Dietary cholesterol is secreted in intestinally derived chylomicrons during several subsequent postprandial phases in healthy humans[1,2]

Gabrielle Beaumier-Gallon, Christophe Dubois, Michelle Senft, Marie-France Vergnes, Anne-Marie Pauli, Henri Portugal, and Denis Lairon

ABSTRACT

Background: The process of intestinal absorption and chylomicron resecretion of dietary cholesterol in humans is poorly understood.

Objective: The present study aimed to test the hypothesis that dietary cholesterol ingested during a given meal is resecreted into chylomicrons (and plasma) during several subsequent postprandial periods.

Design: Seven healthy subjects ingested 3 comparable mixed test meals (at 0, 8, and 24 h) containing a given amount of fat (49 g) and cholesterol (157 mg); blood samples were taken 3 and 6 h after each test meal and 48 and 72 h after the beginning of the experiment. Heptadeuterated dietary cholesterol was present in the first test meal only, enabling its specific determination with use of gas chromatography–mass spectrometry. Chylomicrons, LDL, and HDL were isolated and lipids were quantified.

Results: In apolipoprotein B-48–containing chylomicrons, deuterated cholesterol concentrations were moderate after the first meal (1.3×10^{-4} mmol/L), reached a maximum after the second meal (2.4×10^{-4} mmol/L), and were still elevated after the third meal (1.7×10^{-4} mmol/L). In plasma, LDL and HDL cholesterol enrichment in deuterated cholesterol was lower than in chylomicrons and plateaued after 24–48 h. Estimates of newly secreted exogenous deuterated cholesterol in chylomicrons indicate that 30.7%, 55.2%, and 14.1% of the total was secreted after the first, second, and third meals, respectively.

Conclusion: Ingested dietary cholesterol is secreted by the small intestine in chylomicrons into the circulation during ≥3 subsequent postprandial periods in healthy humans. This likely results from a complex multistep intestinal processing of cholesterol with dietary fat as a driving force. *Am J Clin Nutr* 2001;73:870–7.

KEY WORDS Lipid, digestion, absorption, chylomicron, postprandial metabolism, men, heptadeuterated cholesterol

INTRODUCTION

Studies in humans and nonhuman primates showed that diets rich in cholesterol, at least among responders and hyperresponders, lead to increased concentrations of fasting plasma and LDL cholesterol and presumably to susceptibility to coronary artery disease (1–3). There is evidence that plasma and LDL-cholesterol concentrations in humans are linked to intestinal absorption of cholesterol (4) and of a potential role of individual susceptibility based on different genetic backgrounds. Despite the fact that the overall extent of exogenous cholesterol absorption is low in humans ($\bar{x}$: ≈55%; range: 30–80%) (5), small variations in absorption can have metabolic effects (6).

The complexity of this matter, especially in the postprandial state (7), is due partly to the existence of 2 main sources of cholesterol in the intestinal lumen: biliary cholesterol (1–2 g/d) and dietary cholesterol (0–1 g/d). These 2 sources compete for distribution in emulsified lipid droplets, dispersion in vesicles and mixed micelles, and uptake of cholesterol by the enterocyte brush border. The process of intestinal absorption of cholesterol is likely a saturable one (8, 9), but it is not clear whether cholesterol moieties from the 2 origins are absorbed to the same velocity or to the same extent (10). Few data suggest that, in humans (11) and animals (12), resecretion of absorbed cholesterol into the lymph could be a slow process with limited influence from the amount of dietary cholesterol ingested during short-term follow-up. Along these lines, only limited changes in chylomicron lipid concentrations were observed 0–7 h postprandially in healthy humans after ingestion of high compared with low amounts of dietary cholesterol (13). Moreover, in a previous study (14) we observed, after ingestion by healthy subjects of a single fat-containing mixed meal, that the occurrence of ingested dietary deuterated cholesterol in chylomicrons is slow and progressive until 7 h postprandially. This indicated that resecretion of dietary cholesterol into chylomicrons is not concomitant, and thus eventually delayed, compared with that of bulk cholesterol and triacylglycerol. Thus, the mechanisms and regulations involved in dietary cholesterol absorption remain largely unknown (10, 15).

Therefore, the present study aimed to test for the first time our hypothesis (14) that dietary cholesterol ingested during a given meal is resecreted into chylomicrons (and then plasma) after several subsequent meals. Because dietary fat is a key factor involved in the regulation of the absorption of lipid moieties and

[1] From Unité 476, Human Nutrition and Lipids, INSERM (National Institute of Health and Medical Research), and Laboratoire de Chimie Analytique, Faculté de Pharmacie, Université de Méditerrannée, Marseille, France.

[2] Reprints not available. Address correspondence to D Lairon, Unité 476-INSERM, 18 Avenue Mozart, 13009 Marseille, France. E-mail: lairon@marseille.inserm.fr.

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cholesterol, of resecretion at the basal side of enterocytes (16), and of the occurrence of chylomicrons in the lymphatic (11) and circulatory (13) systems, 3 comparable test meals containing a fixed amount of fat (49 g) and cholesterol (157 mg) were ingested by healthy subjects within 24 h.

SUBJECTS AND METHODS

Subjects and experimental protocol

Seven healthy male volunteers aged 20–28 y participated in the study after giving written, informed consent to a protocol approved by the University Hospital Medical Ethics Committee. None of the subjects had any digestive or metabolic disease, as checked by medical history and fasting blood measurements. None of them had taken medications that interfere with lipid metabolism for several months before or during the experimental period. Fasting blood lipid, apolipoprotein (apo) A-1 and B, and insulin concentrations were within the reference ranges. All subjects had an *E3*/*E3* genotype. Dietary intakes were calculated from 3-d dietary records with use of GENI (Micro6, Nancy, France). Mean (±SD) total energy intake was 10713 ± 1676 kJ/d, with 40.8 ± 3.5% of energy as fat (along with 495 ± 30 mg/d as cholesterol), 15.6 ± 1.6% as protein, and 43.6 ± 4.0% as carbohydrate. The subjects had comparable dietary patterns and ingested a light, standardized dinner the evening before the first study day, as described previously (13), to limit possible interferences from the previous meal.

During the first 24-h study period, the subjects ingested 3 comparable fat- and cholesterol-containing mixed test meals. Time 0 marked the beginning of the experiment; intake of the first test meal began at ≈0800. The other 2 test meals were consumed 8 and 24 h later on the grounds that 2–3 meals (or their nutrient equivalent) are usually ingested during a 24-h period. The exact meal timing was a compromise between practical and physiologic constraints. The test meals were ingested within 20 min. At 14 h, the subjects ingested a light snack (2111.4 kJ) containing no fat or cholesterol to prevent hunger and hypoglycemia.

The 3 experimental test meals consisted of commercially available food (bread, pasta, tomato sauce, sunflower oil, margarine, egg white and egg yolk, yogurt, and water) and provided ≈3403 kJ with 11.7% protein, 54% fat, and 34.3% carbohydrate. Each meal provided 49 g fat and 157 mg cholesterol. In the first test meal only, 80 mg heptadeuterated [$^2H_7$] cholesterol, carefully dissolved in fat as described previously (14) and mixed with 77 mg unlabeled cholesterol (from egg yolk), was present. In contrast, in the second and third test meals, cholesterol was present as unlabeled cholesterol (egg yolk) only. Previous data from our laboratory (17) showed that most ingested egg yolk cholesterol is found in emulsified triacylglycerol droplets in the human stomach and duodenum contents.

Sampling and sample preparation

After the subjects fasted overnight, a baseline (time 0) blood sample was collected before the first test meal was consumed. Ten blood samples were then obtained 3, 6, 8, 11, 14, 24, 27, 30, 48, and 72 h after the first test meal was consumed. Thus, as already described in normolipidemic subjects with comparable intakes of fat and cholesterol (13, 18), we obtained for each test meal *1*) fasting blood, *2*) postprandial blood after 3 h (ie, triacylglycerol peak), and *3*) postabsorptive blood after 6 h.

Blood was collected in tubes containing EDTA, and a protease inhibitor cocktail was added as reported previously (18). Plasma was separated from whole blood by centrifugation (at 10°C for 10 min at 1547 × *g*). For all plasma samples, the chylomicron fraction (hereafter referred to as chylomicrons) containing intestinally derived chylomicrons plus large chylomicron remnants was isolated according to methods described previously (18, 19). Briefly, 1.5 mL plasma was underlayered with 1.5 mL (0.9%) NaCl and ultracentrifuged (at 15°C for 6 min at 35000 × *g*) in a TLX 100 ultracentrifuge with a 100.3 rotor (Beckman, Palo Alto, CA). Triacylglycerol-rich lipoproteins (TRLs), ie, the chylomicron fraction plus VLDL particles and remnants; LDL; and HDL were isolated by sequential flotation as adapted from methods described previously (18, 19). Plasma (1.5 mL) was underlayered with a 1.5-mL KBr solution [density (*d*) = 1.006 g/L] and ultracentrifuged at 412000 × *g* for 2 h and 40 min in the 100.3 rotor. After the floating TRLs were collected, the same procedure was used, but at *d* = 1.063 g/L for LDL and *d* = 1.12 g/L for HDL. From the TRL fraction, intestinally derived apo B-48– containing TRL particles (apo B-48 TRLs) were subsequently separated from apo B-100–containing TRLs (apo B-100 TRLs) by immunoaffinity chromatography with 2G8 monoclonal antibody (Mona, Moscow), which did not cross-react with apo B-48 (20), according to the procedure described by Cohn et al (21). Briefly, 2G8 monoclonal antibody was coupled to CNBr-activated Sepharose (1 mg/g gel) as recommended by Pharmacia (Orsay, France) and the coupled gel suspended in the phosphate-buffered saline (PBS; pH 7.4, 0.02% NaN_3) was divided into aliquots in 1.5-mL Eppendorf tubes (Eppendorf, Hamburg, Germany). After centrifugation at 130 × *g* for 5 min at 10°C, the supernatant fluid was removed from the tube and TRLs (≈300 μg protein) were added to ≈0.5 mL packed gel. After incubation for 1 h at room temperature and centrifugation at 130 × *g* for 5 min at 10°C, ≈0.5 mL supernatant fluid (unbound apo B-48 fraction) was collected. Then, 2 × 1 mL PBS was added for washing. Lipids were measured in ≈2.5 mL of a pooled apo B-48–containing fraction. As checked by sodium dodecyl sulfate–polyacrylamide gel electophoresis (SDS-PAGE) separation and quantification by densitometry (22), the unbound apo B-48 fraction contained only minor amounts of contaminant apo B-100 (5–8% of total apo B) in such samples obtained in normolipidemic subjects (23).

Analytic determinations

Plasma and lipoprotein triacylglycerols (24) and total cholesterol (25) were routinely measured by enzymatic procedure with kits purchased from BioMerieux (Marcy l'Etoile, France) and spectrophotometry with 96-well microplates (iEMS Reader, Labsystem, Cergy-Pontoise, France). Lipoprotein fraction purity was assayed by isoelectrofocusing and SDS-PAGE electrophoresis by using a Phast System apparatus (Pharmacia). Apo E genotyping was performed according to the method of Hixson and Vernier (26).

[$^2H_7$]Cholesterol (98% total enrichment) was supplied by Eurisotop (St Aubin, France), ergosterol was supplied by Fluka (Buchs, Switzerland), and cholesterol and epicoprostanol were supplied by Sigma (St Quentin-Fallavier, France).

Total [$^2H_7$]cholesterol and cholesterol were measured in a single run with an improved gas chromatography–mass spectometric method involving 2 internal standards (epicoprostanol and ergosterol) and with use of an HP 5890 series II gas chromatograph

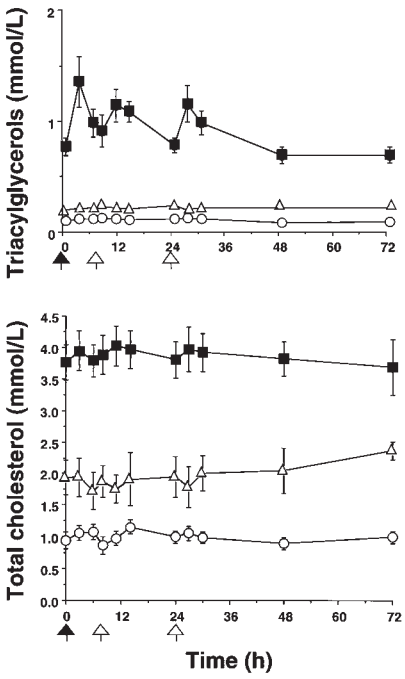

FIGURE 1. Mean (±SEM) changes over time in concentrations of plasma (■), LDL (△), and HDL (○) triacylglycerols and total cholesterol. After an overnight fast, the subjects ingested 3 comparable test meals (with 49 g fat and 157 mg cholesterol) at 0, 8, and 24 h (arrows); blood samples were taken 3 and 6 h after each meal. additional samples were taken at 48 and 72 h. The first test meal was the only source of [$^2H_7$]dietary cholesterol (filled arrow) and the second and the third meals contained unlabeled cholesterol only (open arrows). $n = 7$.

interfaced to a HP 5972 A mass spectrometer as described previously (27). Aliquots (200 μL) of serum and lipoprotein fraction samples were added together with one internal standard and a hydrolysis reagent to glass-stoppered test tubes and incubated at 70°C for 1 h. After being cooled, the second internal standard, distilled water, and hexane were added. After begin vortex mixed and centrifuged at 1500 × *g* for 10 min at room temperature, the supernatant fluid was evaporated to dryness. Next, a derivatizing reagent (bistrimethylsilyltrifluoroacetamide and chlorotrimethylsilane; Sigma) was added and left for 1 h at room temperature. After hexane was added and mixed, 1 μL of the extract was injected on the gas chromagographic column. Single ion monitoring was performed on the following fragments: free [$^2H_7$] cholesterol, mass-to-charge ratio (*m/z*) = 336; epicoprostanol, *m/z* = 370; endogenous free cholesterol, *m/z* = 329; and ergosterol, *m/z* = 363. Free [$^2H_7$]cholesterol initially present in the sample was quantified essentially by using the same method but without saponification as reported (28). Values obtained from chylomicrons prepared either as apo B-48 TRLs (immunoaffinity method) or as floating large TRL particles (ultracentrifigation method) were not corrected for recovery.

Calculations

Total and free [$^2H_7$]cholesterol and cholesterol concentrations in plasma and lipoprotein fractions were calculated by dividing the *m/z* values of the time-dependent evolution by the *m/z* values of the internal standards (336/370 and 329/363, respectively). The equations used were reported in detail previously (27). Esterified [$^2H_7$]cholesterol and esterified endogenous cholesterol concentrations were calculated from total and free moieties. The isotopic enrichments of cholesterol were calculated as the tracer/tracer + tracee ratios.

According to human data obtained by other researchers (29), labeled cholesterol in plasma is secreted into bile after a lag time of ≈6 h. This means that after the first test meal period (6 h) and up to the last time point (72 h), [$^2H_7$]cholesterol secreted in the form of intestinally derived TRLs has 3 origins: plasma cholesterol, bile cholesterol derived from plasma, and newly absorbed or secreted dietary cholesterol from the small intestine. The maximum isotopic enrichment of bile cholesterol was assumed to be (at the most) equal to plasma total cholesterol as measured herein at apparent steady state after 48 h. The contribution of plasma and bile to chylomicron [$^2H_7$]cholesterol is potentially overestimated this way, given that lower levels of enrichment were reported previously in bile cholesterol than in plasma cholesterol in humans with bile fistulae (29), but direct measurement of biliary cholesterol enrichment was not possible. This maximum (and somewhat overestimated for bile cholesterol) isotopic enrichment (29) was substracted from measured values of isotopic enrichment of apoB48-TRL cholesterol to calculate minimal actual values for apo B-48 TRLs newly secreted exogenous deuterated cholesterol at each time point after 6 h (isotopic enrichment × apo B-48 TRL cholesterol concentration) (*see* Discussion).

Statistics

Results are expressed as means ± SDs ($n = 7$). The 0–6, 8–14, and 24–30-h areas under the curve (AUCs) after each test meal were calculated by the trapezoidal method as described previously (18). The statistical significance ($P < 0.05$) of the differences observed between the 3 test-meal AUCs was assessed by using one-way analysis of variance for repeated values and Tukey's test. Correlation coefficients were obtained by linear regression analyses. STATVIEW PLUS (Abacus Concepts, Berkeley, CA) and SIGMASTAT for WINDOWS (Jandel Scientific, Erkrath, Germany) were used.

RESULTS

Postprandial changes in plasma and lipoprotein triacylglycerol and total cholesterol concentrations

As shown in **Figure 1**, plasma triacylglycerols increased 3 h after ingestion of each fat-containing test meal (up to 1.32 ± 0.6, 1.10 ± 0.4, and 1.12 ± 0.43 mmol/L, respectively); there were subsequent declines to baseline after 6–8 h. LDL- and HDL-trigacylglycerol concentrations showed only minor variations postprandially. Concomitantly (**Figure 2**), intestinally derived apo B-48 TRL triacylglycerol increased markedly 3 h after the 3 test meals (up to 0.60 ± 0.12, 0.40 ± 0.09, and 0.49 ± 0.09 mmol/L, respectively) over the initial fasting value (0.2 ± 0.04 mmol/L) and further declined to baseline values. Incremental changes in apo B-48 TRL triacylglycerols accounted for most of the changes in plasma triacylglycerols (76.9%, 76.6%, and 80.6%, respectively, after the 3 test meals). Overall, as shown in **Table 1**, the 0–6-h AUCs for plasma triacylglycerol or apo B-48 TRL triacylglycerol were comparable after the 3 test meals, as expected.

Concentrations of plasma total cholesterol (Figure 1) showed transient marginal changes over fasting values after each test

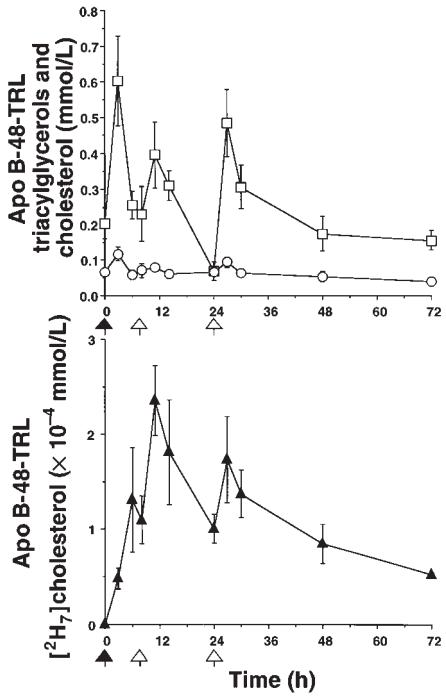

FIGURE 2. Mean (±SEM) changes over time in concentrations of apolipoprotein (apo) B-48–containing triacylglycerol-rich lipoprotein (TRL) triacylglycerol (□), total cholesterol (○), and [$^2H_7$]cholesterol (▲). After an overnight fast, the subjects ingested 3 comparable test meals (with 49 g fat and 157 mg cholesterol) at 0, 8, and 24 h (arrows); blood samples were taken 3 and 6 h after each meal. Additional samples were taken at 48 and 72 h. The first test meal was the only source of [$^2H_7$]cholesterol (filled arrow) and the second and the third meals contained unlabeled cholesterol only (open arrows). Intestinally derived apo B-48 TRLs were isolated by immunoaffinity and [$^2H_7$]cholesterol and cholesterol were quantified with use of gas chromatography–mass spectrometry (*see* Methods). $n = 7$.

meal ($\bar{x}$: 0.16 mmol/L), with negligible to moderate changes in LDL- or HDL-cholesterol concentrations postprandially. As observed for apo B-48 TRL triacylglycerols but with less amplitude, the concentrations of apo B-48 TRL total cholesterol temporarily increased 3 h after each test meal to 0.12 ± 0.03, 0.08 ± 0.02, and 0.10 ± 0.02 mmol/L, respectively, over baseline. The 0–6-h AUCs for plasma total cholesterol or apo B-48 TRL total cholesterol were comparable after the 3 test meals (Table 1).

Occurrence of dietary deuterated cholesterol in lipoproteins and plasma

As shown in Figure 2, dietary [$^2H_7$]cholesterol increased stepwise in the apo B-48 TRL fraction 3 and 6 h after the first test meal, which provided this deuterated molecule; the [$^2H_7$] cholesterol concentration was maximum (1.3 ± 0.550 × 10^{-4} mmol/L) at 6 h. Three hours after the second test meal (at 11 h), which provided unlabeled cholesterol only, a marked increase in apo B-48 TRL [$^2H_7$]cholesterol concentration was observed (2.35 ± 0.370 × 10^{-4} mmol/L); there was a subsequent decline 6 h after intake of the meal (at 14 h) and a more marked decline after 16 h (at 24 h). Three hours after the third test meal (at 27 h), the apo B-48 TRL [$^2H_7$]cholesterol concentration increased again to 1.73 ± 0.450 × 10^{-4} mmol/L and then

TABLE 1
Areas under the curve (AUC) for triacylglycerol and cholesterol responses after the 3 test meals[1]

0–6-h AUC	Meal 1	Meal 2	Meal 3
Plasma			
Triacylglycerols (mmol·h/L)	6.77 ± 1.21	5.88 ± 0.58	5.91 ± 0.64
Total cholesterol (mmol·h/L)	22.92 ± 1.98	23.64 ± 1.94	23.47 ± 2.04
[$^2H_7$]Cholesterol (× 10^{-3} mmol·h/L)	2.50 ± 0.46[a]	18.56 ± 1.68[b]	33.95 ± 2.89[c]
Apo B-48 TRL[2]			
Triacylglycerols (mmol·h/L)	2.49 ± 0.45	2.02 ± 0.34	2.21 ± 0.39
Total cholesterol (mmol·h/L)	0.53 ± 0.08	0.40 ± 0.05	0.44 ± 0.03
[$^2H_7$]Cholesterol (× 10^{-4} mmol·h/L)	3.48 ± 0.94[a]	11.42 ± 1.88[b]	8.76 ± 1.80[b]

[1] Values within a row with different superscript letters are significantly different, $P < 0.05$.
[2] Apolipoprotein B-48–containing triacylglycerol-rich lipoprotein particles.

declined after 6 h (at 30 h) to much lower values 48 and 72 h after the first test meal. Overall (Table 1), the 0–6-h AUCs for apo B 48 TRL [$^2H_7$]cholesterol concentrations after the second and third test meals were 11.4 and 8.8 mmol·h/L, ie, significantly higher than the value obtained after the first test meal (3.5 mmol·h/L). Comparable time courses of [$^2H_7$]cholesterol occurrence were obtained with the apo B-48 TRL isolated by immunoaffinity as given or with the chylomicron fraction obtained classically by ultracentrifugation (data not shown). Overall, ≈93% of apo B-48 TRL [$^2H_7$]cholesterol was in the form of chylomicrons as defined by ultracentrifugation.

The occurrence of dietary [$^2H_7$]cholesterol in plasma and cholesterol-rich lipoproteins is shown in **Figure 3**. A slow and progressive increase in the plasma concentration of [$^2H_7$]cholesterol was observed until a maximum value (6.26 ± 1.60 × 10^{-3} mmol/L) was reached 24–48 h after ingestion of the first test meal. As shown in Table 1, we observed a marked and stepwise

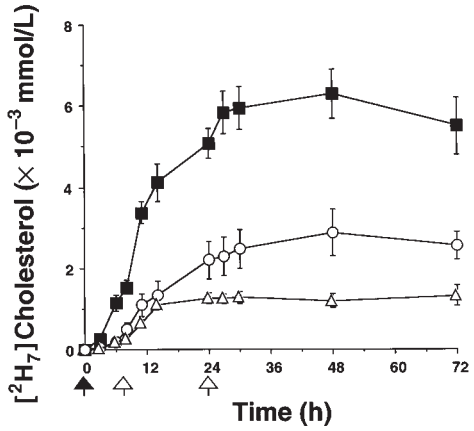

FIGURE 3. Mean (±SEM) changes over time in concentrations of [$^2H_7$] cholesterol in plasma (■), LDL (O), and HDL (△) particles. After an overnight fast, the subjects ingested 3 comparable test meals (with 49 g fat and 157 mg cholesterol) at 0, 8, and 24 h (arrows); blood samples were taken 3 and 6 h after each meal. Additional samples were taken at 48 and 72 h. The first test meal was the only source of [$^2H_7$]cholesterol (filled arrow) and the second and the third meals contained unlabeled cholesterol only (open arrows). [$^2H_7$]Cholesterol and cholesterol were quantified with use of gas chromatography–mass spectrometry (*see* Methods). $n = 7$.

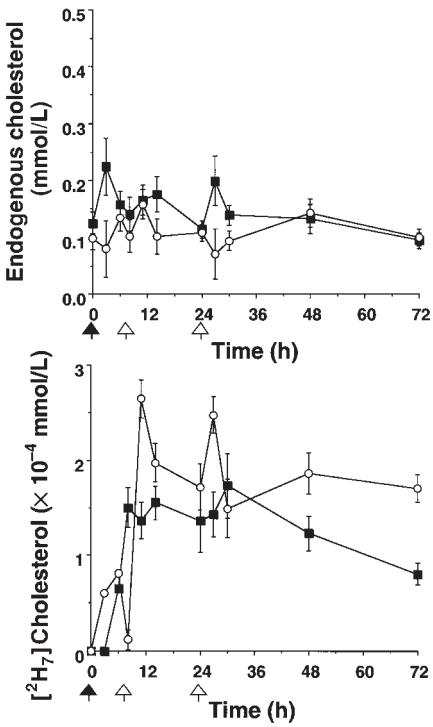

FIGURE 4. Mean (±SEM) changes over time in concentrations of endogenous and [$^2H_7$]cholesterol in chylomicrons as free (■) and esterified (O) cholesterol. After an overnight fast, the subjects ingested 3 comparable test meals (with 49 g fat and 157 mg cholesterol) at 0, 8, and 24 h (arrows); blood samples were taken 3 and 6 h after each meal. Additional samples were taken at 48 and 72 h. The first test meal was the only source of [$^2H_7$]cholesterol (filled arrow) and the second and the third meals contained unlabeled cholesterol only (open arrows). Intestinally derived apo B-48 TRLs were isolated by immunoaffinity and [$^2H_7$]cholesterol and cholesterol were quantified with use of gas chromatography–mass spectrometry (*see* Methods). $n = 7$.

increase in the 0–6-h AUCs for plasma [$^2H_7$]cholesterol concentration after the 3 subsequent meals. The increase in LDL [$^2H_7$]cholesterol over time (Figure 3) paralleled that of total plasma but was ≈3-fold lower (maximum: $2.85 \pm 0.580 \times 10^{-3}$ mmol/L). The increase in HDL [$^2H_7$]cholesterol was comparable with that in LDL until 24 h, when the concentration of [$^2H_7$]cholesterol in HDL reached its maximum value ($\approx 1.25 \pm 0.130 \times 10^{-3}$ mmol/L) and then plateaued.

From a particular set of gass chromatography–mass spectrometry measurements, we separately determined free cholesterol and cholesterol esters in the chylomicron fraction prepared by ultracentrifugation (**Figure 4**) and plasma (data not shown) for unlabeled (endogenous) and [$^2H_7$] moieties. Endogenous cholesterol peaked postprandially in chylomicrons mostly as free cholesterol (73%, 66%, and 74% after the first, second, and third test meals, respectively). Conversely, most chylomicron [$^2H_7$]cholesterol was in the form of esterified moeities postprandially (63–66%). After the second and third test meals (at 14 and 27 h), higher concentrations of [$^2H_7$]cholesterol esters than of free [$^2H_7$]cholesterol (2.64–2.47 compared with $1.36–1.430 \times 10^{-4}$ mmol/L) were measured in the chylomicron fraction, whereas similar values were found in the fasting state (1.71 ± 0.25 and $1.36 \pm 0.340 \times 10^{-4}$ mmol/L, respectively, at 24 h).

Tracer enrichment

The changes in the isotopic enrichment of total cholesterol with [$^2H_7$]cholesterol, the tracer/tracer + tracee ratio, are shown in **Figure 5**. In the plasma, the enrichment of cholesterol with [$^2H_7$]cholesterol was slow and progressive until a plateau was reached after 24–48 h (1.640×10^{-3}). Comparable maximum values were measured in LDL cholesterol (1.390×10^{-3} mmol/L) and HDL cholesterol (1.330×10^{-3}) (data not shown). Conversely, in the apo B-48 TRL fraction (Figure 5), an abrupt increase up to 2.250×10^{-3} in enrichment was observed 6 h after the first test meal. After a subsequent decline in the postabsorptive state, [$^2H_7$]cholesterol increased dramatically after the second test meal up to 3.00×10^{-3} and after the third test meal up to 1.840×10^{-3}. After 48 and 72 h, the enrichment of cholesterol with [$^2H_7$]cholesterol in the apo B-48 TRL fraction (1.60×10^{-3}) was comparable with that in whole plasma, indicating that an isotopic equilibrium was finally attained.

DISCUSSION

This study was performed to test the hypothesis that dietary cholesterol ingested during a given meal is secreted from the small intestine into the circulation during several subsequent postprandial periods. This hypothesis originated from earlier observations (11) and the recent observation made in healthy humans by our group (14) that, after a single meal, the appearance of dietary deuterated cholesterol in chylomicrons was delayed and not concomitant with bulk cholesterol and triacylglycerol present.

To achieve this goal, we used [$^2H_7$]cholesterol to trace ingested dietary cholesterol. The suitability of this method was established previously (14, 27, 30, 31). Given that reasonable amounts of fat (49 g) and cholesterol (157 mg) were ingested by the subjects as part of 3 full mixed meals over 24 h, a physiologic increase in postprandial triacylglycerols and chylomicrons

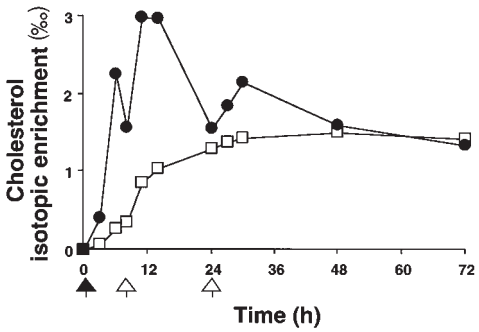

FIGURE 5. Mean changes over time in the isotopic enrichment of total cholesterol in plasma (□) and apolipoprotein B-48–containing triacylglycerol-rich lipoproteins (●). The isotopic enrichments were calculated as the tracer/tracer + tracee ratios with tracer as [$^2H_7$]cholesterol and tracee as unlabeled (endogenous) cholesterol. After an overnight fast, the subjects ingested 3 comparable test meals (with 49 g fat and 157 mg cholesterol) at 0, 8, and 24 h (arrows); blood samples were taken 3 and 6 h after each meal. Additional samples were taken at 48 and 72 h. The first test meal was the only source of [$^2H_7$]cholesterol (filled arrow) and the second and the third meals contained unlabeled cholesterol only (open arrows). Intestinally derived apo B-48 TRLs were isolated by immunoaffinity and [$^2H_7$]cholesterol and cholesterol were quantified with use of gas chromatography–mass spectrometry (*see* Methods). $n = 7$.

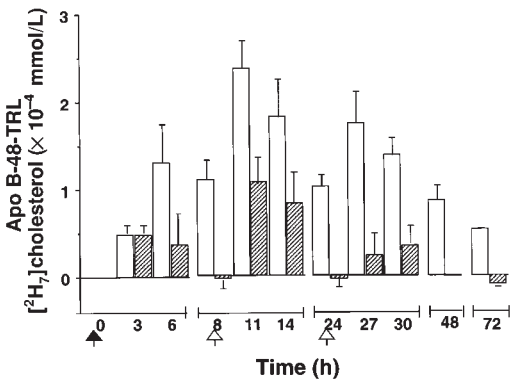

FIGURE 6. Mean (±SEM) measured and calculated changes in the concentration of [$^2H_7$] cholesterol in apolipoprotein (apo) B-48–containing triacylglycerol-rich lipoproteins (TRL). The total [$^2H_7$]cholesterol concentrations in apo B-48-TRLs (open bars) are those measured as shown in Figure 2 (bottom). The so-called newly secreted dietary [$^2H_7$]cholesterol (hatched bars) was calculated by substracting from the total measured [$^2H_7$]cholesterol the overestimated contribution of plasma and bile after 6 h (28) by using (maximum) isotopic enrichment values of plasma measured at steady state equilibrium at 48 h. After an overnight fast, the subjects ingested 3 comparable test meals (with 49 g fat and 157 mg cholesterol) at 0, 8, and 24 h (arrows); blood samples were taken 3 and 6 h after each meal. Additional samples were taken at 48 and 72 h. The first test meal was the only source of [$^2H_7$]cholesterol (filled arrow) and the second and the third meals contained unlabeled cholesterol only (open arrows). Intestinally derived apo B-48 TRLs were isolated by immunoaffinity and [$^2H_7$]cholesterol and cholesterol were quantified with use of gas chromatography–mass spectrometry (*see* Methods). $n = 7$.

was expected to occur (13, 14), which has direct relevance to healthy human beings.

As expected from previous postprandial studies (13, 14, 18), plasma triacylglycerols and chylomicron triacylglycerols increased markedly 3 h after the 3 comparable test meals and then returned to baseline values after 6–8 h, leading to overall comparable postprandial 0–6-h changes. Nevertheless, after the first test meal supplying [$^2H_7$]cholesterol, a progressive and limited occurrence of [$^2H_7$]cholesterol was observed in intestinally derived chylomicrons. These data closely agree with those obtained after a single meal (14), and establish the idea of a reduced bioavailability of dietary cholesterol in the short term in healthy humans.

The novel key observation made was that the enrichment of chylomicron cholesterol in [$^2H_7$]cholesterol from dietary origin was maximized after the second meal and still high after a third meal. The fact that the measured cholesterol enrichment in [$^2H_7$]cholesterol was higher in chylomicrons than in the plasma, LDL, or HDL supports the view that chylomicron particles are the initial carriers of dietary cholesterol into the circulation. Nevertheless, it appears that dietary cholesterol has a different time course of occurrence in chylomicrons than does bulk cholesterol or triacylglycerols, as illustrated by the strong correlation found between total cholesterol and triacylglycerols in apo B-48 TRL ($r = 0.74$, $P = 0.0007$) but not between [$^2H_7$]cholesterol and total cholesterol. At the postprandial peaks, the concentration of chylomicron [$^2H_7$]cholesterol esters was particularly elevated, whereas that of free [$^2H_7$]cholesterol was unchanged, indicating that cholesterol from the diet is incorporated mainly in chylomicrons as esterified moieties. This suggests that different pools can contribute to secretion of either free or esterified cholesterol in chylomicrons. When in the circulation, and given the short half-life of chylomicrons, particle surface free [$^2H_7$]cholesterol is rapidly transferred to other particles, preferentially nascent HDL (32), leading to the observed rapid input of [$^2H_7$]cholesterol in HDL. Most [$^2H_7$]chylomicron cholesterol, mainly as esterified [$^2H_7$]cholesterol in remnant particles, is taken up rapidly by the liver (and other tissues), leading to resecretion of [$^2H_7$]cholesterol–enriched VLDL, then LDL on lipolysis, as observed herein. After liver uptake of [$^2H_7$]cholesterol from both sources, secretion of free [$^2H_7$]cholesterol and bile acids occurs in bile (29).

The data we obtained after the first test meal and previously after a single meal (14) establish that most chylomicron cholesterol moieties are not of dietary origin in this case. The low contribution of dietary cholesterol to lymph cholesterol was shown previously in humans (11) and animals (12) in the short term. Thus, other sources of cholesterol in the small intestine and lymph must likely be involved, eg, biliary free cholesterol (≈1–2 g/d), which could eventually be more rapidly or efficiently absorbed than is dietary cholesterol (10), newly synthesized or stored intracellular cholesterol (16), or cholesterol from filtrated lipoproteins through enterocytes (33).

After the second and third test meals, which supplied no deuterated cholesterol but only unlabeled cholesterol, a marked increase in chylomicron [$^2H_7$]cholesterol was observed, likely resulting from a new input of deuterated cholesterol into chylomicrons. The fact that chylomicron cholesterol enrichment in deuterated cholesterol was higher than that in plasma or in LDL or HDL cholesterol and was somewhat lower after the third than after the second test meal indicates that not only endogenous (bile or plasma) [$^2H_7$] cholesterol is responsible for this further increase after the second and third meals. This new input of exogeneous [$^2H_7$]cholesterol was estimated more quantitatively (*see* Methods) by substracting from measured apo B-48 TRL [$^2H_7$]cholesterol values the maximum possible contribution by endogeneous plasma total cholesterol (29). The resulting values obtained for calculated newly secreted exogeneous [$^2H_7$]cholesterol in apo B-48 TRLs are shown in **Figure 6**. The data show that, after the second and third test meals, ≥46% and 13% of apo B-48 TRL [$^2H_7$]cholesterol, respectively, is still the newly secreted exogeneous type. Calculation of 0–6-h AUC for apo B-48 TRL cholesterol after each test meal showed that the second meal ($4.5 \pm 1.3 \times 10^{-4}$ mmol·h/L) induced a significantly higher secretion than did the first ($2.5 \pm 0.6 \times 10^{-4}$ mmol·h/L) and third ($1.1 \pm 0.5 \times 10^{-4}$ mmol·h/L) meals. On the basis of these AUC values, the first, second, and third test meals resulted in the secretion of 30.7%, 55.2%, and 14.1% of total secreted exogenous [$^2H_7$]cholesterol as apo B-48 TRLs over 3 subsequent postprandial periods, respectively. Given that apparent steady state equilibrium for plasma and chylomicron cholesterol isotopic enrichments was attained after 24–48 h, it is likely that only negligible amounts of ingested deuterated cholesterol are secreted after further meals. Nevertheless, one cannot rule out the possibility that meals with different compositions would alter to some extent the values calculated in this article.

On the whole, the present finding provides an experimental basis for explaining the progressive enrichment of human plasma with labeled dietary cholesterol observed until 24–48 h by many authors (10, 30, 31) and in this article, as well as the reported moderate effects of graded amounts of dietary cholesterol on postprandial TRLs after a single test meal (13).

The present data suggest that, under usual conditions in healthy subjects, secretion of dietary cholesterol from the small

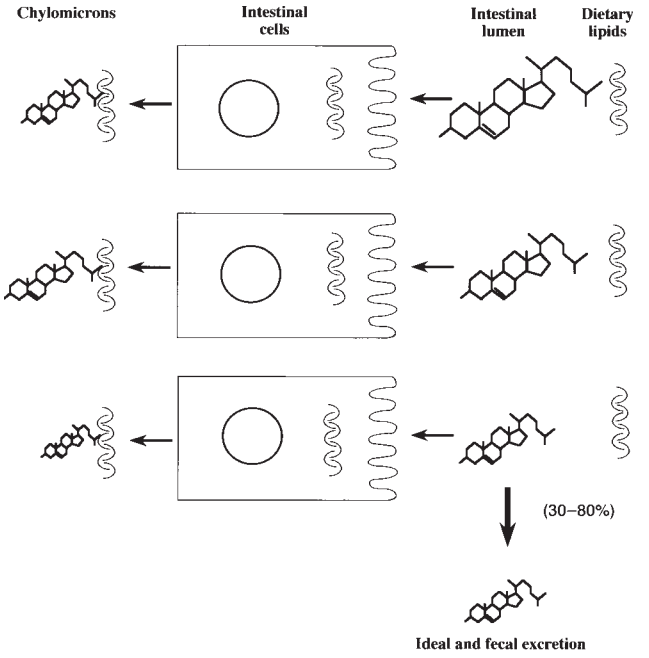

FIGURE 7. The waves model for the processing of dietary cholesterol in the human small intestine. Top: ingested dietary cholesterol is resecreted as chylomicrons to a low extent (about one-third of total resecreted) as driven by the first lipid wave (mainly fatty acids) resulting from digestion of a given meal containing fat and cholesterol. Middle: a second wave resulting from the next fat-containing meal generates resecretion by the small intestine of about one-half of the total dietary cholesterol resecreted. Bottom: a third lipid wave leads to the resecretion of the remaining absorbed cholesterol as chylomicrons. Given limited knowledge, it is not yet known whether this suggested mechanism operates at the apical border or at some site within the enterocyte controlling chylomicron cholesterol secretion, or both. The exact rates of cholesterol apical uptake by the small intestine during these subsequent steps is not known but the overall extent of absorption of dietary cholesterol is ≈55% (range: 30–80%) in humans (5), the remaining part being excreted in the feces. The molecular-structure symbol represents dietary cholesterol (molecule size reflects relative amount present). The wavy symbol represents a lipid wave, consisting of fatty acids or fatty acid–containing lipids, which are generated in the gut lumen via dietary lipid hydrolysis and then uptaken apically by the enterocyte, where it directs chylomicron assembly and further secretion at the basolateral side.

intestine into the circulation is a slow and multistep process driven by digestion, assimilation, and resecretion of dietary fats because a nonfat test meal does not generate chylomicron secretion (13, 18). Because our experimental design did not allow us to evaluate whether this delayed occurrence in chylomicrons was due to a delayed uptake or to resecretion of dietary cholesterol by the small intestine, further studies should be done to solve this question. Our observations might also support the possibility that cholesterol is absorbed further down the small intestine.

It was shown decades ago that exogenous cholesterol absorption is definitively low and variable in humans (5, 10), whereas that of dietary fat is usually almost total. This implies that different steps control the availability of cholesterol from the digestive tract, making the overall process relatively inefficient. In fact, cholesterol absorption is most likely a saturable process (8), but it is not well known how this process is controlled (10). The first important step is the competition between exogenous cholesterol and biliary cholesterol for distribution in emulsified droplets, unsoluble material, and dispersed structures, such as vesicles and mixed micelles in the intestinal lumen (17). The second step is the uptake by the enterocyte brush border membrane of cholesterol molecules carried by the dispersed structures. Nevertheless, previous (11, 14) and present data do suggest that dietary cholesterol is not preferentially or quickly incorporated into chylomicrons. The suggestion of a role of the SR-B1 scavenger receptor in cholesterol uptake by the intestinal mucosae (34) implicates a role of specific carrier proteins, such as the biliary anionic polypeptide fraction (35). Within the enterocyte, the different mechanisms involved in resecretion of absorbed cholesterol at the basal side are not fully understood. Nevertheless, a relevant observation made by using human intestine Caco-2 cells (16) is that plasma membrane cholesterol is preferentially secreted in TRLs, whereas newly absorbed cholesterol is not. This possible mechanism is fully consistent with the delayed appearance of dietary cholesterol in chylomicrons we observed in humans. Such a delayed resecretion into the circulation of the fat-soluble vitamin β-carotene (36), and triacylglycerols to a much lower extent (37), were also reported.

Our novel finding in healthy humans, along with some other pieces of available information, leads us to propose a new "waves model." As illustrated in **Figure 7**, the waves of secretion may reflect absorption or incorporation of absorbed cholesterol into chylomicrons, or both, given limited knowledge. This model proposes that fat ingested in a given meal will generate, under digestion and almost-complete absorption in the gut, a fatty acid flux (first wave), stimulating intestinal uptake of an unknown part of the cholesterol present and postprandial resecretion in chylomicrons of a minor part of absorbed cholesterol moieties only. A next fat-containing meal will generate a second wave, enabling further cholesterol uptake and noticeable resecretion in chylomicrons. Three succesive fatty acid waves seem necessary to allow resecretion in chylomicrons of ≈50% (range: 30–80%) of dietary cholesterol ingested from a given meal. Further studies are needed to precisely evaluate the relative importance of the different limiting steps potentially involved in this complex process.

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