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Acute ingestion of different dietary fatty acid species modulates postprandial lipid responses in New Zealand white rabbits

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Although several investigations have linked the degree of fatty acid saturation to plasma lipid responses in the postprandial state, further evaluation is necessary. In this study, we compared the effect of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids on postprandial lipid metabolism using complementary in vivo and in vitro approaches. Fat (10 g) cholesterol (0.5 g) test meals that provided either lard (SFA), olive oil (MUFA), or sunflower oil (PUFA) were ingested by chow-fed New Zealand white rabbits ($n = 8$). In addition, hepatic uptake of triglyceride-cholesterol-rich lipoproteins (TCRL) isolated from rabbits chronically ingesting SFA, MUFA, or PUFA diets was measured using freshly isolated chow-fed rabbit hepatocytes. Whatever dietary fatty acids ingested, postprandial triglyceridemia and occurrence of radiolabelled dietary lipids in plasma were not markedly different. Conversely, SFA induced higher postprandial cholesterolemia and phospholipemia than MUFA ($P < 0.05$) whereas PUFA prevented postprandial cholesterol increase. TCRL disappearance from cultured liver cell media was delayed with SFA-rich TCRL and faster with PUFA whereas MUFA-rich TCRL showed an intermediate figure. From these data, we conclude that SFA, MUFA, and PUFA elicited different postprandial plasma and lipoprotein lipid responses. The fatty acid composition of TCRL had a major impact on their subsequent metabolism, especially uptake by cultured hepatocytes. The SFA-induced hypercholesterolemia could be related to an altered hepatic uptake whereas a faster clearance and hepatic uptake could explain the cholesterol-lowering effect of PUFA in rabbits. MUFA, like PUFA, accelerate uptake by hepatocytes but favor cholesterol ester enrichment of TCRL. (J. Nutr. Biochem. 10:458–466, 1999)

Keywords: dietary lipids; postprandial; lipoprotein clearance; rabbits

Introduction

It has been shown repeatedly that fatty acid species included in the diet alter fasting blood cholesterol levels in both humans and experimental animals. In fact, consumption of saturated fatty acid (SFA) is associated with hypercholesterolemia whereas dietary fat providing monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids have opposite effects.[1,2] Recently, in a meta-analysis of human studies, Gardner and Kraemer[3] summarized the lowering effect of MUFA or PUFA on fasting plasma and low density lipoprotein (LDL) cholesterol concentrations associated with SFA-rich diet replacement.

These studies reported the effect of various dietary fats as assessed in the postabsorptive state and thus, did not consider that humans spend most of the day time in the postprandial state. In fact, it has been suggested during the last 2 decades that postprandial changes in lipid metabolism, especially triglyceride-rich lipoprotein (TRL) accumu-

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lation, are related to atheroma deposition and incidence of cardiovascular diseases.[4–7] In addition to LDL, very low density lipoprotein (VLDL) and chylomicron remnants are thought to be atherogenic lipoprotein fractions. Several studies in humans and animals provided concordant data that showed that acute or chronic (n-6) PUFA versus SFA ingestion results in an enhanced clearance of chylomicron remnants and lower concentration of VLDL[8–10]. In two other recent studies, no evidence was found that a SFA meal alters chylomicron remnant clearance compared with (n-6) PUFA in healthy humans.[11,12] Although most postprandial studies were designed to determine the acute effects of dietary SFA, MUFA, and (n-6) PUFA on postprandial triglyceridemia or TRL, little is know about postprandial plasma and lipoprotein cholesterol responses. To date, only a few studies have been aimed to link fatty acid composition of TRL or chylomicrons with subsequent hepatic metabolism of cholesterol.[13,14]

Thus, in this study, we examined plasma and hepatic postprandial lipid metabolism using complementary approaches. The New Zealand white (NZW) rabbit was chosen because of its wide use in lipid nutrition and because it was used in two previous postprandial studies.[4,15] Among usual animal models, and as compared with humans, it is known that the rabbit displays markedly increased postabsorptive cholesterolemia and triglyceridemia when fed high-fat or high-cholesterol diets. This was basically ascribed to an exacerbated postprandial lipid response.[4] Such a remarkable sensitivity to lipids makes this species a useful model despite some metabolic discrepancies regarding humans. Changes in plasma lipid parameters were measured in chow-fed rabbits after ingestion of a single meal rich in either SFA, MUFA, or PUFA. In addition, to investigate how fatty acid species carried by triglyceride-rich lipoprotein enriched in cholesterol (TCRL) influence lipid metabolism, we studied the in vitro uptake of these TCRL using freshly isolated hepatocytes.

Methods and materials

Animals and diets

Seventeen male NZW rabbits (Elevage Scientifique des Dombes, Romans, France), aged 14 to 15 weeks and weighing 2.71 ± 0.05 kg at the beginning of the experiment, were used. They were housed individually at 20°C and had free access to water and food. They were exposed to a 12-hour light-dark cycle. One group of six rabbits was chronically fed 150 g/d of a regular commercial low-fat, no-cholesterol diet (LF) in granular form (U.A.R 112) from U.A.R (Usine d'Alimentation Rationnelle, Villemoison-Sur-Orge, France). The diet contained (g/kg): cellulose, 160; protein, 130; carbohydrate, 495; vegetable fat, 27; mineral and vitamin mixture, 80; and water, 105. They were given three different test meals as described below.

To study TCRL uptake and metabolism by liver cells, we needed postprandial model TCRL particles enriched in either SFA, MUFA, or PUFA. To get such particles, three groups of three donor rabbits each were chronically fed for 4 weeks high-fat, high-cholesterol diets (HF) prepared by U.A.R. These diets consisted of the commercial diet (921 g/kg) supplemented with cholesterol (3.5 g/kg) and fat (75 g/kg) either in the form of lard, olive oil, or sunflower oil. The LF and HF diets provided 220 and 240 kJ/kg, respectively.

Test meals

To study the acute effect of the ingestion of SFA, MUFA, or PUFA on postprandial metabolism, each of the six rabbits fed the control diet was challenged with three oral fat loads in a random sequence. On the morning of the experiment day (weeks 2, 4, and 6), after an overnight food deprivation, chow-fed rabbits were given a test meal (55 g) as an intrabuccal bolus with a syringe within 20 minutes as described previously.[4] Three test meals provided either lard (L-TM), olive oil (OO-TM), or sunflower oil (SO-TM). The test meals contained cholesterol 0.5 g, [^{3}H]-cholesterol 740 kBq/g cholesterol, and [^{14}C]-triolein 37 kBq/g fatty acid (CEA, Gif-sur-Yvette, France) dissolved in chloroform-methanol (2:1, v/v). Phospholipids as soyabean lecithins (0.5 g) and fat (10.0 g) were added after the organic solvent was evaporated to dryness under nitrogen. Then, casein 4.0 g, cellulose 5.0 g, carbohydrate 15.5 g, mineral and vitamin mixture 2.8 g, and water 18.2 g were added and blended.

To generate postprandial TCRL used for cell experiments, chronically high-fat, high-cholesterol fed rabbits (three per group) ingested a test meal containing the same fat source that was included in their daily diet.

Analytical determinations

Blood was collected from the ear artery in tubes containing lithium-heparin. Blood samples were obtained either in the post-absorptive state (after an overnight food deprivation) or 10, 20, 25, and 35 hours after test meal ingestion. The bleeding time points were determined from previous studies.[4,15] In fact, rabbits have a long lasting postprandial period related to their digestive physiology. To adequately store plasma samples, a cocktail of inhibitors was added as previously reported.[16] Blood was spun for 15 minutes at 910 g at room temperature.

Triglycerides were determined by an enzymatic procedure[17] using commercial kits (Kits BioMérieux, Marcy l'Etoile, France). Total cholesterol was assayed by the cholesterol oxidase method[18] with kits purchased from BioMérieux. Radiolabelled dietary and plasma lipids were measured by liquid scintillation counting in dual label mode with an autoquench correction with a Packard 1600TR equipment (Packard, Meriden, CT USA).

Lipoproteins were separated from plasma using a sequential ultacentrifugation micromethod [435,680 g at 15°C in thickwall polycarbonate tubes in a TL100 Beckman 100.2 rotor (Palo Alto, CA USA)] as previously described.[19] Briefly, 0.3 mL plasma (overnight postabsorptive or 20-h postprandial peak samples) were overlayed with 0.7 mL KBr solution at a density (d) of 1.006 g/mL. After 2 hours of centrifugation, TCRL that included chylomicrons and VLDL were collected in 0.4 mL supernatant. Then, LDL were isolated with the same method but in KBr solution at a density of 1.063 g/mL after 3 hours of ultracentrifugation. High density lipoproteins (HDL) were isolated in KBr solution at a density of 1.21 g/mL after 6 hours of ultracentrifugation. Lipoprotein lipids were adjusted for recovery [i.e., triglycerides (95.5 ± 3.2%), esterified cholesterol (89.1 ± 4.7%), and free cholesterol (93.3 ± 2.6%)].

Cholesteryl ester transfer protein

To compare the acute effects of fatty acid species included in the test meals, the cholesteryl ester transfer protein (CETP) activity of chow-fed rabbits was measured in the postabsorptive state (0 h) and in the postprandial state (20 h). CETP activity was measured as the ability of lipoprotein-deficient rabbit plasma ($d > 1.25$ g/mL fraction) to facilitate the transfer of radiolabelled cholesteryl ester from human HDL_3 to human apoB-containing lipoproteins, as previously described.[20] After incubation for 2 hours at 37°C, the

transfer of cholesteryl ester was stopped by the precipitation method for apoB-containing lipoprotein using dextrane sulfate (0.1%) and $MgCl_2$ (50 mmol/L).[21] The CETP transfer activity was expressed in μg/h/ml plasma) cholesteryl ester transferred from HDL to apoB-containing lipoproteins.

Uptake of postprandial TCRL by isolated rabbit liver cells

The aim of this set of experiments was to establish a possible relationship between postprandial cholesterol responses observed in vivo and the uptake of TCRL specifically enriched with either SFA, MUFA, or PUFA by cultured hepatocytes.

Hepatocytes of rabbits fed the control diet were prepared by perfusion of a liver lobe with collagenase (0.5 g/L) as described previously.[22] After isolation and washing, cells were suspended in William's medium E supplemented with fetal calf serum (10%), glutamine 0.1 mg/mL, kanamycin 0.1 mg/mL, penicillin 20 units/mL, and streptomycin 0.1 mg/L and seeded on collagen-coated dishes (Costar, Cambridge, MA USA; 10^5 cells/cm^2). Dishes were incubated at 37°C in an atmosphere containing 5% CO_2 for 2 to 3 hours. Then the medium was removed by aspiration and cell monolayers were washed with William's medium E supplemented as above but without fetal calf serum. Experimental additions to culture medium were made 24 hours after this point and represented time zero.

Postprandial TCRL that was used ($d \leq 1.019$ g/mL fraction obtained as described above) for the cell culture experiments were isolated from plasmas of rabbits fed the three high-fat, high-cholesterol diets for 4 weeks and 20 hours after test meal consumption (triglyceride postprandial peak). TCRL were dialyzed at 4°C against culture medium and filtered through a 0.22 μm membrane prior to addition to hepatocyte culture. Cholesterol, triglyceride, and protein concentrations were measured using enzymatic procedures.[17,18,23] Fatty acid species carried by TCRL were characterized and quantified by gas liquid chromatography (Perkin Elmer Autosystem XL with a flame ionization detector, St. Quentin en Yvelines, France) after lipid extraction[24] and fatty acid methylation.[25]

Postprandial TCRL were added to the liver cell media at time zero. At 4, 8, and 12 hours after TCRL addition, 0.8 mL culture cell medium was collected for analytical determinations. The disappearance of TCRL was calculated as the percentage of radiolabelled lipids remaining in the cultured cell media. Liver cell viability was controlled in every dish during the experiment and estimated at approximately 90%. At the end point (24 h), liver cell media were removed from dishes. Hepatocytes were washed four times with 5 mL cold 0.15 mol/L saline before being scraped from their dishes and resuspended in 2 mL saline. Lipids were extracted with chloroform/methanol (2:1, v/v).[26] TCRL neutral lipids (triglycerides and free and esterified cholesterol) were extracted[26] and then separated by two-step thin-layer chromatography (TLC) on silica gel plates (Merck-Clevenot, Nogent sur Marne, France).[27] The amount of lipids present was quantified against standard curves by using videodensitometry and BioLab software package, as previously described.[28] The total bile acid content in culture medium was assayed by an established enzymatic method.[29]

Statistical analysis

The statistical significance ($P < 0.05$) of the differences observed between the experimental meals [individual time points or area under the curve (AUC)] or radiolabelled plasma lipid concentrations was assessed by using analysis of variance (ANOVA) for repeated values and Fisher's test.[30] The statistical significance ($P < 0.05$) of the differences observed between culture cell medium concentrations was assessed by using ANOVA for factorial nonrepeated values and Fisher's test. The areas under the 0 to 35-hour curves were calculated by the trapezoidal method. Linear regression analysis were performed. Stat-View II (Abacus, Berkeley, CA USA) microcomputer program was used.

Results

Postprandial plasma lipids and lipoproteins

In rabbits fed the low-fat, no-cholesterol diet, the overnight postabsorptive triglyceride and total cholesterol lipoprotein concentrations, as well as plasma lipid concentrations, which were measured on weeks 2, 4, and 6, remained unchanged during this study period (data not shown).

As is shown in *Figure 1A*, a triglyceride peak was observed 20 hours after meal intake and did not differ among rabbits ingesting lard (SFA, 1.14 ± 0.14 mmol/L), olive oil (MUFA, 1.17 ± 0.08 mmo/L), or sunflower oil (PUFA, 1.29 ± 0.14 mol/L). On the contrary, total cholesterol 0 to 35-hour responses were significantly different between test meals and plateaued at 25 to 35 hours (*Figure 1B*). Acute lard ingestion induced a higher increase in total cholesterol concentration (+123.9 ± 18.3% at 35 h) than olive oil (+75.9 ± 5.7% at 35 h) compared with postabsorptive values whereas sunflower oil (+26.6 ± 16.7% at 35 h) did not significantly modify total cholesterol concentrations postprandially.

When compared with postabsorptive values, TCRL triglyceride concentrations, and to a much less extent LDL triglyceride concentrations, significantly increased postprandially (*Figure 2A*). Nevertheless, fat-cholesterol meals providing lard, olive oil, or sunflower oil did not induce different responses. HDL triglyceride concentrations did not change postprandially after olive oil ingestion whereas lard, and more markedly, sunflower oil, increased HDL triglycerides. As shown in *Figure 2B*, total cholesterol concentrations rose to significantly higher values in TCRL 20 hours after meal intake, compared with postabsorptive values with a lower incremental TCRL-cholesterol rise after sunflower oil than lard or olive oil. Ingestion of sunflower oil did not alter LDL cholesterol concentrations postprandially whereas lard, and to a lesser extent, olive oil, increased LDL cholesterol. As shown in *Figure 2B*, olive oil only decreased significantly HDL cholesterol concentration in the postprandial period.

Postprandial occurrence of dietary lipids in the plasma

The addition of radiolabelled esterified fatty acids and cholesterol to the test meals allowed the post-meal follow up of lipids from dietary origin in the plasma as illustrated in *Figure 3*. [^{14}C] Oleic acid-containing lipids (essentially triglycerides[4]) peaked 20 to 25 hours postprandially (*Figure 3A*). Olive oil, lard, and sunflower oil generated comparable dietary fatty acid enrichment in the circulation with the exception of a higher level with sunflower oil at 25 hours. Plasma 0 to 25-hour concentrations of cholesterol from dietary origin did not show any difference between meals (*Figure 3B*). Conversely, at the postprandial end-point (35 h), the concentration of plasma radiolabelled cholesterol was lower after sunflower oil ingestion than lard, with

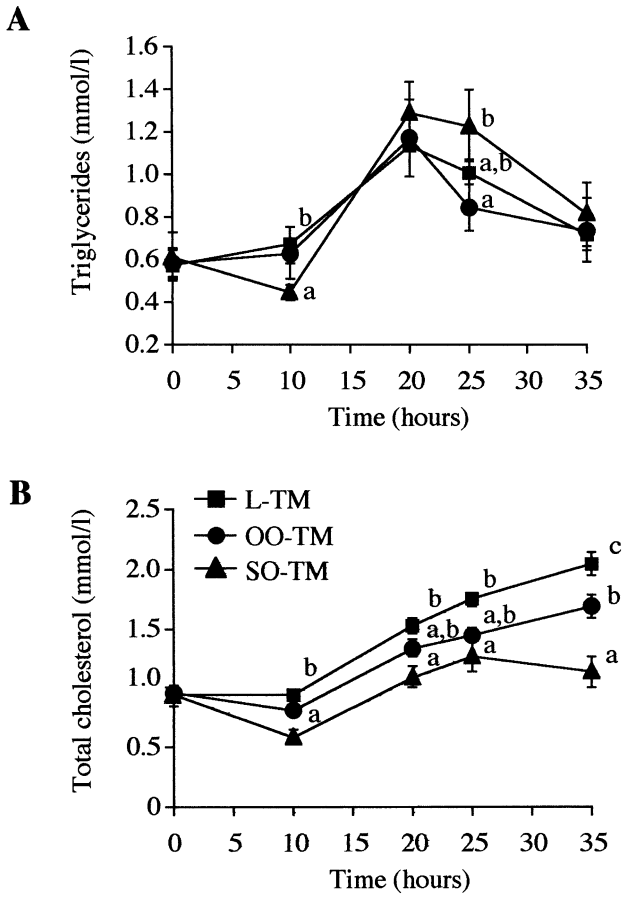

Figure 1 Plasma 0 to 35-h triglyceride *(A)* and total cholesterol *(B)* responses to the fat (10 g), cholesterol (0.5 g) test meals providing either lard (L-TM), olive oil (OO-TM), or sunflower oil (SO-TM). Rabbits ($n = 6$) fed a control diet were randomly given test meals on weeks 2, 4, and 6. Values are means ± SEM represented by vertical bars. Different letters indicate significant differences between test meals at a given time point (analysis of variance for repeated values and Fisher's test, $P < 0.05$). *Significantly different from the postabsorptive values.

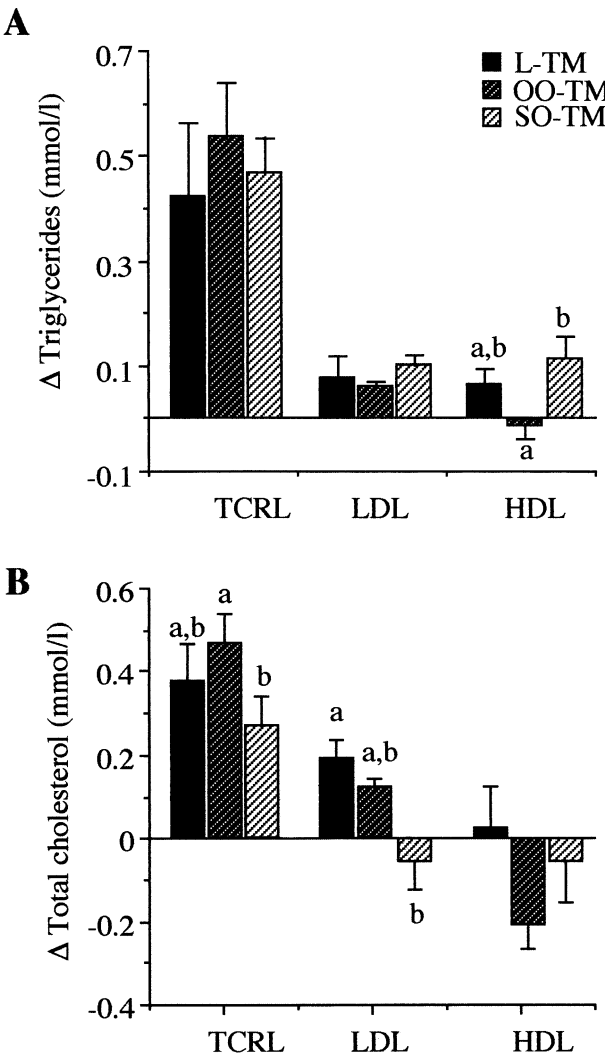

Figure 2 Postprandial (20 h) lipoprotein lipid concentration changes in rabbits ($n = 6$) fed the control diet. The fat (10 g), cholesterol (0.5 g) test meals, containing either lard (L-TM), olive oil (OO-TM), or sunflower oil (SO-TM), were randomly performed on weeks 2, 4, and 6. *(A)* Triglycerides, *(B)* total cholesterol. Data are concentration changes (Δ) from postabsorptive values and are expressed as mmol/L. Values are means ± SEM represented by vertical bars. In the postprandial state, triglyceride, cholesterol-rich lipoproteins (TCRL) (d < 1.019 g/mL) include chylomicrons and very low density lipoproteins. Different letters indicate significant differences between test meals (analysis of variance for repeated values and Fisher's test, $P < 0.05$). *Indicates a significant difference with the postabsorptive values. LDL, low density lipoproteins; HDL, high density lipoproteins.

intermediate figure exhibited by olive oil. Nevertheless, cumulated 0 to 35-hour plasma responses (AUCs) of dietary [^{3}H]-cholesterol to the three meals were not different.

Plasma CETP activity

The mean plasma CETP activity measured in the postabsorptive state (0 h) was 118.3 ± 5.9 μg/(h.mL) of esterified cholesterol transferred from HDL to apoB-containing lipoproteins. Acute ingestion of olive oil, but not lard or sunflower oil, led to a slight but significant postprandial increase (approximately 10%) in plasma CETP activity over postabsorptive value. Moreover, as compared with the value obtained 20 hours postprandially after the olive oil containing test meal [130.4 ± 4.5 μg/(h.mL)], the sunflower oil test meal [114.1 ± 5.6 μg/(h.mL)] significantly lowered CETP activity in the postprandial state. An intermediate value of CETP activity was measured 20 hours after lard intake [i.e., 127.0 ± 5.9 μg/(h.mL)].

Time course of TCRL lipid uptake by cultured liver cells

Postprandial TCRL used for the cell culture experiments were isolated from plasma of donor rabbits fed the three high-fat, high-cholesterol diets for 4 weeks and 20 hours after consumption of a test meal containing the same fat they were chronically fed (i.e., lard, olive oil, or sunflower oil). Chronic ingestion of SFA, MUFA, or PUFA and test meals led to accumulation of TCRL specifically enriched in the corresponding fatty acid species (*Table 1*). With the aim

of comparing uptake and metabolism of cholesterol in the form of different TCRL, we elected to normalize the amount of TCRL cholesterol provided to cultured cells. Thus, TCRL total cholesterol concentrations were adjusted to determine comparable final total cholesterol concentrations (comparable to in vivo postprandial incremental concentrations). Total and free cholesterol, triglyceride, and protein concentrations are given in *Table 2*. Protein concentrations of the three different TCRL preparations were not markedly different, suggesting the presence of a comparable number of particles. The uptake of postprandial TCRL [at 20 hours triglyceride peak from rabbits chronically fed lipid rich diets providing lard (L-TCRL), olive oil (OO-TCRL), or sunflower oil (SO-TCRL)] by chow-fed rabbit hepatocytes was determined (*Figure 4*). TCRL triglyceride disappearance tended to be lower when hepatocytes were incubated with lipoproteins isolated from lard-fed rabbits compared with olive oil- and sunflower oil-fed animals (*Figure 4A*), whereas TCRL total cholesterol uptake by hepatocytes was not different after 4 hours (*Figure 4B*) but was significantly higher after 8 and 12 hours with SO-TCRL, and to a lesser extent, OO-TCRL.

Incubation of hepatocytes with OO-TCRL or SO-TCRL for 24 hours induced a significant and comparable increase in secreted bile acid concentration over baseline without TCRL addition (*Table 3*). On the contrary, L-TCRL did not noticeably stimulate bile acid secretion by hepatocytes.

The intracellular triglyceride content was significantly higher after 24 hours incubation with postprandial OO-TCRL or L-TCRL (*Table 4*). Incubation with all three TCRL slightly increased intracellular contents in free cholesterol. On the contrary, when hepatocytes were incubated 24 hours with L-TCRL, the esterified cholesterol content increased 1.8-fold over baseline, a figure significantly higher than that induced by incubation with OO-TCRL or SO-TCRL.

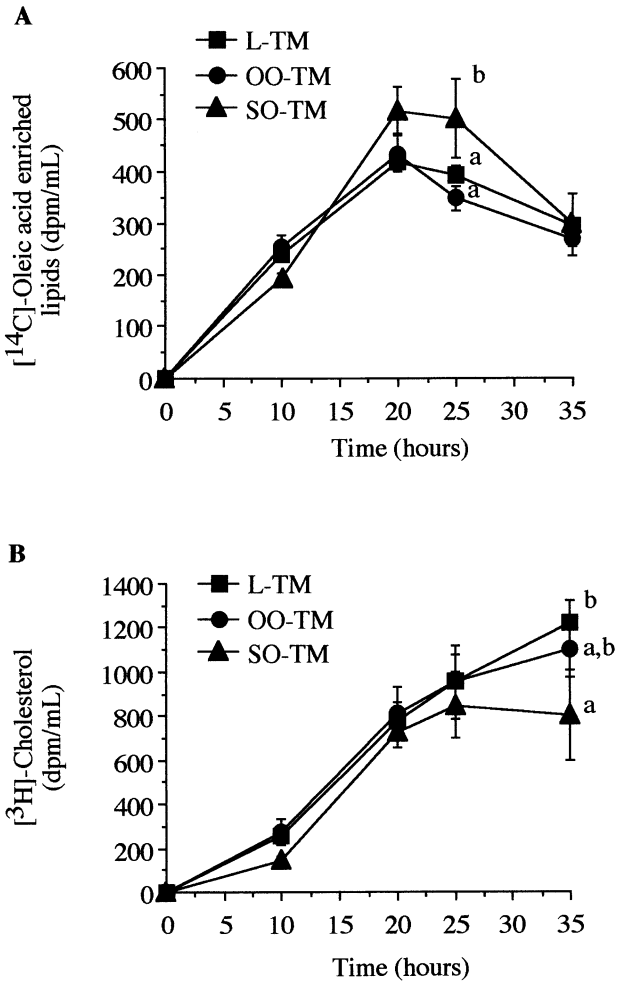

Figure 3 Plasma 0 to 35-h [^{14}C]-oleic acid containing lipid responses *(A)* and [^{3}H]-cholesterol responses *(B)* to the fat (10 g), cholesterol (0.5 g) test meals providing either lard (L-TM), olive oil (OO-TM), or sunflower oil (SO-TM). Rabbits ($n = 6$) fed the control diet were randomly given test meals on weeks 2, 4, and 6. Values are means ± SEM represented by vertical bars. Different letters indicate significant differences between test meals at a given time point (analysis of variance for repeated values and Fisher's test, $P < 0.05$).

Discussion

This study aimed to compare the respective effects of three sources of edible fat—lard, olive oil, and sunflower oil—providing predominantly SFA, MUFA, and PUFA, respectively, on postprandial lipid metabolism in the rabbit. We selected the rabbit model because of its remarkable sensitivity to dietary lipids and exacerbated postprandial response.[4,15] Complementary experimental approaches such as acute feeding of fat, postprandial follow-up after test meals, TCRL uptake, and metabolism by cultured isolated hepatocytes were used.

The first worthwhile observation was that acute feeding of lard, olive oil, or sunflower oil generated overall comparable hypertriglyceridemic responses as well as increase in TCRL triglycerides or [^{14}C]-oleic acid-containing lipids in the plasma after test meals providing the same quantity of nutrients. These data are not in agreement with previous studies obtained in humans[10] and rats.[8] These previous studies showed exacerbated triglyceride increases in the plasma postprandially after acute ingestion of SFA compared with PUFA. Similarly, other authors have reported a delayed catabolism of chylomicrons obtained in coconut oil-fed rats compared with safflower-fed ones, due to a lower stimulation of lipoprotein lipase in adipose tissue.[9] Conversely, other authors recently showed in healthy humans that chronic feeding of a diet rich in SFA or (n-6) PUFA elicited comparable triglyceride postprandial responses, although SFA tended to increase apo-B100-containing TCRL.[11]

The postprandial changes in plasma total cholesterol were altered by the kind of fat ingested by the rabbit. Sunflower oil feeding did not noticeably increase plasma total cholesterol postprandially whereas olive oil feeding increased it slightly and lard feeding more markedly. At the same time, accumulation of radiolabelled dietary cholesterol in the plasma was comparable except for a lower figure after sunflower oil at the end point (35 h). Postprandial lipoprotein analysis indicated that sunflower oil reduced total cholesterol in TCRL and LDL. This suggests that sunflower oil can reduce the postprandial rise in plasma

Table 1 Fatty acid composition of triglyceride, cholesterol-rich lipoproteins (TCRL) isolated postprandially from rabbits chronically fed either lard, olive oil, or sunflower oil

Fatty acids (%)	L-TCRL	OO-TCRL	SO-TCRL
C 14:0	1.05	0.98	0.77
C 16:0	26.70	20.36	16.81
C 16:1	3.71	0.48	0.62
C 18:0	9.52	6.28	7.80
C 18:1	33.23	42.90	23.75
C 18:2	18.46	20.46	37.80
C 18:3	1.10	1.44	3.14
C 20:4	1.17	1.06	3.44
Others	5.06	6.04	5.87

Lipoproteins were isolated from hypercholesterolemic rabbits chronically fed lard (L-TCRL), olive oil (OO-TCRL), or sunflower oil (SO-TCRL), as noted in the animals and diets section, in the postprandial state (20 h triglyceride peak samples). Composition of test meals is detailed in the Test meals section. The fatty acid content of the three TCRL fractions, measured using gas liquid chromatography, is expressed as percentage of total fatty acid species.

Table 2 Lipid and protein concentrations in cultured hepatocyte media

Lipids	L-TCRL	OO-TCRL	SO-TCRL
Total cholesterol (mmol/L)	1.85	1.85	1.85
Esterified cholesterol (mmol/L)	1.22	1.27	1.17
Free cholesterol (mmol/L)	0.63	0.58	0.68
Triglycerides (mmol/L)	0.32	0.77	0.62
Protein (g/L)	0.31	0.35	0.28

Triglyceride, cholesterol-rich lipoproteins (TCRL) were isolated from hypercholesterolemic rabbits chronically fed lard (L-TCRL), olive oil (OO-TCRL), or sunflower oil (SO-TCRL), as noted in the Animals and diets section, in the postprandial state (20 h triglyceride peak samples). Test meal composition is detailed in the Test meals section.

total cholesterol because of a faster clearance of TCRL from the plasma, as evidenced by the similar occurrence of radiolabelled dietary lipids in the plasma after intestinal absorption of the three types of fat. The fact that olive oil lowered the postprandial rise in total and esterified cholesterol (data not shown) compared with lard was essentially due to a drop in HDL cholesterol, suggesting that olive oil feeding could stimulate the HDL cholesterol uptake by tissue postprandially or alternatively could stimulate the CETP-mediated esterified cholesterol transfer from HDL to TCRL.[20] The finding of a positive correlation ($r = 0.68$, $P = 0.008$) between postprandial HDL esterified cholesterol and CETP activity gives weight to the second possibility.

These data show that acute ingestion of dietary fatty acids can alter postprandial levels of plasma and lipoprotein total cholesterol although effects on plasma triglycerides and TCRL triglycerides are marginal. Complementary experimental approaches were developed to highlight some potential mechanisms involved.

Given that comparable postprandial accumulations of dietary lipids and cholesterol were observed with various sources of fat, it appeared that an important difference in fat handling by the small intestine could not play a major role in the difference that was observed, as has been suggested by other authors.[8,31–33] Thus, the metabolic responses elicited by different dietary fats could likely result from different handlings of TCRL particles in the circulation. From studies[34] in humans and rats,[8] it has been suggested that fatty acid species could modulate the lipoprotein lipase activity. Although not measured in this study, it is unlikely

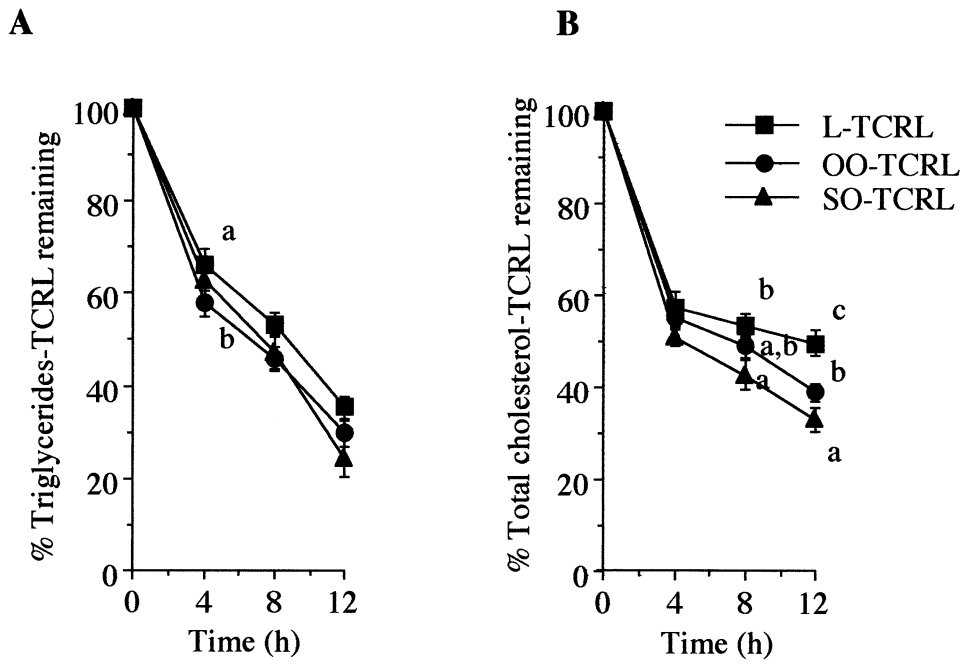

Figure 4 Kinetic of disappearance of triglyceride, cholesterol-rich lipoproteins (TCRL) triglycerides *(A)* and TCRL cholesterol *(B)* during incubation with cultured hepatocytes. The amount of TCRL lipids remaining in the culture medium is shown as the fraction (%) the dose dispensed to cells at time zero. TCRL were isolated from rabbits in the postprandial state (20 h), chronically fed either lard (L-TCRL), olive oil (OO-TCRL), or sunflower oil (SO-TCRL). Values represent means ± SEM. Hepatocytes were isolated from rabbit fed on a control diet for 4 weeks. Different letters indicate significant differences between groups of dishes (analysis of variance for factorial nonrepeated values and Fisher's test, $P < 0.05$).

Table 3 Bile acid concentration in hepatocyte culture medium

	Bile salt secreted (24 h) (mg/L)
TCRL free	26.1 ± 2.8[a]
L-TCRL	31.1 ± 3.1[a,b]
OO-TCRL	38.3 ± 3.8[b,c]
SO-TCRL	41.6 ± 1.8[c]

Rabbit hepatocytes (10^6 cells = 10^5 cells/cm^2) were incubated with triglyceride, cholesterol-rich lipoproteins (TCRL) isolated in the postprandial state from hypercholesterolemic rabbits chronically fed lard (L-TCRL), olive oil (OO-TCRL), or sunflower oil (SO-TCRL). Values are bile acid concentrations in culture media without added TCRL or 24 hours after TCRL addition and represent means ± SEM of six replicates.
[a,b,c]Different letters indicate a significant difference between treatment (analysis of variance for factorial nonrepeated values and Fisher's test, $P < 0.05$).

that acute ingestion of fat could alter the level of endovascular lipoprotein lipase within hours.

The second step involved in clearing particles from the circulation is the uptake by peripheral tissue, especially the liver. To evaluate the role of dietary fatty acids in this process, we incubated TCRL isolated postprandially from rabbits fed the different fats with cultured hepatocytes obtained from donor chow-fed rabbits. Postprandial TCRL are a mixture of intact chylomicrons and β-VLDL and their remnants. Given the low level of hepatic lipase in the rabbit,[35] one can assume that the disappearance of TCRL lipids from the incubation media essentially accounts for uptake by hepatocytes, especially under conditions mimicking in vivo postprandial incremental rise in cholesterol concentration. The data obtained showed that the disappearance of postprandial TCRL cholesterol was higher from sunflower oil and olive oil TCRL than from lard TCRL. Given that the uptake by hepatocytes of postprandial TCRL triglycerides was higher with TCRL from olive oil-fed, and even more markedly, sunflower oil-fed, rabbits than with TCRL from lard-fed rabbits, it seems likely that MUFA and PUFA stimulate the uptake by cultured hepatocytes of respective postprandial TCRL. This observation fits well with post-meal data obtained in animals that show a reduced cholesterol accumulation in the plasma after olive oil or sunflower oil ingestion. Similar data have also been obtained by other authors studying chylomicron clearance in rats receiving different dietary fats.[13] In rats, MUFA- or SFA-enriched chylomicrons showed a reduced rate of clearance compared with PUFA-enriched chylomicrons.[13] In contrast, using the perfused liver model, chylomicrons rich in SFA, MUFA, or n-6 PUFA had comparably reduced rates of clearance than chylomicrons rich in n-3 PUFA or butter fat.[36]

We observed that the rate of uptake of postprandial TCRL total cholesterol and triglycerides were not superimposable. Given that TCRL are heterogenous (i.e., a mixture of cholesterol ester-rich endogenous β-VLDL and dietary cholesterol-rich chylomicrons,[4] such differences observed could be due to somewhat different ways of uptake of these lipoprotein fractions by hepatocytes. It has already been shown that β-VLDL preferentially binds the LRP hepatic receptor[37] whereas chylomicron remnant uptake could be mediated, at least in part, by the LDL receptor.[38] Recently, a new hepatic receptor called LSR, which is activated by free unsaturated fatty acids, has been identified.[39] Although the effect of dietary fats on the in vivo regulation of the expression of the LSR is unknown, the down-regulation of the LDL receptor by palmitate compared with oleate or 25-hydroxycholesterol has been demonstrated using human HepG2 cells.[40] In this study, we observed that the level of hepatocyte free cholesterol, which down-regulates the expression of the LDL receptor at cell surface,[41] was more increased 20 hours after incubation with L-TCRL than with OO-TCRL or SO-TCRL. This could explain the higher rate of cellular uptake of OO-TCRL and SO-TCRL compared with that of L-TCRL. A mechanism that might lower accumulation of free cholesterol within hepatocytes has been identified: When hepatocytes were incubated with OO-TCRL, and even more noticeably, SO-TCRL, the amount of bile salts secreted was significantly higher. Comparable data have been obtained by others[42] after feeding diets rich in PUFA, MUFA, or SFA to mice for 3 weeks. Altogether, the data obtained with cultured hepatocytes illustrate the cascade by which sunflower oil can prevent the increase in cholesterolemia during the postprandial period.

In conclusion, by using complementary experimental approaches, we showed that acute ingestion of dietary fatty acids in the rabbit modulates TCRL metabolism in the postprandial state. SFA were shown to modulate the hepatic uptake by isolated hepatocytes, leading to an increase in cholesterolemia postprandially. Conversely, oleic acid could slow TCRL lipolysis but stimulates uptake by isolated

Table 4 Triglyceride and free and esterified content of rabbit hepatocytes without added triglyceride, cholesterol-rich lipoproteins (TCRL) or 24 hours after TCRL addition in culture medium

Lipids (μg/10^6 cells)	TCRL free (n = 6)	L-TCRL (n = 6)	OO-TCRL (n = 6)	SO-TCRL (n = 6)
Triglycerides	150.2 ± 10.3[a]	219.5 ± 14.7[b]	206.0 ± 8.8[b]	150.6 ± 23.8[a]
Total cholesterol	155.4 ± 6.8[a]	246.4 ± 6.2[b]	199.2 ± 4.8[b,c]	182.2 ± 3.2[c]
Free cholesterol	65.1 ± 4.8[a]	85.5 ± 1.9[b]	81.6 ± 2.9[b]	75.4 ± 3.7[a,b]
Esterified cholesterol	90.3 ± 7.9[a]	160.9 ± 8.7[b]	117.6 ± 5.8[c]	106.8 ± 2.9[c]

The rabbit hepatocytes (10^6 cells = 10^5 cells/cm^2) were incubated with TCRL isolated from hypercholesterolemic rabbits, chronically fed lard (L-TCRL), olive oil (OO-TCRL), or sunflower oil (SO-TCRL) in the postprandial state. Values are means ± SEM of six replicates.
[a,b,c]Different letters indicate a significant difference between treatment (analysis of variance for factorial nonrepeated values and Fisher's test, $P < 0.05$).

hepatocytes, thus limiting the plasma accumulation of cholesterol ester-enriched TCRL. Finally, linoleic acid-rich fat favors TCRL clearance and hepatic uptake and prevents postprandial rise in cholesterolemia by stimulating bile salt secretion.

As recently reviewed,[43] our knowledge of the effects of individual dietary fatty acids on postprandial lipid metabolism in humans is limited and the data somewhat conflicting. Despite known species differences in the regulation of lipid metabolism, the present data indicate that acute ingestion of different fatty acids can generate different accumulations of TCRL postprandially, at least in individuals sensitive to dietary fat. It is noteworthy that the postprandial changes observed in this animal model are in agreement with the hypercholesterolemic effect of SFA reported after chronic intake in humans. Further studies are required to fully establish the long-term effects of fatty acids on postprandial metabolism.

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