Intestinal Cholesterol Uptake: Comparison Between Mixed Micelles Containing Lecithin or Lysolecithin

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ABSTRACT

The aim of our study was to define the mechanism by which cholesterol uptake is inhibited by lecithin but not by lysolecithin. The work compared the cholesterol uptake by everted rat jejunal sacs from bile salt-lecithin-cholesterol or bile salt-lysolecithin-cholesterol micelles. The micellar size and the cholesterol saturation were measured.

The size or molecular weight increases when the lecithin concentration rises, and the cholesterol uptake decreases and leads to zero when the micelles contain more than 30% lecithin. The size of bile salt-lysolecithin-cholesterol micelles is smaller than that of lecithin micelles in comparable molar ratios. Consistent with this result is the fact that, for a given phospholipid concentration, cholesterol uptake is greater in the presence of lysolecithin than in the presence of lecithin. The diffusion rate of the micelles through the unstirred water layer decreases when micellar size increases. However, the comparison of uptakes from lecithin or lysolecithin micelles similar in size and in cholesterol saturation showed that the cholesterol uptake is still lower for lecithin micelles. This shows that with larger micelles some factor other than micellar size and cholesterol content of the micelles is important. We observe that lysolecithin absorption is 15-fold greater than lecithin absorption. We suggest that lysolecithin absorption results in a rapid supersaturation with cholesterol leading to cholesterol absorption. *Lipids* 20:145-150, 1985.

INTRODUCTION

Several publications have demonstrated that the intestinal absorption of cholesterol and fatty acids from mixed micelles is partially inhibited by the presence of lecithins but not by that of lysolecithins (1-3). This inhibition seems to occur at the level of lipid uptake by the intestinal mucosa, but the mechanism remains to be elucidated. Two main hypotheses have been advanced as a function of current knowledge on lipid absorption. These are, first, that the rate of absorption depends on the velocity of micelle diffusion through the unstirred water layer. The inclusion of lecithins in the micelles increases micellar size and so decreases their rate of diffusion toward the epithelium (4,5). The importance of this diffusion velocity has been determined for other types of micelles (6,7). Second, the absorption of lipolysis products depends on their partition coefficient between the micellar phase and the juxta-membranous aqueous phase (8). This would explain why the absorption of cholesterol from mixed micelles of bile salts, fatty acids, monoolein and cholesterol depends on both the quantity of cholesterol present in the micelles and the degree of saturation of the micelles. Lecithins may increase the capacity of bile salt micelles to solubilize lipids, thus decreasing the release of monomers (9).

The role of these two parameters is not yet clearly established. The inhibitory effect of lecithins exists in conditions in which micellar size would not be excessively modified (4). In this case it is difficult to determine if the effect of lecithins on the solubilization of cholesterol can explain the results obtained, because mixtures with a highly variable composition have been used without determining the solubility limit of cholesterol in the mixtures.

In the present work we attempted to determine if the inhibitory effect of lecithin on intestinal cholesterol absorption could be explained by changes in micellar size and solubilization of cholesterol. This was done by in vitro comparisons of intestinal cholesterol uptake from mixed micelles of bile salt-lecithin-cholesterol and bile salt-lysolecithin-cholesterol, each with known size and degree of cholesterol saturation. The first step was the determination of conditions for optimal uptake of cholesterol from bile salt-lecithin-cholesterol micelles.

MATERIALS AND METHODS

Chemicals

Sodium taurocholate was purchased from Calbiochem, L α phosphatidylcholine (lecithin), L α lysophosphatidylcholine (lysolecithin) and

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cholesterol were 99% pure and were obtained from Sigma Chemical Co., St. Louis, Missouri. [4-^{14}C]-cholesterol (40-50 mCi · mmole^{-1}) and ^{3}H-cholesterol (50 Ci · mmole^{-1}) were purchased from CEA-France, and were found to be greater than 98-99% pure. [1-^{14}C] phosphatidylcholine and ^{3}H-inulin obtained from Amersham-France SA and [1-^{14}C] lysophosphatidylcholine from New England Nuclear, Boston, Massachusetts, were 97% and 98% pure. The radiochemical purity of the compounds was ascertained by thin layer chromatography (TLC).

Preparation of Mixed Micelles

The mixed micelles taurocholate-lecithin-cholesterol or taurocholate-lysolecithin-cholesterol were prepared by the coprecipitation method (10). The appropriate amounts of bile salt, lecithin, lysolecithin and cholesterol were dissolved in chloroform:methanol (2:1, v/v) in order to reach a final concentration of 10 mM bile salt with various lipid/bile salt ratios. The solvent was evaporated in vacuo over phosphorus pentoxide for 24 hrs, then the dried mixtures were dissolved in Krebs-Ringer bicarbonate (Ca^{++} omitted) pH 7.4. Micellar solubilities of cholesterol were determined as previously described (10) or from Carey's tables (11).

Molecular Weight Determination

Analytical ultracentrifugation was performed at 20 C in a Spinco-Beckman ultracentrifuge, model E, with speed and temperature controls. Sedimentation coefficient was measured using a double sector capillary cell with Schlieren optics. The Yphantis method (12) was used for the determination of micelle weights. The partial specific volume of mixed micelles was experimentally measured in a Parr microdensimeter (13).

Gel Filtration. The 8 mm x 100 mm glass column was packed with Ultrogel Aca 34 to a total bed volume of 37.7 ml. Eluted with 0.01 M Tris HCl, pH 7.5, 0.02% Na N_3, 8 mM Na taurocholate, blue dextran and vitamin B_{12} were used to determine the void volume (vo) and the total volume (vt).

The K_{AV} of the micellar elution volume was calculated as follows: $K_{AV} = (ve - vo)/(vt - vo)$ (14). This method gives an indirect estimation of the relative sizes of the different mixtures. The chemical determination of lecithin/bile salt/cholesterol peak obtained after chromatographic filtration showed the same molar ratio as the initial micellar solution. This method has been used for mixed micelles (4).

Cholesterol Uptake

The preparation of everted sacs has been described previously (6). Briefly, male Wistar rats (250-280 g) were fasted 12 hrs before experimentation and were killed by decapitation. The small intestine was removed and rinsed with cold saline and immediately everted over a glass rod. A 10-15 cm segment distal to the ligament of Treitz was used in this experiment. Sacs 1.5 cm long from this segment were tied off sequentially and kept in cold buffer solution until used. Incubation was made immediately at 37 C for 5 min and for a stirring rate of 750 rev/min^{-1} in micellar solutions which contained ^{14}C-cholesterol or ^{14}C-lecithin or ^{14}C-lysolecithin and trace amounts of ^{3}H-inulin as radiolabeled volume marker. Following incubation, sacs were removed, rinsed in cold saline and dried overnight at 60 C. Sacs were weighed and solubilized with Soluène-350 and Dimilume-30 (Packard Instrument, Downers Grove, Illinois) and the liquid scintillation counting was carried out using a TRI-CARB 300 C counter (Packard Instrument). The kinetic of cholesterol uptake has been measured between 3 and 15 min. A linear relationship exists between the amount of cholesterol uptake and time. Therefore, a 5-min incubation time was chosen because 5 min are sufficient for the unstirred water layer to become uniformly labeled with the nonpermeant marker and not too long to damage the membrane. The data were expressed as nmol of cholesterol, lecithin or lysolecithin per 100 mg tissue dry weight and per 5 min incubation. The results were given as means ± SE and were compared by using Student's t-test.

RESULTS AND DISCUSSION

Effect of Lecithin Concentration on Cholesterol Uptake

The different taurocholate-lecithin-cholesterol mixtures were all saturated with cholesterol. Thus, the cholesterol concentration varied in each mixture and increased in parallel with that of the lecithins. It is known that uptake depends not only on the degree of saturation of the micelles, but also on the cholesterol concentration in the mixtures. In these conditions, we should observe an uptake which is proportional to the quantity of cholesterol and thus to lecithins present. The results in Table 1, however, show that the actual situation is more complex. Cholesterol uptake increases with lecithin concentration only when lecithin concentration is lower than 2.1 mM. This increase seems to be related to the low concentration of cholesterol solubilized in micelles

TABLE 1

Effect of Varying Concentrations of Lecithin on Cholesterol Uptake

Concentration in micellar mixtures				
Lecithin (mM)	Cholesterol (mM)	n	Cholesterol uptake (nmoles·100mg^{-1}·5min^{-1})	Apparent adherent fluid volume (μl·100mg^{-1}·5min^{-1})
1.20	0.21	12	8.40 ± 1.02	43.83 ± 2.86
2.10	0.38	12	17.65 ± 0.80	54.30 ± 2.33
2.42	0.45	15	12.40 ± 2.15	57.79 ± 2.34
3.00	0.57	21	5.53 ± 0.82	60.81 ± 5.09
4.40	0.75	12	0.91 ± 0.52	62.55 ± 3.55
6.00	0.75	12	0	54.15 ± 2.16

The taurocholate concentration is kept constant (10 mM), and cholesterol concentrations used correspond to maximal cholesterol solubilities determined by Carey's table.
Values are mean ± SE. n = number of animals.

containing less than 2.1 mM lecithins. On the contrary, when lecithin concentration increases from 2.1 mM to 6.6 mM, cholesterol uptake decreases in spite of the increasing amount of cholesterol solubilized by the micelles. Cholesterol uptake is completely abolished for lecithin concentration greater than 4.4 mM. The decrease of cholesterol uptake seems well explained by changes in micellar size. The results in Table 2 clearly show that, when lecithin varies from 2.1 mM to 6.0 mM, the size of the taurocholate-lecithin-cholesterol micelles doubles. The apparent molecular weight increases from 50,000 for 1.2 mM lecithin concentration to 124,000 for 6 mM lecithin concentration in mixed micelles. The results obtained by gel filtration method (K_{AV}) confirmed the changes of micellar size observed by analytical ultracentrifugation. It is to be noted that the methods used furnish only an apparent mean molecular weight and do not account for eventual structural changes as a function of concentration or polydispersity. Prior work has shown that this type of change is not extensive in our conditions (15). The values obtained after ultracentrifugation indicate that the molecular weight of the mixed micelles is slightly higher than published values (16). The different results are difficult to compare, because the experimental conditions are not exactly the same. It should be noted that our results obtained with ultracentrifugation and gel filtration are in entire agreement in terms of the micellar size change as a function of lecithin concentration. In addition, the migration buffer for ultracentrifugation or gel filtration included 8 mM taurocholate, reducing the risks of micellar rearrangement. For polydispersity it appears from the data of Mazer et al. (17) that in the concentration range we studied with lecithin two micellar species coexist, simple bile salt micelles and mixed micelles. Simple bile salt micelles have a negligible solubilization power for cholesterol and do not interfere directly with cholesterol uptake. Mixed micelles which contain the cholesterol have, for a low lecithin concentration, a very low index of polydispersity (20%) and the mean molecular weight must be meaningful. The overall result is consistent with a major influence of micellar size (above 63,000) on cholesterol uptake for a lecithin range between 2.1 mM and 4.4 mM. On the contrary, below this value the effect of micellar size becomes negligible in contrast to the effect of the quantity of cholesterol solubilized which becomes predominant. The result obtained appears to be explicable without incurring a specific effect of lecithins except for modifications of micellar structure, even though such an effect cannot be excluded (18).

In order to more precisely examine the possible existence of specific inhibition of choles-

TABLE 2

Effect of Varying Concentrations of Lecithin on Apparent Molecular Weight of Mixed Micelles Taurocholate-Lecithin-Cholesterol

Concentration lecithin (mM)	Ultracentrifugation			Gel filtration
	V[a]	S[b]	aMW[c] daltons	KAV[d]
1.20	0.928	1.91	50,000	–
2.10	0.927	1.98	63,000	0.75
2.42	–	–	–	0.70
3.00	0.927	2.24	73,000	0.60
4.40	–	–	–	0.52
6.00	0.924	2.54	124,000	0.37

[a]Partial specific volume.
[b]Sedimentation coefficient.
[c]Apparent molecule weight.
[d]KAV = (elution volume - void volume) (total volume - void volume).

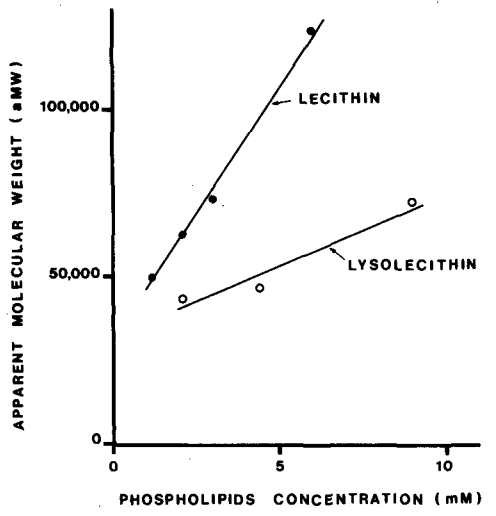

FIG. 1. Apparent molecular weight of mixed bile salt-lysolecithin-micelles versus mixed bile salt-lecithin micelles is determined by analytical ultracentrifugation. The bile salt concentration is 10 mM and mixed micelles are saturated with cholesterol.

terol uptake, we investigated cholesterol uptake from micellar bile salt-lysolecithin-cholesterol mixtures.

Cholesterol Uptake from Bile Salt-Lysolecithin-Cholesterol Mixtures

Before studying cholesterol uptake it was necessary to determine the cholesterol solubilizing capacity and micellar size of bile salt-lysolecithin mixed micelles.

The solubility limit of cholesterol in micellar taurocholate-lysolecithin cholesterol mixtures depends on lysolecithin concentration. It is 0.21 mM of cholesterol for 2.1 mM of lysolecithin, 0.27 mM for 3.30 mM, 0.35 mM for 4.40 mM and 0.60 mM for 9.00 mM. The solubility remains lower than in the case of lecithins in comparable molar ratios.

Concerning the micellar size, Figure 1 shows the variation of micellar size related to lecithin or lysolecithin concentrations. The slopes of these two curves are very different and show that in the case of lysolecithins the size of the micelles does not change significantly when the lysolecithin concentration increases. In all cases, the size of mixed micelles containing lysolecithins is smaller than that of lecithin micelles in comparable molar ratios.

It is likely that the structure of this type of mixture is similar to that obtained with lecithins. Lysolecithins are more water soluble than lecithins and are distributed between the micellar and aqueous phase in the presence of bile salts. Solubilized cholesterol, however, must be completely inside the bile salt-lysolecithin-cholesterol micelles. It appears that lysolecithins alone can incorporate cholesterol only in an insoluble lamellar phase at a lysolecithin/cholesterol ratio 1/1 (19). In the presence of bile salts, lysolecithins and cholesterol are found in the micellar phase (20).

The results of cholesterol uptake from bile salt-lysolecithin mixed micelles are given in Table 3. It appears that cholesterol uptake depends on both cholesterol saturation of the micelle and on micellar size.

From micelles of small size (lysolecithin between 2.1 and 4.4 mM), cholesterol uptake seems to depend on cholesterol saturation alone. For a given cholesterol concentration (0.21 mM) when lysolecithin concentration increases from 2.1 to 3.3 mM, micellar size is unchanged, and uptake and saturation of cholesterol vary in parallel: cholesterol uptake decreases by 19% and cholesterol saturation by 22%. Similarly, cholesterol saturation decreases by 40% and cholesterol uptake by 43% when lysolecithin concentration increases from 2.1 to 4.4 mM.

For higher lysolecithin concentration (9.0 mM), cholesterol uptake decreases in spite of a

TABLE 3

Effect of Varying Concentrations of Lysolecithin on Cholesterol Uptake

Concentration in micellar mixtures					
Lysolecithin (mM)	Cholesterol (mM)	Cholesterol saturation (%)	n	Cholesterol uptake (nmoles·100mg^{-1}·5min^{-1})	Apparent adherent fluid volume (μl·100mg^{-1}·5min^{-1})
2.1	0.21	100	8	21.96 ± 0.96	57.45 ± 3.81
3.3	0.21	78	8	16.64 ± 1.64	61.81 ± 3.11
4.4	0.21	60	7	12.47 ± 1.67	56.81 ± 3.68
9.0	0.60	100	11	12.21 ± 1.17	55.43 ± 5.39

The taurocholate concentration is kept constant at 10 mM.
Values are mean ± SE. n = number of animals.

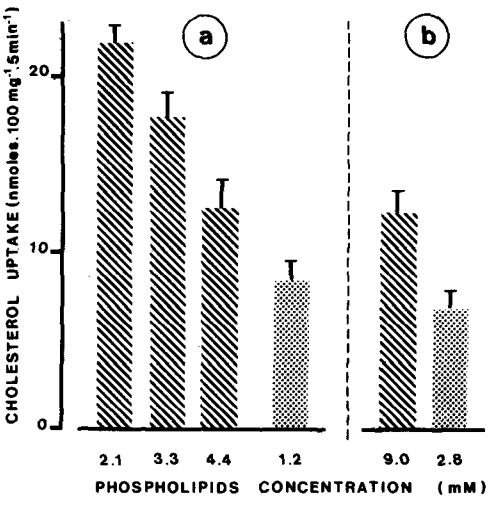

FIG. 2. Effect of lysolecithin (▒▒) and lecithin (▨) on cholesterol uptake. For all micelles, the bile salt concentration is kept constant (10 mM). In part (a), the cholesterol concentration is 0.21 mM for all mixtures and the apparent molecular weight is about 50,000. In part (b), for the two micelles, the cholesterol concentration is 0.54 mM and the size is 70,000. It must be emphasized that cholesterol content of the micelles in parts a and b is very different. This explains why cholesterol uptake from phospholipid micelles is not very much decreased in b compared to a, in spite of the larger size of the micelles.

higher cholesterol content (0.6 mM). This is consistent with the corresponding results with lecithins, showing the importance of micellar size.

Comparative Effect of Lecithins and Lysolecithins on Intestinal Cholesterol Uptake

A comparison of Tables 1 and 3 shows that cholesterol uptake is generally higher in the presence of lysolecithins than lecithins. From this general result, it is difficult to be certain if the difference is due only to a change of micellar size and cholesterol saturation degree or to a specific influence of lecithins on cholesterol uptake. To answer this question, it is necessary to compare cholesterol uptake in two groups of micelles, similar in size and in quantity of cholesterol. This is obtained in two cases.

1) Figure 2a shows the result obtained from mixed micelles containing either lecithins (1.2 mM) or lysolecithins (2.1 mM to 4.4 mM). Both series are small size micelles (50,000) and contain the same amount of cholesterol (0.21 mM). In this case, cholesterol uptake is significantly higher (+48%) in the presence of lysolecithins (4.4 mM) than in the presence of lecithins ($2p \leq 0.01$). This difference is even greater (+160%) if we compare this phenomenon with lysolecithin mixed micelles with the same saturation degree as lecithin (2.1 mM lysolecithin versus 1.2 mM lecithin). The comparison between the effects of lecithin (1.2 mM) and lysolecithin (3.3 mM) on cholesterol uptake after an incubation time from 3 to 15 min is performed to check the linearity of the results (see methods). At 15 min cholesterol uptake is 1.90-fold greater in the presence of lysolecithin, this ratio remaining constant after 3 and 5 min.

2) Figure 2b shows the result from mixed micelles with a larger micellar size (70,000) which is obtained with 2.1 mM lecithin and 9 mM lysolecithin. Both exhibit the same size, the same cholesterol saturation and the same quantity of cholesterol (0.54 mM). Cholesterol uptake from lysolecithin micelles is significantly higher (77%) than in the presence of lecithin. This shows that the effect of lecithins on the size of mixed micelles and their degree of cholesterol saturation is not sufficient to explain their effect on intestinal absorption of lipids.

The greater uptake from lysolecithin micelles cannot result from a breakdown of the intestinal barrier due to a detergent effect of lysolecithin (21) for several reasons. The lysolecithin concentration we used in our experiments was much lower than that which Bolin et al. (22) considered as having a damaging effect on the intestinal membrane. To confirm this observation in presence of bile salt, we have measured the diffusion volume of ^{3}H-inulin. In the case of intact membrane, ^{3}H-inulin does not cross the enterocyte membrane, and the diffusion volume corresponds to adherent fluid in contact with the membrane. In the case of modifications of membrane permeability, the diffusion volume will include the adherent fluid plus ^{3}H-inulin uptake and will appear increased. The results of adherent fluid volumes are shown in Tables 1 and 3. Whatever the phospholipid concentrations, there is no significant difference between lecithin and lysolecithin. Furthermore, the average of adherent fluid volumes are the same as those determined from an incubation medium containing ^{3}H-inulin without micelles ($58.00 \pm 3.84\ \mu l \cdot 5min^{-1}$). In all of our experimental conditions, the results cannot be influenced significantly by changes of membrane permeability.

Lecithin and Lysolecithin Uptake from Mixed Micelles

The differential behavior of lysolecithins and lecithins nevertheless remains explicable without special mechanisms for cholesterol uptake occurring, if we consider the relative rates of absorption of lecithins and lysolecithins. We measured the uptakes of lysolecithins and lecithins in the same experimental conditions as used for cholesterol. We used ^{14}C-lecithin or

^{14}C-lysolecithin and ^{3}H-inulin as radiolabeled volume marker. The comparison is made with mixed micelles containing 0.21 mM of cholesterol and 1.2 mM lecithin or 4.4 mM lysolecithin. Lecithin uptake (20.11 ± 3.77 nmoles · 100 mg^{-1} · 5 min^{-1} for 24 experiments) is very slight in comparison to that of lysolecithin (345 ± 14 nmoles · 100 mg^{-1} · 5 min^{-1} for 12 experiments). This difference is highly significant ($2p \leqslant 0.01$). As shown above, lysolecithin absorption is not due to a possible membrane injury. We studied the influence of the presence of lysolecithins on lecithin uptake. Lecithin absorption is unchanged when studied in the presence of 9.0 mM of lysolecithin (18.20 ± 1.80 nmol·5 min^{-1} instead of 21.93 ± 2.83 nmol·5 min^{-1} without lysolecithin).

The observed difference between lysolecithin and lecithin uptake seems to explain the results, if we consider that cholesterol absorption is a simple partitioning between a saturated micellar phase and an aqueous phase in contact with the epithelium. In presence of lecithin, these micelles should remain stable with no tendency toward supersaturation with cholesterol, because the lecithins are not absorbed to a great extent. As a result of the considerable absorption of lysolecithins, on the other hand, the bile salt-lysolecithin-cholesterol micelles should tend toward supersaturation with cholesterol leading to cholesterol absorption. The dissociation of mixed micelles in different components during absorption after diffusion through the unstirred water layer seems to be a determinant factor for rate of cholesterol uptake (23). The release of lysolecithin and its uptake lead to a cholesterol supersaturation, located near the membrane; this behavior does not occur with lecithins which are not absorbed.

Our results are thus compatible with the hypothesis that lecithins decrease lipid absorption by modifying the equilibrium and the stability of mixed micelles.

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[Received March 26, 1984]