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New model to study cholesterol uptake in the human intestine in vitro

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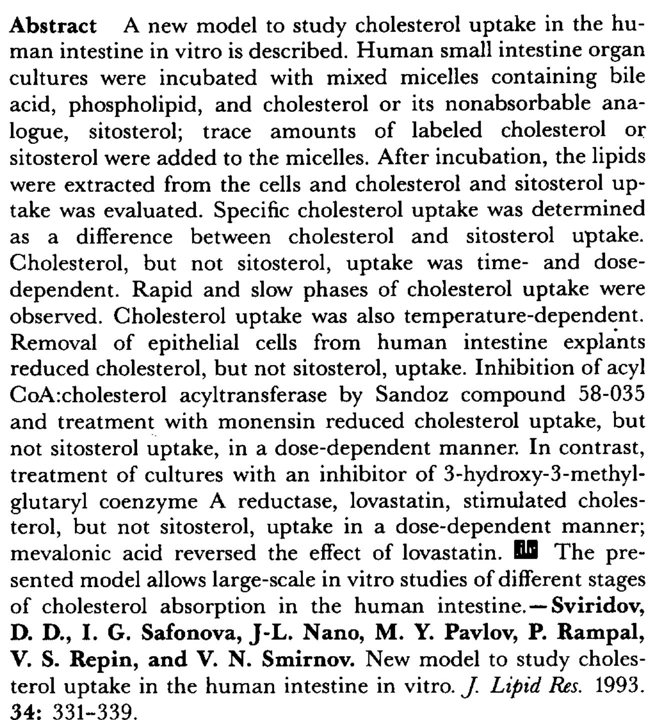

Abstract A new model to study cholesterol uptake in the human intestine in vitro is described. Human small intestine organ cultures were incubated with mixed micelles containing bile acid, phospholipid, and cholesterol or its nonabsorbable analogue, sitosterol; trace amounts of labeled cholesterol or sitosterol were added to the micelles. After incubation, the lipids were extracted from the cells and cholesterol and sitosterol uptake was evaluated. Specific cholesterol uptake was determined as a difference between cholesterol and sitosterol uptake. Cholesterol, but not sitosterol, uptake was time- and dose-dependent. Rapid and slow phases of cholesterol uptake were observed. Cholesterol uptake was also temperature-dependent. Removal of epithelial cells from human intestine explants reduced cholesterol, but not sitosterol, uptake. Inhibition of acyl CoA:cholesterol acyltransferase by Sandoz compound 58-035 and treatment with monensin reduced cholesterol uptake, but not sitosterol uptake, in a dose-dependent manner. In contrast, treatment of cultures with an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, lovastatin, stimulated cholesterol, but not sitosterol, uptake in a dose-dependent manner; mevalonic acid reversed the effect of lovastatin. The presented model allows large-scale in vitro studies of different stages of cholesterol absorption in the human intestine.—**Sviridov, D. D., I. G. Safonova, J-L. Nano, M. Y. Pavlov, P. Rampal, V. S. Repin, and V. N. Smirnov.** New model to study cholesterol uptake in the human intestine in vitro. *J. Lipid Res.* 1993. **34:** 331–339.

Supplementary key words cholesterol absorption • cholesterol transport • sitosterol • monensin • ACAT inhibitor • lovastatin • human intestine organ culture

Absorption of cholesterol in the gut is an important source of cholesterol in humans. It is estimated that consumption of dietary cholesterol is about 0.5–2.0 g/day (1–3) and contribution of endogeneous (mostly biliary) cholesterol is about 0.7–1.5 g/day (2, 3). Taking into consideration that the effectiveness of cholesterol absorption is about 50% (1, 2), this results in 0.6–1.8 g of cholesterol absorbed per day. De novo cholesterol synthesis contributes about 1.0 g/day (1), i.e., up to 60% of body total cholesterol input is due to dietary and biliary cholesterol absorption in the gut. In hypercholesterolemia, when cholesterol synthesis is suppressed, the portion of absorbed cholesterol in total cholesterol input could be even higher. Restriction of cholesterol consumption and/or absorption results in a significant reduction of plasma cholesterol content (for review see 4). Further studies of mechanisms of cholesterol absorption as well as a search for inhibitors of this process are important for the control of dyslipoproteinemias and prevention and treatment of atherosclerosis.

Absorption is usually defined as "the transfer of a substance from the lumen of gastrointestinal tract to either lymph or circulatory system" (5). According to this definition, cholesterol absorption is a multistep process that includes hydrolysis of cholesteryl esters in the gut lumen, formation of mixed micelles, transport of cholesterol into enterocytes, its reesterification, and assembling and secretion of lipoproteins (chylomicrons and nascent high density lipoproteins) (1, 3). While the process of cholesterol absorption is relatively well studied as a whole by physiologists, the data about individual steps of cholesterol absorption are still scanty and are mainly derived from utilization of inhibitors at certain stages, which are believed to be specific. Lack of knowledge about individual steps is due mainly to absence of a satisfactory model that allows study of cholesterol absorption using methods of cell biology and biochemistry.

In the present study we propose a new in vitro model that allows the study of individual steps of cholesterol absorption. The model uses human intestine organ culture. While many steps of cholesterol absorption can be studied using

Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; BSA, bovine serum albumin; DMPC, dimyristoylphosphatidylcholine; DMSO, dimethylsulfoxide; HBSS, Hanks' balanced salt solution; HDL, high density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein; MEM, minimum essential medium; PBS, phosphate-buffered saline.

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this model, in the present study we focused on one: transport of cholesterol from the gut lumen into enterocyte.

MATERIALS AND METHODS

Human intestine organ culture

A segment of middle jejunum was taken at autopsy from children (from several days old to 10 years of age) within 1 h of death. The predominant causes of death were congenital heart and brain defects. The study was approved by Scientific Council of Cardiology Research Center and by Medical Council of the 1st Moscow Children's Hospital where autopsies were performed. The intestinal segment was oriented longitudinally, thoroughly washed with cold Hanks' balanced salt solution (HBSS), and explants were cut off from the mucosa surface under a dissecting microscope. The size of explants was about 2 × 2 mm. Explants were placed in the wells of 24-well plates (Nunc, Denmark); the mucosa was oriented facing up, and 0.5 ml of medium was added just to cover the mucosal surface of the explant. The media consisted of Trowell T-8 medium containing 10% NCTC-135 medium, 2 mM L-glutamine, 2.5 μg/ml fungizone (all reagents from Flow, Irvine, U.K.), 100 units/ml polimixin B (GIBCO, Paisley, Scotland), and 2% Ultroser SF (Serva, Heidelberg, Germany). Medium was gassed with 5% CO_2, 95% O_2. The dishes were incubated for indicated periods of time at 37°C in a humidified atmosphere of 5% CO_2, 95% O_2. All manipulations were carried out under sterile conditions.

Preparation of mixed micelles

To prepare stock solutions, cholesterol (Sigma, St. Louis, MO) (final concentration 1 mM), β-sitosterol (Janssen, Belgium) (final concentration 1 mM), and dimyristoyl-phosphatidylcholine (DMPC) (Sigma) (final concentration 6 mM) were dissolved in chloroform-methanol 2:1 (v/v) mixture. The final cholesterol solution contained 40 μl of stock cholesterol solution, 400 μl of stock DMPC solution, and 0.8 μCi of [4-^{14}C]cholesterol (Amersham, Bucks, U.K., sp act 50-60 mCi/mmol) in 4 ml of chloroform-methanol mixture. The solvent was evaporated, 4 ml of complete culture media containing 6.5 mM sodium taurodeoxycholate (Sigma) was added, and the mixture was vigorously shaken for 2 h at room temperature. The resulting solution was sterilized by filtration and aliquots were counted to determine the actual radioactivity and sterol concentration. The final sitosterol solution was prepared as described above, but stock β-sitosterol solution and β-[4-^{14}C]sitosterol (Amersham, sp act 50-60 mCi/mmol) were substituted for the stock cholesterol solution and labeled cholesterol, respectively.

Lovastatin (Merck, Sharp & Dohme, Rahway, NJ) was prepared as a 10 mM solution in dimethylsulfoxide (DMSO). Sandoz compound 58-035 (Sandoz, Switzerland) was prepared at 13 mM in DMSO; monensin (Sigma) was prepared at 5 mM in DMSO; mevalonic acid (lactone, Sigma) was prepared at 0.1 M in MEM prior to the experiment.

Cholesterol uptake assay

Human intestine explants were washed and cholesterol- or sitosterol-containing micelles were added to parallel incubations in the serum-free media. Final sterol concentrations were 2 μM and specific activities were 0.02 μCi/ml. Samples were incubated for the indicated periods of time at 37°C. After incubation explants were washed with complete medium, removed, and weighed. Lipids from explants were extracted according to Folch, Lees, and Sloane Stanley (6) and radioactivity was counted using a dioxane-based scintillation cocktail and a "Rack-beta" beta-counter (LKB, Bromma, Sweden).

To calculate the amount of cholesterol specifically taken up, the amount of sitosterol taken up was substracted from the amount of cholesterol taken up. Results are expressed as μmol of sterol per g of tissue (wet weight).

Cholesterol esterification and synthesis assays

To determine the rate of cholesterol esterification, organ culture of human small intestine was incubated with [^{14}C]oleate-bovine serum albumin (BSA) complex for 2 h at 37°C in an atmosphere of 5% CO_2, 95% O_2. To prepare the [^{14}C]oleate-BSA complex, 100 μCi [1-^{14}C]oleic acid (Amersham, sp act 60 mCi/mmol) in toluene was mixed with 1.4 mg of KOH and the toluene was evaporated. Dulbecco phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+} (1.5 ml) containing 4.24 mg BSA (essentially fatty acid-free, Sigma) was added and the mixture was shaken vigorously. The complex was stored at −20°C; 50 μl of this solution was added to each explant (final concentration approximately 5 μCi/ml). After incubation, explants were washed and weighed and lipids were extracted according to Folch et al. (6). After evaporation of the solvent, lipids were dissolved in 50 μl chloroform-methanol 2:1 (v/v). Cholesteryl esters were isolated by thin-layer chromatography on Kiselgel plates (Merck, Darmstadt, Germany) developed in petroleum ether-ethyl ester-acetic acid 85:15:1 (v/v/v) and corresponding spots were counted.

Determination of the rate of cholesterol synthesis was performed as described in a previous study (7).

All experiments were performed in triplicate or quadriplicate and reproduced at least twice. Means ± standard errors of mean (SEM) are presented at the figures. Statistical significances were calculated according to a two-tailed Student's *t*-test.

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RESULTS

Organ culture of human small intestine taken at autopsy was cultivated under conditions previously described for the intestine biopsy organ culture (7). The micrograph of the cross-section of the intestinal explant after an 8-h incubation under the conditions of organ culture is shown in **Fig. 1.** No signs of cell dystrophy or loosening of mucosal epithelium were found during this time. Morphological examination of explants after different times of cultivation showed that they remained morphologically unchanged for at least 36 h; however, all experiments lasted for no longer than 6 h. In addition to anatomical studies, the rate of cholesterol synthesis by explants after different times of incubation was evaluated. It was demonstrated that the time-course of [^{14}C]acetate incorporation into cholesterol by human small intestine organ culture was essentially linear (**Fig. 2**), and the rate of cholesterol synthesis was constant for at least 8 h ($2.25 \pm 0.31 \times 10^5$ dpm/g tissue per h).

Incubation of explants with cholesterol-containing micelles should result in specific cholesterol uptake as well as in nonspecific adsorption of micelles on damaged lateral and basal surfaces of explants, and, possibly, in nonspecific sterol uptake. To discriminate between specific and nonspecific processes, uptake of cholesterol was compared with the uptake of sitosterol, presented in the same type of micelles. Sitosterol differs from cholesterol

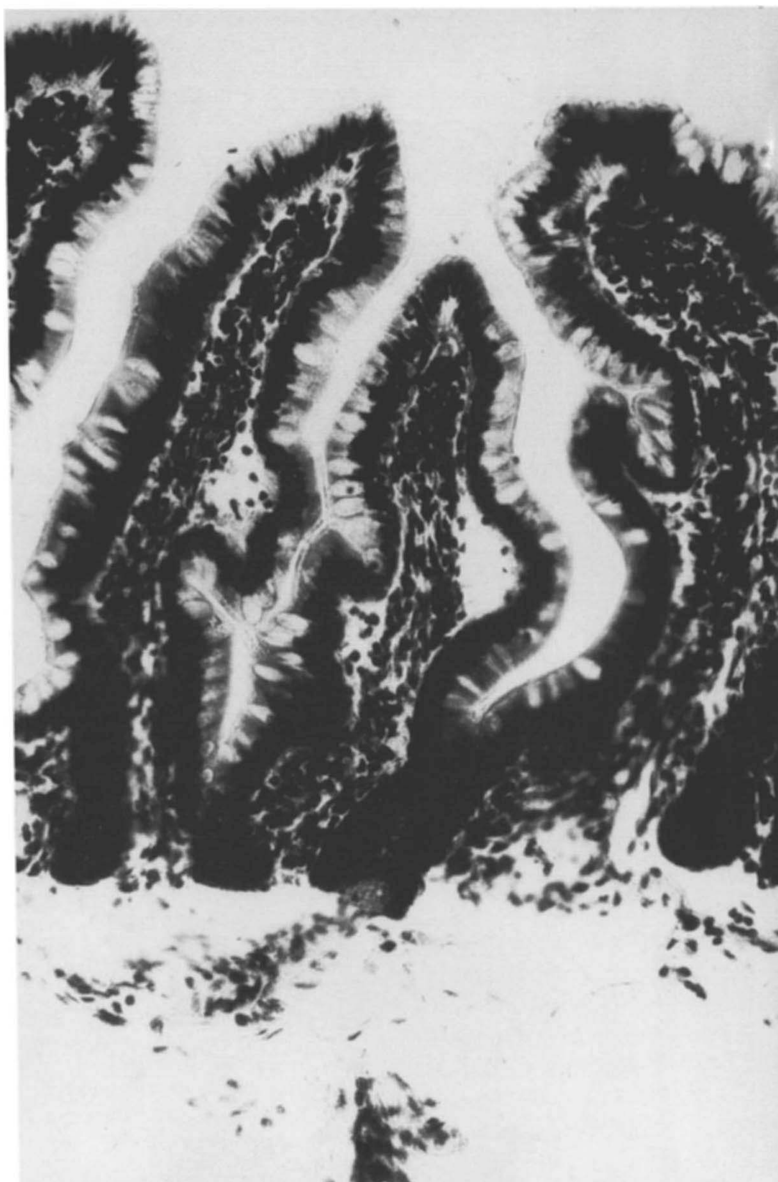

Fig. 1. Photomicrograph of the cross-section of human small intestine explant cultivated for 8 h under the conditions of organ culture (see Materials and Methods). Hematoxylin-eosin staining; magnification × 300.

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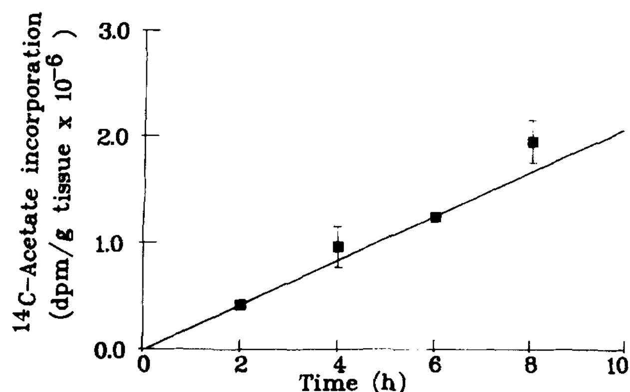

Fig. 2. Time-course of cholesterol synthesis by human small intestine organ culture. Human small intestine organ cultures were incubated with [^{14}C]acetate (final concentration 20 μCi/ml) for the indicated periods of time under conditions described in Materials and Methods. After incubation, cultures were washed, weighed, and saponified by incubation for 2 h at 100°C in 5 M KOH in 50% ethanol. Cholesterol was precipitated with digitonin. Each point represents mean ± SEM of quadruplicate determinations.

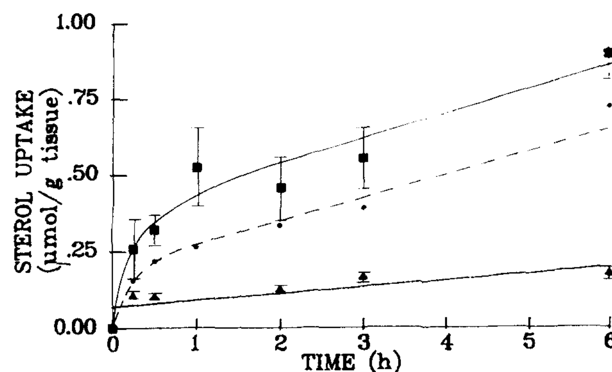

Fig. 3. Time-course of cholesterol uptake by human small intestine organ culture. Human small intestine organ cultures were incubated with [^{14}C]cholesterol- or [^{14}C]sitosterol-containing micelles (final sterol concentration 2 μM; final specific activity 0.02 μCi/ml) for the indicated periods of time under conditions described in Materials and Methods. After incubation, cultures were washed and lipids were extracted and counted. Specific cholesterol uptake was determined as the difference between labeled cholesterol and sitosterol uptake. Each point represents mean ± SEM of triplicate determinations; (■), [^{14}C]cholesterol uptake; (▲), [^{14}C]sitosterol uptake; (●), specific cholesterol uptake.

by an additional ethyl residue at C_{24}, but, in contrast to cholesterol, it is not absorbed in the gut (8, 9). Uptake of sitosterol was considered as nonspecific uptake, and specific cholesterol uptake was determined as a difference between the uptake of cholesterol and sitosterol.

Time courses of [^{14}C]cholesterol and [^{14}C]sitosterol uptake by human small intestine organ culture are presented in **Fig. 3.** Total and specific [^{14}C]cholesterol uptake rates were time-dependent. Rapid cholesterol uptake (about 260 nmol/g per h) occurred during the first 0.5-1 h of incubation and was followed by a decreased rate of cholesterol uptake and this rate (about 88 nmol/g per h) remained relatively constant during the next 5 h. In contrast, [^{14}C]sitosterol uptake showed little time-dependence; after initial uptake of 100 nmol/g of [^{14}C]sitosterol during first 15 min, the amount of [^{14}C]sitosterol in the explant increased by only 80 nmol/g during next 5.75 h, i.e., with an average rate 14 nmol/g per h. As a result, after 6 h of incubation, uptake of [^{14}C]sitosterol was about 20% of total [^{14}C]cholesterol uptake and 25% of specific cholesterol uptake.

The dose-dependencies of [^{14}C]cholesterol and [^{14}C]sitosterol uptake by human small intestine organ culture are presented in **Fig. 4.** Total and specific [^{14}C]cholesterol uptake rates were dose-dependent; however, saturation of cholesterol uptake was not reached up to a concentration of 4 μM. In contrast, [^{14}C]sitosterol uptake was not dose-dependent and remained constant in the tested concentration range. At maximum sterol concentration (4 μM), uptake of [^{14}C]sitosterol consisted 22% of total [^{14}C]cholesterol uptake and 28% of specific cholesterol uptake.

Several additional experiments were performed to study the properties of cholesterol uptake in the human small intestine organ culture. To study whether the intestinal epithelium plays a central role in the control of cholesterol uptake, [^{14}C]cholesterol and [^{14}C]sitosterol uptake were evaluated in the intact explants and in the explants where epithelium was removed by scraping. Microscopic examination showed that scraping removes 70-80% of epithelial cells. It was demonstrated that [^{14}C]sitosterol uptake increased by 17% ($P < 0.05$) while [^{14}C]cholesterol uptake fell by 46% ($P < 0.001$) (**Table 1**). As a result, specific cholesterol uptake in the explants without epithelial cells was only 19% of that in the intact explants.

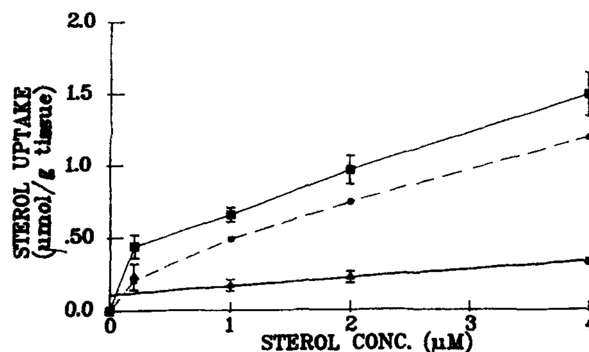

Fig. 4. Dose-dependence of cholesterol uptake by human small intestine organ culture. Human small intestine organ cultures were incubated with indicated concentrations of [^{14}C]cholesterol- or [^{14}C]sitosterol-containing micelles for 2 h under the conditions described in Materials and Methods. After incubation, cultures were washed and lipids were extracted and counted. Specific cholesterol uptake was determined as the difference between labeled cholesterol and sitosterol uptake. Each point represents mean ± SEM of triplicate determinations; (■), [^{14}C]cholesterol uptake; (▲), [^{14}C]sitosterol uptake; (●), specific cholesterol uptake.

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TABLE 1. Properties of cholesterol uptake in the organ culture of human small intestine

Condition	[14C]Cholesterol Uptake	[14C]Sitosterol Uptake	Specific Cholesterol Uptake
		μmol/g	
A: Control	1.33 ± 0.09	0.47 ± 0.01	0.86 ± 0.05
B: Epithelium removed	0.71 ± 0.09[a]	0.55 ± 0.03[b]	0.16 ± 0.04[a]
C: 4°C	0.58 ± 0.07[a]	0.28 ± 0.02[b]	0.30 ± 0.03[a]
D: Monensin, 25 μM	1.07 ± 0.05[b]	0.52 ± 0.05	0.55 ± 0.04[b]

Human small intestine organ culture was incubated with [14C]cholesterol- or [14C]sitosterol-containing micelles (final sterol concentration 2 μM) for 2 h at 37°C in the atmosphere 5% CO_2, 95% O_2. Changes made in the incubations were: A) no changes; B) epithelium was removed by scraping prior to incubation; C) incubation was performed at 4°C instead of 37°C; D) cultures were preincubated with 25 μM monensin for 45 min prior to incubation. After incubation, explants were removed and weighed, and lipids were extracted and counted. Each value represents mean ± SEM of quadruplicate determinations. Specific cholesterol uptake was determined as the difference between [14C]cholesterol and [14C]sitosterol uptake.
[a] $P < 0.001$ (vs. control).
[b] $P < 0.05$ (vs. control).

In order to determine whether cholesterol uptake is an energy-dependent process, [14C]cholesterol and [14C]sitosterol uptake was measured at 4°C. At this low temperature [14C]cholesterol uptake decreased by 56% ($P < 0.001$) and [14C]sitosterol uptake decreased by 40% ($P < 0.05$) (Table 1). As a result, specific cholesterol uptake decreased by 65% ($P < 0.001$).

The necessary property of a model to study cholesterol uptake is an ability to respond to different stimuli that can regulate cholesterol absorption. Several types of stimuli were tested to study whether this model could respond to them. First, human intestine organ culture was preincubated with 25 μM monensin, an agent that disrupts the Golgi complex and blocks secretory and endocytic activity (10). This should prevent incorporation of absorbed cholesterol into lipoproteins and their secretion, and increase cholesterol concentration in the cells. It was demonstrated that [14C]cholesterol uptake by human intestine organ culture decreased by 20% ($P < 0.05$), while uptake of [14C]sitosterol increased slightly after treatment of explants with monensin for 45 min at 37°C (Table 1). As a result, specific cholesterol uptake decreased by 36% ($P < 0.05$).

Another stimulus that can affect cholesterol uptake is specific inhibition of acyl CoA:cholesterol acyltransferase (ACAT), an enzyme that catalyzes reesterification of cholesterol in enterocytes. Inhibition of ACAT results in an increase of intracellular free cholesterol level and affects many aspects of cholesterol and lipoprotein metabolism, e.g., down-regulates 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase (11) and low density lipoprotein (LDL) receptor (11) and up-regulates the high density lipoprotein (HDL) receptor (12). It was also demonstrated in in vivo experiments that inhibition of ACAT reduces cholesterol absorption in the gut (13). ACAT inhibitor, Sandoz compound 58-035, effectively inhibited [14C]oleic acid incorporation into cholesteryl esters in the human small intestine organ culture (**Fig. 5A**). The effect was pronounced at an inhibitor concentration of 10 μM and saturated at 40 μM with 70% inhibition of cholesterol esterification. Both total and specific [14C]cholesterol uptake were inhibited in a dose-dependent manner after preincubation of explants with ACAT inhibitor (Fig. 5B). Effects of 58-035 on cholesterol uptake and cholesterol esterification saturated at the same concentration of the inhibitor. Maximum 30% and 40% inhibition of, respectively, total and specific [14C]cholesterol uptake was observed. [14C]sitosterol uptake was not affected after preincubation of explants with ACAT inhibitor (Fig. 5B). A good positive correlation was observed between effects of the ACAT inhibitor on cholesterol esterification and uptake in the human small intestine explants ($r = 0.94$; $P < 0.001$).

Another compound that has an opposite effect on cell intracellular cholesterol content is the HMG-CoA reductase inhibitor, lovastatin. It was demonstrated in previous studies that lovastatin effectively inhibits cholesterol synthesis in a variety of cells in vitro (14) including human intestinal explants (7). Lovastatin has an effect opposite to that of ACAT inhibitors on the expression of LDL (15) and HDL (12) receptors; there are indications that the lovastatin analogue, simvastatin, can affect cholesterol absorption (16). Dose-dependence of the effect of lovastatin on cholesterol uptake in the human intestine organ culture is presented in **Fig. 6**. Both total and specific [14C]cholesterol uptake were stimulated in a dose-dependent manner after preincubation of human small intestine explants with lovastatin (Fig. 6). Total and specific [14C]cholesterol uptake were stimulated to a maximum of 80% and 180%, respectively; the effect was saturated at a lovastatin concentration of 0.02 nM. [14C]sitosterol uptake was not affected by lovastatin. There was a

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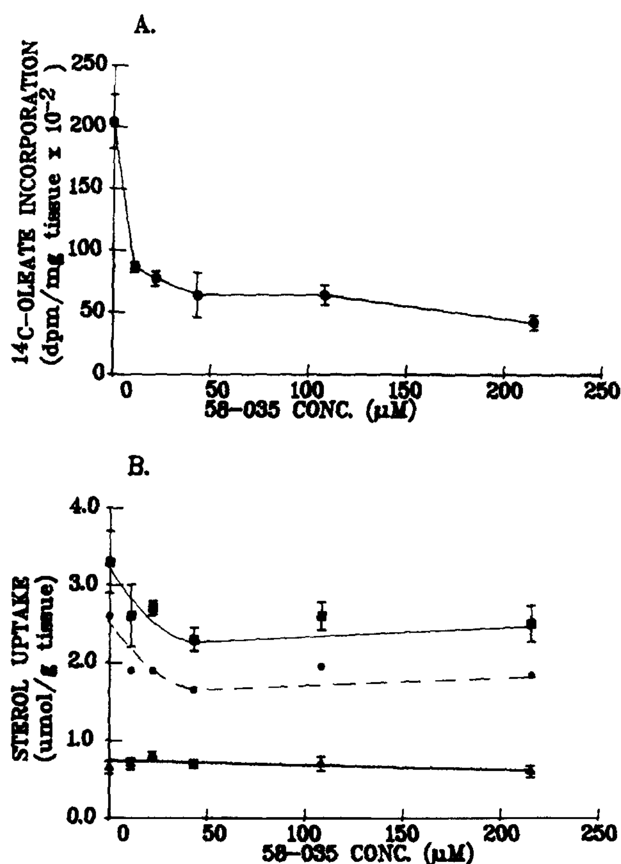

Fig. 5. Effect of Sandoz compound 58-035 on cholesterol esterification (A) and cholesterol uptake (B) by human small intestine organ cultures. A: Cultures were preincubated with indicated concentrations of Sandoz compound 58-035 for 3 h at 37°C. Then 50 μl of [^{14}C]oleic acid-BSA complex was added (approximate final activity 5 μCi/ml) and cultures were incubated for an additional 2 h at 37°C. After incubation, explants were washed; lipids were extracted and analyzed by thin-layer chromatography as described in Materials and Methods. Each point represents mean ± SEM of triplicate determinations. B: Cultures were preincubated with indicated concentrations of Sandoz compound 58-035 for 3 h at 37°C. Then [^{14}C]cholesterol- or [^{14}C]sitosterol-containing micelles (final sterol concentration 2 μM; final activity 0.02 μCi/ml) were added and cultures were incubated for an additional 2 h under the conditions described in Materials and Methods. After incubation, cultures were washed and lipids were extracted and counted. Specific cholesterol uptake was determined as the difference between labeled cholesterol and sitosterol uptake. Each point represents mean ± SEM of triplicate determinations; (■), [^{14}C]cholesterol uptake; (▲), [^{14}C]sitosterol uptake; (●), specific cholesterol uptake.

good negative correlation between effects of lovastatin on cholesterol synthesis and cholesterol uptake in the human intestine organ culture ($r = -0.72$, $P < 0.05$).

To test further whether the effect of lovastatin on cholesterol uptake is related to its ability to inhibit cholesterol synthesis, simultaneous addition of lovastatin and mevalonic acid, which is a product of the reaction inhibited by lovastatin, was studied. It was demonstrated that the addition of mevalonic acid completely reversed the effect of lovastatin on cholesterol uptake in the human intestine organ culture (**Table 2**). Mevalonic acid alone did not affect cholesterol uptake (Table 2).

DISCUSSION

Cholesterol absorption in both humans and laboratory animals has been intensively studied during last decade; however, a majority of these studies used physiological in vivo models (9, 13, 17-24). The principal scheme of these studies included feeding (17-22) or infusion (9, 13, 23, 24) of labeled cholesterol into gut with the subsequent analysis of the labeled cholesterol content in the lymph and feces. While these studies provided important information about absorption of different sterols (9, 17, 18, 20), impaired cholesterol absorption in certain diseases (17, 18) and phenotypes (19), the effect of diet composition on cholesterol absorption (22, 24) and attenuation of cholesterol absorption by pharmacological agents (13, 21), physiological models possess certain limitations, which makes necessary the search for a more simple in vitro model to study cholesterol absorption. Several in situ and in vitro models have been developed recently. Chijuwa and Linscheer (25) described an in situ model that includes perfusion of isolated loops of rat intestine, and Mayer et al. (26) and Reynier et al. (27) used everted intestinal sacs to study cholesterol absorption in rats. Use of pieces of intestine (28), isolated cells (8), and membrane preparations (8, 29, 30) to study cholesterol absorption has also been described. However, to our knowledge, no in vitro model to study cholesterol absorption in the human intestine has been developed.

In the present paper we describe a new model to study cholesterol uptake in the human intestine in vitro, using human small intestine organ culture. Cultures were incubated with mixed micelles that contained bile acid, phospholipid, and sterol (cholesterol or β-sitosterol) in

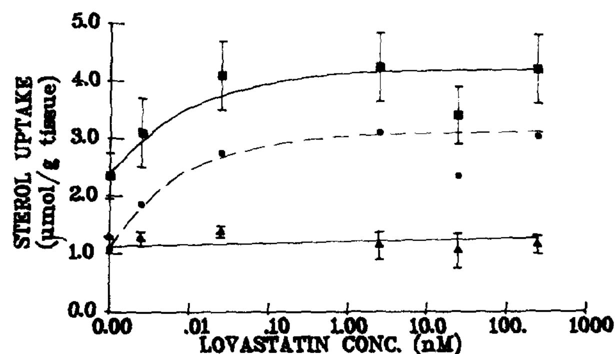

Fig. 6. Effect of lovastatin on cholesterol uptake in human intestine organ culture. Human small intestine organ cultures were preincubated with indicated concentrations of lovastatin for 3 h at 37°C. Then [^{14}C]cholesterol- or [^{14}C]sitosterol-containing micelles were added (final sterol concentration 2 μM; final activity 0.02 μCi/ml) and cultures were incubated for an additional 2 h under conditions described in Materials and Methods. After incubation, cultures were washed and lipids were extracted and counted. Specific cholesterol uptake was determined as the difference between labeled cholesterol and sitosterol uptake. Each point represents mean ± SEM of triplicate determinations; (■), [^{14}C]cholesterol uptake; (▲), [^{14}C]sitosterol uptake; (●), specific cholesterol uptake.

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TABLE 2. Effect of lovastatin, mevalonic acid, and their combination on cholesterol uptake in the organ culture of human small intestine

Addition	[14C]Cholesterol Uptake	[14C]Sitosterol Uptake	Specific Cholesterol Uptake
		μmol/g	
None	2.34 ± 0.35	1.28 ± 0.01	1.06 ± 0.1
Lovastatin	4.25 ± 0.09[a]	1.13 ± 0.20	3.12 ± 0.2[a]
Mevalonic acid	1.98 ± 0.13	0.80 ± 0.01	1.18 ± 0.09
Lovastatin and mevalonic acid	2.44 ± 0.37	1.31 ± 0.04	1.13 ± 0.18

Human small intestine explants were preincubated at 37°C for 2 h with no additions or with lovastatin (2 nM), or with mevalonic acid (lactone) (9 mM), or both. After preincubation, [14C]cholesterol- or [14C]sitosterol-containing micelles (final sterol concentration 2 μM) were added and cultures were incubated for an additional 2 h at 37°C in the atmosphere 5% CO_2, 95% O_2. After incubation, explants were removed and weighted, and lipids were extracted and counted. Each value represents mean ± SEM of quadruplicate determinations. Specific cholesterol uptake was determined as the difference between [14C]cholesterol and [14C]sitosterol uptake.
[a] $P < 0.01$ (vs. control).

proportions similar to those in the gut. Trace amounts of [14C]cholesterol or [14C]sitosterol were added to the micelles to monitor the movement of the sterols into the cells. A dual-isotope technique was not used, and cholesterol- and sitosterol-containing micelles were added to the parallel incubations because sitosterol can attenuate absorption of cholesterol (31). Specific cholesterol uptake was defined as a difference between uptake of labeled cholesterol, which represents both specific and nonspecific uptake, and that of its nonabsorbable analogue, sitosterol, which represents only nonspecific uptake.

Several lines of evidence suggest that uptake of cholesterol, but not sitosterol, is a specific process. Cholesterol uptake was time- and dose-dependent, while sitosterol uptake was not (Figs. 3 and 4). Rapid and slow phases of cholesterol uptake were observed. It could be assumed that the rapid phase corresponds to an initial influx of cholesterol into the cell, while the slow phase corresponds to cholesterol uptake accompanied with the efflux of excess cholesterol with synthesized lipoproteins (32). Temperature-dependence of cholesterol uptake indicates that it is an energy-dependent process (Table 1). However, because sitosterol uptake was also moderately decreased at low temperature, the effect of physicochemical changes of micelles or cell membranes on cholesterol uptake at low temperature cannot be excluded. Damaging of epithelial layer integrity resulted in a loss of the specific uptake of cholesterol, while uptake of sitosterol increased (Table 1), i.e., no other cells in the intestine organ culture can discriminate cholesterol from sitosterol.

Other evidence for the specificity of cholesterol uptake in the model system is regulation of the process. Treatment of human intestinal explants with monensin results in decreased cholesterol, but not sitosterol uptake. Two explanations of the effect of monensin are possible: *i)* specific transport of cholesterol may be related to the endocytic pathway that is inhibited by monensin (10), and *ii)* monensin inhibits secretion of lipoproteins (10) which could result in the increase of cellular cholesterol content and subsequent down-regulation of cholesterol uptake.

An effective way to decrease cholesterol absorption tested in several studies is an inhibition of cholesterol esterification (13, 33–35). As free cholesterol has limited ability to be incorporated into chylomicrons and then secreted, as compared to cholesteryl esters, inhibition of ACAT results in an increase of intracellular free cholesterol content and down-regulation of cholesterol absorption. It was demonstrated in this model that treatment of cells with ACAT inhibitor, Sandoz compound 58-035, results in inhibition of both cholesterol esterification and cholesterol uptake (Fig. 5). These data are in accordance with other studies (13, 33).

Another pharmacological agent tested in our study was lovastatin, an inhibitor of cholesterol biosynthesis. Inhibition of cholesterol biosynthesis results in a decrease of intracellular free cholesterol content, and, if cholesterol uptake responds to it, should have an effect opposite to that of the ACAT inhibitor. Indeed, inhibition of cholesterol biosynthesis results in a dose-dependent stimulation of cholesterol uptake by human intestine explants. This is in contrast to data of Ishida et al. (16), who demonstrated that inhibition of cholesterol biosynthesis had no effect or decreased cholesterol absorption in rabbits in vivo.

In the present study the model was used predominantly for the investigation of only one stage of cholesterol absorption: transport of cholesterol from gut lumen into enterocytes. However, possible applications of this model are not limited to this stage only. Investigation of the effect of ACAT and HMG-CoA reductase inhibitors on cholesterol uptake gives some insight on the metabolism of absorbed cholesterol in the enterocytes; secretion of lipoproteins by human intestine organ culture was studied in our previous study (32). Certain limitations of this model should also be mentioned. Thus, the effect of plasma constituents (e.g., hormones and lipoproteins) and drugs and nutritives that are poorly soluble in the culture media can

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hardly be studied in this model. Another disadvantage is that the duration of experiments is limited.

In addition to human intestine organ culture taken at autopsy, biopsy specimens taken at routine endoscopy examination were also used to study cholesterol uptake in pathological situations. Preliminary results indicate that, with respect to cholesterol uptake, hypo- and hyperresponders can be found among healthy subjects (I. G. Safonova and D. D. Sviridov, unpublished observation).

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