Intestinal absorption and lymphatic transport of cholesterol in the rat: influence of the fatty acid chain length of the carrier triglyceride

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ABSTRACT This paper deals with the effect of the fatty acid chain length of dietary triglyceride on the intestinal uptake and lymphatic transport of exogenous and endogenous cholesterol in the rat. This question seemed of interest as the chain length of the monoglyceride and fatty acids formed in the intestinal lumen from the triglyceride fed could be expected to affect the concentration of cholesterol in the micellar or isotropic phase of intestinal content.

Feeding rats medium- or short-chain triglycerides (C_{12} to C_2) did not affect the lymphatic transport of endogenous cholesterol from the intestine compared to the fasting state.

The extent of lymphatic transport of cholesterol added to these fats increased proportionally with chain length (C_6–C_{18}) of the component fatty acids. The uptake of exogenous cholesterol into the intestinal wall was similarly related to the chain length of the carrier triglyceride, with the exception of triacetin, which gave a much higher intestinal uptake than lymphatic transport.

When cholesterol was fed in octadecane, negligible amounts only were transported to the thoracic duct lymph. This again indicates the importance of the polar split products of dietary fat for cholesterol absorption.

SUPPLEMENTARY KEY WORDS triacetin · coconut oil · medium-chain triglycerides · triolein · octadecane

In previous communications (1, 2) the quantitative aspects of sterol absorption in the rat have been defined. The uptake of cholesterol into the intestinal cell hadthe characteristics of a passive diffusion process. The limiting factor for the rate of absorption of cholesterol was its rate of transport across the intestinal cell. The extent of absorption was related to the concentration of sterol in the micellar phase of intestinal content and the rate of transport across the intestinal cell. The cholesterol concentration in the micellar phase has earlier been found to be dependent on the presence therein of the hydrolytic products of the carrier fat in which the sterol was fed (3, 10). In the experiments discussed above, cholesterol was fed in triolein, but it was obvious that the composition of the carrier fat could affect the extent of sterol absorption. The chain length of the triglyceride used could be expected to greatly affect the interaction of its hydrolytic products with the bile salt solution of intestinal content, which in turn would affect the concentration of sterol in its micellar phase.

In the experiments to be discussed here, cholesterol dissolved in triglycerides with different fatty acid chain lengths was fed to rats, and the uptake of cholesterol into the intestinal wall and its transport to the lymph of the thoracic duct were determined. In another series of experiments cholesterol dissolved in octadecane was also fed and its lymphatic transport determined.

METHODS

The triglycerides used in these experiments were: hydrogenated coconut oil (HCOCO) (Karlshamn's Oil Refinery, Karlshamn, Sweden), mean chain length of the fatty acids 13.3; medium chain triglyceride (MCT) (Drew Chemical Corp., New York), mean fatty acid chain length 8.4; and tricaproin, tributyrin, and triacetin from Fluka (Buchs, Switzerland). Cholesterol-4-^{14}C and cholesterol-^{3}H were obtained from the Radiochemical Centre, Amersham, Bucks., England. Their radiopurity, checked by thin-layer chromatography,

Abbreviations: MCT, medium-chain triglycerides; HCOCO, hydrogenated coconut oil.

was found to be better than 98%. Cholesterol was dissolved in the triglyceride to a concentration of 50 μmoles/0.8 ml at 37°C, with the exception of triacetin, for which a solution of only 5 μmoles/0.8 ml could be obtained. In some experiments trace amounts of labeled cholesterol (0.03 μmole) were dissolved in the same volume of triglycerides.

Thoracic duct- and stomach-cannulated rats of the Sprague-Dawley strain (Anticimex, Stockholm) were maintained as previously described (1). 0.8 ml of the glyceride mixtures was administered intragastrically 18–24 hr after the operation.

The triolein figures given here are taken from a previous paper (1).

In the experiments in which the intestinal wall was analyzed, the rats were killed 6 hr after feeding (by intubation) of 0.8 ml of the different oils, with 0.03 μmole of cholesterol. The intestinal wall was carefully removed from the animal and washed through with 10 ml of a solution 2.4 mM in sodium taurodeoxycholate and 0.15 M in NaCl. The intestine was then passed with gentle pressure, between two fingers to remove residual content. The intestinal wall was saponified and the non-saponifiable fraction was isolated as previously described (4).

Cholesterol was precipitated as the digitonide and quantitatively determined (5). Total lipid ester bonds of lymph were determined as described previously (6). Aliquots of samples for radioactivity assay were taken to dryness in counting vials. 15 ml of toluene-based scintillation solution was added and radioactivity was determined to a confidence level of at least 95% in a Packard spectrometer of the 4000 series. No correction for quenching was needed. In general, the figures given represent the mean of five replicate experiments.

In the results and in the discussion the transport of cholesterol and ester bonds in the thoracic duct lymph is generally expressed in μmoles/hr, which is the mean transport ± SD per hr during the 4th, 5th, and 6th hr after administration. Statistical analysis has been performed by testing the difference between two means by a Rank sum test (7).

RESULTS

The transport of cholesterol in the thoracic duct lymph (Fig. 1) during the 4th, 5th, and 6th hr did not increase over the fasting value (0.87 ± 0.06 μmole/hr) when 0.8 ml of triacetin, tricaproin, MCT, or HCOCO was fed alone. This is in contrast to a 100% increase to 1.7 ± 1.0 μmoles/hr when 0.8 ml of triolein was fed (1). When 50 μmoles of cholesterol was fed in tricaproin, MCT, or HCOCO the transport values for cholesterol during the same interval were 1.4 ± 0.2, 2.0 ± 0.7, and 3.6 ± 1.6 μmoles/hr, respectively. The comparable figure obtained when 50 μmoles of cholesterol was fed in 0.8 ml of triolein was 4.9 ± 1.2 μmoles/hr (1).

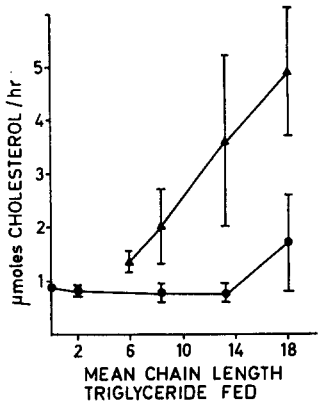

FIG. 1. Lymphatic transport of cholesterol in μmoles per hr ± SD (n = 5) during the 4th, 5th, and 6th hr after feeding, by intubation, 0.8 ml of triglyceride of different fatty acid chain lengths ●—● and the same oils with 50 μmoles of cholesterol in 0.8 ml of triglyceride ▲—▲. The value corresponding to zero chain length refers to the fasting state.

When triacetin or tricaproin was used as carrier fat for the cholesterol (Fig. 2), there was no increase in the ester bond transport over the fasting value. When MCT, HCOCO, and triolein were fed, the ester-bond transport in the lymph increased approximately in direct proportion to the chain length of the triglyceride (Fig. 2). This figure also shows that the number of μmoles of

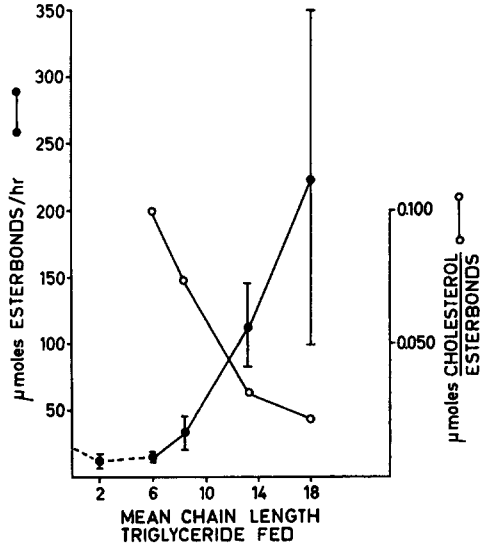

FIG. 2. Lymphatic transport of ester bonds ●—● in μmoles per hr ± SD (n = 5) during the 4th, 5th, and 6th hr after feeding 50 μmoles of cholesterol dissolved in 0.8 ml of triglycerides with different fatty acid chain lengths. For triacetin only 5 μmoles of cholesterol in 0.8 ml of oil was fed (because of low solubility). The value corresponding to zero chain length refers to the fasting state. ○—○, μmoles of cholesterol transported/μmole of ester bond.

cholesterol transported per μmole of ester bond decreases with increasing chain length of the triglyceride fed.

Fig. 3 shows the percentage of exogenous cholesterol (calculated from the radioactivity of the lymph cholesterol by differential specific activities) transported in the lymph during 8 and 24 hr after feeding 50 μmoles of cholesterol in the different triglycerides. It is seen that the percentage of exogenous cholesterol transported in the lymph increases with increasing chain length of the glyceride from tricaproin to triolein. The percentages for triacetin are more than double those for tricaproin. Because only 5 μmoles of cholesterol was fed with triacetin, the total amount of cholesterol transported in this case was lower than for tricaproin. The rates of transport of total, exogenous, and endogenous cholesterol in these experiments are given in Fig. 4. The rate of transport of total, as well as exogenous, cholesterol increases with the chain length of the triglyceride from tricaproin on up. Compared to the fasting state (7.3 ± 0.7 μmoles), the transport of endogenous cholesterol is significantly increased ($P < 0.025$) during 8 hr after feeding 50 μmoles of cholesterol dissolved in HCOCO (16.2 ± 7.3 μmoles) or triolein (13.5 ± 3.1 μmoles).

(When 50 μmoles of cholesterol was fed in 0.8 ml of octadecane, the lymphatic transport of exogenous cholesterol in 24 hr was 0.45 and 0.65% in two experiments.)

The percentage of radioactivity recovered in the small intestinal wall was maximal 6 hr after feeding labeled cholesterol in triolein (unpublished data). Fig. 5 shows the percentage of radioactivity recovered in the small intestinal wall 6 hr after labeled cholesterol dissolved in the various triglycerides had been fed. The curve has a shape similar to that for the lymphatic transport (Fig. 3). Feeding cholesterol in triacetin gives a percentage recovery of labeled cholesterol in the intestinal wall that is even higher than that found after feeding triolein.

The lymph flow produced by these rats during 8 hr after administration of the test meal was affected by the

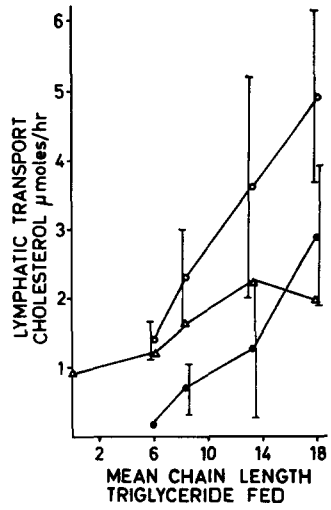

FIG. 4. Lymphatic transport of total ○—○, exogenous ●—●, and endogenous △—△ cholesterol in μmoles per hr ± SD (n = 5) during the 4th, 5th, and 6th hr after feeding rats 50 μmoles of cholesterol in 0.8 ml of various triglycerides.

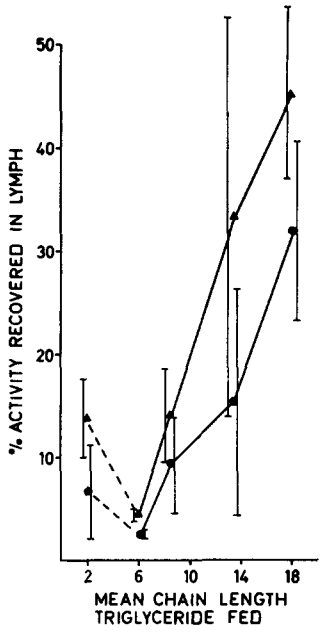

FIG. 3. Percentage of dietary cholesterol ± SD (n = 5) recovered in thoracic duct lymph during the 8 hr (●—●) and 24 hr (▲—▲) after feeding rats 0.8 ml of triglycerides, of different chain lengths, containing 50 μmoles of radioactive cholesterol. In the triacetin experiment only 5 μmoles of cholesterol was fed.

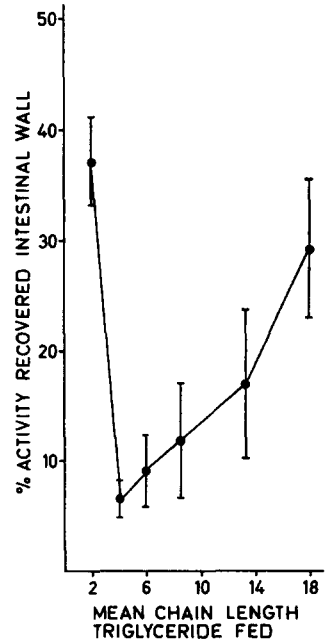

FIG 5. Percentage of radioactive cholesterol ± SD recovered in the small intestinal wall 6 hr after feeding rats 0.8 ml of triglycerides with different chain lengths and 0.03 μmole of cholesterol-4-^{14}C.

chain length of the triglyceride fed and also its cholesterol content. When 0.8 ml of MCT was fed, the lymph flow was the same as in the fasting state, 10.6 ± 6.0 ml and 11.9 ± 6.8 ml respectively. With 50 μmoles of cholesterol included in MCT the lymph flow significantly ($P < 0.005$) increased to 19.2 ± 6.4 ml. For HCOCO and triolein fed alone, a similar increase compared to the fasting state was found, to 20.3 ± 5.5 ml and 16.6 ± 4.7 ml, respectively. Addition of 50 μmoles of cholesterol to these oils had no further significant effect on the volume of lymph produced ($P > 0.20$).

DISCUSSION

In the experiments presented in this study the effect of the chain length of the fed glyceride on the intestinal uptake and lymphatic transport of cholesterol has been determined. The different glycerides have been fed in a constant volume of 0.8 ml; the molar amounts fed therefore vary widely, from 800 μmoles for triolein to 4000 μmoles for triacetin.

The increase in total fat transport (measured as ester bonds) via the lymphatic pathway with increasing chain length of the glyceride fed (Fig. 2) is well established (8, 9). According to Bloom, Chaikoff, and Reinhardt (8), about 50% of C_{12} fatty acid is transported in the lymph.

The short- and medium-chain triglycerides when fed alone did not affect the lymphatic transport of cholesterol, which remained on the fasting level (Fig. 1). A long-chain triglyceride such as triolein, however, increased it by approximately 100%. Inclusion of cholesterol in the fed glyceride increased the lymphatic transport of cholesterol for triglycerides with chain lengths of C_6 or higher. The increase in cholesterol transport was directly proportional to the chain length of the carrier triglyceride from tricaproin to triolein. This relationship was found both for total and exogenous (dietary) cholesterol (Fig. 4). These results are at variance with those reported by Vahouny and Treadwell (9), who found little change in lymph cholesterol transport when cholesterol was fed in tributyrin, trilaurin, or triolein. Their experimental conditions, however, were very different from ours; equimolar amounts, approximately 350 μmoles of triglyceride and 130 μmoles of cholesterol, were fed emulsified in 3 ml of a solution containing 540 μmoles of sodium taurocholate.

The uptake of dietary cholesterol into the intestinal mucosa, measured 6 hr after feeding, was related to the chain length of the glyceride in the same way as was the lymphatic transport. These results indicate that cholesterol absorbed into the mucosa is further transported to the lymph independently of the chain length of the glyceride fed, the possible exception being triacetin.

Uptake of fat from the intestine takes place mainly from its micellar or isotropic phase (10–14), which is continuously generated from the oil phase by the action of pancreatic lipase (15). The uptake of cholesterol into the intestinal cell has the characteristics of a passive diffusion process and reflects the concentration of cholesterol in the micellar phase of intestinal content (1, 16).

The effect of the chain length of the glyceride fed on the uptake of cholesterol into the intestinal mucosa is most probably mediated through an effect of the hydrolytic products of the glyceride on the transfer of cholesterol to the micellar phase of intestinal content. Such an effect has been demonstrated in experiments in vitro, in which the partition of cholesterol to the isotropic phase had a maximum for C_{12} fatty acid (3). In vivo, however, the rate of absorption of the hydrolytic split products of the glyceride will also be of importance for the composition of the isotropic phase.

When cholesterol is fed dissolved in a long-chain hydrocarbon such as octadecane, it is absorbed in negligible quantities only (as is the hydrocarbon). Addition of a long-chain triglyceride such as triolein to the hydrocarbon oil results in definite absorption of both the cholesterol and hydrocarbon (unpublished data). These results again indicate the importance of the hydrolytic products of the glyceride for the conversion on nonglyceride fat to a physical form available for absorption.

The results obtained in this investigation do not speak in favor of a direct relationship between the lymphatic transport of cholesterol and ester bond; the ratio of cholesterol to ester bond in lymph decreases with increasing chain length of the fatty acid of the glyceride fed (Fig. 2). An interesting observation is the effect of feeding cholesterol with shorter-chain glycerides (C_6–C_8) on the volume of the lymph produced. Cholesterol fed with these glycerides significantly increases the lymph flow without increasing the ester bond transport.

Feeding cholesterol with triacetin results in a cholesterol absorption pattern different from that expected by extrapolation of the results obtained with the other glycerides. The uptake into the cell was (on a percentage basis) more important than was the lymphatic transport. This anomalous behavior of triacetin might depend on the fact that triacetin is hydrolyzed at a relatively much lower rate by pancreatic juice enzymes (17) and therefore might be absorbed without previous hydrolysis.

The results of previous investigations (1, 2) indicate that for cholesterol, intestinal absorption is quantitatively directly related to transport via the lymphatic pathway. Although not definitely established, this most probably is also the case when cholesterol is fed in shorter-chain triglycerides. The decreased absorption of cholesterol

when fed with shorter-chain triglycerides therefore might be one factor leading to the decreased plasma cholesterol level produced by feeding MCT (18). Even though differences between species might be important, the hypercholesterolemic effect of coconut fat and trilaurin demonstrated for several species, including man, is most probably not mediated by an increased absorption of exogenous or endogenous cholesterol via the intestinal tract.

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