Pancreatic lipase/colipase-mediated triacylglycerol hydrolysis is required for cholesterol transport from lipid emulsions to intestinal cells

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This study tested the hypothesis that dietary cholesterol uptake by intestinal cells is dependent on the structure and composition of the lipid carriers in the extracellular milieu. In *in vivo* experiments with female C57BL/6 mice, cholesterol absorption from phospholipid/triacylglycerol emulsions was significantly reduced by administration of tetrahydrolipstatin, an inhibitor of pancreatic lipase. This inhibitor had no effect on the absorption of cholesterol from phospholipid vesicles. The importance of pancreatic-lipase-mediated triacylglycerol hydrolysis for cholesterol transport from emulsions to intestinal cells was confirmed by *in vitro* experiments with rat IEC-6 intestinal cells. Cellular uptake of cholesterol from emulsions with a phospholipid/triacylglycerol molar ratio of < 0.3 could be stimulated by pancreatic lipase/colipase hydrolysis of the core neutral lipids. However, pancreatic lipase/colipase was ineffective in hydrolysing triacylglycerols in emulsions with a phospholipid/triacylglycerol molar ratio of > 0.3. Phospholipase A₂-mediated

hydrolysis of the surface phospholipids was necessary prior to triacylglycerol hydrolysis in these phospholipid-rich emulsions and to the stimulation of cholesterol transport from these particles to IEC-6 cells. The data also revealed that minimal triacylglycerol hydrolysis was sufficient to significantly increase cholesterol transport from lipid emulsions to the intestinal cells. Thus the products of triacylglycerol hydrolysis, namely monoacylglycerol and non-esterified fatty acids, are key determinants in mediating cholesterol transport from lipid emulsions to intestinal cells. Taken together, these results support the hypothesis that remodelling of the surface and core components of lipid carriers is necessary prior to absorption of dietary cholesterol from the gastrointestinal tract.

Key words: dietary fat, digestion, lipid vesicles, phospholipase A₂.

INTRODUCTION

Epidemiological studies have clearly established a direct relationship between cholesterol absorption efficiency, plasma cholesterol levels and coronary heart disease [1]. Increased dietary fat and cholesterol intake has also been associated with increased incidence of other diseases, such as cancer, diabetes and obesity [2–4]. Thus there is considerable interest in designing effective dietary and/or therapeutic strategies to lower cholesterol absorption efficiency and reduce morbidity and mortality due to these debilitating diseases. Unfortunately, the effort is hampered due to incomplete information regarding the mechanism of fat and cholesterol absorption. Additional studies to identify the proteins that are necessary for cholesterol transport from the intestinal lumen to the mucosal cells would provide valuable information towards this goal.

Dietary intake of cholesterol is usually associated with fat consumption. Most dietary cholesterol is unesterified, while 5–10% is esterified with fatty acids as cholesteryl esters. The process of fat digestion begins in the stomach, with a fraction of the triacylglycerols being hydrolysed by pre-duodenal lipases [5–7]. The dietary lipids are also emulsified in the stomach by the action of peristalsis [8]. However, lipid digestion in the stomach is incomplete, and the dietary cholesterol is delivered to the intestinal lumen as crude lipid emulsion particles in association with the undigested triacylglycerols and phospholipids [9–11]. Within the lumen of the intestine, the triacylglycerols, phospholipids and cholesteryl esters in the lipid emulsion are hydrolysed further by pancreatic enzymes, including pancreatic lipase,

phospholipase A₂ and cholesterol esterase. The digested products are mixed with phospholipids and bile salts in micellar structures, or complexed with phospholipids in vesicles [9,10]. Whether cholesterol delivered to the lumen as lipid emulsions can be transported directly to the mucosal cells, or whether its redistribution to lipid vesicles and bile salt mixed micelles is necessary prior to absorption, remains unclear.

The purpose of the present study was to investigate the possibility that pancreatic-lipase-mediated hydrolysis of the triacylglycerols in the lipid emulsion particles is necessary prior to cholesterol transport to the intestinal mucosal cells.

MATERIALS AND METHODS

Materials

C57BL/6 inbred mice were obtained from Harlan Bioproducts (Indianapolis, IN, U.S.A.). LM485 mouse chow was purchased from Teklad (Madison, WI, U.S.A.). The rat intestinal cell line IEC-6 was purchased from American Type Culture Collection (Manassas, VA, U.S.A.). Dulbecco's modified Eagle's medium and fetal bovine serum were purchased from Life Technologies, Inc. (Gaithersburg, MD, U.S.A.). Phosphatidylcholine, cholesterol, triolein, taurocholate, pig pancreatic lipase, colipase and phospholipase A₂ were products of Sigma (St. Louis, MO, U.S.A.). Radiolabelled cholesterol and triolein were obtained from NEN Life Science Products (Boston, MA, U.S.A.). [¹⁴C]Sitosterol was a product of Amersham Life Science

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(Arlington Heights, IL, U.S.A.). Human pancreatic lipase was purchased from Calbiochem and Novachem Corp. (La Jolla, CA, U.S.A.). Tetrahydrolipstatin was generously donated by Hoffman-La Roche (Basel, Switzerland).

Cholesterol absorption in mice

Female C57BL/6 mice (10–12 weeks old) were housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle and were fed a basal Teklad LM485 diet containing 5.7% (w/w) fat, without cholesterol. All experimental protocols involving the handling of animals were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Cincinnati, in compliance with the National Institutes of Health guidelines. Cholesterol absorption studies were performed using the procedure described previously [12,13]. Briefly, the mice were transferred to metabolic cages 24 h prior to the beginning of experiments. Each mouse received, by stomach gavage, a bolus test meal of 50 μ l of an emulsified test meal containing 1.3 GBq/litre [3 H]cholesterol (1.85 TBq/mmol), 260 MBq/litre [β - 14 C]sitosterol (2 TBq/mmol), 2 g/litre cholesterol, 8 g/litre egg phosphatidylcholine and 50 g/litre triolein, or a 50 μ l meal consisting of the radiolabelled sterols in phospholipid vesicles without triolein. Immediately after the administration of the test meal, half of the mice in each group received an oral dose of tetrahydrolipstatin (50 μ g/g body weight), while the other half of the animals in each group received vehicle solution without the inhibitor. The mice were returned to their metabolic cages, where they had free access to food and water. Faeces were collected over a 24 h period. The samples were homogenized in water and then extracted with an equal volume of chloroform/methanol (2:1, v/v). An aliquot of the organic phase from each sample was used for scintillation counting to determine the amount of the radioactive sterols excreted in the faeces. Counting efficiency was determined using the channel ratio method based on external standards. Cholesterol absorption efficiency was calculated as a percentage of the administered dose absorbed after normalizing for recovery based on the amount of the non-absorbable [β - 14 C]sitosterol in each sample, using the formula described by Grundy et al. [14], as follows: $\{1 - [(\text{H-Bq}/^{14}\text{C-Bq}) \text{ in faeces}] / [(\text{H-Bq}/^{14}\text{C-Bq}) \text{ administered}]\} \times 100$.

Cholesterol uptake by rat IEC-6 intestinal cells

The IEC-6 cells were grown in high-glucose (4.5 g/litre) Dulbecco's modified Eagle's medium supplemented with 5% (v/v) fetal bovine serum, 4 μ g/ml insulin, 100 units/ml penicillin, 100 μ g/ml streptomycin and 2 mM L-glutamine. The cells were maintained in 75 cm² tissue culture flasks at 37 °C in a humidified incubator with 10% CO₂. For experiments, IEC-6 cells between passages 18 and 28 were plated into 24-well dishes at a density of 1.5×10^5 cells/well in the above medium. Cholesterol uptake by the IEC-6 cells was assessed at confluency.

A lipid emulsion was prepared by mixing 382.6 nmol of cholesterol (which included 3.85×10^7 d.p.m. of [14 C]cholesterol), 28.3 μ mol of triolein (which included 3.3×10^7 d.p.m. of [3 H]triolein) and 5.19 μ mol of egg-yolk phosphatidylcholine in organic solvent. After evaporating the solvent to dryness under a nitrogen stream, 500 μ l of deionized water was added, and the sample was emulsified by sonication for 5 min in a Bransonic-32 bath sonicator. Immediately before adding to the cell culture, the lipid emulsion was diluted 40-fold into medium containing 0.1 mg/ml BSA with or without human pancreatic lipase and porcine pancreatic colipase. The IEC-6 cells in 24-well plates were incubated with 190 μ l of the reaction mixture for 1 h at 37 °C. The reaction was stopped by removal of the medium. Cells were

washed twice with ice-cold PBS. A cell extract was prepared by incubation for 10 min with 200 μ l of 1 M NaOH. A 150 μ l sample of the cell lysate was used for liquid scintillation counting to determine the amount of [14 C]cholesterol taken up by the intestinal cells.

Determination of triacylglycerol and fatty acid uptake by IEC-6 cells

Non-esterified fatty acids released from hydrolysis of the triacylglycerols in the lipid emulsion were assessed as the sum of the non-esterified fatty acids present in the incubation medium and those taken up by the IEC-6 cells. The amount of non-esterified fatty acids present in the medium was determined by adjusting the pH of the incubation medium to 10.5 with 5 volumes of 50 mM sodium carbonate, followed by extraction with 15 vol. of methanol/chloroform/heptane (28:25:20, by vol.), as described [15]. Phase separation was achieved after centrifugation for 15 min at 1500 g. An aliquot of the aqueous phase (750 μ l) was subjected to liquid scintillation counting to determine the amount of [3 H]oleate present in the incubation medium. Cell-associated [3 H]oleate, taken up after triacylglycerol hydrolysis, was determined using the same procedure as described above for determination of cell-associated [14 C]cholesterol.

Statistical analyses

Cholesterol absorption efficiencies in control and inhibitor-fed mice were compared by Student's *t* test. All other data were analysed for statistical significance using two-factorial ANOVA. A *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

The pancreatic lipase inhibitor tetrahydrolipstatin has been shown previously to reduce triacylglycerol absorption by rat intestine [16] and to improve postprandial lipaemia in human subjects [17]. Furthermore, intraduodenal infusion experiments performed in rats showed that the inhibitor was also effective in reducing intestinal absorption of cholesterol when the substrate was administered in emulsified triolein [16]. However, the impact of tetrahydrolipstatin administration on cholesterol absorption in intact animals has not been explored previously. This study examined the effect of tetrahydrolipstatin on cholesterol absorption *in vivo*. Ten age-matched female C57BL/6 mice were fed by stomach gavage a test meal containing [3 H]cholesterol and [β - 14 C]sitosterol, as described in the Materials and methods section. Five of these mice also received an oral dose of tetrahydrolipstatin at 50 μ g/g body weight immediately after the test meal. The other five mice received vehicle solution without the inhibitor. Faeces were collected over a 24 h period to determine the amount of radiolabelled sterols not absorbed from the diet. The results showed that a single dose of tetrahydrolipstatin was sufficient to significantly reduce cholesterol absorption when the substrate was provided as an emulsion with phospholipids and triacylglycerols (Table 1). Interestingly, inhibition of pancreatic lipase by tetrahydrolipstatin had no effect on cholesterol absorption when the substrate was provided as a vesicular complex with phospholipids (Table 1). These data support the hypothesis that lipolysis of the lipid emulsion is required for effective cholesterol absorption from these particles.

Consideration was given to the fact that tetrahydrolipstatin is a β -lactone that can decompose rapidly in the stomach after gastric infusion. Therefore the importance of triacylglycerol hydrolysis for cholesterol transport from lipid emulsion particles

Table 1 Effect of inhibition of pancreatic lipase on cholesterol absorption by C57BL/6 mice

Each mouse received, by stomach gavage, a bolus emulsified test meal of 50 μ l containing 1.3 GBq/litre [3 H]cholesterol (1.85 TBq/mmol), 260 MBq/litre [β - 14 C]sitosterol (2 TBq/mmol), 2 g/litre cholesterol, 8 g/litre egg phosphatidylcholine and 50 g/litre triolein, or a 50 μ l meal consisting of the radiolabelled sterols in phospholipid vesicles without triolein. Half of the mice in each group also received 50 μ g/g body weight of tetrahydrolipstatin (THL), while the other animals in each group received vehicle solution without inhibitor. Cholesterol absorption efficiency was calculated as the percentage of the administered dose found in the faeces after a 24 h period after normalizing for recovery based on the amount of non-absorbable [β - 14 C]sitosterol in each sample. Results are means $\pm$ S.E.M. ($n = 5$); * significantly different from mice without THL ($P < 0.05$)

Substrate	Cholesterol absorption (%)
Emulsions	76.2 $\pm$ 4.3
Emulsions + THL	61.0 $\pm$ 3.3*
Vesicles	77.9 $\pm$ 5.6
Vesicles + THL	73.2 $\pm$ 3.3

Figure 1 Effects of pancreatic lipase and colipase on triacylglycerol hydrolysis and lipid transport from emulsions to rat IEC-6 intestinal cells

The IEC-6 cells were plated in 24-well dishes at a density of 1.5×10^5 cells/well and then incubated with 190 μ l of medium containing 3.80 nmol of [14 C]cholesterol, 282 nmol of [3 H]triolein and either 200 μ g/ml ($\bullet$, $\blacktriangle$, $\blacksquare$) or 800 μ g/ml ($\circ$, $\triangle$) egg phosphatidylcholine. Pancreatic lipase and colipase were added in a molar ratio of 3:2 at the concentrations indicated. Incubation was continued for 1 h at 37 $^{\circ}$ C. Triolein hydrolysis was determined from measurement of non-esterified fatty acids released into the medium ($\bullet$, $\circ$) and oleate taken up by the IEC-6 cells ($\blacksquare$). Cholesterol uptake ($\blacktriangle$, $\triangle$) by the IEC-6 cells was determined by liquid scintillation counting of the cell lysate. Error bars represent S.D. values from three separate experiments with triplicate determinations.

to intestinal mucosal cells was examined directly *in vitro* using rat IEC-6 intestinal cells. When IEC-6 cells were incubated with lipid emulsions containing 7.4 μ g/ml cholesterol, 1.25 mg/ml triolein and 200 μ g/ml phosphatidylcholine, only small amounts of cholesterol were found to be incorporated into the cells in the absence of pancreatic lipase and colipase (Figure 1). The inclusion of pancreatic lipase, and its activator colipase, in the incubation medium resulted in a concentration-dependent increase in triacylglycerol hydrolysis and a concomitant increase in cholesterol and oleate uptake by the IEC-6 cells (Figure 1). The requirement of triacylglycerol hydrolysis for cholesterol transport was further supported by the observation that pancreatic lipase alone, without colipase activation of its enzymic activity, was incapable of triacylglycerol hydrolysis and did not stimulate cholesterol or fatty acid uptake by the IEC-6 cells (Figure 2). Moreover, at a constant concentration of 0.21 μ M pancreatic lipase in the

Figure 2 Effect of colipase on pancreatic-lipase-dependent triacylglycerol hydrolysis and lipid transport from emulsions to rat IEC-6 intestinal cells

The IEC-6 cells were plated in 24-well dishes at a density of 1.5×10^5 cells/well and then incubated with 190 μ l of medium containing 3.80 nmol of [14 C]cholesterol, 282 nmol of [3 H]triolein and either 200 μ g/ml ($\bullet$, $\blacktriangle$, $\blacksquare$) or 800 μ g/ml ($\circ$, $\triangle$) egg phosphatidylcholine. Pancreatic lipase was added at a concentration of 0.21 μ M, with the colipase present at the concentrations indicated. Incubation was continued for 1 h at 37 $^{\circ}$ C. Total triacylglycerol hydrolysis was determined based on measurement of non-esterified fatty acids released into the medium ($\bullet$, $\circ$) and oleate taken up by the IEC-6 cells ($\blacksquare$). Cholesterol uptake ($\blacktriangle$, $\triangle$) by the IEC-6 cells was determined by liquid scintillation counting of the cell lysate. Error bars represent S.D. values from three separate experiments with triplicate determinations.

Table 2 Effect of taurocholate on pancreatic-lipase/colipase-stimulated cholesterol transport

IEC-6 cells were plated in 24-well dishes at a density of 1.5×10^5 cells/well and then incubated for 1 h at 37 $^{\circ}$ C with 190 μ l of medium containing 3.43 nmol of [14 C]cholesterol, 800 μ g/ml egg phosphatidylcholine, 282 nmol of [3 H]triolein, 0.21 μ M pancreatic lipase and 0.14 μ M colipase, with or without taurocholate. Cholesterol uptake by the IEC-6 cells was determined by liquid scintillation counting of the cell lysate. The data represent means $\pm$ S.D. from two separate experiments with triplicate determinations; * significantly different from control at $P < 0.01$.

Taurocholate (mM)	Cholesterol uptake (pmol/dish)
0	10.9 $\pm$ 3.2
5	20.5 $\pm$ 10.0*

incubation medium, colipase-concentration-dependent triacylglycerol hydrolysis and IEC-6-cell uptake of both fatty acids and cholesterol were observed (Figure 2). Interestingly, pancreatic lipase/colipase was incapable of hydrolysing triacylglycerols in emulsions with a high concentration of phospholipids (Figures 1 and 2). Under these conditions, pancreatic lipase/colipase also failed to stimulate cholesterol uptake by the IEC-6 cells (Figures 1 and 2). The addition of taurocholate, a bile salt that has been reported previously to promote cholesterol absorption *in vivo* [18], increased pancreatic lipase/colipase stimulation of cholesterol transport from phospholipid-rich emulsions to IEC-6 cells by 2-fold (Table 2). However, it is important to note that this amount of stimulated cholesterol transfer from phospholipid-rich emulsions was substantially lower than the pancreatic lipase/colipase-mediated cholesterol transport from phospholipid-poor emulsions (compare data in Table 2 and Figure 1).

The relationship between triacylglycerol and phospholipid concentrations in the lipid emulsion and pancreatic lipase/colipase-stimulated cholesterol uptake by IEC-6 cells was explored further by incubating cells with emulsions containing a

Figure 3 Effect of phosphatidylcholine on pancreatic-lipase/colipase-catalysed triacylglycerol hydrolysis and cholesterol transport from lipid emulsions to rat IEC-6 intestinal cells

The IEC-6 cells were plated in 24-well dishes at a density of 1.5×10^5 cells/well and then incubated with 190 μl of medium containing 3.80 nmol of [14 C]cholesterol and 282 nmol of [3 H]triolein, with egg phosphatidylcholine at the concentrations indicated. Pancreatic lipase was added at a concentration of 0.21 μM, and colipase was included at a lipase/colipase molar ratio of 3:2. Incubation was continued for 1 h at 37 °C. Cholesterol uptake by IEC-6 cells (upper panel) and triacylglycerol hydrolysis (lower panel) in the presence of pancreatic lipase and colipase (●) was compared with that observed in their absence (○). Error bars represent S.D. values from three separate experiments with triplicate determinations.

constant concentration of triolein (1.25 mg/ml; 1.41 mM) with various concentrations of phosphatidylcholine, ranging from 50 to 800 μg/ml (approx. 0.065–1.04 mM). Results showed that cholesterol transfer from the lipid emulsion to IEC-6 cells was minimal in the absence of pancreatic lipase, regardless of the lipid composition of the emulsions (Figure 3, upper panel). The addition of pancreatic lipase/colipase to the incubation medium resulted in the effective hydrolysis of the triacylglycerols in lipid emulsions containing low concentrations of phosphatidylcholine (Figure 3, lower panel), resulting in concomitant cholesterol transfer from these particles to the IEC-6 cells. Pancreatic lipase/colipase addition did not result in triacylglycerol hydrolysis or an increase in cholesterol transport when high concentrations of phosphatidylcholine were used to prepare the lipid emulsion substrate (Figure 3). The addition of high levels of pancreatic lipase and colipase failed to overcome the inhibitory effects of phosphatidylcholine on triacylglycerol hydrolysis and cholesterol transport to the intestinal cells (results not shown).

Figure 4 Effect of triolein on cholesterol transport to rat IEC-6 intestinal cells (upper panel) and lipid hydrolysis in lipid emulsions (lower panel)

The IEC-6 cells were plated in 24-well dishes at a density of 1.5×10^5 cells/well and then incubated with 190 μl of medium containing 3.80 nmol of [14 C]cholesterol, 200 μg/ml egg phosphatidylcholine and [3 H]triolein at the concentrations indicated. Cholesterol uptake by IEC-6 cells (upper panel) and triacylglycerol hydrolysis (lower panel) after a 1 h incubation period with 0.21 μM pancreatic lipase and 0.14 μM colipase (●), with 0.21 μM phospholipase A₂ (○) or with both pancreatic lipase/colipase and phospholipase A₂ (▲) were determined as described in the legend to Figure 1. Error bars represent S.D. values from three separate experiments with triplicate determinations.

Additional experiments were performed to evaluate the impact of varying the triacylglycerol concentration in lipid emulsions with a constant phosphatidylcholine concentration of 200 μg/ml on cholesterol transfer to IEC-6 cells. Results showed that pancreatic lipase/colipase was ineffective in stimulating cholesterol transport to IEC-6 cells from emulsions containing low levels of triolein (Figure 4, upper panel). This ineffectiveness was similar to the inability of pancreatic lipase/colipase to catalyse triacylglycerol hydrolysis in these particles (Figure 4, lower panel). Hydrolysis of the phospholipid on the surface of these emulsions by phospholipase A₂ significantly increased the ability of pancreatic lipase/colipase to hydrolyse the triacylglycerols in the core of these particles (Figure 4, lower panel), and resulted in the activation of cholesterol transport to the IEC-6 cells (Figure 4, upper panel). In contrast, the addition of the bile salt taurocholate in the absence of phospholipase A₂ increased cholesterol transport from these particles to the intestinal cells only slightly (Table 3). Interestingly, pancreatic lipase/colipase was capable of hydrolysing triacylglycerols in emulsions containing higher concentrations of triolein (Figure 4, lower panel),

Table 3 Effect of taurocholate on cellular cholesterol uptake from emulsions with low triolein concentrations

IEC-6 cells were plated in 24-well dishes at a density of 1.5×10^5 cells/well and then incubated for 1 h at 37 °C with 190 μ l of medium containing 3.43 nmol of [14 C]cholesterol, 800 μ g/ml egg phosphatidylcholine and 89.8 nmol of [3 H]triolein, in the presence or absence of 0.21 μ M pancreatic lipase/0.14 μ M colipase or 0.21 μ M pancreatic phospholipase A₂, with or without taurocholate at 5 mM. Cholesterol uptake by the IEC-6 cells was determined by liquid scintillation counting of the cell lysate. The data represent means $\pm$ S.D. from two separate experiments with triplicate determinations; * significantly different from no-taurocholate control at $P < 0.01$.

Lipolytic enzymes added	Cholesterol uptake (pmol/dish)	
	No taurocholate	5 mM Taurocholate
Pancreatic lipase/colipase	15.7 $\pm$ 1.9	39.5 $\pm$ 2.9*
Phospholipase A ₂	17.8 $\pm$ 9.3	79.5 $\pm$ 19.7*
Lipase/colipase + phospholipase A ₂	56.8 $\pm$ 2.8	146 $\pm$ 34.0*

Table 4 Effects of monoacylglycerol and fatty acids on cellular cholesterol uptake from lipid emulsions

IEC-6 cells were plated in 24-well dishes at a density of 1.5×10^5 cells/well and then incubated for 1 h at 37 °C with 190 μ l of medium containing 3.43 nmol of [14 C]cholesterol, 800 μ g/ml egg phosphatidylcholine and 226 nmol of triolein, in the presence or absence of monoacylglycerol and oleate at the concentrations indicated. Cholesterol uptake by the IEC-6 cells was determined by liquid scintillation counting of the cell lysate. The data represent means $\pm$ S.D. from two separate experiments with triplicate determinations; * significantly different from no-addition control at $P < 0.001$.

Additions		Cholesterol uptake (pmol/dish)
Monoacylglycerol (nmol)	Oleate (nmol)	
0	0	9.4 $\pm$ 1.5
17	34	33.6 $\pm$ 8.6*
34	68	61.0 $\pm$ 18.1*

resulting in increased cholesterol transport to the IEC-6 cells (Figure 4, upper panel). The addition of phospholipase A₂ and bile salt to the incubation medium augmented the induction of triacylglycerol hydrolysis and cholesterol transport by pancreatic lipase/colipase (Figure 4 and Table 3).

The requirement for lipid digestion prior to cholesterol transport from lipid emulsions to rat IEC-6 cells suggested that the lipolytic digestion products, i.e. monoacylglycerol and non-esterified fatty acids, may be important in mediating cholesterol uptake by intestinal cells. This possibility was examined by preparing the cholesterol substrate in emulsion particles containing various concentrations of monoacylglycerol and non-esterified fatty acids. Results showed that monoacylglycerol and non-esterified fatty acids increased cholesterol transport from lipid emulsion particles to the rat IEC-6 cells in a concentration-dependent manner, even in the absence of pancreatic lipase/colipase (Table 4). Interestingly, the level of cellular cholesterol uptake induced by monoacylglycerol and non-esterified fatty acids was similar to that observed with pancreatic-lipase/colipase-treated lipid emulsions (Figure 1 and Table 4).

DISCUSSION

This study utilized rat IEC-6 cells to document for the first time that cholesterol in phospholipid/triolein emulsions is not available for uptake by intestinal mucosal cells. Cellular uptake of

cholesterol from emulsions with a phospholipid/triacylglycerol molar ratio of < 0.3 could be stimulated by pancreatic lipase/colipase hydrolysis of the core neutral lipids. However, pancreatic lipase/colipase was ineffective in hydrolysing the triacylglycerols in emulsions with a phospholipid/triacylglycerol molar ratio of > 0.3 . As a consequence, pancreatic lipase/colipase was incapable of stimulating cholesterol transport from these emulsions to the intestinal cells. Under these conditions, the addition of phospholipase A₂, which catalysed the hydrolysis of phospholipids on the surface of the emulsions, was necessary prior to triacylglycerol hydrolysis by pancreatic lipase/colipase and cholesterol transfer to the IEC-6 cells. Taken together, these observations suggest that remodelling of the surface and core components in lipid emulsions is a required step before cholesterol can be transported from these particles to the intestinal cells. These *in vitro* data are consistent with the *in vivo* observations that inhibition of pancreatic lipase (Table 1) and/or phospholipase A₂ [19] resulted in a significant decrease in the absorption of dietary cholesterol through the gastrointestinal tract.

The requirement for phospholipid hydrolysis before cholesterol can be taken up by intestinal cells is not a surprising finding. Early studies showed that cholesterol output to the lymphatics was higher when duodenal infusion was performed with lipid emulsions containing low levels of phosphatidylcholine compared with that observed with higher levels of phospholipids [20]. In addition, human subjects receiving an intraduodenal infusion of phosphatidylcholine were also found to absorb cholesterol to a lower extent compared with subjects infused with the same amount of cholesterol and safflower oil with a similar level of fatty acids [21]. However, despite these observations, the precise mechanism by which a high phospholipid concentration results in reduced cholesterol absorption is not completely understood. One possibility is that the addition of phospholipid enhances the solubility of cholesterol in micelles and shifts the partition coefficient for cholesterol diffusion away from the cell membrane [20]. It is also possible that an increase in the phospholipid concentration may lead to an increased packing density of the surface component of the lipid emulsion, thereby reducing the accessibility of the dietary cholesterol in the lipid emulsion particles for uptake by the intestinal mucosal cells. The *in vivo* infusion experiments could not be used to test the latter hypothesis, due to the presence of both bile salt micelles and lipid emulsions in the intestinal lumen. Thus the effects on phospholipid emulsions could not be differentiated from the effects on micelles. The present study used an *in vitro* cell culture model and demonstrated that increasing the phospholipid concentration on the surface of the lipid emulsion reduced its capacity for cholesterol transport to intestinal cells. Moreover, the data revealed that the mechanism is related to the inhibition of hydrolysis of the core triacylglycerols in these lipid particles by pancreatic lipase/colipase. The results of the present study also showed that bile salt micelles were incapable of overcoming phospholipid inhibition of cellular cholesterol uptake from emulsion particles. These observations are consistent with results reported by Armand et al. [22], which showed that bile salt micelles were unable to alter lipid emulsion particle size and composition. More importantly, these results support the hypothesis that dietary cholesterol, which exists in the intestinal lumen in emulsion particles with phospholipids and triacylglycerols, may be absorbed via different mechanisms than is biliary cholesterol, which is primarily associated with bile salt mixed micelles [23].

It is important to note that, under the experimental conditions described here, cholesterol is not transported from lipid emulsion particles to the intestinal cells without prior hydrolysis of some

of the triacylglycerols in the core. The lipid composition of the emulsions used in these experiments ranged from a phospholipid/triacylglycerol molar ratio of 0.05 to one of 0.74. Thus a significant amount of the cholesterol is predicted to be located on the surface of the emulsion particles [24,25]. However, the cholesterol is not taken up by the IEC-6 cells. These results suggest that cholesterol transport to intestinal mucosal cells is not a simple process of cholesterol diffusion from the surface of the lipid carrier to the cell membrane, and that the structure and composition of the cholesterol carrier in the extracellular milieu is an important determinant of cholesterol uptake by intestinal mucosal cells.

The results of the present experiments also showed that hydrolysis of phospholipids on the surface of the lipid carrier by phospholipase A₂, which has been shown previously to stimulate intestinal cholesterol absorption from vesicular and micellar substrates [26,27], is not sufficient for intestinal cholesterol absorption from emulsified substrates (Figure 4). In contrast, minimal hydrolysis of the triacylglycerol in the core compartment is sufficient to induce significant cholesterol transport to the intestinal cells in culture. The data presented in Figure 4 clearly show that significant cholesterol transport could be achieved after < 1% of the triacylglycerol emulsions in the triolein-rich emulsion (4 mg/ml triolein) was hydrolysed by pancreatic lipase/colipase. In contrast, cholesterol in emulsions containing a lower concentration of triolein (1.25 mg/ml) was not significantly taken up by the IEC-6 cells without triacylglycerol hydrolysis. These data are interpreted to indicate that the triacylglycerol concentration in emulsions is not, in itself, the primary factor in determining intestinal cholesterol absorption from lipid emulsions. Taken together, the data are consistent with the hypothesis that products of triacylglycerol hydrolysis, namely monoacylglycerol and/or non-esterified fatty acids, are key determinants in mediating cholesterol transport to the intestinal cells. These lipolytic products may facilitate cholesterol transport by altering the structure and composition of the surface of these lipid carriers. Alternatively, the monoacylglycerol and non-esterified fatty acids generated from pancreatic-lipase/colipase-mediated triacylglycerol lipolysis may form micelles that serve as necessary intermediates for cholesterol transport to the intestinal cells. The results showing efficient lipase-independent cholesterol transport from emulsions containing monoacylglycerol and non-esterified fatty acids are consistent with this hypothesis. Regardless of the precise mechanism, the data in the present paper clearly document

that partial hydrolysis of the triacylglycerol into monoacylglycerol and non-esterified fatty acids is required for dietary cholesterol that is transported as emulsified substrates to be absorbed by intestinal cells. The inhibition of triacylglycerol digestion will thus impact on the absorption efficiency of dietary cholesterol, in addition to its well-documented effect on fat absorption.

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