High Cholesterol Intake Modifies Chylomicron Metabolism in Normolipidemic Young Men[1]

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ABSTRACT Whether the consumption of egg yolk, which has a very high cholesterol content without excess saturated fats, has deleterious effects on lipid metabolism is controversial. Absorbed dietary cholesterol enters the bloodstream as chylomicrons, but the effects of regular consumption of large amounts of cholesterol on the metabolism of this lipoprotein have not been explored even though the accumulation of chylomicron remnants is associated with coronary artery disease (CAD). We investigated the effects of high dietary cholesterol on chylomicron metabolism in normolipidemic, healthy young men. The plasma kinetics of a chylomicron-like emulsion, doubly-labeled with ^{14}C-cholesteryl ester (^{14}C-CE) and ^{3}H-triolein (^{3}H-TG) were assessed in 25 men (17–22 y old, BMI 24.1 ± 3.4 kg/m^2). One group ($n = 13$) consumed 174 ± 41 mg cholesterol/d and no egg yolk. The other group ($n = 12$) consumed 3 whole eggs/d for a total cholesterol intake of 804 ± 40 mg/d. The nutritional composition of diets was the same for both groups, including total lipids and saturated fat, which comprised 25 and 7%, respectively, of energy intake. Serum LDL and HDL cholesterol and apoprotein B concentrations were higher in the group consuming the high-cholesterol diet ($P < 0.05$), but serum triacylglycerol, apo AI, and lipoprotein (a) did not differ between the 2 groups. The fractional clearance rate (FCR) of the ^{14}C-CE emulsion, obtained by compartmental analysis, was 52% slower in the high-cholesterol than in the low-cholesterol group ($P < 0.001$); the ^{3}H-TG FCR did not differ between the groups. Finally, we concluded that high cholesterol intakes increase the residence time of chylomicron remnants, as indicated by the ^{14}C-CE kinetics, which may have undesirable effects related to the development of CAD. J. Nutr. 136: 971–976, 2006.

KEY WORDS: • *egg yolk* • *dietary cholesterol* • *chylomicron metabolism* • *lipid emulsion* • *young men*

In most foodstuffs, a high cholesterol content is found in association with a high saturated fat content. There are, however, a few remarkable exceptions such as egg yolk in which a low percentage of energy from saturated fat coexists with high amounts of cholesterol. Eggs are inexpensive and widely consumed; they are nutritionally rich due to their vitamin and protein content. It is, therefore, important to study egg yolk and the effects of isolated cholesterol consumption, apart from those of saturated fats, on plasma lipids. Although this topic has been discussed for decades, it continues to be one of most controversial issues in nutrition. Isolated dietary cholesterol has been described as being either deleterious (1–3) or without effect (4–7) on plasma lipid concentration and coronary artery disease (CAD)[3] incidence.

In contrast to the other lipoproteins that are routinely evaluated in clinical practice, one aspect that has remained unexplored is the effect of dietary cholesterol on the chylomicron metabolism. Chylomicrons are triacylglycerol-rich lipoproteins synthesized in the intestine; they carry absorbed dietary fats and cholesterol in the circulation. Because chylomicrons are non-steady-state lipoproteins and their concentration in the plasma depends on lipid absorption rates, it is very difficult to evaluate this metabolism. Chylomicrons share a common catabolic pathway with liver-produced VLDL (8). Like VLDL, chylomicron triacylglycerol is broken down into glycerol and fatty acids by lipoprotein lipase action on the capillary wall, triggered by apolipoprotein (apo) CII. The lipolysis products are then absorbed by body tissues, especially muscle and adipose tissue where these compounds are reesterified and stored (8,9). This pathway is fundamental for the organism's energetic economy because fat constitutes its greatest storage energy source. Once lipolysis takes place, the resulting smaller particles called chylomicron remnants unbind from the enzyme and return to the circulation. They are then sequestered into the space of Disse to be taken up by liver cells through various receptor mechanisms, mainly LDL receptors

[1] Supported by Programa de Apoio ao Desenvolvimento Científico da Faculdade de Ciências Farmacêuticas (FCF-UNESP), Araraquara, Brazil; Grant PADC 94/15-I, and by Fundação do Amparo à Pesquisa do Estado de São Paulo (FAPESP), São Paulo, Brazil. Grant FAPESP 99/01229-2.

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[3] Abbreviations used: apo, apolipoprotein; AUC, area under the curve; CAD, coronary artery disease; CE, cholesteryl ester; CHD, coronary heart disease; FCR, fractional clearance rate; HCD, high cholesterol diet HDL-C, HDL cholesterol; LCD, low cholesterol diet LDL-C, LDL cholesterol; LI, lipolysis Index; Lp(a), lipoprotein (a); LRP, receptor-related protein; NCEP, National Cholesterol Education Program; P:S, polyunsaturated:saturated fatty acid ratio; RT, residence time; TG, triacylglycerol.

Manuscript received 13 April 2005. Initial review completed 6 May 2005. Revision accepted 14 December 2005.

and receptor-related protein (LRP) (8–10). Apo E is the main ligand of chylomicron remnants to the hepatic receptors (11). Of all the lipoprotein classes, chylomicrons possess the fastest plasma clearance mechanism with a half-life of ~15–20 min (8,9). Accumulating evidence supports a relation between delayed chylomicron catabolism in the plasma and accelerated progression of atherosclerosis (12–15).

In this study, the effects of the consumption of high daily amounts of cholesterol on the chylomicron catabolism were evaluated in young men. For this purpose, we used a method for the determination of the plasma kinetics of chylomicron-like emulsions. This method offers a straightforward and integrated view of intravascular chylomicron metabolism. The emulsion is doubly-labeled with radioactive cholesteryl esters (^{14}C-CE) and triacylglycerols (^{3}H-TG). After i.v. injection into the subjects, determination of the plasma decay curve for the TG in the emulsion allows us to track the chylomicron lipolysis process. The CE curve, on the other hand, traces the remnant removal. Because chylomicrons and VLDL share a common intravascular catabolic pathway, we assumed that the method would also provide a glimpse of the overall triglyceride-rich lipoprotein metabolism.

SUBJECTS AND METHODS

Subjects. Male volunteers (n = 25) were selected from 200 students of the Agricultural Technical School in the city of Jau, São Paulo, Brazil. They were 17–22 y old with a BMI of 24.1 ± 3.4 kg/m^2. Inclusion criteria were serum total cholesterol concentration <5.43 mmol/L and LDL cholesterol (LDL-C) <3.62 mmol/L. Exclusion criteria were consumption of >10 cigarettes/d, weekly alcoholic intake >60 mL, or 700 mL of beer or 240 mL of wine; current use of prescription pharmaceuticals, and a sedentary life style. The Scientific and Ethics Committee of the Heart Institute of the Medical School Hospital (INCOR-HCFMUSP), University of São Paulo, approved the study, and informed written consent was obtained from each subject after the design and the objective of the study were explained.

Diets and dietary treatment. Meals were prepared using fresh ingredients following a cyclical menu composed of a variety of foods such as beef, chicken, fish, dairy products, fruits, green vegetables, cereals, legumes and desserts. Meal energy content was calculated for each subject taking into account the dietary history and physical activity of each participant, according to National Cholesterol Education Program (NCEP) guidelines (16). The composition of all diets related to the total energy content was 60% carbohydrate, 15% protein and 25% total lipid, with 7% saturated fatty acids and 18% monounsaturated and polyunsaturated fatty acids (1:1). The nutritional composition of the planned diets was evaluated using the software Diet Therapy, Brand-Brazil Company, version 1.0. Two types of diets, according to the cholesterol content, were prepared: *1*) a low-cholesterol diet (LCD), prepared as described above, plus the whites of 3 eggs/d; and *2*) a high-cholesterol diet (HCD), prepared as before, plus 3 whole eggs (white and yolk)/d.

All participants consumed the LCD or HCD for a 15-d period. Daily meals were supplied by the school cafeteria, including the weekends. A 15-d weighed food record was used to determine intakes of total energy and macronutrients. A digital scale was used to weigh the meals (Nutri Scale, TBW). Any leftovers were measured and subtracted from the record. Samples of all meals were analyzed chemically by AOAC methods (17), including the following: *1*) moisture (losses at 105°C), *2*) protein (Micro Kjeldahl), *3*) lipids (Soxhlet method), *4*) ash (fixed mineral residue method), and 5) Nifext fraction methodology. Dietary cholesterol and fiber were estimated using the Table of Brazilian Food Composition of the University of São Paulo, Brazil (18).

Total energy intake was estimated by the sum of macronutrients that were obtained by the 15-d weighed food record (**Table 1**), in which 1 g protein or carbohydrate is equal to 16.7 kJ and 1 g lipid is equal to 37.7 kJ.

TABLE 1

Energy and nutrients intake for the 15-d dietary treatment of the LCD and HCD groups[1,2]

	LCD		HCD	
Cholesterol, *mg/d*	174 ± 41		804 ± 40*	
Total fat,[3] *g/d*	105 ± 18	(25 ± 4)	111 ± 15	(24 ± 3)
Saturated fat, *g/d*	28 ± 4	(7 ± 1)	32 ± 5	(7 ± 1)
P:S ratio	2.2 ± 0.2		2.1 ± 0.2	
Carbohydrates, *g/d*	580 ± 43	(62 ± 2)	640 ± 33	(61 ± 2)
Protein, *g/d*	131 ± 21	(14 ± 1)	154 ± 19	(15 ± 2)
Fiber, *g/d*	26 ± 3		27 ± 2	
Total energy,[4] *MJ/d*	15.8 ± 1.7		17.5 ± 1.4	

[1,2] Values are means ± SD, n = 13 (LCD) or 12 (HCD). *Different from LCD, $P < 0.05$ (Student's t test).

[3] Nutrients expressed as g/d are followed, in parentheses, by the percentage of total energy intake.

[4] To convert MJ to kcal, multiply by 239.

Preparation and plasma kinetics of the emulsion. The chylomicron-like emulsion (final composition: triolein 76.5 ± 4.1%, free cholesterol 1.9 ± 0.3%, cholesterol oleate 11.2 ± 3.0% and phosphatidylcholine 10.4 ± 1.3% with a size range from 80–110 nm) was obtained from lipid mixtures emulsified by ultrasonic irradiation and purified by ultracentrifugation (24191 × g; 30 min) in density gradients as described previously (15). ^{14}C-cholesteryl oleate and ^{3}H-triolein (Amersham) were added to the lipid mixtures for determination of plasma kinetics. The emulsion was finally sterilized by passage through a 0.2-μm filter.

Determination of chylomicron-like emulsion plasma kinetics was performed in all of the men 1 d after the dietary intervention (d 16); blood samples for biochemical analysis of lipoproteins and apolipoproteins were taken after a 12-h fast, preceding the injection of the labeled emulsion. The emulsion total lipid (3 mg; 200–300 μL), containing 74 kBq (2 μCi) ^{14}C-cholesterol oleate and 148 kBq (4 μCi) ^{3}H-glycerol trioleate, was injected as a bolus into the forearm vein of each subject. Blood samples were collected from a vein of the other forearm perfused with a sterile solution of 0.15 mol/L NaCl at preestablished times of 2, 4, 6, 10, 15, 20, 31, 45, and 60 min after the injection. Plasma samples were separated from the blood. Each plasma sample (100 μL) was placed into a counter vial and 7 mL of scintillating solution (PPO: dimetil-POPOP:tritonX-100:toluene, 5 g:0.5 g:333 mL:667 mL) was added. Radioactivity was then counted in a Packard 1660 TR spectrometer.

Compartmental analysis. The removal of the chylomicron-like emulsion from the plasma was evaluated by compartmental analysis as previously described (19). Briefly, 4 compartments were employed to estimate the kinetic parameters for both ^{14}C-CE and ^{3}H-TG tracers. Plasma hydrolysis and removal of native chylomicrons as well as, chylomicron-like emulsions displayed a rapid initial decay followed by a slow removal phase. The $k_{j,i}$ constant represents the transfer or fractional clearance rate (FCR) from compartment i to compartment j. The kinetics of ^{14}C-CE and ^{3}H-TG metabolism are represented by compartments 1–4 and 5–8, respectively. The ^{14}C-CE label particle injected is represented by compartment 1. A fraction ($k_{3,1}$) of this particle is removed directly to the extravascular space. Another fraction ($k_{2,1}$) of the injected particle is transformed inside the plasma (compartment 2) and it is removed subsequently ($k_{3,2}$) to the extravascular space. Similar consideration is applied to compartments 5 and 6, and $k_{7,5}$, $k_{6,5}$, $k_{7,6}$, relative to the ^{3}H-TG of the particle. For both labels, the rapid decay curves are associated with $k_{3,1}$ and $k_{7,5}$, respectively. The model (**Fig. 1**) also takes into account the recirculation of the radioactive tracers in plasma in the form of newly synthesized VLDL (expressed by $k_{4,3}$ and $k_{8,7}$ for the ^{14}C and ^{3}H, respectively). The fractional catabolic rate, the FCR of the radiolabeled material, is essentially the inverse of the area under the activity-time curve (19) for compartments 2 (^{14}-CE) and 6 (^{3}H-TG), respectively, which more truly represent the chylomicron particles. The residence time (RT) was calculated by the area under the curve

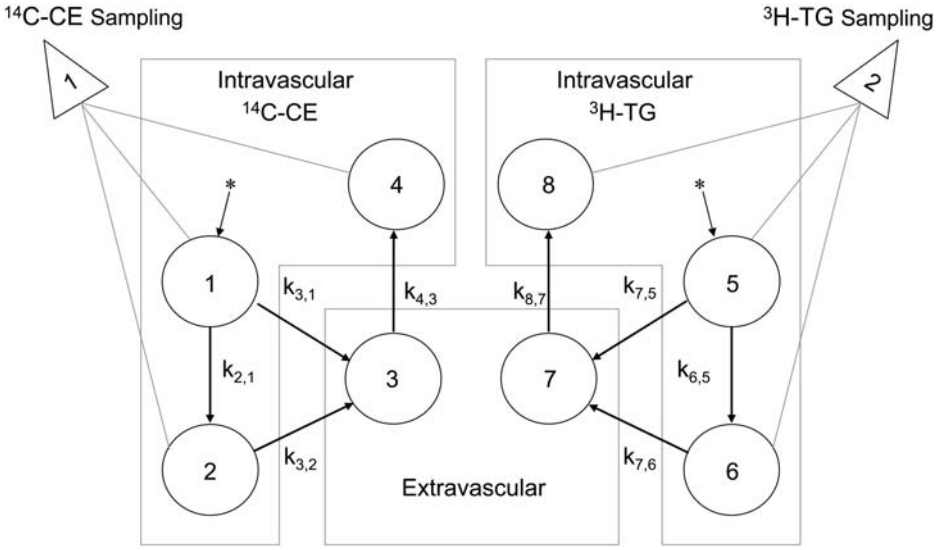

FIGURE 1 Schematic model for chylomicron-like emulsion kinetic analysis. The model consists of 8 discrete pools, 4 for each label, ^{14}C-CE and ^{3}H-TG; 6 pools are in the intravascular space (1, 2, 4 and 5, 6, 8) and 2 pools in the extravascular space (3 and 7). It is assumed that the intravascular pool is in dynamic equilibrium with the extravascular pool. This model assumes that the input of ^{14}C-CE and ^{3}H-TG occurs in the intravascular pool 1 and pool 5, respectively. A fraction of the labeled lipid is removed from pool 1 and pool 5 to the extravascular pool 3 and 7, respectively, and another fraction of the injected lipid is converted in compartments 2 and 6. Those pools are captured by the extravascular compartments 3 and 7, respectively. Radiolabeled lipids return to the intravascular space chemically or functionally modified, constituting compartments 4 and 8, respectively. The $k_{j,i}$ values represent the fractional turnover rates (FCR, min^{-1}), which are associated with the half-life time (min), by the formula T½ = 0.693/$k_{j,i}$.

(AUC) extended to an infinite time divided by the injected label (20). All calculations were performed using computer software (21).

The injected radiation dose in each experiment was below the annual radionuclide for the individual (1 mSv) intake limit as determined by the International Commission on Radiological Protection. The equivalent dose for ^{14}C-CE was 0.0411 mSv and for ^{3}H-TG, 0.0025 mSv as described elsewhere (15).

Serum lipid and apolipoprotein determinations. Plasma total cholesterol (CHOD-PAP; Roche) and triacylglycerol (Triglycerid Rapid; Roche) were determined by enzymatic methods on a Cobas Bio analyzer. HDL cholesterol (HDL-C) was measured after precipitation of the VLDL and LDL with HDL Reagent ROCHE (method phosphotungsten acid:$MgCl_2$) in the same automatic equipment. VLDL and LDL-C were estimated by the Friedewald formula (22). Apo A1 and apo B were determined by an immunoturbidimetric assay (Roche) on a Cobas MIRA analyser. Lipoprotein (a) [Lp(a)] concentrations were measured using a commercial ELISA (Biopool).

Statistical analysis. Data are expressed as means ± SD. Differences of $P < 0.05$ were considered significant. All sets of data were tested for normality before statistical tests. Most variables were analyzed by Student's *t* test. TG, apo A, apo B, Lp(a) concentrations, the kinetics parameters $k_{2,1}$, $k_{7,6}$, RT-TG and lipolysis index (LI) were analyzed by the non-parametric Mann-Whitney Rank Sum Test. Total energy intake data were not tested because they were adjusted to individual needs. Sigma Stat v. 3.11 for Windows^{R} was used to perform statistical calculations.

RESULTS

The energy and nutrients intakes during the experimental period did not differ between the LCD and HCD groups (Table 1). Indeed, both diets were characterized by low fat and low saturated fat composition, according to the NCEP guidelines (16). They differed only in terms of their cholesterol content ($P < 0.05$). Physical characteristics, serum lipids, and apolipoproteins of the LCD and HCD groups did not differ at baseline (**Table 2**).

In the HCD group, the addition of cholesterol (3 yolk eggs/d) increased the serum total cholesterol, LDL-C, and HDL-C concentrations compared with the LCD group. However, triglyceride and Lp(a) concentration did not differ (**Table 3**). Apo B was greater in the HCD group than in the LCD group, but apo A1 did not differ between them.

The decay curve of the emulsion ^{3}H-triolein in the 2 groups (**Fig. 2***A*) did not differ, whereas the emulsion ^{14}C-CE (Fig. 2*B*) was slower in the HCD group.

The $k_{2,1}$ and $k_{6,5}$ compartmental parameters (**Table 4**), according to the model adopted in this study, represent the transformation of the injected particles in another subpopulation of particles (19), represented by compartments 2 and 6, respectively. After dietary treatment, there was no difference in the parameters $k_{2,1}$ and $k_{6,5}$ between the LCD and HCD groups (Table 4).

In contrast, the parameter $k_{3,2}$, which corresponds to the fraction from the remaining ^{14}C-CE taken up by the hepatic

TABLE 2

Baseline characteristics of the LCD and HCD groups[1,2]

	LCD	HCD
n	13	12
Age, *y*	19.4 ± 1.9	18.4 ± 1.9
Smokers, *cigarettes/d*	4.5 ± 4.9	1
Body weight, *kg*	71.3 ± 13.9	73.6 ± 11.7
BMI,[2] *kg/m*	23.3 ± 4.5	24.6 ± 3.9
Physical activity, *h/wk*	17 ± 4	17 ± 7
Serum		
Total cholesterol,[3] *mmol/L*	4.40 ± 0.76	4.12 ± 0.64
LDL-C, *mmol/L*	2.38 ± 0.69	2.48 ± 0.58
HDL-C, *mmol/L*	1.26 ± 0.24	1.32 ± 0.38
Triacylglycerol, *mmol/L*	2.38 ± 0.69	1.60 ± 0.57
Lp(a), *mmol/L*	1.35 ± 1.25	1.50 ± 1.29
Apo AI, *g/L*	1.95 ± 0.29	2.13 ± 0.58
Apo B, *g/L*	1.22 ± 0.17	1.12 ± 0.19
Diet		
Energy intake,[4] *MJ/d*	16.5 ± 0.47	16.2 ± 1.63
Dietary cholesterol, *mg/d*	465 ± 135	460 ± 106
Saturated fatty acids, *% MJ/d*	19 ± 2	19 ± 2
Dietary lipids, *% MJ/d*	25 ± 4	25 ± 2

[1,2] Values are means ± SD.
[3] To convert serum lipid concentrations from mmol/L to mg/dL, divide by 0.02586.
[4] To convert MJ to kcal, multiply by 239.

TABLE 3

Post-treatment serum biochemical variables of the LCD and HCD groups[1,2]

	LCD	HCD
n	13	12
Total cholesterol,[3] *mmol/L*	3.50 ± 0.47	4.71 ± 0.72*
LDL-C, *mmol/L*	2.22 ± 0.72	3.09 ± 0.62*
HDL-C, *mmol/L*	0.99 ± 0.28	1.27 ± 0.18*
LDL-C/HDL-C	2.5 ± 1.3	2.4 ± 0.4
Triacylglycerol, *mmol/L*	1.41 ± 0.21	1.70 ± 0.49
Lp(a), *mmol/L*	1.33 ± 1.09	1.55 ± 1.24
Apo AI, *g/L*	1.68 ± 0.15	1.86 ± 0.34
Apo B, *g/L*	1.02 ± 0.25	1.28 ± 0.20*

[1,2] Values are means ± SD. *Different from LCD, $P < 0.05$ [Student's *t* test for TC, HDL-C, LDL-C, and the Mann-Whitney test forTG, apo AI, apo B, Lp(a)].

[3] To convert serum lipid concentrations from mmol/L to mg/dL, divide by 0.02586.

cells (compartment 3) (19), was 49% slower in its removal to the extravascular compartment in the HCD group ($P < 0.05$). Furthermore, the parameter $k_{3,1}$ suggests that the direct removal of a fraction of the ^{14}C-CE-injected particles by the liver or other tissues was 51% lower in the HCD group ($P < 0.05$). These results show a delayed plasma removal of ^{14}C-CE with

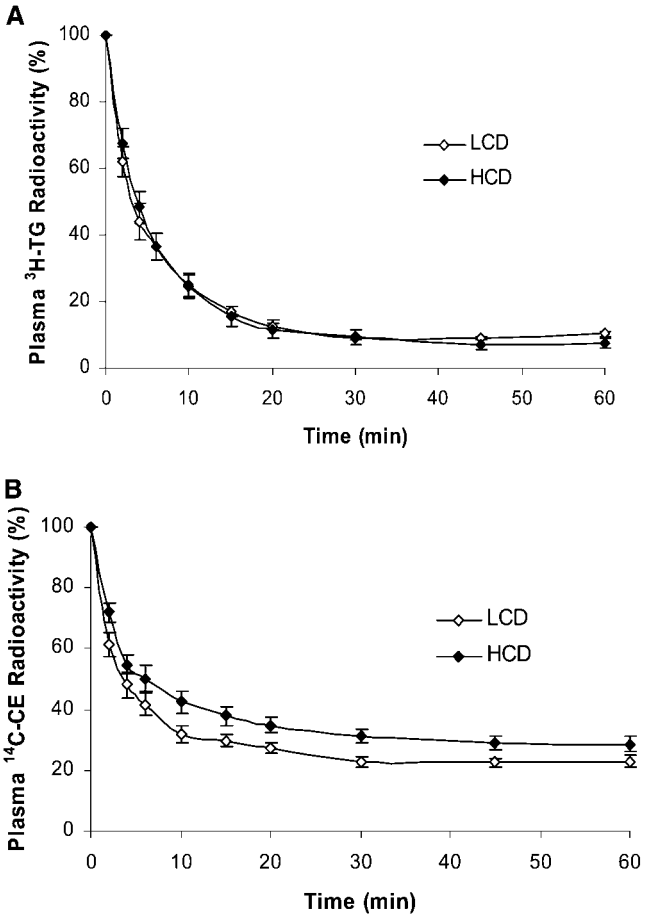

FIGURE 2 Plasma decay curves of the ^{3}H-TG (*upper panel*) and ^{14}C-CE (*lower panel*) emulsions in healthy normolipidemic young men after 15 d of consuming a LCD or a HCD. Values are means ± SD, $n = 13$ (LCD) or 12 (HCD).

TABLE 4

Chylomicron emulsion lipids ^{14}C-CE and ^{3}H-TG plasma kinetic parameters for post-treatment of the LCD and HCD groups[1,2]

Parameter[2]	LCD	HCD
n	13	12
$k_{3,1}$	0.407 ± 0.296	0.198 ± 0.071*
$k_{3,2}$	0.0340 ± 0.0133	0.0173 ± 0.0108*
$k_{2,1} = k_{6,5}$	0.401 ± 0.587	0.152 ± 0.081
$k_{4,3}$	0.00380 ± 0.00155	0.00313 ± 0.00203
$k_{7,5}$	0.326 ± 0.169	0.215 ± 0.130
$k_{7,6}$	0.084 ± 0.025	0.118 ± 0.126
$k_{8,7}$	0.00198 ± 0.00054	0.00115 ± 0.00063*
FCR–^{14}C-CE, min^{-1}	0.0702 ± 0.0165	0.0334 ± 0.0177*
FCR–^{3}H-TG, min^{-1}	0.143 ± 0.040	0.139 ± 0.053
RT–^{14}C-CE, *min*	15.2 ± 4.1	54.1 ± 50.4*
RT–^{3}H-TG, *min*	7.57 ± 2.14	8.62 ± 4.01
LI,[2] *%*	1.09 ± 0.56	2.50 ± 1.62

[1] Values are means ± SD. *Different from LCD, $P < 0.05$ (Student's *t* test for $k_{3,1}$, $k_{3,2}$, $k_{4,3}$, $k_{7,5}$, $k_{8,7}$, FCR-^{14}C-CE, FCR-^{3}H-TG, RT-^{14}C-CE, and the Mann-Whitney test for $k_{2,1}$, $k_{7,6}$, RT-TG, LI).

[2] Compartmental parameters: k_{ji}, FCR, RT, and LI, as indicated in Figure 1.

consumption of a HCD that is associated with higher levels of serum TC and LDL-C compared with the LCD group.

The parameters $k_{7,5}$, and $k_{7,6}$, which are related to the release of fatty acids and the removal of the ^{3}H-TG residual of the emulsion remnant (19), respectively, were not affected by the dietary intervention. Furthermore, the parameter $k_{6,5}$ indicates that the HCD did not affect ^{3}H-TG lipolysis or the removal of fatty acids from the emulsion to extravascular tissues (23).

Because CE removed from compartments 1 and 2 is supposedly incorporated into hepatic VLDL (compartment 3), the parameter $k_{4,3}$ represents the ^{14}C-CE in the VLDL pool recycled to the plasma (compartment 4) (19). According to the experimental data, the parameter $k_{4,3}$ was not affected by the HCD (Table 4). Recirculation of ^{3}H-TG was also considered in the VLDL secretion (compartment 8), with parameter $k_{8,7}$ representing the transfer rate of the ^{3}H-fatty acids to the VLDL pool (19). In fact, the parameter $k_{8,7}$ (Table 4) suggests that the HCD slowed down the degree of recirculation of ^{3}H-fatty acids in the VLDL pool by 62% ($P < 0.05$).

This result was confirmed by the decrease in the FCR of the emulsion ^{14}C-cholesteryl oleate in the HCD group, which was ~52% lower than in the LCD group (Table 4, $P < 0.001$). In contrast, the 2 groups did not differ in the FCR for the emulsion ^{3}H-triolein. Correspondingly, the ^{14}C-CE plasma RT in men that consumed the HCD was almost 1 h, whereas with those that consumed the LCD, the ^{14}C-CE was removed in ~15 min. The LI did not differ between the 2 groups.

DISCUSSION

In this study, supplementation of a low-fat diet with a high cholesterol dose comprising 3 egg yolks/d decreased the removal from the plasma of the emulsion cholesteryl esters yet it did not modify the removal of the emulsion triacylglycerol. It can thus be assumed that the high cholesterol diet led to a diminished remnant clearance while the removal of triglyceride was not affected.

The cholesterol plasma pool is regulated by feedback between dietary cholesterol intake and hepatic cholesterol synthesis. As documented in rats by Nervi and Dietschy (24), chylomicron remnants are the main lipoproteins that effectively

downregulate cholesterol synthesis by the liver. This is because this lipoprotein has the ability to transfer cholesterol esters rapidly into the hepatocyte, reaching maximal storage, and thus, simultaneously decreasing the mobilization of cholesterol from the plasma lipoproteins, which has consequences for the process of atherogenesis.

A high-cholesterol diet clearly enhances LDL-C levels. It is a key point that cholesterol supplementation was added to a diet with a low-fat, high polyunsaturated:saturated fat ratio. A high-fat diet with a low polyunsaturated:saturated fat ratio has a powerful hypercholesterolemic effect, which is quantitatively far more important than the high cholesterol intake. This effect is achieved either by the downregulation of LDL receptors, which impairs the removal of the lipoprotein from the plasma (25,26) or by an increase in the synthesis of the LDL precursor lipoprotein VLDL (27). Therefore, the introduction of a high-cholesterol diet together with a high-fat high polyunsaturated: saturated fatty acid (P:S) ratio could mask the eventual effects of the cholesterol supplementation.

The decline in the removal of remnants from the plasma elicited by the intake of a high-cholesterol diet may be explained by downregulation that was caused by the cholesterol intake of the receptors involved in the removal of those lipoproteins from the circulation. Studies in animals suggest that the LDL receptor represents the main pathway for chylomicron remnant removal (9,10). Another receptor, the LRP, may also remove chylomicron remnants from the plasma and appears to act as a backup to the LDL receptor (28). In our recent study, we showed that the removal from the circulation of emulsion remnants is negatively correlated with the concentrations of LDL-C, which suggests that the 2 lipoproteins share the LDL receptor (29).

In several studies, the delayed remnant clearance or accumulation of postprandial triacylglycerol was associated with an increased risk of developing CAD. This has been suggested by different methodological approaches using oral fat-load tests, in which chylomicron remnants were traced by retinyl palmitate or apo B48. With the emulsion approach used here, we showed that the removal of both emulsion cholesteryl esters and triacylglycerol was delayed in patients with CAD compared with subjects without the disease (30). Recently, we also showed that a defective chylomicron-like emulsion metabolism is associated with the angiographic severity of CAD and that this metabolism can predict the evolution to severe angina among patients undergoing secondary prevention therapy for CAD (19). Therefore, a high-cholesterol diet could be proatherogenic not only because of the increase in plasma LDL-C, but also due to the delay in the removal of chylomicron remnants.

The issue of chylomicrons and postprandial lipoproteins and egg-yolk ingestion was also investigated in young normolipidemic men by Ginsberg et al. (31); in that study, cholesterol supplementation was given by increasing cholesterol dose periods together with consumption of a low-fat, NCEP and AHA step 1 diet regimen that was essentially no different from that used in the current study. Similar to our study, they found that egg yolk feeding increased LDL-C. The removal of remnants was measured by the oral fat-load test with the addition of retinyl palmitate as a chylomicron label. The AUC of the appearance-disappearance of the retinyl esters in the plasma did not differ among the 0, 1, 2, or 4 eggs/d dietary periods.

As assessed by the chylomicron-like emulsion kinetics method, the intestinal absorption component of the chylomicron metabolism, which is a very important source of experimental variation, is bypassed and a straightforward analysis of the emulsion removal from plasma is allowed. A doubly labeled emulsion with radioactive triacylglycerol and cholesteryl esters allows a high-resolution view of this metabolism in which the 2 main events, lipolysis and remnant removal, are traced simultaneously. This methodological approach permits a better discrimination between experimental situations, which may explain the identification of metabolic alterations elicited by high-cholesterol intake not otherwise perceived in the study of Ginsberg et al. (31).

Because chylomicrons and VLDL share a common metabolic pathway, such as lipoprotein lipase and in part the receptor mechanisms, our results suggest that the VLDL route is altered, decreasing the efficiency of its removal. This is confirmed by the accumulation of LDL particles after high-cholesterol intake documented by the increase of LDL-C and apo B. Those finding are in agreement with our previous study in which the emulsion cholesteryl ester FCR correlated negatively with LDL-C (30).

The finding that HDL-C was increased by a high cholesterol intake is in line with the results reported by Ginsberg et al. with normolipidemic young men (31) and women (32). Our results agree with a recent meta-analysis (6), which found that the addition of 100 mg/d dietary cholesterol increased HDL-C by 0.008 mmol/L. Although LDL-C also increased with consumption of a high-cholesterol diet (Table 3), the ratio of LDL-C:HDL-C was 0.1 units lower compared with the group fed low cholesterol, perhaps suggesting an increased risk of CHD. Similar responses to dietary cholesterol were also discussed in recent papers (4,33,34).

In conclusion, this study showed that a daily intake of 3 eggs during a 2-wk period impaired the chylomicron remnants as evaluated by the chylomicron-like emulsion method, whereas the lipolysis of these lipoproteins remained unaffected. Due to the common metabolic pathway, the removal of products resulting from VLDL catabolism may also be diminished, which explains the greater LDL-C and apo B concentrations after cholesterol intake.

LITERATURE CITED

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