Macrophage cholesterol efflux elicited by human total plasma and by HDL subfractions is not affected by different types of dietary fatty acids[1–3]

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ABSTRACT

Background: Plasma HDL concentrations and composition, important predictors of coronary heart disease, are modified by fatty acids (FAs) in high-fat diets.

Objective: Following the National Cholesterol Education Program Adult Treatment Panel III recommendation that 25%–30% of total calorie intake be in the form of fat, we compared the results of the intake of 30% of energy as fat in diets enriched with *trans*, polyunsaturated, or saturated FAs. These dietary effects on the composition and ability of HDL_2, HDL_3, and total plasma to efflux cholesterol from mouse peritoneal macrophages that previously were loaded with LDL-acetylated 14C-cholesteryl ester were evaluated by using ultracentrifugally isolated lipoproteins.

Design: After a 2-wk run-in period, 30 healthy persons (9 M, 21 F), were randomly distributed among 3 groups ($n = 10$/group) and fed for 4 wk with either an 8.3% *trans* FA, a 14.6% polyunsaturated FA, or a 13.2% saturated FA diet. The 3 diets had similar proportions of monounsaturated FAs.

Results: The percentage of radioactive cell cholesterol removal did not vary among these diets, possibly because of the small difference in the composition of the HDL fraction elicited by the different diets. However, the percentage was consistently higher for HDL_3 than for HDL_2.

Conclusion: Differences in the cell cholesterol efflux with these diets were not observed, probably because the changes in the HDL composition were quite modest as a result of the limitation of the fat intake to 30% of total calories and because of the rigorous control of the proportions of FAs in the experimental diets used in this investigation. *Am J Clin Nutr* 2007;86:1270–7.

KEY WORDS Dietary fatty acids, HDL, cholesterol efflux, HDL composition, *trans* fatty acids

INTRODUCTION

trans Fatty acids (TFAs) have shown a stronger correlation with coronary heart disease than have saturated fatty acids (SFAs) (1–3). As to the total plasma cholesterol concentration, some investigations have shown higher cholesterol concentrations with SFA diets than with TFA diets (4–10), whereas others have failed to show any differences (11–14)—eg, when the main SFA components were palmitic (12, 13) or stearic (14) acid—or have displayed a higher total cholesterol concentration with a TFA diet than with an SFA diet (15, 16). With respect to the other dietary fatty acids (FAs), studies have also shown further conflicting results for total cholesterol: higher with TFA diets than with polyunsaturated FA (PUFA) diets (5, 6, 10, 14, 17), similar with TFA and monounsaturated FA (MUFA) diets (11, 13, 16, 18), and higher with TFA diets than with MUFA diets (4, 12).

TFA diets have the inconvenience of lowering HDL cholesterol (4–8). There are no clear explanations for the differences found in these studies, because several factors may have been involved, such as sequences of experimental diets, amounts and proportions of fats fed, duration of the experiments, and differences in the sex and age of the subjects investigated. From our point of view, necessary constraints on the proportions of FAs were neglected in most, if not all, of the studies mentioned above. Moreover, investigations on human plasma lipoproteins to date have dealt with >30% of total calories as fat (4, 7, 8, 12, 13, 14, 19), which could exacerbate the effects of fats on all lipoprotein classes. Thus, the specific effects on these lipoproteins of the different dietary FAs may have been missed for lack of rigorous pairing of the FA proportions in the diets.

The inverse correlation between plasma HDL-cholesterol concentrations and the incidence of coronary heart disease has long been recognized in humans (20–22). HDL plays a critical role in cholesterol efflux from the arterial intima cells as the first step of reverse cholesterol transport (RCT) (23, 24). Nonetheless, results in animals (25–27) and humans (28–31) have been contradictory. Some studies have shown an increased cell cholesterol efflux with diets rich in MUFA or PUFA (28–30), but they failed to control for the dietary FA composition.

The goal of the present investigation was to elucidate the effects on lipoprotein composition and rates of cell cholesterol efflux for whole plasma and HDL subfractions during TFA-, SFA-, and PUFA-enriched diets in healthy persons, both in fasting and postprandial conditions. The total fat intake represented

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TABLE 1
Baseline characteristics of study participants[1]

Characteristics	Study group		
	TFA diet (n = 10)	PUFA diet (n = 10)	SFA diet (n = 10)
M/F	3/7	3/7	3/7
Weight (kg)	65.8 ± 9.6[2]	65.5 ± 14.0	65.5 ± 11.4
BMI (kg/m^2)	24.7 ± 4.3	24.6 ± 3.2	24.1 ± 2.7
Age (y)	36 ± 10	34 ± 7	36 ± 9
Total cholesterol (mg/dL)[3]	144 ± 22	150 ± 32	156 ± 24
Triacylglycerol (mg/dL)[3]	105 ± 57	111 ± 59	88 ± 19
LDL cholesterol (mg/dL)[3]	82 ± 20	90 ± 30	99 ± 19
HDL cholesterol (mg/dL)[3]	41 ± 17	38 ± 6	40 ± 11
LDL/HDL	2.1 ± 0.8	2.5 ± 0.9	2.6 ± 0.9

[1] TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Characteristics of the diet groups were compared by using one-factor ANOVA. Nonsignificant changes were detected.
[2] $\bar{x}$ ± SD (all such values).
[3] Biochemical values were measured during the fasting state.

30% of the energy intake, as is recommended by National Cholesterol Education Program Third Adult Treatment Panel (NCEP-ATPIII) guidelines (32). Furthermore, the composition of dietary FAs was rigorously controlled to maintain similar proportions of MUFAs.

SUBJECTS AND METHODS

Subject recruitment

Thirty healthy employees at the University of São Paulo Medical School were recruited for the investigation. Body mass index (in kg/m^2) was <30. Baseline characteristics were blood sugar concentrations <100 mg/dL in the fasting condition and <140 mg/dL 2 h after a glucose tolerance test. Plasma concentrations were <200 and <150 mg/dL for LDL cholesterol and triacylglycerol, respectively. Subjects with chronic diseases and subjecs who were taking medications that could interfere with the lipid metabolism were not included.

All subjects provided written informed consent. The Ethics in Research Committee of the Hospital of the University of São Paulo Medical School approved the study protocol.

Experimental design

Subjects were advised to eat diets that corresponded to the NCEP-ATPIII recommendation (32)—ie, diets consisting of a maximum of 30% of energy as fat, <10% of energy as SFA, and <300 mg cholesterol/d during a 2-wk run-in period in which sunflower oil was used in meal preparation. They were then paired by sex, age, and body mass index and randomly distributed to the TFA-, PUFA-, or SFA-enriched diet. Baseline characteristics of the participants, assessed immediately before starting these experimental diets, are shown in **Table 1**.

At the end of the run-in period and and the end of the 4-wk experimental period, participants were submitted to meal tests, called basal and final tests, respectively, which were compared statistically as time-dependent. Blood samples were drawn after an overnight fasting period and 4 h after the ingestion of a standardized meal consisting of a beef or chicken pancake with rice and vegetables containing the same type of fat mixture eaten during the preceding 4-wk experimental period.

Diets

On a weekly basis, participants received frozen diets (Condieta; São Paulo, SP, Brazil) that were sufficient for their main daily meals—namely, lunch and dinner. For breakfast, they were instructed to consume a fat-free meal. Compliance was ensured by dietary counseling after weekly personal interviews with a nutritionist. Individual body weight was monitored weekly, and it remained stable. All experimental diets had 30% of energy as fat, and the composition of the custom-made fat used (**Table 2**) provided two-thirds of the total daily fat intake. The oil composition was carefully calculated to provide minimal variations of oleic acid and maximal differences in the proportions of TFA, PUFA, and SFA in the experimental diets. For instance, the ratio of PUFA to SFA (PUFA:SFA) in the TFA, PUFA, and SFA oils was 0, 6.37, and 0.22, respectively, compared with 3.52 of the oil at baseline (basal oil). In addition, the *trans* fat contained 36% TFA and 27% SFA, whereas the saturated fat contained 45% SFA (Table 2).

Biochemical analyses

Blood samples were collected in tubes containing EDTA (10 μL EDTA/mL). Plasma was immediately separated by centrifugation (3000 rpm, 15 min, 4 °C; RT6000B; Sorvall Instruments, DuPont Co, Newton, CT), and the following preservatives were added: 0.25% chloramphenicol plus 0.5% gentamycin (20 μL/

TABLE 2
Fatty acid composition and sources of the fats used in the preparation of the diets[1]

Fatty acids	Fats and oils used in the experimental diets			
	Basal	TFA	PUFA	SFA
trans Fat (g/100 g total fatty acids)	0	36	0	1
PUFA (g/100 g total fatty acids)	60	0	51	10
SFA (g/100 g total fatty acids)	17	27	8	45
Monounsaturated (g/100 g total fatty acids)	22	36	41	43
PUFA/SFA	3.52	—	6.37	0.22
Sources	Sunflower oil	Hydrogenated soybean oil	46% rapeseed oil, 54% sunflower oil	12% olive oil, 88% palm oil

[1] TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Analysis was performed with the use of gas liquid chromatography.

TABLE 3
Plasma total cholesterol and triacylglycerol obtained before (Basal) and after (Final) the TFA-, PUFA-, and SFA-enriched diets in the fasting (FP) and postprandial (PP) periods[1]

	TFA diet (n = 10)		PUFA diet (n = 10)		SFA diet (n = 10)	
	Basal	Final	Basal	Final	Basal	Final
	mg/dL		*mg/dL*		*mg/dL*	
Total cholesterol						
FP	142 ± 23	151 ± 27	150 ± 32	152 ± 33	156 ± 23	166 ± 20
PP	142 ± 22	150 ± 28	146 ± 29	154 ± 33	153 ± 23	161 ± 16
Triacylglycerol						
FP	107 ± 59	91 ± 38	111 ± 60	99 ± 45	88 ± 19	96 ± 15
PP	117 ± 70	108 ± 66	126 ± 65	111 ± 49	98 ± 31	97 ± 26

[1] All values are $\bar{x}$ ± SD. TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Means were analyzed by repeated-measures ANOVA. There were no significant 2- or 3-factor interactions, and the main effect of period was significant for triacylglycerol (PP>FP, $P = 0.012$).

mL), 2 mmol benzamidine/L (5 μL/mL), 10 mmol phenyl-methyl-sulfonyl fluoride/L (0.5 μL/mL), and aprotinin (0.5 μL/mL).

Isolation of lipoprotein density classes

HDL_2 and HDL_3 were isolated by density gradient ultracentrifugation (33, 34) and dialyzed against phosphate-buffered saline (PBS; 0.9% NaCl, 0.04% EDTA, and 0.01% NaN_3; pH 7.0), and protein concentrations were measured in all samples (35). The lipoprotein chemical composition was determined by using enzymatic kits to measure total cholesterol and triacylglycerol (Roche Diagnostics Corporation, Indianapolis, IN), phospholipids and apolipoprotein (apo) A-II (Wako Chemicals, Richmond, VA), and apo A-I (Biotécnica Indústria e Comércio LTDA, Varginha, Brazil).

TABLE 4
HDL_2 composition obtained before (Basal) and after (Final) the TFA-, PUFA-, and SFA-enriched diets in the fasting (FP) and postprandial (PP) periods[1]

	TFA diet (n = 10)		PUFA diet (n = 10)		SFA diet (n = 10)		Interactions and main effects (P)
	Basal	Final	Basal	Final	Basal	Final	
	mg/dL		*mg/dL*		*mg/dL*		
Total cholesterol							0.009[3]
FP	23 ± 13[2]	25 ± 10	23 ± 6	22 ± 5	28 ± 12	24 ± 8	
PP	22 ± 13	27 ± 9	23 ± 8	25 ± 8	26 ± 11	19 ± 7	
Triacylglycerol							0.041[4]
FP	12 ± 7	9 ± 7	10 ± 7	8 ± 5	14 ± 4	10 ± 4	
PP	11 ± 5	9 ± 3	10 ± 4	11 ± 5	14 ± 9	10 ± 5	
Phospholipid							0.007[3]
							0.048[5,6]
FP	33 ± 17	39 ± 12	30 ± 12	30 ± 11	37 ± 14	29 ± 11	
PP	35 ± 15	42 ± 12	30 ± 12	35 ± 14	35 ± 20	26 ± 9	
Apo A-I							0.002[3]
FP	34 ± 18	40 ± 14	32 ± 14	36 ± 15	42 ± 15	33 ± 14	
PP	32 ± 19	46 ± 15	36 ± 14	44 ± 18	41 ± 16	30 ± 14	
Apo A-II							0.001[3]
							0.006[5,6]
							0.03[5,7]
FP	15 ± 5	17 ± 4	15 ± 6	15 ± 6	16 ± 4	11 ± 4	
PP	14 ± 7	18 ± 5	16 ± 6	17 ± 7	15 ± 5	10 ± 4	
Lipids/Apo A[8]							0.005[3]
							0.009[5,9]
FP	1.5 ± 0.7	1.3 ± 0.1	1.4 ± 0.4	1.2 ± 0.1	1.3 ± 0.1	1.5 ± 0.2	
PP	1.7 ± 0.5	1.2 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.3 ± 0.3	1.5 ± 0.5	

[1] TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Apo, apoligoprotein. Values were analyzed by repeated-measures ANOVA. There were no significant differences among the diets at Basal testing. There were no significant 3-factor interactions.
[2] x ± SD (all such values).
[3] Time × diet interaction.
[4] Main effect of time.
[5] Contrasts were Bonferroni-corrected.
[6,7,9] Final: [6] *trans* > SFA; [7] PUFA > SFA; [9] SFA > PUFA.
[8] (Total cholesterol + triacylglycerol + phospholipids)/Apo A (A-I + A-II).

TABLE 5
Total HDL composition obtained before (Basal) and after (Final) the TFA-, PUFA-, or SFA-enriched diets in the fasting (FP) and postprandial (PP) periods[1]

	TFA diet ($n = 10$)		PUFA diet ($n = 10$)		SFA diet ($n = 10$)		
	Basal	Final	Basal	Final	Basal	Final	*P*
Total cholesterol							0.016[3]
FP	29 ± 14[2]	31 ± 10	30 ± 7	29 ± 6	34 ± 13	30 ± 7	
PP	29 ± 14	33 ± 9	29 ± 10	30 ± 8	31 ± 12	25 ± 7	
Triacylglycerol							
FP	16 ± 7	13 ± 9	13 ± 7	12 ± 6	17 ± 4	12 ± 4	
PP	14 ± 5	12 ± 4	14 ± 4	14 ± 6	17 ± 11	12 ± 5	
Phospholipid							0.007[3]
							0.015[4,5]
FP	40 ± 18	49 ± 12	36 ± 14	35 ± 12	41 ± 14	34 ± 12	
PP	44 ± 17	50 ± 13	38 ± 16	42 ± 18	39 ± 21	30 ± 10	
Apo A-I							0.001[3]
							0.039[4,5]
FP	49 ± 18	59 ± 14	45 ± 16	51 ± 18	55 ± 16	42 ± 19	
PP	48 ± 22	62 ± 16	52 ± 18	59 ± 23	50 ± 16	37 ± 15	
Apo A-II							0.001[3]
							<0.001[4,5]
							0.06[4,6]
FP	21 ± 5	24 ± 4	20 ± 7	20 ± 7	20 ± 5	14 ± 4	
PP	21 ± 7	24 ± 5	22 ± 8	22 ± 9	18 ± 6	12 ± 40.03[5,7]	
Lipids/Apo A[7]							0.007[3]
							0.003[4,8]
							<0.001[4,9]
FP	1.2 ± 0.3	1.1 ± 0.1	1.3 ± 0.3	1.1 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	
PP	1.3 ± 0.2	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.1	1.3 ± 0.3	1.4 ± 0.4	

[1] TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Apo, apolipoprotein. Values were analyzed by repeated-measures ANOVA. There were no significant 3-factor interactions.
[2] $\bar{x}$ ± SD (all such values).
[3] Time and diet interaction.
[4] Contrasts were Bonferroni-corrected.
[5,6,8,9] Final: [5] *trans* > SFA; [6] PUFA > SFA; [8] SFA > *trans*; [9] SFA > PUFA.
[7] (Total cholesterol + triacylglycerol + phospholipids)/Apo A (A-I + A-II).

Cell culture

Macrophages were obtained from the abdominal cavity of Swiss mice. Cells were seeded into 24-well plates in RPMI medium (R5886-500ML; Sigma-Aldrich, St Louis, MO) supplemented with penicillin and 10% fetal bovine serum and were incubated at 37 °C in a 95% air and 5% CO_2 atmosphere for 24 h. Cells were then overloaded with acetylated LDL and cholesterol (4-^{14}C) in DMEM (Sigma-Aldrich) containing 0.1% FA-free albumin (FAFA, A60003; Sigma-Aldrich) for 24 h. After being washed with PBS, cells were incubated for 5 h with DMEM-FAFA containing HDL_2, HDL_3, or total plasma in a concentration of 50 μg/mL. The medium was centrifuged at 1500 rpm for 10 min at 4 °C (RT 6000B; Sorvall Instruments) to remove cell debris, and radioactivity was measured in a β-scintillation counter (LS6000-TA8; Beckman Instruments, Palo Alto, CA). Cells were then washed twice with saline solution, and radioactive radiation was measured after extraction of the cells with a hexane:isopropanol solution [3:2 (vol:vol)]. The percentage of cell cholesterol efflux was calculated as medium cholesterol radioactivity/(medium cholesterol radioactivity + cell cholesterol radioactivity) × 100.

Statistical analysis

For all analyses, we used NCSS 2004 statistical software (version 2004; NCSS, Kaysville, UT). The results were treated by analyses of repeated measures, with diet as a between-subject factor and time (final compared with basal) and postprandial status as within-subject factors.

RESULTS

Plasma total cholesterol and triacylglycerol were not affected by the diets (TFA, PUFA, or SFA), by time (basal compared with final tests), or by the period (fasting compared with postprandial status) according to the repeated-measures analyses (**Table 3**), except for the triacylglycerol concentration, which was significantly ($P = 0.012$) higher in the postprandial period, independent of the diets and of time. The chemical composition of the ultracentrifugally separated HDL_2, HDL_3, and HDL (as the sum of HDL_2 and HDL_3) is shown in **Table 4**, **Table 5**, and **Table 6**). In Table 4, the TFA diet increased the concentrations of HDL_2 total cholesterol, phospholipid, and apo A-I and apo A-II and decreased the ratio of lipids to apo A (lipids:apo A) over time. In

TABLE 6
HDL_3 composition obtained before (Basal) and after (Final) the TFA-, PUFA-, and SFA-enriched diets in the fasting (FP) and postprandial (PP) periods[1]

	TFA diet (n = 10)		PUFA diet (n = 10)		SFA diet (n = 10)		
	Basal	Final	Basal	Final	Basal	Final	P
	mg/dL		*mg/dL*		*mg/dL*		
Total cholesterol							—
FP	5.5 ± 1.8[2]	6.3 ± 2.2	7.3 ± 2.4	6.4 ± 2.7	6.0 ± 2.0	6.3 ± 1.6	
PP	6.6 ± 2.3	6.1 ± 1.2	6.6 ± 3.2	5.3 ± 2.4	5.4 ± 2.2	6.5 ± 1.7	
Triacylglycerol							—
FP	4.2 ± 2.1	3.7 ± 4.1	3.0 ± 1.3	3.2 ± 1.8	3.0 ± 1.2	2.0 ± 0.6	
PP	3.3 ± 1.4	3.0 ± 1.7	3.4 ± 1.4	3.6 ± 1.8	3.2 ± 2.4	1.9 ± 0.9	
Phospholipids							0.002
FP	7.0 ± 2.6	9.6 ± 2.7[3]	5.7 ± 4.0	5.7 ± 4.2	4.8 ± 2.4	4.5 ± 2.5[3]	
PP	8.9 ± 2.7	8.0 ± 1.8[3]	7.5 ± 4.2	6.8 ± 4.1	4.2 ± 2.3	3.9 ± 2.6[3]	
Apo A-I							<0.001
FP	14.3 ± 4.8	18.6 ± 3.6[3]	13.0 ± 6.8	14.5 ± 6.8[4]	12.7 ± 5.2	9.4 ± 6.2[3,4]	
PP	16.2 ± 4.0	15.9 ± 2.4[3]	16.0 ± 6.4	14.9 ± 6.1[4]	9.1 ± 5.4	7.2 ± 2.6[3,4]	
Apo A-II							<0.001
FP	6.1 ± 2.0	7.1 ± 1.7[3]	5.3 ± 3.2	4.9 ± 2.7[4]	4.1 ± 2.0	2.9 ± 1.6[3,4]	
PP	6.9 ± 1.9	5.7 ± 1.2[3]	5.9 ± 2.4	5.2 ± 2.6[4]	2.7 ± 2.0	2.0 ± 1.5[3,4]	
Lipids/Apo A							<0.001
FP	0.9 ± 0.3	0.8 ± 0.1[3]	1.0 ± 0.4	0.9 ± 0.5[4]	0.9 ± 0.3	1.3 ± 0.5[3,4]	
PP	0.8 ± 0.2	0.8 ± 0.1[3]	0.9 ± 0.4	0.9 ± 0.4[4]	1.5 ± 1.1	1.4 ± 0.4[3,4]	

[1] TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Apo, apolipoprotein. Values were analyzed by repeated-measures ANOVA. There were no significant 2- or 3-factor interactions for all the variables. The main effects were different for the diet, but not for time and period.
[2] $\bar{x}$ ± SD (all such values).
[3,4] Multiple comparisons of diets were significantly different from respective basal values using the combinations of periods: [3]TFA diet × SFA diet, [4] PUFA diet × SFA diet.

contrast, the SFA diet decreased the concentrations of total cholesterol, phospholipid, and apo A-I and apo A-II and increased lipids:apo A over time. The PUFA diet decreased only lipids:apo A over time. No changes were detected among the diets in the basal period. In the final period, compared with the SFA diet, phospholipds and apo A-II were higher with the TFA diet and apo A-II was higher and lipids:apoA was lower with the PUFA diet. The HDL_2-triacylglycerol concentration did not differ with diet according to the 3-factor interaction analyses, but decreased over time (final < basal, $P = 0.041$).

Similar to the HDL_2 subfraction shown above, the total HDL was changed over time by the diets (Table 5). Over time, the TFA diet increased total cholesterol, phospholipids, apo A-II, and apo A-II and decreased lipids:apo A, whereas the SFA diet decreased total cholesterol, phospholipids, apo A-I, and apo A-II. In the final period, compared with the SFA diet, phospholipids, apo A-I, and apo A-II were higher and lipids/apo A was lower with the TFA diet, whereas apo A-II was higher and lipids:apo A was lower with the PUFA diet. No changes were observed in the triacylglycerol concentrations.

Some changes in the HDL_3 subfraction were elicited by the diets (Table 6. When time and period were considered together, compared with the SFA diet, concentrations of phospholipids, apo A-I, and apo A-II were higher and lipids:apo A was lower with the TFA diet, and apo A-I and apo A-II were higher and lipids:apo A was lower with the PUFA diet.

The percentage of removal of mouse peritoneal macrophage cholesterol was investigated by using the HDL_2 and HDL_3 fractions as cell cholesterol acceptors, and the results are shown in both the fasting and fed periods (**Table 7**). The data did not differ significantly by repeated-measures analyses, which means that the TFA, PUFA, and SFA diets did not influence the ability of HDL_2 or HDL_3 to remove macrophage cholesterol either during fasting or postprandially. As expected, HDL_3 displayed greater efficiency in removing cellular cholesterol than did HDL_2.

DISCUSSION

MUFA seems to be the major determinant of HDL composition, enhancing the lipid content of HDL_3 more than do PUFA and SFA (29); it is also the main determinant of HDL_2 composition, including the components apo A-I and apo A-II (36), both after a single meal (28) and after a 3-wk dietary period (36). Furthermore, investigations that compared the PUFA and SFA diets did not show modifications in total cholesterol, phospholipids (11, 37, 38), unesterified and esterified cholesterol, triacylglycerol, protein, and apo A-I contents of HDL (11, 37). Only one study investigated the effect of TFA on HDL composition, and no differences were found between the TFA diet and the SFA or PUFA diet (11), except for a lower Apo A-I–HDL concentration with the TFA diet than with the SFA diet (16).

In the present study, perhaps because of the use of the recommended 30% of energy as fat and the similarity of proportions of oleic acid in the 3 experimental diets, we found no major differences over time in plasma total cholesterol and triacylglycerol concentrations (Table 3). Both in HDL_2 and in total HDL, TFA increased total cholesterol, phospholipids, and apo A-I and apo A-II and decreased lipids:apo A but did not change the triacylglycerol concentration over time. In contrast, SFA decreased

TABLE 7
Percentage of the HDL_2, HDL_3, and total plasma removal of radioactive cholesterol from mouse peritoneal macrophages before (Basal) and after (Final) the TFA-, PUFA-, or SFA-enriched diets in the fasting (FP) and postprandial (PP) periods[1]

	TFA diet (n = 10)		PUFA diet (n = 10)		SFA diet (n = 10)	
	Basal	Final	Basal	Final	Basal	Final
	%		%		%	
HDL_2						
FP	21.6 ± 8.6	19.8 ± 6.7	17.7 ± 4.2	16.6 ± 4.8	19.9 ± 3.0	18.1 ± 3.6
PP	22.3 ± 9.2	20.7 ± 6.4	17.3 ± 3.2	16.6 ± 3.7	20.2 ± 3.8	18.6 ± 2.0
HDL_3						
FP	28.6 ± 10.0	27.8 ± 6.7	27.8 ± 6.7	21.8 ± 8.2	29.2 ± 3.4	26.8 ± 9.2
PP	30.0 ± 8.1	31.2 ± 4.3	24.4 ± 8.0	27.4 ± 4.3	25.9 ± 7.0	27.9 ± 3.0
Plasma						
FP	36.2 ± 5.9	35.9 ± 5.6	36.7 ± 4.9	36.7 ± 4.6	36.4 ± 7.6	37.2 ± 6.6

[1] All values are $x \pm$ SD. TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Values were analyzed by repeated-measures ANOVA There were no significant 2- or 3-factor interactions, and there was no significant main effect of period, diet, or time for any variable. Measurements of total plasma were carried out only in the FP.

total cholesterol, phospholipids, and apo A-I and apo A-II of HDL_2 and total HDL over time (Tables 4 and 5). In addition, both SFA and PUFA decreased lipids:apo A of the HDL_2 subfraction only (Table 4). HDL_2 is the subfraction known to be more responsive to dietary interventions (28, 36). Despite these modifications, the specific efficiency of the removal of radioactive cholesterol from mouse peritoneal macrophages by HDL_2 and HDL_3 did not differ—either before (basal) or after (final) the TFA, PUFA, and SFA diets—among the 3 diets or in the fasting and postprandial periods (Table 7). Our contention is that previous studies had not adequately investigated the capability of removing cell cholesterol in humans (28- 31) and in animals (25, 26, 39) after various FAs were consumed and when different FAs were added in vitro (27). In hamsters submitted to a fat-rich Western-type diet or to a low-fat, high-fiber diet, the flow of esterified cholesterol (EC) to the liver, as measured by the kinetics of plasma radioactive HDL, was not modified, despite the lower plasma EC concentration attained after the low-fat diet (39). In addition, SFA-, MUFA-, and PUFA-enriched diets fed to monkeys for 21 wk modified the HDL phospholipid content without altering the HDL-mediated cholesterol efflux from hepatoma cells (25).

An in vivo assay of the RCT system was performed in rats receiving either TFA, MUFA (oleic), or SFA (palmitic) in which radioactive cholesterol was delivered to the liver as acetylated LDL, and the reappearance of the label into plasma and into HDL was determined (26). Plasma radioactivity in the TFA-fed rats was higher than that in their SFA-fed counterparts, and this difference was ascribed to higher concentrations of LDL (3H)cholesterol. Despite differences in cholesterol, phospholipid concentrations, and in the FA composition of the HDL particles, the amount of label belonging to HDL did not differ among the experimental groups, which suggests that the consumption of these diets resulted in HDL populations with similar capacities with respect to the RCT system (26).

In another study, apo A-I and different FAs were incubated in vitro with macrophages already loaded with radioactive cholesterol, and the activity of the ABCA1 membrane transporter was stimulated by cyclic AMP (27). When compared with the SFA group, both the PUFA and MUFA groups had lower cell cholesterol removal efficiency, ABCA1 expression, and apo A-I binding; however, these effects could not be ascribed to modifications of the HDL particles in vivo (27). In human studies, we argued on several grounds that results with cell cholesterol efflux using the influences of different dietary FAs are also controversial. When compared with SFA or PUFA, MUFA enhanced the HDL_3 fraction's ability to remove Fu5AH (hepatoma cell) cholesterol (28); however, this study was carried out by using blood drawn after a single oral fat load. Nevertheless, similar results were observed in women who had eaten MUFA-, PUFA-, and SFA-rich diets for 7 wk (29), but the in vitro removal system used cultured fibroblasts incubated with HDL_3. Montoya et al (30) carried out a similar protocol in humans fed the aforementioned diets for 5 wk and found an increased total serum ability to remove Fu5AH cholesterol in those subjects following the PUFA-enriched diet. Nonetheless, because total plasma has several components that could be modified by different FAs from the diet—such as enzymes and other proteins (eg, lecithin-cholesterol acyltransferase, phospholipid transfer protein, and cholesterol ester transfer protein)—that are known to regulate the metabolism of lipoproteins, this result did not attribute to HDL any specificity to HDL about the cholesterol-removal capacity.

Another study (31), in which serum and hepatoma cells were used, can be criticized on the same grounds. In that investigation, the cell cholesterol efflux process was carried out with the use of an NCEP-ATPIII diet enriched with cholesterol, but this result may be attributed to the fact that the plasma HDL-cholesterol concentration increases with that diet. In addition, the abovementioned cells do not mirror the conditions that prevail in the atherosclerotic intima, which is rich in macrophages that contain the ABCA1 transporter as the major exporter of cholesterol to HDL (40).

Subsequent steps involved in the RCT have been partially investigated. Subbaiah et al (41) found in vitro that the TFA-rich diet inhibits lecithin-cholesterol acyltransferase activity in humans. Another investigation reported that the PUFA diet raised the plasma cholesterol ester transfer protein concentration only in the postprandial period (42). In addition, the TFA diet seems to enhance cholesterol ester transfer protein activity more than

does the MUFA diet (19) or the SFA and PUFA diets (43), an effect that likely is responsible for the dietary fat–induced changes in HDL composition observed here. Therefore, in addition to the removal of cell cholesterol by the HDL fractions investigated here, further steps of the RCT system must be thoroughly re-evaluated under the effect of dietary fats. In other words, although epidemiologic trials have shown a strong association between cardiovascular disease and the consumption of TFA, our data show that the proatherogenic effect of TFAs is not related to a faulty efficiency of macrophage cholesterol efflux. In addition, a lack of significant lipoprotein composition differences in HDL-cholesterol efflux rates by cells is likely attributed to our limiting the total fat intake to 30% of total energy and simultaneously controlling the proportions of FAs in the experimental diets.

The authors' responsibilities were as follows—VB and AMPL: contributed to the study concept and design, performed the assays, and contributed to the data collection, interpretation of the results, and writing of the manuscript; ERN: contributed to the study design; MP: supervised the cholesterol efflux method; VSN: contributed to the data analyses; ECRQ: contributed to the interpretation of the results and to writing of the manuscript; and all authors: reviewed the final manuscript. None of the authors had a financial or personal conflict of interest.

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