP5050A series mass-selective detector. The oven temperature program was as follows: 180°C for 1 min, 20°C/min to 250°C, 5°C/min to 290°C, and 290°C for 30 min. Helium was used as the carrier gas, the total run time being 42.5 min. The gas chromatograph was operated in the constant flow mode, with the flow rate set to 1.5 ml He/min. The injector was operated in the split ratio of 1:5 and was kept at 300°C, and the detector transfer line was kept at 250°C. The mass spectrometer was operated in the electron impact (EI) mode (70 eV). A quantitative analysis was performed by the internal standard method for mass spectrometry in the selected ion monitoring mode. Figure 1 shows a representative chromatogram, using a programmed multiple-selection ion detector for cholesterol and oxysterols isolated from the lymph of rats that had been fed on a diet containing oxysterols. The oxysterols were grouped into seven ‘time windows’: Group 1: 17–20 min (5α-cholestan); Group 2: 20–27 min (7α-hydroxycholesterol), Group 3: 27–29.7 min (7β-hydroxycholesterol, β-epoxycholesterol, α-epoxycholesterol), Group 4: 29.7–32 min (unidentified compounds), Group 5: 32–34.2 min (cholestanetriol); Group 6: 34.2–36.5 min (7-ketocholesterol, 25-hydroxycholesterol); Group 7: 36.5–40 min (27-hydroxycholesterol: not detected in the lymph). The monitored ion during the chromatographic run was varied as a function of time, and the characteristic ion for each oxysterol was recorded. To quantify the oxysterol, calibration curves measuring the peak area of oxysterol versus that of the internal standard were used. Peak identification was confirmed by the relative retention time and a mass spectral comparison with authentic standards, as well as with the NIST (National Institute of Standards and Technology)/EPA (Environmental Protection Agency)/NIH mass spectral database library (Shimadzu, Kyoto, Japan). Table 2 shows the ions monitored, relative retention times, correlation coefficients for the calibration curves, response factors for the monitored ions, detection limit, and coefficient of variation for repeated injection.

![Fig. 1. Representative GC-MS Chromatogram of Cholesterol and Oxysterols in Rat Lymph.](image)

**Statistics.** A statistical analysis was carried out with Statcel (Excel 2000). Variance among the groups was first checked according to the Bartlett test, and statistical differences were analyzed by Student’s *t*-test or Fisher’s PLSD method.

**Results.**

There were no significant differences in the consumption of the diets for 30 min between the cholesterol- and oxysterol-fed rats (mean ± SEM: 4.94 ± 0.52 g and 5.25 ± 0.94 g for the cholesterol and oxysterol groups, respectively, *n* = 5). All the oxysterols that were identified in the diet were detected in the lymph collected for 7 h (Fig. 1). Diet-derived oxysterols (7-ketocholesterol, α-epoxycholesterol, β-epoxycholesterol, and 7β-hydroxycholesterol) reached a maximum output rate in the lymph at 5 h (Fig. 2). The rest of the oxysterols (7α-hydroxycholesterol, cholestanetriol, and 25-hydroxycholesterol) also reached a maximum output rate in

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**Table 2.** Relative Retention Time against 5α-Cholestan (RRT), Monitored Ions (MI), Response Factor (RF), Correlation Coefficient of the Calibration Curve (R), Detection Limit (DL), and Coefficient of Variation within one day (CV-w) and between Days (CV-b)

<table>
<thead>
<tr>
<th>Sterols</th>
<th>RRT</th>
<th>MI</th>
<th>RF</th>
<th>R²</th>
<th>DL</th>
<th>CV-w</th>
<th>CV-b</th>
</tr>
</thead>
<tbody>
<tr>
<td>5α-Cholestan</td>
<td>1</td>
<td>217</td>
<td></td>
<td></td>
<td>0.4</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td>7α-Hydroxycholesterol</td>
<td>1.34</td>
<td>456</td>
<td>2.441</td>
<td>0.998</td>
<td>0.7</td>
<td>2.7</td>
<td>0.8</td>
</tr>
<tr>
<td>7β-Hydroxycholesterol</td>
<td>1.56</td>
<td>456</td>
<td>3.500</td>
<td>0.999</td>
<td>0.3</td>
<td>3.0</td>
<td>9.1</td>
</tr>
<tr>
<td>β-Epoxycholesterol</td>
<td>1.58</td>
<td>474</td>
<td>0.152</td>
<td>0.999</td>
<td>5.7</td>
<td>3.2</td>
<td>2.5</td>
</tr>
<tr>
<td>α-Epoxycholesterol</td>
<td>1.61</td>
<td>474</td>
<td>0.127</td>
<td>0.998</td>
<td>3.0</td>
<td>2.4</td>
<td>7.5</td>
</tr>
<tr>
<td>Cholestanetriol</td>
<td>1.84</td>
<td>403</td>
<td>0.581</td>
<td>0.997</td>
<td>2.0</td>
<td>1.1</td>
<td>8.4</td>
</tr>
<tr>
<td>7-Ketocholesterol</td>
<td>1.93</td>
<td>472</td>
<td>0.421</td>
<td>0.997</td>
<td>2.0</td>
<td>5.8</td>
<td>9.1</td>
</tr>
<tr>
<td>25-Hydroxycholesterol</td>
<td>1.97</td>
<td>131</td>
<td>4.659</td>
<td>0.997</td>
<td>1.0</td>
<td>3.6</td>
<td>7.4</td>
</tr>
<tr>
<td>27-Hydroxycholesterol</td>
<td>2.12</td>
<td>456</td>
<td>0.306</td>
<td>0.996</td>
<td>2.4</td>
<td>3.0</td>
<td>5.9</td>
</tr>
</tbody>
</table>

* RF = [weight (oxysterol)/area (oxysterol)]/[weight (5α-cholestan)/area (5α-cholestan)]. DL is defined as the concentration giving a signal-to-noise ratio of 3:1. CV was determined after repeated injection of each sterol in a standard mixture (*n* = 3).
Table 3. Consumption of Dietary Oxysterols, and Lymphatic Transport and Recovery in the Rat*  

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Consumption of oxysterol in 30 min</th>
<th>Lymphatic transport of oxysterols</th>
<th>Recovery of oxysterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol</td>
<td>Before (nmol/h)</td>
<td>After (nmol/h)</td>
</tr>
<tr>
<td>7α-Hydroxycholesterol</td>
<td>0.83 ± 0.15 ab</td>
<td>0.472 ± 0.364 ab</td>
<td>27.7 ± 6.72 ab</td>
</tr>
<tr>
<td>7β-Hydroxycholesterol</td>
<td>2.18 ± 0.39 cd</td>
<td>0.385 ± 0.238 cd</td>
<td>87.5 ± 20.7 cd</td>
</tr>
<tr>
<td>β-Epoxycholesterol</td>
<td>5.51 ± 0.98 ab</td>
<td>1.562 ± 0.442 ab</td>
<td>117 ± 29.9 ab</td>
</tr>
<tr>
<td>α-Epoxycholesterol</td>
<td>3.96 ± 0.71 ab</td>
<td>1.515 ± 0.295 ab</td>
<td>360 ± 96.4 ab</td>
</tr>
<tr>
<td>Cholestanetriol</td>
<td>0.21 ± 0.04 bc</td>
<td>0.369 ± 0.271 bc</td>
<td>10.2 ± 3.74 bc</td>
</tr>
<tr>
<td>7-Ketocholesterol</td>
<td>0.59 ± 1.71 d</td>
<td>0.782 ± 0.510 bc</td>
<td>531 ± 187 bc</td>
</tr>
<tr>
<td>25-Hydroxycholesterol</td>
<td>0.90 ± 0.16 d</td>
<td>0.148 ± 0.148 bc</td>
<td>16.6 ± 5.85 cd</td>
</tr>
</tbody>
</table>

*a The rats consumed a diet containing oxysterols for 30 min, and the lymph was collected before and after the diet consumption.

Di ſ erent superscripts in the same column show signi ſ icant di ſ erence at \( P < 0.05 \).

The apparent lymphatic recovery of oxysterols was calculated according to their consumption. Each value is the mean ± SEM for 5 rats.

Discussion

The present study shows that the mass of oxysterols transported into the lymph of the rats for 7 h after consuming an oxysterol-containing diet was almost proportional to that in the diet. According to the review by Brown and Jessup, estimates of the extent to which oxysterols are absorbed in the various systems vary greatly (from 6% to 93%). Bascul et al. have reported that most (93%) of α-epoxycholesterol was absorbed in rats after radioactive α-epoxycholesterol had been administered and the \( {^{14}C \text{C}^{1H}} \) ratios in the plasma and feces were measured for 2 days. In the present study, the lymphatic recovery of oxysterols over 7 h was the highest for α-epoxycholesterol (10.5%), followed by 7-

Cholesterol, β-epoxycholesterol, and 25-hydroxycholesterol.

Figure 3 shows the lymph flow and cumulative transport of sterols (cholesterol and oxysterols) and triacylglycerols. There was no significant difference in the lymph flow between the rats fed on the diet containing cholesterol or oxysterols (Fig. 3(A)). The cholesterol-fed rats, in comparison with the oxysterol-fed rats, had greater transport of cholesterol, reflecting the amount of cholesterol in the diet (Fig. 3(B)). The oxysterol transport in the rats fed on the diet containing oxysterols was much smaller than the cholesterol transport, but there was a steady increase with time. In contrast to the cholesterol transport, the oxysterol group had increased triacylglycerol transport 5 h after starting to consume the diet compared with the cholesterol group (Fig. 3(C)). Figure 4 shows the lymphatic output rate of cholesterol (A) and triacylglycerols (B). In the cholesterol group, the maximum output rate of cholesterol was 5 h after consuming the food, and the corresponding value for triacylglycerols was 1 h. In the oxysterol group, the maximum output rates of cholesterol and triacylglycerols were after 1 h and 5 h, respectively.

Fig. 2. Lymphatic Output Rate of Oxysterols.

Each value is the mean ± SEM, n = 5. Values at each time assigned with di ſ erent letters are signi ſ icantly different at \( P < 0.05 \).

The lymph at 5 h. In the basal lymph collected prior to the consumption of the oxysterols, 7 kinds of oxysterols were detected: β-epoxycholesterol and α-epoxycholesterol were the predominant species, followed by 7-ketocholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol, cholestanetriol and 25-hydroxycholesterol (Table 3); 27-hydroxycholesterol was not detected. After the consumption of the oxysterols, the lymphatic transport of oxysterols mirrored the consumed ones, but α-epoxycholesterol and 7-ketocholesterol were the most abundant, although the latter was more prevalent in the diet. The output rate of the oxysterols reached a maximum at 5 h (Fig. 2). The estimated recovery in the lymph of the individual oxysterols for 7 h was the highest for α-epoxycholesterol, followed by 7-ketocholesterol, cholestanetriol, 7β-hydroxycholesterol, 7α-hydroxycholesterol, β-epoxycholesterol, and 25-hydroxycholesterol.
Fig. 3. Lymph Flow and Cumulative Transport of Sterols and Triacylglycerols in Rats Fed on a Diet Containing Cholesterol and Oxysterols.

Unshaded and shaded squares represent rats fed on a diet containing cholesterol and oxysterols, respectively. The lymphatic oxysterol transport in rats fed on the cholesterol diet was very small compared to that in the rats fed on the oxysterol diet. Therefore, the oxysterol transport in the cholesterol diet group is not shown (B).

Each value is the mean ± SEM, n = 5. Values at each time assigned with asterisks are significantly different at P < 0.05.

Fig. 4. Lymphatic Output Rate of Cholesterol and Triacylglycerols in Rats Fed on a Diet Containing Cholesterol and Oxysterols.

Unshaded and shaded squares represent rats fed on a diet containing cholesterol and oxysterols, respectively. Each value is mean ± SEM, n = 5. Values at each time assigned with asterisks are significantly different at P < 0.05.

ketocholesterol (5.8%), cholestanetriol (5.2%), 7β-hydroxycholesterol (4.8%), 7α-hydroxycholesterol (3.5%), β-epoxycholesterol (2.2%) and 25-hydroxycholesterol (1.8%). All the oxysterols reached a maximum output rate at 5 h. In the rats fed on the diet containing cholesterol, the lymphatic recovery of cholesterol was 15–20%, and its output rate reached a maximum at 5 h. Therefore, it is likely that the rat intestine discriminated the amount of cholesterol and α-epoxycholesterol from that of the other oxysterols in the luminal phase.

Others have also reported that the lymphatic recovery of oxysterols, which had been prepared by heating cholesterol, but had not been purified prior to administering to rats, differed according to the type. According to Osada et al.,21 who administered an oxysterol-triolein emulsion stabilized with taurocholate and albumin into the stomach of rats, the percentage recovery of oxysterols over 24 h was the highest for 7β-hydroxycholesterol (41.5%), followed by β-epoxycholesterol (32.3%), 7α-hydroxycholesterol (30.4%), α-epoxycholesterol (27.5%), cholestanetriol (15.2%) and 7-ketocholesterol (12.0%). According to Vine et al.,3 who administered an oxysterol-triolein emulsion into the stomach of rats, the percentage recovery of oxysterols over 12 h was the highest for 7β-hydroxycholesterol (7.9%), followed by 7-ketocholesterol (5.8%) and α-epoxycholesterol (5.5%). In their experiment, they were unable to detect β-epoxycholesterol in the lymph. These results indicate that, although the absorption of oxysterols differs according to type, the findings are not consistent, possibly due to the methods used for the preparation and administration of the oxysterols. Therefore, to clarify the differences in their absorbability, further research is required by using individual oxysterols.

The results of the present study show that dietary supplementation with oxysterols, in comparison with cholesterol, resulted in increased transport of triacylglycerols in the lymph. The maximum output rate was reached after 5 h. In the rats fed on a diet containing cholesterol, the lymphatic output rate of triacylglycerols was reached after 1 h and maintained throughout the period of lymph collection. Vine et al.3 have also reported that the administration of an oxysterol-triolein emulsion increased the lymphatic transport of triacylglycerols. Their oxysterol preparation, in comparison to ours, contained more unreacted cholesterol (69.3%) and less oxysterols (30.7%) and included smaller amounts of unknown compounds (1.6%). Our oxysterol preparations included 4.2% cholesterol, 54.5% oxysterols and 41.2% unknown compounds. Taken together, it appears that oxysterols were responsible for the...
increased triacylglycerol transport in the lymph. In contrast to the present results and those of Vine et al., Osada et al. have reported that the administration of an oxysterol-triolein emulsion that had been stabilized with taurocholate and albumin decreased the lymphatic transport of triacylglycerols. The reason for this discrepancy is not clear.

The effects of oxysterols on the triacylglycerol synthesis or secretion in tissue culture systems have been reviewed by Schroepfer. Although their results varied according to the cell type, Bhadra et al. reported an increased incorporation of [14C]oleic acid into triacylglycerols in human endothelial cells when incubated with α-epoxycholesterol. Carlson et al. have observed an increased secretion of [3H]glycerol-labeled triacylglycerols from Hep G2 cells when incubated with 25-hydroxycholesterol. If the oxysterols administered to the rats in the present experiment had behaved in a manner similar to those cells, the increase in the lymphatic transport of triacylglycerols might be attributable to an increased resynthesis of triacylglycerols in the enterocytes. Alternatively, some oxysterols may increase the luminal absorption of fatty acids or triacylglycerols, because physiological oxysterols (22-hydroxycholesterol, 26-hydroxycholesterol and 7α-hydroxycholesterol) are a ligand for LXR, a nuclear transcription factor, which is known to be involved in lipid absorption in the intestine by activating the expression of ABCA1. In any event, it remains to be determined which oxysterol was responsible for the increased lymphatic transport of triacylglycerols.

In the present experiment, the basal lymph contained considerable amounts of α- and β-epoxycholesterol and cholestanetriol. Furthermore, there was no detectable amount of 27-hydroxycholesterol in the basal lymph. According to Breuer and Björkhem, rats can produce 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol and 27-hydroxycholesterol in vivo, but not α- and β-epoxycholesterol and cholestanetriol. Therefore, α- and β-epoxycholesterol and cholestanetriol found in the basal lymph might have been derived from the diet. Cholestanetriol might have been derived from α- and β-epoxycholesterols, since Maerker et al. have shown that cholestanetriol was easily formed when epoxycholesterols were incubated with gastric juice. Alternatively, we cannot neglect the possibility that the oxysterols found in the basal lymph might have been produced during the preparation.

Acknowledgments

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