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Effects of increasing amounts of dietary cholesterol on postprandial lipemia and lipoproteins in human subjects

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Abstract Our aim was to determine the effects of increasing amounts of dietary cholesterol (0–710 mg) on the postprandial plasma lipid responses and lipoprotein changes in normolipidemic human subjects. Ten subjects were fed five different test meals in a random order: one meal did not contain fat or cholesterol while the four others contained a fixed amount of lipids (45 g) and 0, 140, 280, and 710 mg cholesterol, respectively. Fasting and post-meal blood samples were obtained for 7 h. Large and small triglyceride-rich lipoproteins (TRL), low density (LDL), and high density (HDL) lipoproteins were isolated. Compared to the no-fat, no-cholesterol meal, the fat-enriched meals raised ($P < 0.05$) plasma triglycerides, phospholipids, and free cholesterol and lowered cholesteryl esters postprandially. The meals containing zero or 140 mg cholesterol generally elicited comparable postprandial plasma and lipoprotein lipid responses. The meals providing 280 or 710 mg cholesterol significantly increased postprandial plasma phospholipids and large TRL triglycerides and decreased plasma esterified cholesterol. The lipid composition of the large TRLs and the concentrations of the small TRL lipid components were not altered postprandially by cholesterol intake. On the other hand, LDL free cholesterol increased after 3 h, LDL cholesteryl esters dropped after 3 and 7 h, HDL cholesteryl esters dropped after 3 h, and HDL phospholipids increased 7 h after ingesting meals highly enriched in cholesterol. Blood insulin, apoA-I and apoB were not altered postprandially by cholesterol intake. Thus, the data show that ingesting more than 140 mg cholesterol per meal significantly alters the postprandial lipoprotein response in healthy subjects.—**Dubois, C., M. Armand, N. Mekki, H. Portugal, A-M. Pauli, P-M. Bernard, H. Lafont, and D. Lairon.** Effects of increasing amounts of dietary cholesterol on postprandial lipemia and lipoproteins in human subjects. *J. Lipid Res.* 1994. **35:** 1993–2007.

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Numerous studies have shown that dietary lipids, in addition to genetic susceptibilities, affect serum and lipoprotein cholesterol levels in animal models as well as in human subjects. A strong body of information has established that dietary saturated fatty acids play detrimental roles in lipid metabolism status by increasing blood cholesterol and altering the lipoprotein pattern (1). Literature concerning the effects of dietary cholesterol is more controversial. Experimental evidence obtained in animal models has shown that dietary cholesterol alters normal fasting blood lipid patterns (1) basically by suppressing the activity of the LDL apoB, E receptor (2, 3). Because humans seem less sensitive to dietary cholesterol than other animal species, and given the individual high- or low-responder status to dietary cholesterol (4–6), contradictory data have been obtained. Nevertheless, carefully controlled studies have consistently shown that increasing dietary cholesterol increases blood cholesterol levels (7–10).

Given the high prevalence of coronary heart disease (11, 12) and usual food consumption patterns in industrialized countries, expert committees and consensus conferences have given sound nutritional recommendations to lower fat intake to 30% of energy and dietary cholesterol below 300 mg per day (11, 12). However, the quantitative relationship between cholesterol intake and blood parameters is still debated (8, 13) and data obtained from epidemiological surveys, dietary manipulations, or drug therapy trials do not always support concepts based solely on fasting parameters as recently discussed (1, 14, 15).

Since the claim of Zilversmit (16) that atherosclerosis could be a postprandial phenomenon, much knowledge has been obtained on postprandial lipemia during the last decade. For instance, recent data support the concept that

Abbreviations: TRL, triglyceride-rich lipoproteins; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; AUC, area under the curve.

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myocardial infraction (17, 18) or some fasting dyslipidemic conditions (19–22) are associated with abnormal postprandial patterns. On the basis that duration of the postprandial hyperlipidemic state represents most of the day-time (21–29), the most recent concept proposes that fasting lipoprotein levels, and especially fasting HDL cholesterol, might not be seen as responsible for protection against atherosclerosis but rather as a dependent marker of the postprandial metabolism of triglyceride-rich lipoprotein (14, 15, 25, 28). Thus, alterations in the postprandial response after ingestion of selected food components, and especially lipids, should be regarded as an early metabolic event.

Although detailed studies have recently reported numerous basic mechanisms involved during alimentary lipemia (23–25, 28, 30, 31), little information is available in humans on the effects of varying the amounts of lipid nutrients. A dose-response relationship has been shown in normolipidemic subjects for postprandial triglyceridemia and dietary triglycerides in the range 40–120 g fat (32) and recently down to 30 g (29). Concerning the effects of increasing amounts of dietary cholesterol, very limited information is now available. In the dog (33), ingestion of graded amounts (1.0–6.3 g) of dietary cholesterol was reported to elicit a dose-related increase in the cholesterol content of lymph chylomicron without any effect on lymph triglyceride. In a recent study (34), lymph-cannulated rats were fed fat emulsions containing increasing amounts of cholesterol (0–100 mg/g fat): only relatively large doses of dietary cholesterol (30 mg/g fat and above) induced changes in chylomicron composition. To our knowledge, no data concerning the effects of dietary cholesterol on postprandial lipemia are available in humans.

The present study was therefore performed in healthy human subjects to evaluate the effects of test meals with increasing amounts of dietary cholesterol (0–710 mg) on postprandial plasma lipids and lipoproteins.

SUBJECTS AND METHODS

Subjects

Ten adult male volunteers (ages 22–33 years) participated in the study after giving written informed consent to a protocol approved by the Medical Ethics Committee (Regional University Hospital Center, Marseille). None suffered from any digestive or metabolic disease as checked by medical history and fasting blood parameters. None was obese (body mass index: 18.8–25.2) and body weights did not vary noticeably during the experiment. None had taken medications that interfere with lipid metabolism for months. Among ten subjects, eight exhibited an E3/E3 phenotype and two an E3/E2 phenotype, as determined by isoelectrofocusing (35, 36). Fasting blood concentrations were in the normal range, with the following overall mean (± SEM) values: plasma triglycerides (1.03 ± 0.05 mmol/l), phospholipids (2.42 ± 0.04 mmol/l), total cholesterol (4.27 ± 0.08 mmol/l), esterified (3.21 ± 0.06 mmol/l) and free cholesterol (1.06 ± 0.02 mmol/l), LDL-cholesterol (2.74 ± 0.11 mmol/l), HDL-cholesterol (1.28 ± 0.06 mmol/l), apoA-I (1.26 ± 0.06 g/l), apoB (0.83 ± 0.03 g/l), and insulin (41.1 ± 2.2 pmol/l). As shown in **Table 1**, the mean fasting lipid parameters were very close and not different ($P < 0.05$) at the time of the five test meals.

The subjects were instructed not to deviate from regular habits and, especially, to avoid excess alcohol consumption or exercise, given their reported influence on lipid metabolism. The usual basal diet of each subject was monitored through a 3-day food recall during the first and last week of experiment and calculations were made with the GENI software package (Micro 6, Nancy, France) for a Macintosh microcomputer (Apple Corporation Inc., Cupertino, CA). The food data base used in this program is based on the nutrient tables of Paul and Southgate (37) and Feinberg, Favier, and Ireland-Ripert (38). The subjects relied on a typical Western diet, with a moderate

TABLE 1. Mean fasting plasma lipids before ingestion of the five experimental meals

	Experimental Meal (g fat–mg cholesterol)				
	0–0	45–0	45–140	45–280	45–700
			mmol/l		
Triglycerides	1.03 ± 0.15	0.98 ± 0.12	1.02 ± 0.12	1.05 ± 0.17	1.06 ± 0.16
Phospholipids	2.42 ± 0.10	2.45 ± 0.14	2.42 ± 0.11	2.39 ± 0.12	2.40 ± 0.10
Total cholesterol	4.20 ± 0.24	4.18 ± 0.27	4.27 ± 0.29	4.29 ± 0.28	4.30 ± 0.25
LDL-cholesterol	2.70 ± 0.18	2.68 ± 0.19	2.78 ± 0.24	2.79 ± 0.22	2.77 ± 0.18
HDL-cholesterol	1.27 ± 0.13	1.28 ± 0.11	1.25 ± 0.09	1.29 ± 0.09	1.30 ± 0.06

Values are means ± SEM of 10 subjects. In a given row, values were statistically compared (ANOVA) and did not show any significant difference ($P < 0.05$).

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energy consumption (mean: 9928 kJ/d, 2375 kcal/d); proteins, carbohydrates, and fats accounted for 14.7%, 39.5%, and 45.8% of energy, respectively. The P/S ratio was 0.40, the mean daily cholesterol intake was 439 mg, and the total dietary fiber intake was 14.1 g/day. Mean alcohol intake was 11.8 ± 5.2 g/day. No noticeable changes in food consumption were recorded during the experiment period.

Test meals

The five experimental test meals were presented in a random order. The interval between two test meals was 7-10 days. To take into account the possible effect of the preceding meal, the subjects were asked to have a light dinner prior to 21:00 the evening before an experiment (27, 39).

The five experimental meals consisted of commercially available food. The basal meal without any fat or cholesterol added (subsequently called no-fat, no-cholesterol meal; 0-0) contained 4 slices of white bread, 60 g of wheat semolina (cooked and hydrated with 120 ml water), cooked egg white (95 g), one 0%-fat yogurt (125 ml), a cup of coffee (150 ml), and a glass of tap water (100 ml). The nutrient and energy content of the basal meal is given in **Table 2.** The four other test meals were all enriched with triglycerides (20 g as sunflower margarine and 20 g as olive oil in a dressing with 8.4 g mustard and 5 g vinegar). Three of them were enriched with cholesterol. The different amounts (142, 284, and 710 mg) of dietary cholesterol (Table 2) were provided in the form of cooked egg yolk (batch cholesterol content, 1.32 g/100 g). The increased extra amounts of triglycerides and lecithins provided by the egg yolk supplements were taken into account by adding extra olive oil triglycerides (6.6 to 11 g) and soybean lecithins (1.76 to 4.4 g) to some meals. Along a similar line, extra proteins supplied by egg yolk (16 g/100 g) were taken into account by adding extra egg white proteins (5.2 to 8.6 g) to some meals. The nutrient and energy content of the five whole experimental meals, obtained from analyses of duplicate meals, are given in Table 2. The 0-0 meal contained only traces of triglycerides and no cholesterol. The fat-enriched meals (45-0 to 45-700) contained about 45 g triglycerides and 4.3 g phospholipids and variable amounts of cholesterol from zero up to 710 mg. The fatty acid composition of the test meals was determined by separation and quantification of the fatty acid methyl esters by gas chromatography using a DI 200 Delsi unit (Suresnes, France) equipped with a 0.22 mm × 25 m BPX 70 capillary column, 0.25 μm film thickness (SGE, Villeneuve St. Georges, France), at 160°C for 6 min and 160 to 197°C at 1°C/min. The fatty acid composition (%) of typical fat-enriched meals (45-700) was as follows: < C16, 0.29; C16:0, 17.05; C16:1, 1.44; C18:0, 4.87; C18:1, 49.88; C18:2, 24.18; C18:3, 0.87; C:20, 0.88; > C20, 0.53. The protein, carbohydrate and energy contents of the four fat-enriched meals were comparable. The total dietary fiber supply, as calculated from the food data base, was 2 g per meal. During the 7-h postprandial period, participants were allowed to drink 300 ml water.

After the subjects fasted overnight, an antecubital vein was catheterized with an intravenous cannulae equipped with disposable obturators (Jelco-Critikon, Chatenay-Malabry, France). A baseline fasting blood sample (0 h) was collected. The subjects ingested the test meal within 20 min and blood samples (15-20 ml) were obtained every hour for 7 h (27, 29, 39).

Analytical determinations

Blood was collected in tubes containing EDTA and an inhibitor cocktail was added as reported (40). Plasma was separated by centrifugation (10°C, 10 min, 910 *g*). The large triglyceride-rich lipoprotein fraction (S_f > 1000), subsequently called large TRL, containing chylomicrons plus large chylomicron remnants was isolated by flotation (20, 21, 27) from 2 ml serum layered under 3 ml 0.9% NaCl (ultracentrifugation for 1.6 × 10^6 *g*-min at 10°C in a Beckman (Palo Alto, CA) 40.3 rotor).

From eight subjects, aliquots (2 ml) of the resulting infranatants of plasma samples (fasting, 3 h postprandial peak and 7 h postprandial samples) were used for separation of lipoprotein classes (small-sized chylomicron rem-

TABLE 2. Nutrient composition of the test meals

Meal	Dry Weight	Proteins	Carbohydrates	Lipids	Triglycerides	Cholesterol	Energy
			g/meal			*mg/meal*	*kJ/meal*
Basal meal (0-0)	145.7	30.5	109.4	2.1	traces	0	2421
Fat, cholesterol-enriched meals							
45-0	207.2	41.0	112.5	48.3	44.1	0	4383
45-140	212.4	45.2	114.2	49.9	45.6	142	4542
45-280	209.6	43.7	110.5	49.4	45.1	284	4437
45-700	215.0	37.9	121.3	50.1	45.7	710	4545

Nutrients were assayed by conventional methods from duplicate meals. Triglycerides were calculated by subtracting the phospholipids measured as lipid phosphorus from total lipids.

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nants + very low density lipoproteins, small TRL: d≤ 1.006 g/ml); LDL, low density lipoproteins: 1.006 ≤d≤1.063 g/ml; and HDL, high density lipoproteins: 1.063 ≤d≤1.21 g/ml) by ultracentrifugation on a KBr discontinuous gradient (200,000 *g* for 24 h at 10°C in a Beckman SW 41 rotor) as previously described (29, 39, 41). In one subject with representative plasma fasting and postprandial values, lipoproteins were isolated from fasting and hourly postprandial samples. Lipoprotein lipid concentrations were adjusted for ultracentrifugation recovery. On the whole, the lipoprotein (small TRL, LDL, HDL) triglyceride recovery was 0.97 ± 0.06 in the fasting samples and 0.83 ± 0.03 and 0.82 ± 0.05 in the 3-h and 7-h postprandial samples, respectively. Comparable figures were obtained for lipoprotein cholesterol recovery (0.96 ± 0.02, 0.86 ± 0.01, and 0.87 ± 0.02 in the 0-h, 3-h, and 7-h samples, respectively).

Triglycerides were determined by an enzymatic procedure (42) using commercial kits (BioMerieux, Marcy l'Etoile, France). Total and free plasma cholesterol were assayed by the cholesterol oxidase method (43) with kits purchased from BioMerieux (Marcy l'Etoile, France). Plasma phospholipids were assayed by an enzymatic procedure (44) with commercial kits (BioMerieux, Marcy l'Etoile, France). Total plasma apoA-I (45) and apoB (46) were assayed by laser-nephelometry (Behring Werke A. G., Marburg, Germany) in the fasting samples and postprandial samples with the highest triglyceride level after a given meal (2–3 h), and 7-h samples. Insulin was assayed by an immuno-enzymatic method (47) with commercial kits (Boehringer Mannheim, Mannheim, Germany).

Statistical analysis

In this randomized study, each subject consumed the five experimental meals and served as his own control. The values (mean ± SEM) were expressed as variations of concentration over baseline (fasting baseline values being zero). The area under the 0-7 h curve (AUC) was calculated by the trapezoidal method (21, 27, 32). The statistical significance ($P < 0.05$) of the differences observed between the experimental meals (individual time-points or AUCs) was assessed by using analysis of vari-

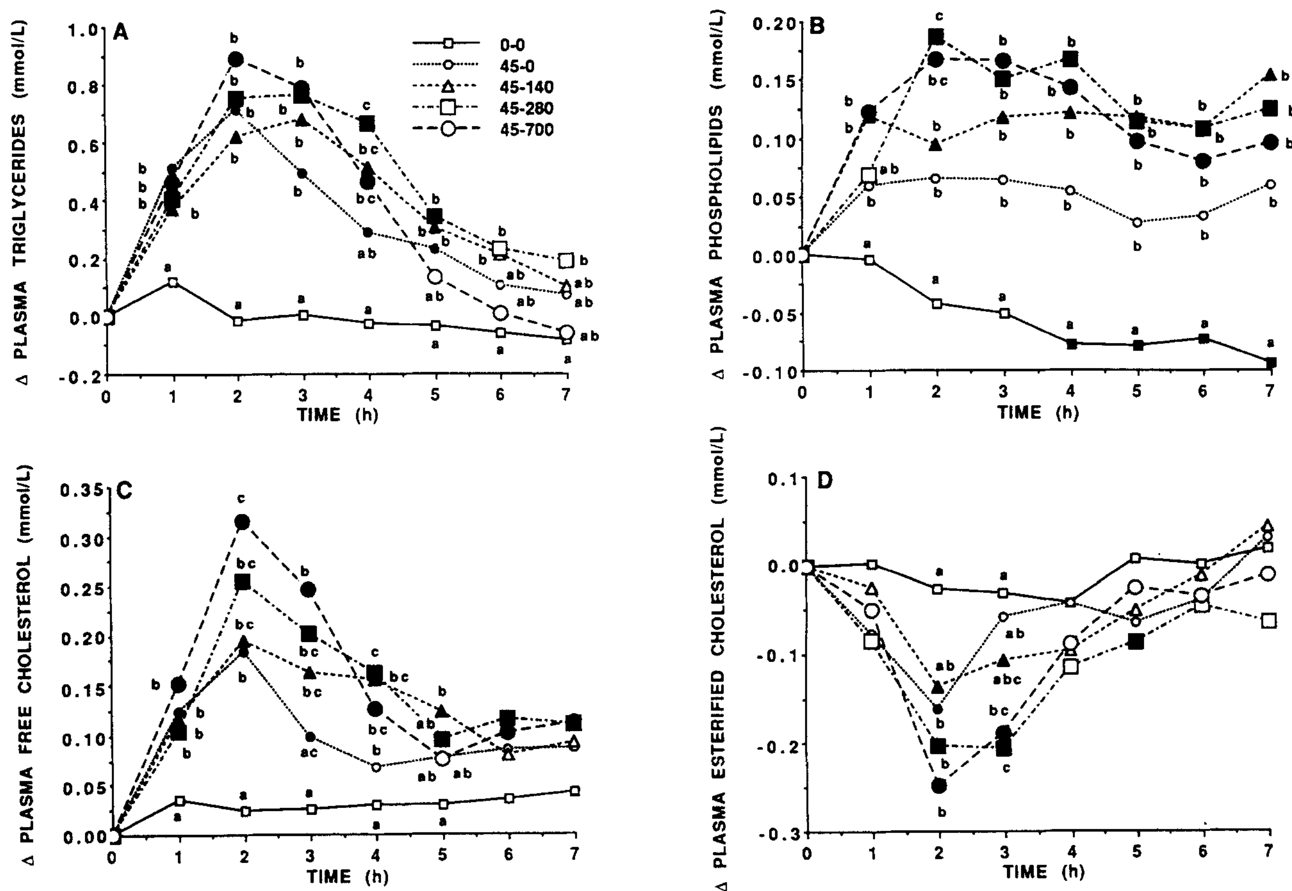

Fig. 1. Plasma triglyceride (A), phospholipid (B), free cholesterol (C), and esterified cholesterol (D) 0–7 h responses to the five test meals. Values [concentration changes (Δ) from fasting values] are means of 10 subjects. For a given test meal, a filled symbol indicates that the corresponding value is significantly different (ANOVA, $P < 0.05$) from the fasting (0 h) value. Different letters indicate significant differences (ANOVA, $P < 0.05$) between meals at a given time-point. 0-0. basal meal without lipids; 45-0 to 45-700. meals with 45 g triglycerides and 0, 140, 280, and 710 mg cholesterol, respectively.

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TABLE 3. Areas under the 0-7 h postprandial curves (AUCs) for plasma and large TRL lipids

	Test Meals				
	0-0	45-0	45-140	45-280	45-700
			mmol/l • h		
Δ Plasma TG	-0.05 ± 0.05^{a}	2.40 ± 0.22^{b}	2.76 ± 0.75^{b}	3.25 ± 0.96^{b}	2.72 ± 0.66^{b}
Δ Plasma PL	-0.37 ± 0.24^{a}	0.34 ± 0.24^{b}	$0.76 \pm 0.26^{b,c}$	0.86 ± 0.24^{c}	0.83 ± 0.20^{c}
Δ Plasma FC	0.20 ± 0.09^{a}	0.68 ± 0.12^{b}	$0.88 \pm 0.22^{b,c}$	$0.99 \pm 0.22^{b,c}$	1.08 ± 0.21^{c}
Δ Plasma EC	-0.07 ± 0.15^{a}	-0.42 ± 0.20^{a}	$-0.40 \pm 0.21^{a,b}$	-0.77 ± 0.18^{b}	-0.64 ± 0.19^{b}
Δ Large TRL TG	0.45 ± 0.26^{a}	1.89 ± 0.16^{b}	$2.17 \pm 0.29^{b,c}$	3.29 ± 0.59^{c}	2.71 ± 0.31^{c}
Δ Large TRL PL	0.08 ± 0.05^{a}	0.37 ± 0.04^{b}	0.44 ± 0.09^{b}	0.51 ± 0.15^{b}	0.46 ± 0.07^{b}
Δ Large TRL FC	0.02 ± 0.007^{a}	0.08 ± 0.004^{b}	0.09 ± 0.02^{b}	0.12 ± 0.03^{b}	0.10 ± 0.02^{b}
Δ Large TRL EC	0.03 ± 0.05^{a}	0.26 ± 0.04^{b}	0.29 ± 0.05^{b}	0.27 ± 0.07^{b}	0.21 ± 0.06^{b}

Values (area (mmol/l • h) under the 0-7 h curve) are means ± SEM of 10 subjects. Values bearing the same superscript letter (*a, b, c*) in the same row are not significantly different (ANOVA for repeated measurements, $P < 0.05$). TG, triglycerides; PL, phospholipids; EC, esterified cholesterol; FC, free cholesterol; TC, total cholesterol; Large TRL, large triglyceride-rich lipoproteins; 0-0, basal meal; 45-0, 45-140, 45-280, and 45-700, meals containing 45 g triglycerides and 0, 140, 280, and 710 mg cholesterol, respectively.

ance (ANOVA) for repeated values. This statistical method was used to assess the significance ($P < 0.05$) of the differences observed between the 0-7 h time-point values for a given test meal. Correlation coefficients were obtained from linear regression analyses. The StatView II micro-computer program (Abacus, Berkeley, CA) was used (48).

RESULTS

Postprandial plasma responses

The blood insulin responses to the five meals exhibited a significant ($P < 0.05$) rise over baseline 1-3 h postprandially with a maximum increase (100-222 pmol/l over baseline) after 1 h. The 0-7 h AUCs of the five meals were in the range 266 to 416 (pmol/l)•h and did not significantly differ (data not shown).

The plasma triglyceride responses are shown in **Fig. 1A.** No change over baseline was observed after ingesting the zero-fat meal (0-0) whereas the four meals containing 45 g triglycerides elicited 0-7 h curves with significant increases from 1 to 5 h and comparable maximum values after 2-3 h. At this time-point, triglyceridemia approximately doubled without respect to the amount of cholesterol present in the meal. The plasma triglyceride 0-7 h AUCs exhibited overall comparable values after ingestion of the four meals supplying 45 g triglycerides (**Table 3**).

As shown in Fig. 1B, the postprandial plasma phospholipid pattern was greatly influenced by the type of meal. In the absence of triglycerides in the test meal, there was a measurable decrease in plasma phospholipids, which led to concentrations significantly below baseline 4-7 h after ingestion of the meal, thus giving a negative cumulative 0-7 h postprandial variation (Table 3). Addition of 45 g triglycerides to the meal reversed this response to a slightly positive one from 1 to 4 h in the absence of dietary cholesterol. Addition of cholesterol to the test meal induced a significant 1-7 h postprandial phospholipid rise with a tendency to a higher but unsignificant (with one exception) response. The plasma phospholipid 0-7 h AUCs had variable amplitudes depending on the amount of cholesterol in the meal, i.e., significantly greater AUC values were obtained after ingestion of 280 or 700 mg cholesterol than after triglycerides alone (Table 3).

The postprandial time-courses of variation of plasma free cholesterol are shown in Fig. 1C. When triglycerides were not in the meal, there was no postprandial change in plasma free cholesterol concentration for 7 h. Conversely, in the presence of 45 g triglycerides and different amounts of cholesterol, plasma free cholesterol showed a positive significant postprandial 1-4 h peak over baseline with a significantly higher maximum value 2 h after meal intake. At this time-point, the free cholesterol response was influenced by the amount of cholesterol in the diet, i.e., a significantly higher maximum value was given by the test meal (45-700) containing 700 mg cholesterol compared to a test meal without cholesterol (45-0). The two other cholesterol-enriched meals (45-140 and 45-280) gave intermediate values. Adding triglycerides to the meal induced an overall 3.4-fold increase in 0-7 h free cholesterol variations. The influence of dietary cholesterol on the overall 0-7 h plasma free cholesterol response was clearly shown (Table 3) by the AUC values: a stepwise increase in the free cholesterol AUC was observed when dietary cholesterol increased.

As shown in Fig. 1D, the hourly postprandial changes in plasma esterified cholesterol were almost absent without triglycerides in the meal (0-0), exhibited significantly negative figures when only triglycerides were added (45-0), and showed maximum significant drops 2 and 3 h after ingestion of meals containing both triglycer-

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ides and cholesterol (45-280 and 45-700). The 0-7 h AUCs obtained (Table 3) showed that adding triglycerides to the meal led to an overall 5.7-fold decrease in cholesteryl esters and that ingesting 280 or 700 mg dietary cholesterol elicited maximum postprandial decreases in plasma cholesteryl esters.

After fatty test meals with different amounts of cholesterol, the overall 0-7 h lipid changes (AUCs) were highly associated: triglycerides correlated with phospholipids (r = 0.709, $P < 0.05$), free cholesterol (r = 0.85, $P < 0.01$), and cholesteryl esters (r = − 0.88, $P < 0.001$); phospholipids correlated with free cholesterol (0.60, $P < 0.01$) and esterified cholesterol (r = − 0.79, $P < 0.05$); and free cholesterol correlated with cholesteryl esters (r = − 0.78, $P < 0.05$).

ApoA-I and apoBs were assayed in the plasma samples 3 h and 7 h postprandially. The postprandial changes were very limited for both apolipoproteins and the values were not significantly different from baseline after all test meals (data not shown).

Postprandial large triglyceride-rich lipoprotein changes

The occurrence of large TRL (chylomicrons and large chylomicron remnants) in the plasma is shown in **Fig. 2.** As expected (Fig. 2A), almost no large TRL triglycerides appeared after the fat-depleted basal meal (0-0) whereas large TRL triglycerides peaked 2-3 h postprandially after ingestion of meals containing 45 g triglycerides. The maximum large TRL triglyceride increases (2-3 h) were significantly greater after the 45-700 meal than after the 45-0 and 45-140 meals. This difference was confirmed by the observation that values exhibited by the large TRL triglyceride 0-7 h AUCs (Table 3) after the 45-280 and 45-700 meals were significantly greater than the value given by the fat-meal without cholesterol (45-0).

Large TRL phospholipids (Fig. 2B) exhibited postprandial responses comparable to those given by triglycerides, characterized by a 2-3 h postprandial increase markedly stimulated by dietary triglycerides and addi-

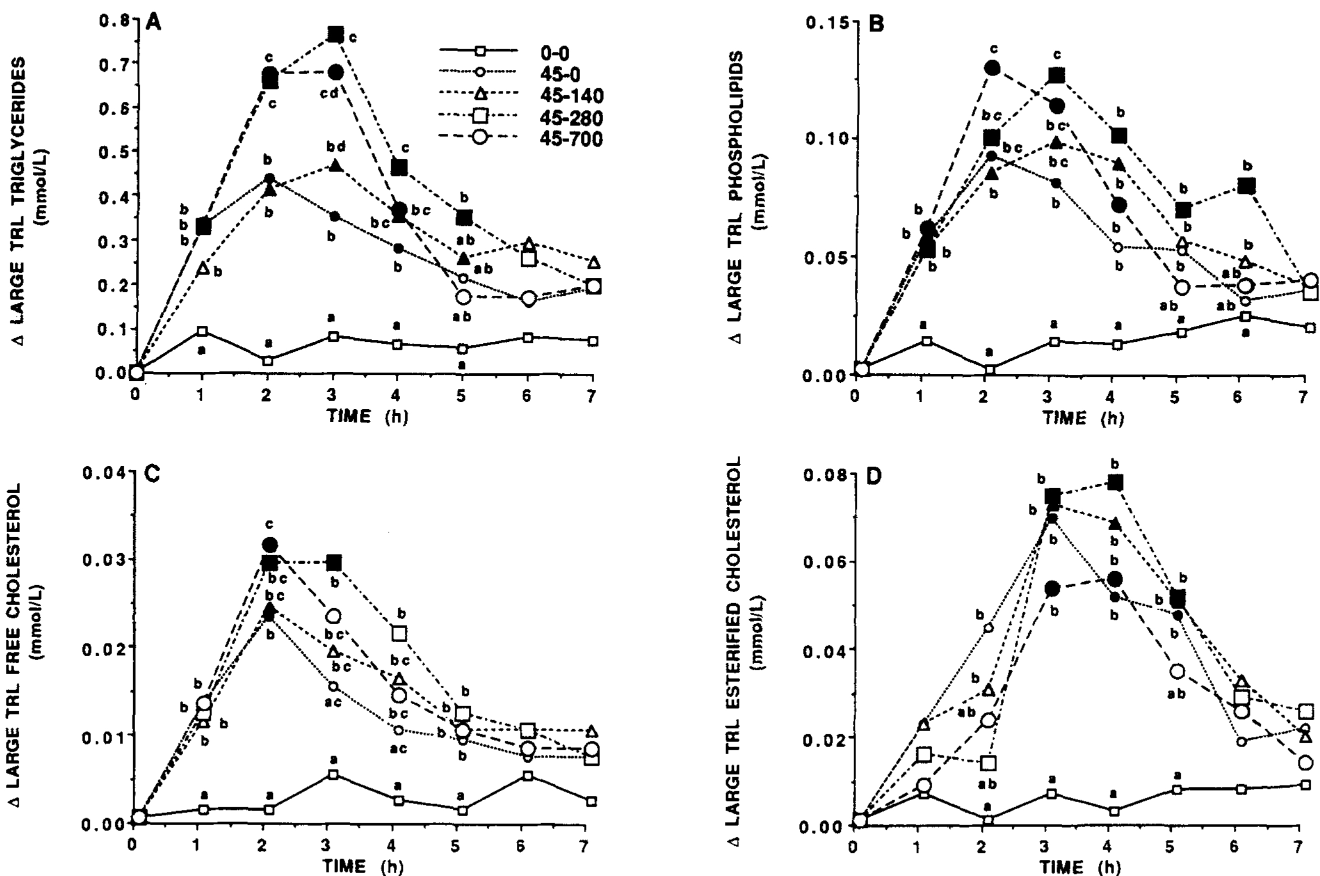

Fig. 2. Large triglyceride-rich lipoprotein (large TRL) triglyceride (A), phospholipid (B), free cholesterol (C), and esterified cholesterol (D) 0-7 h responses to the five test meals. Values [concentration changes (Δ) from fasting values] are means of 10 subjects. For a given test meal, a filled symbol indicates that the corresponding value is significantly different (ANOVA, $P < 0.05$) from the fasting (0 h) value. Different letters indicate significant differences (ANOVA, $P < 0.05$) between meals at a given time-point.

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tionally by a high amount of dietary cholesterol as supplied by the 45-280 meal (after 3 h) and the 45-700 meal (after 2 h). The 0-7 h AUCs exhibited by large TRL phospholipids (Table 3) did not differ significantly after the four meals providing 45 g triglycerides and increasing amounts of cholesterol.

The occurrence of large TRL free cholesterol in the postprandial plasma was close to that exhibited by phospholipids (Fig. 2C). Significant changes over baseline were only observed when the test meals contained triglycerides. The maximum rise was observed 2-3 h postprandially and significantly higher values were given by the two test meals with the highest amounts of cholesterol. The large TRL free cholesterol 0-7 h AUCs obtained after the four fat-meals with different amounts of cholesterol did not differ significantly (Table 3).

The postprandial changes exhibited by large TRL cholesteryl esters (Fig. 2D) were only observed after the fat-containing meals and no marked influence of the amount of dietary cholesterol in the meal was observed in the time-course (maximum at 3-4 h) or the amplitude of overall variation.

The relative lipid composition of the large TRL fraction is given in **Table 4.** No significant change in the lipid composition of this fraction was induced by increasing amounts of dietary cholesterol. Moreover, the surface to core lipid ratios (w/w) were not different between meals and fell in the following ratios at 3 h: phospholipids/triglycerides: 0.14-0.21; phospholipids + free cholesterol/triglycerides + cholesteryl esters: 0.16-0.20. The overall 0-7 h AUC triglyceride/phospholipid ratios in large TRLs did not exhibit significant changes after the five experimental meals (range: 4.97-5.90).

Postprandial lipoprotein changes

In order to check the time-course of lipoprotein class responses, the small TRL (VLDL + small chylomicron remnants), LDL, and HDL were isolated from each hourly postprandial plasma sample obtained after the five meals in one representative subject. As already observed on plasma and chylomicron samples, the changes exhibited by lipoprotein classes were most noticeable (data not shown) after 3 h for triglycerides (small TRL, LDL, HDL), 2-3 h for phospholipids and free cholesterol (small TRL, LDL), and 3 h for esterified cholesterol (small TRL, LDL, HDL). Given the data obtained herein and other previous data from the literature (23, 25, 28, 39, 41), we analyzed postprandial lipoproteins 3 h and 7 h after meal intake.

Significant increases over baseline were observed 3 h but not 7 h postprandially for small TRL triglycerides, after ingesting the 45 g triglyceride meals as shown in **Fig. 3.** The postprandial rise over baseline (mean baseline: 0.39 ± 0.04 mmol/l) in small TRL triglycerides was not influenced by the amount of cholesterol present in the test meals. The LDL triglycerides and HDL triglycerides were not markedly modified 3 h and 7 h postprandially in any case (Fig. 3).

Significant small TRL phospholipid rises were obtained 3 h after ingestion of a meal containing 45 g triglycerides whatever the cholesterol supply as shown in **Fig. 4A.** After 7 h (Fig. 4B), values close to baseline (mean baseline: 0.21 ± 0.01 mmol/l) were obtained. Conversely, LDL phospholipids (mean baseline: 1.09 ± 0.04 mmol/l) did not markedly change postprandially after the five meals studied. HDL phospholipids (mean baseline: 1.20 ± 0.05 mmol/l) tended to decrease slightly 3 h after the ingestion of the different meals (Fig. 4A) whereas after 7 h (Fig. 4B), HDL phospholipids did not change after the cholesterol-depleted meals (0-0 and 45-0) and significantly increased over baseline to a comparable extent after the three cholesterol-containing meals.

The significant postprandial changes observed in plasma free cholesterol concentration (Fig. 1C) were basically due to changes in small TRL and LDL free cholesterol as shown in **Fig. 5.** Small TRL free cholesterol basically increased over baseline (mean baseline: 0.083 ± 0.01 mmol/l) after 3 h whatever the test meal and came back to baseline after 7 h. LDL free cholesterol slightly but unsignificantly changed 3 h after the 0-0,

TABLE 4. Lipid composition of the large triglyceride-rich lipoproteins recovered 3 h after meals with different cholesterol contents

Meal	TG	PL	EC	FC	Apparent Size[a]
		weight %			*nm*
0-0	81.7 ± 5.0	11.1 ± 3.3	4.8 ± 1.8	2.4 ± 1.6	96 ± 8.5
45-0	72.2 ± 3.4	15.0 ± 1.3	11.4 ± 3.3	1.5 ± 1.3	76 ± 7.2
45-140	75.0 ± 7.3	14.2 ± 4.4	9.3 ± 2.7	1.4 ± 0.4	80 ± 8.2
45-280	80.1 ± 3.6	12.2 ± 1.3	6.3 ± 2.6	1.5 ± 1.5	94 ± 5.3
45-700	81.1 ± 2.2	12.4 ± 1.2	5.1 ± 1.7	1.3 ± 1.4	92 ± 3.8

Values are mean ± SEM of 10 subjects. TG, triglycerides; PL, phospholipids; EC, esterified cholesterol; FC, free cholesterol.

[a]The apparent particle size was calculated from the weight percent lipid composition by using a microcomputer program (53).

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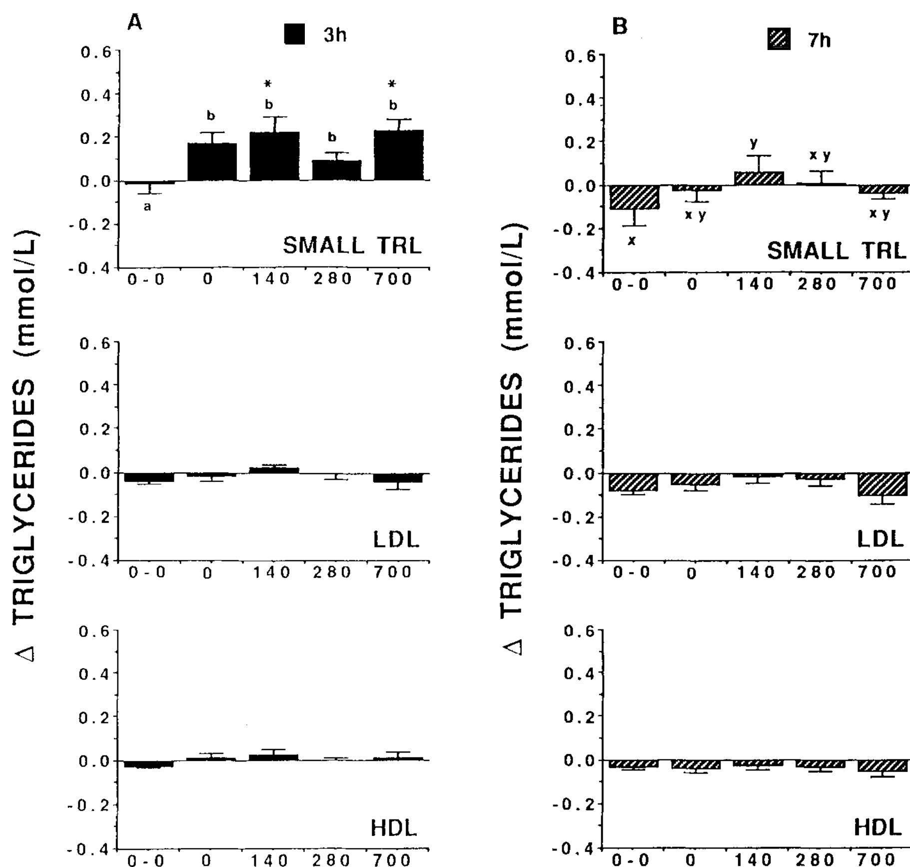

Fig. 3. Changes in lipoprotein triglycerides (3 h, panel A; 7 h, panel B) after intake of the five test meals. Values (concentration changes (Δ) from fasting values) are means ± SEM for 8 subjects. At a given time-point, an asterisk indicates a significant difference (ANOVA, $P < 0.05$) from the corresponding fasting value. Different letters indicate significant differences (ANOVA, $P < 0.05$) between meals. Small TRL, small triglyceride-rich lipoprotein (small chylomicron remnants + very low density lipoproteins); LDL, low density lipoproteins; HDL, high density lipoproteins; 0-0, basal meal without lipids; 45-0 to 45-700, meals with 45 g triglycerides and 0, 140, 280, and 710 mg cholesterol, respectively.

45-0, and 45-140 meals (Fig. 5A) whereas it significantly increased over baseline (mean baseline: 0.67 ± 0.03 mmol/l) after the 45-280 and 45-700 meals. The incremental change induced by the 45-700 meal was significantly greater than the one given by the cholesterol-depleted meal (45-0). Significant variations were no longer observed (except with the 45-280 meal) after 7 h (Fig. 5B). The HDL free cholesterol appeared almost unchanged 3 h postprandially (Fig. 5A) and exhibited significant increases over baseline (0.22 ± 0.02 mmol/l) 7 h after the 45-0 and 45-750 meals (Fig. 5B): no significant differences were found between meal responses.

As shown in **Fig. 6A,** small TRL cholesteryl esters tended to decrease slightly, but not significantly after 3 h and significantly decreased below baseline (mean baseline: 0.28 ± 0.03 mmol/l) 7 h after the 45-0 and 45-140 meals (Fig. 6B). The LDL cholesteryl esters exhibited bimodal variations depending on postprandial time: after 3 h (Fig. 6A), only the 45-280 and 45-700 meals induced significant drops below baseline (mean baseline: 2.07 ± 0.08 mmol/l) in LDL cholesteryl esters, whereas after 7 h (Fig. 6B), only the three other meals (0-0, 45-0, and 45-140) significantly increased cholesteryl esters. HDL cholesteryl esters were significantly decreased below baseline (mean baseline: 1.05 ± 0.04 mmol/l) after 3 h (Fig. 6A) after the three cholesterol-enriched meals only (45-140, 45-280, and 45-700). No marked variations were observed 7 h postprandially (Fig. 6B).

DISCUSSION

In the present study, as in some previous studies (27, 29, 39), the test meals used were based on normally consumed foodstuffs. The amount of triglycerides present in four meals (45 g/1071 kcal) represents a food consumption pattern (105 g fat/2500 kcal or day, 37.8% of energy as fat) well representative of the usual fat intake in most industrialized countries, as illustrated by the food intake pattern of the subjects involved in the study, and can, therefore,

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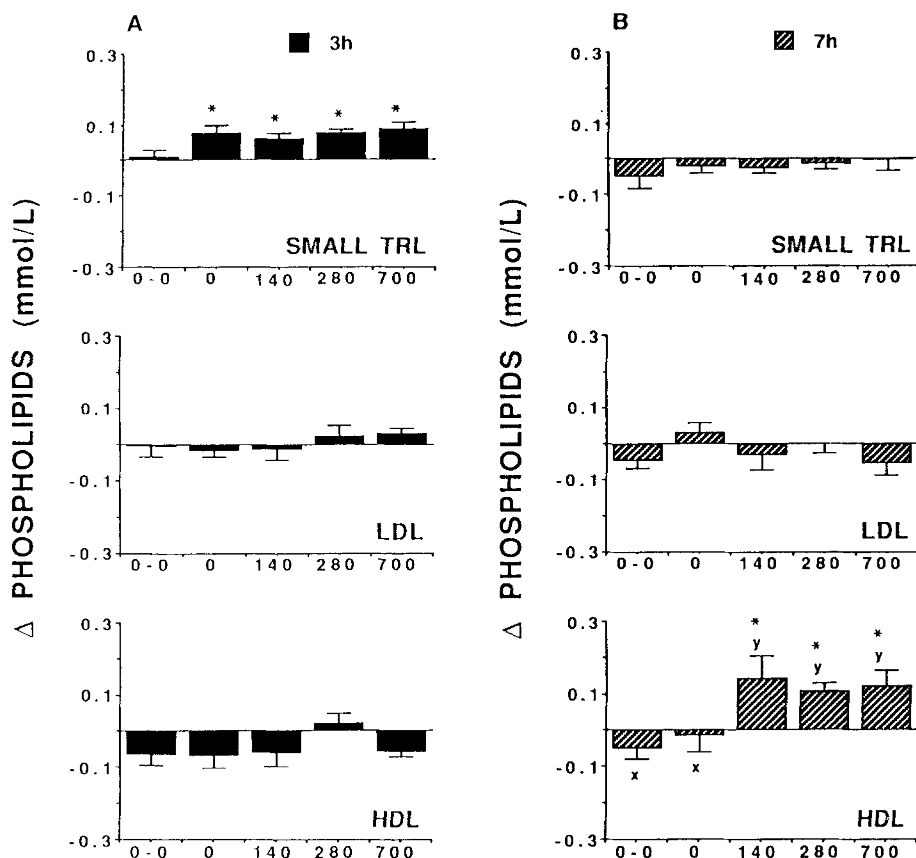

Fig. 4. Changes in lipoprotein phospholipids (3 h, panel A; 7 h, panel B) after intake of the five test meals. Values (concentration changes (Δ) from fasting values) are means ± SEM for 8 subjects. At a given time-point, an asterisk indicates a significant difference (ANOVA, $P < 0.05$) from the corresponding fasting value. Different letters indicate significant differences (ANOVA, $P < 0.05$) between meals. Small TRL, small triglyceride-rich lipoprotein (small chylomicron remnants + very low density lipoproteins); LDL, low density lipoproteins; HDL, high density lipoproteins; 0-0, basal meal without lipids; 45-0 to 45-700, meals with 45 g triglycerides and 0, 140, 280, and 710 mg cholesterol, respectively.

be considered as a physiological fat-meal. Greater amounts of fat have been used in most postprandial studies (22, 23, 25, 27–29, 32, 39, 49–51). In the test meals, dietary fatty acids were basically provided by a mixture of triglycerides (partially hydrogenated sunflower and olive oil) and, to an extent, by phospholipids (egg yolk, soybean lecithins) with an overall P/M/S fatty acid ratio (1.1/2.3/1.0). The increasing amounts of dietary cholesterol (0, 140, 280, and 710 mg) in the test meals were selected to fit the range of daily cholesterol intake and to bracket the recommended maximum daily intake (11, 12) of 300 mg cholesterol (about 120 mg/1000 kcal).

The hardly detectable postprandial response we observed after healthy subjects had ingested a test meal depleted of lipids (and cholesterol) confirms that the small intestine is unable to secrete significant amounts of triglyceride-rich lipoproteins in the absence of dietary fat assimilation (52). Moreover, when triglyceride-rich lipoproteins were not substantially secreted by the intestine into the blood circulation, almost no disturbances could be detected postprandially in the lipoprotein fractions present in the fasting state, i.e., VLDL, LDL, and HDL. This observation means that postprandial changes observed after lipid intake should result directly from the disturbances and remodeling of existing (fasting) lipoproteins induced by the secretion of intestinally derived particles. This is well illustrated by the observation that postprandial changes in plasma lipids (triglycerides, phospholipids, and free and esterified cholesterol) were highly correlated.

The large TRL fraction, composed of native chylomicrons plus large-sized chylomicron remnants, was altered by cholesterol feeding, i.e., only the two highest amounts of dietary cholesterol significantly increased the 2–3 h large TRL triglyceride peak concentration and 0–7 h AUCs. There is no simple way to explain why the two highest doses of dietary cholesterol resulted in significantly higher triglyceride postprandial rises. This might be due to alterations in intestinal secretion of chylomicron and/or plasma clearance. The relative con-

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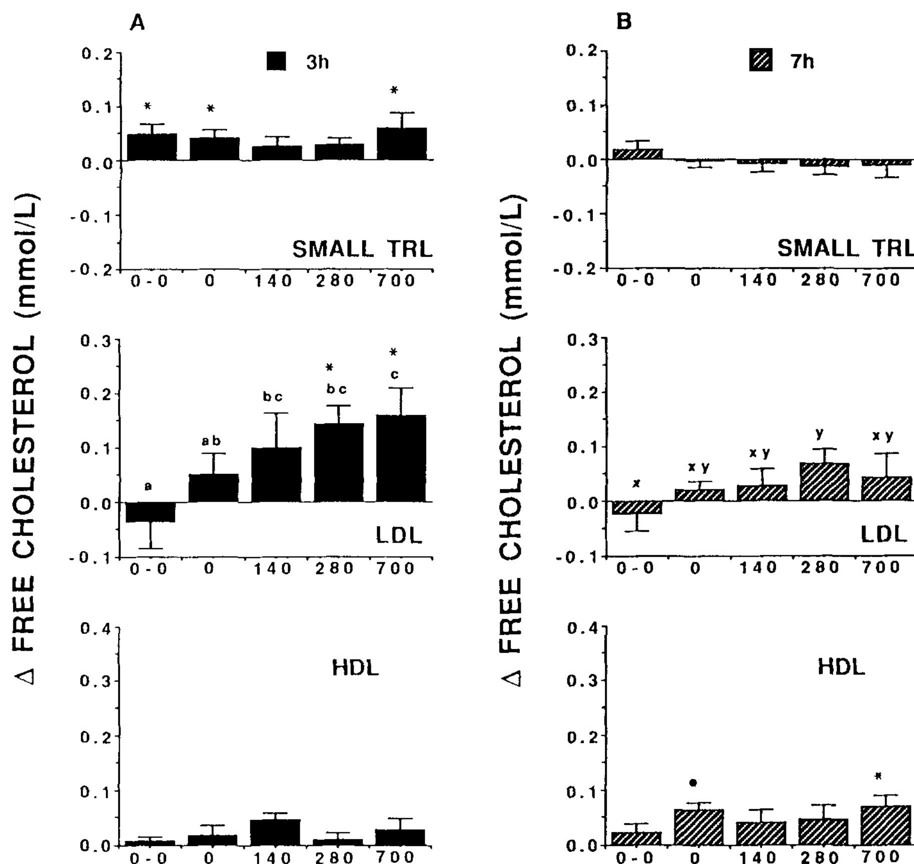

Fig. 5. Changes in lipoprotein free cholesterol (3 h, panel A; 7 h, panel B) after intake of the five test meals. Values (concentration changes (Δ) from fasting values) are means ± SEM for 8 subjects. At a given time-point, an asterisk indicates a significant difference (ANOVA, $P < 0.05$) from the corresponding fasting value. Different letters indicate significant differences (ANOVA, $P < 0.05$) between meals. Small TRL, small triglyceride-rich lipoprotein (small chylomicron remnants + very low density lipoproteins); LDL, low density lipoproteins; HDL, high density lipoproteins; 0–0, basal meal without lipids; 45–0 to 45–700, meals with 45 g triglycerides and 0, 140, 280, and 710 mg cholesterol, respectively.

tent of hydrophobic core lipids (triglycerides plus cholesteryl esters) did not rise with increasing doses of cholesterol and the relative content of surface lipids changed herein only to a slight extent. This was confirmed by the figures given by calculating the apparent size of the large TRL particles based on their lipid percent composition (53) by using a microcomputer program (a generous gift from D. Atkinson and D. M. Small). After the four meals containing 45 g triglycerides, the mean apparent size of the large TRLs ranged from 76 to 94 nm (Table 4) in agreement with literature data (54), and did not exhibit any significant difference between meals containing different amounts of cholesterol, as recently observed on lymph chylomicrons in the rat (34). Acute cholesterol feeding in the rabbit also did not result in a marked change in chylomicron size (55). It is noteworthy that surface lipid composition, which is involved in particle-lipoprotein lipase interactions and particle-receptor binding, exhibited only marginal changes (as indicated by phospholipid and free cholesterol contents) that could not be sufficient to induce different rates of disappearance from plasma (56). In the same line, drastic changes in the protein moiety are not expected from results obtained herein and in the rat (34), although specific modifications in some chylomicron apolipoprotein fraction during intracellular assembly or exchange in plasma could not be ruled out (52). Finally, the question remains open as to whether minor changes in TRL composition could alter lipoprotein lipase activity and/or tissue uptake after a single meal. The present experimental conditions cannot be compared to those where defective removal of TRL remnants have been observed after chronic cholesterol feeding in animals (55) or in heritable hyperlipidemic rabbit (57) or dyslipidemic subjects (19–22). However, a quick postprandial down-regulation of the apoB, E hepatic receptor or the apoE-containing remnant hepatic receptor (58, 59) after a high-cholesterol meal could eventually take place and thus reduce chylomicron remnant uptake.

The large TRL free cholesterol and cholesteryl ester

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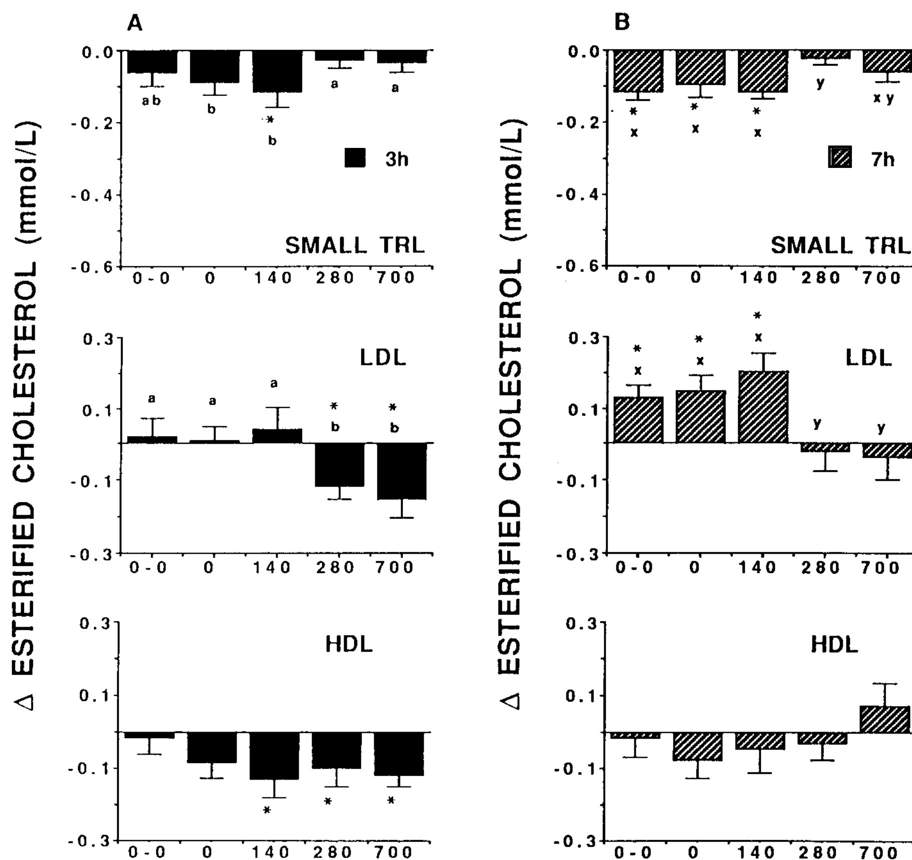

Fig. 6. Changes in lipoprotein esterified cholesterol (3 h, panel A; 7 h, panel B) after intake of the five test meals. Values (concentration changes (Δ) from fasting values) are means ± SEM for 8 subjects. At a given time-point, an asterisk indicates a significant difference (ANOVA, $P < 0.05$) from the corresponding fasting value. Different letters indicate significant differences (ANOVA, $P < 0.05$) between meals. Small TRL, small triglyceride-rich lipoprotein (small chylomicron remnants + very low density lipoproteins); LDL, low density lipoproteins; HDL, high density lipoproteins; 0-0, basal meal without lipids; 45-0 to 45-700, meals with 45 g triglycerides and 0, 140, 280, and 710 mg cholesterol, respectively.

concentration changes over baseline and relative contents were not drastically changed postprandially by the amount of dietary cholesterol. Thus, high levels of cholesterol intake (i.e., 710 mg with 45 g triglycerides) and, presumably, significant intestinal absorption of cholesterol, did not result in substantial cholesterol enrichment in the chylomicron plus large chylomicron remnant fraction. In the only other comparable study performed in the rat (34), although an overall dose–response was found, 20 mg cholesterol/g dietary triglyceride was the minimal effective dose necessary to promote a significant increase in lymph chylomicron cholesterol content; the highest ratio used herein in humans was 15.5 mg/g. In the dog (33), chylomicron composition changes were observed above 10 mg cholesterol/g triglycerides. A second explanation could be that the composition of the TRLs newly secreted into the lymph was influenced mostly by cholesterol exchange from plasma HDL to chylomicrons, as recently shown in chyluric patients (60). A third explanation for this observation could be that native chylomicrons secreted into the lymph could be enriched in cholesterol to some extent due to cholesterol intake, but that the extremely rapid remodeling of particles that occurs when entering the blood stream could lead to only marginal changes in the cholesterol content of the chylomicron-large chylomicron remnant fraction.

Most lipids in the small TRL fraction (VLDL + small chylomicron remnants) were not dramatically altered by increasing dietary cholesterol in the test meals. In contrast, small TRL cholesteryl esters displayed different patterns; with no or a low amount of cholesterol (140 mg), cholesteryl esters decreased after 3 and 7 h, as observed in baboons (61), whereas with high doses of cholesterol they hardly changed. The relative enrichment of small TRLs in cholesteryl esters after high cholesterol intakes (280 and 700 mg) was also observed from the calculated figures given by the whole triglyceride-rich lipoprotein fraction defined as large plus small TRLs in other studies

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(25, 26, 50). This small TRL cholesteryl ester enrichment could result from an increased hepatic secretion of newly synthetized TRL particles as part of particles and cholesterol in the postprandial triglyceride-rich lipoprotein fraction originate from the liver (25, 26, 50). Another possibility could be that the plasma clearance of the small TRL particles would be slowed down, as suggested above for the chylomicron fraction, due to competition for lipolysis and/or the possible involvement of a down-regulation of the hepatic receptors. Finally, the observed relative enrichment of small TRLs in cholesteryl esters might result from an enhanced CETP-mediated transfer of cholesteryl esters from HDL particles to the small TRLs postprandially (14, 24, 62).

The LDL fraction displayed different postprandial changes depending on the level of dietary cholesterol in the test meals. After meals supplying no or a low amount of cholesterol, little changes occurred 3 h postprandially in LDL triglyceride, phospholipid, and cholesterol concentrations. After 7 h, LDL cholesteryl esters markedly increased, likely as a result of a reduced liver uptake after TRL remnant cholesterol input into the liver and down-regulation of hepatic apoB, E receptor activity. This may also result from a late enhanced cholesteryl ester transfer from HDL to LDL at a period of time when the concentration of TRL remnants and VLDL becomes very low. After ingestion of cholesterol-enriched meals (45-280 and 45-700), another pattern was observed; whereas at 3 h postprandially no marked change in LDL triglyceride and phospholipid concentrations was noticed, LDL free cholesterol significantly increased and cholesteryl esters significantly dropped with marginal change in total cholesterol. This increased LDL free cholesterol could result both from the rapid exchange of free cholesterol between lipoprotein particles (63) and the lack of cholesterol esterification at the site of LDL particles. These opposite variations were both correlated with LDL phospholipid changes (r = 0.95, $P < 0.05$ and r = −0.97, $P < 0.02$, respectively). The fact that an increased level of free cholesterol in LDL (and VLDL) blocks the normal CETP-mediated transfer from HDL of cholesteryl esters derived from the LCAT reaction has been shown in several groups of subjects (63). Such a phenomenon could explain the 3 h postprandial drop in LDL cholesteryl esters in addition to competition for transfer in the presence of increased chylomicron-large chylomicron remnants. A postprandial drop in LDL total and esterified cholesterol has been already reported after meals containing triglycerides and 400-600 mg cholesterol (22, 25, 31). On the other hand, it has been shown from studies in humans (31) and baboons (61) that exchanges of free cholesterol between plasma and cells are key events regulating plasma cholesterol levels that are amplified in the postprandial state. We observed herein 3 h after meals containing 280 and 710 mg cholesterol a significant increase in plasma and LDL free cholesterol and significant increases in LDL free cholesterol/phospholipid ratios (0.44 vs. 0.37 and 0.41 vs. 0.36 w/w, respectively). Thus, one may suggest that in those subjects adapted to a usual rather high-fat, high-cholesterol diet, ingesting meals sufficiently enriched in cholesterol could alter the expected movement of cholesterol out of cells into HDL for LCAT reaction, CETP-mediated transfer to TRL, and removal by hepatic uptake.

Postprandial changes displayed by HDL have been already described in detail by others (23, 25, 28, 30, 39, 41, 49, 51, 64). The fact that HDL triglycerides and phospholipids hardly changed herein postprandially after the different meals could be due to the modest amount of triglycerides supplied (45 g/meal) as compared to those used (from 70 to 140 g/meal) in most other studies (22, 23, 25, 28, 32, 39, 41). This was also found in individuals displaying attenuated postprandial lipemia (64). Nevertheless, HDL cholesteryl esters generally dropped 3 h after meal intake in agreement with others as a result of the CETP-mediated transfer to triglyceride-rich particles. A noticeable effect of adding cholesterol to the test meals was a significant increase in HDL phospholipids after 7 h which could originate from the transfer to HDL of the increased chylomicron surface phospholipids as observed postprandially with the 45-280 and 45-700 meals. This could be related to the well-known postprandial alteration in the HDL_3/HDL_2 ratio (30, 54, 64). In turn, the HDL enrichment in phospholipids may enhance the CETP-mediated transfer of cholesteryl esters from HDL particles to triglyceride-rich particles (24), as mentioned above.

The overall link existing between fasting plasma lipids and specific postprandial responses deserves special comment. As almost all subjects participating in the study (8 over 10) exhibited an apoE 3/3 phenotype, the interindividual variability cannot basically result from the documented effect of the apoE E2 or E4 alleles (5, 6). Nevertheless, among these healthy subjects, overall postprandial responsiveness was highly related to several fasting plasma parameters as shown in **Table 5.** Some of these relationships have already been reported by others (21, 25, 28). We confirm herein that even in the range found in subjects exhibiting low plasma triglycerides and cholesterol, the maximum postprandial triglyceride concentration is positively correlated to fasting plasma and VLDL triglycerides and negatively correlated to HDL cholesterol. While fasting triglycerides are correlated to several other postprandial variables, fasting HDL cholesterol is not, thus providing some support for the concept that triglyceride processing is a key parameter and that fasting HDL cholesterol might rather be a dependent variable for triglyceride transport (28). Postprandial changes in plasma and LDL free cholesterol are also positively correlated with fasting triglyceridemia. Among other fasting parameters, plasma phospholipids as well as

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TABLE 5. Linear regression coefficients between fasting and postprandial lipid parameters in normolipidemic subjects

Fasting Concentration	3 h Postprandial Parameters						
	Plasma TG	Plasma PL	Plasma FC	Δ Plasma FC	Δ LDL-FC	Plasma EC	Δ Plasma EC
Plasma TG	0.81 $P < 0.001$	— $P < 0.01$	0.48 $P < 0.001$	0.51* $P < 0.001$	0.61* $P < 0.001$	—	−0.63*
VLDL-TG	0.55* $P < 0.001$	—	—	—	—	—	—
Plasma PL	—	0.95 $P < 0.001$	0.74* $P < 0.001$	0.51 $P < 0.01$	0.53 $P < 0.01$	0.79* $P < 0.001$	— —
Plasma FC	—	0.77 $P < 0.001$	0.91 $P < 0.001$	0.54 $P < 0.001$	0.54* $P < 0.001$	0.63 $P < 0.001$	−0.46 $P < 0.01$
LDL-FC	—	0.66 $P < 0.001$	0.81 $P < 0.001$	— $P < 0.001$	—	0.70	—
Plasma EC	—	0.91 $P < 0.001$	0.76 $P < 0.001$	0.58 $P < 0.001$	0.55* $P < 0.001$	0.94 $P < 0.001$	— —
LDL-TC	—	0.80* $P < 0.001$	0.78 $P < 0.001$	0.57 $P < 0.001$	0.51 $P < 0.01$	0.89 $P < 0.001$	—
HDL−TC	−0.44 $P < 0.001$	—	—	—	—	—	—

Linear regressions were performed with values obtained from eight subjects ingesting the four meals containing 45 g triglycerides. Plasma, VLDL, LDL, or HDL values refer to triglycerides (TG), phospholipid (PL), or cholesterol concentrations (TC, total cholesterol; FC, free cholesterol; EC, esterified cholesterol). Δ Postprandial parameters refer to change at 3 h from fasting baseline concentration. (—) Absence of correlation defined by $r < 0.4$ and $P < 0.01$. An asterisk indicates that the two parameters were significantly correlated ($r > 0.4$ and $P < 0.001$) with the high amounts (280 and 710 mg) of dietary cholesterol but not with the low amounts (0 and 140 mg).

LDL free and esterified cholesterol were found highly correlated with postprandial changes in plasma phospholipids, free and esterified cholesterol, and LDL free and esterified cholesterol. The possible influence of the ingested dose of dietary cholesterol was evaluated by linear regression analysis of data obtained after individual test meals. Highly significant correlations ($P < 0.001$) were found between fasting and postprandial parameters with high doses of dietary cholesterol (280 and 710 mg) and not with zero or 140 mg cholesterol (Table 5) in several occasions such as *1)* fasting triglycerides and 3 h postprandial changes in plasma free and esterified cholesterol and LDL free cholesterol; *2)* fasting phospholipids and postprandial free and esterified cholesterol; and *3)* fasting free or esterified cholesterol and 3 h postprandial change in LDL free cholesterol. This observation suggests that dietary cholesterol exacerbates the postprandial changes influenced by higher fasting triglycerides and phospholipids, and to a lesser extent, higher fasting plasma free or esterified cholesterol.

The present data thus demonstrate that ingesting a meal containing cholesterol in the range 0–140 mg does not alter the postprandial lipoprotein pattern whereas higher amounts in the range 280–710 mg do so significantly. While the role of postprandial lipoproteins in the arterosclerotic process is highly suspect and still actively debated (14, 15, 28), the data obtained herein stress the influence of dietary cholesterol (65, 66) on the postprandial lipoprotein response.

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