Release of Cholesterol From Cell Membranes to Postprandial Plasma From Mildly Hypercholesterolemic Subjects: The Effect of Meals Rich in Olive and Safflower Oils

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Triglyceride-rich lipoproteins increase net transport of cell cholesterol to postprandial plasma from healthy subjects after a meal rich in fat and cholesterol. The aim of the present study was to determine the effect of meals rich in polyunsaturated fats (PUFA) and monounsaturated fats (MUFA) and low in cholesterol on net in vitro transport of cholesterol from red blood cells (RBCs) to postprandial plasma from 21 men with mild to moderate hypercholesterolemia in a randomized, crossover trial. Cholesterol concentration increased by 12% due to accumulation of cell cholesterol in fasted hypercholesterolemic plasma incubated with a 2/1 (vol/vol) excess of RBCs at 37°C for 18 hours. The increase in cell cholesterol in plasma was mainly localized in the low-density lipoprotein (LDL) fraction (64%) and the remainder was approximately equally divided between the very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) fractions. Accumulation of cell cholesterol in the LDL fraction prevented the significant decrease in LDL cholesterol in plasma incubated alone. When RBCs were incubated with postprandial plasma isolated 4 hours and 6 hours after liquid meals rich in safflower and olive oils, the accumulation of cell cholesterol in plasma increased significantly (11%, $P < .004$) above values for fasted plasma and irrespective of the type of fat in the meal. Also, the content of cell cholesterol increased significantly (70%, $P < .001$) in triglyceride (TG)-rich lipoproteins and decreased significantly ($P = .006$) in the LDL fraction, which remained the main ultimate destination of cell cholesterol in postprandial plasma. The increased loss of cell cholesterol to fasted and postprandial plasma was closely correlated ($r > 0.823$, $P < .001$) with the concomitant increase in plasma cholesteryl esters (CE) generated by lecithin cholesterol acyltransferase (LCAT) activity. There was a small (5%), significant ($P < .001$) increase in plasma cholesterol esterification in postprandial plasma. These data suggest that high-fat meals rich in MUFA and PUFA and low in cholesterol may produce a small postprandial increase in the capacity of plasma to accept cell membrane cholesterol that is limited by a concomitant small increase in plasma cholesterol esterification, in hypercholesterolemic subjects. Thus, low-fat, lipid-lowering diets may have a minimal effect on this capacity but will reduce levels of atherogenic LDL cholesterol that appear to be maintained by diffusion of cell cholesterol to plasma.

THE MOVEMENT of cholesterol from peripheral tissues through the blood to the liver is termed reverse cholesterol transport (RCT). In the liver, cholesterol can be excreted from the body and also resecreted into the circulation in lipoproteins. Efflux of cell cholesterol is the first step in RCT and is thought to occur by diverse pathways including active receptor-mediated processes and nonspecific diffusion of cell membrane cholesterol through the surrounding aqueous medium.[1,2] Receptor-mediated cholesterol efflux is rapid and is mediated by a small, apolipoprotein (apo)A1-containing, lipid-poor subfraction of high-density lipoprotein (HDL) with pre β-electrophoretic mobility and an energy-dependent cell transporter of cholesterol and certain other compounds.[2] This pathway is independent of lecithin cholesterol acyltransferase (LCAT), is cell-specific, and occurs only in cholesterol-loaded cells.[2] Human macrophages, aortic smooth muscle cells, and fibroblasts, but not red blood cells (RBCs), can support pre β-HDL–mediated cholesterol efflux.[1] By contrast, the diffusional pathway of cell cholesterol efflux is relatively slow and occurs in all types of cells. Cholesterol efflux from RBCs occurs solely via this pathway and comprises only cell membrane cholesterol. RBCs do not have intracellular membranes and do not synthesize or metabolize cholesterol. Albumin, mature HDL particles (α-HDL), and low-density lipoproteins (LDLs) in the extracellular fluid are acceptors of cholesterol that has diffused from cells. Cell cholesterol initially bound by albumin is redistributed to lipoproteins. Esterification of cell cholesterol in HDL by LCAT activity prevents diffusion of cholesterol back into the cells and thereby enhances net loss of cellular cholesterol (efflux-influx). There is evidence that increased influx of cholesterol from plasma to cells underlies impaired net loss of cholesterol from cultured cells to dilute serum from hyperlipidemic subjects.[3] While HDL transports a portion of esterified cell cholesterol directly to the liver, a portion is transferred to apoB-containing lipoproteins that deliver cholesterol to the liver when they are internalized by receptors on the hepatocytes.

Incubation of cultured human fibroblasts[4] or RBCs[5] with plasma isolated in the postprandial period after a fatty meal increases net loss of cell cholesterol to the medium. Increased postprandial cholesterol esterification and transfer of cholesteryl esters (CE) from HDL to other lipoproteins and especially to triglyceride (TG)-rich lipoproteins, are intimately linked to the accompanying loss of cell cholesterol.[5] Chylomicrons are believed to be the most potent ultimate acceptors of cholesterol transferred from RBCs to postprandial plasma and levels of chylomicrons are closely related to the amount of cell cholesterol transferred.[5] Efficient uptake of chylomicron rem-

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nant particles by hepatic receptors is thought to play a role in promoting RCT in vivo.[5]

The type of fat in a meal can influence postprandial levels of chylomicrons in the blood and the rate of cholesterol efflux from cultured cells to postprandial plasma. Postprandial lipemia and levels of chylomicron remnants are higher at 4 hours after oral fat loading with olive oil compared with safflower oil in healthy, normocholesterolemic men.[6] An oral load of oleic acid–rich fat, but not polyunsaturated fat (PUFA), increases the capacity of postprandial serum to promote efflux of radiolabeled cholesterol from cultured Fu5AH hepatoma cells.[7] Efficient transport of cell cholesterol to plasma is thought to attenuate the accumulation of cholesterol in peripheral tissues such as the artery wall and delay the development of atherosclerosis. Whether net loss of cell cholesterol to postprandial plasma is differentially affected by meals rich in monounsaturated fats (MUFA) and PUFA has not been widely studied. The aim of the present study was therefore to determine the effect of meals rich in olive and safflower oils on the nonspecific, diffusion-mediated cell cholesterol transport estimated by measuring net loss of cell cholesterol from isolated RBCs to postprandial plasma from men with mild to moderate hypercholesterolemia.

MATERIALS AND METHODS

Subjects

Twenty-one men (32 to 59 years of age) with plasma cholesterol levels between 5.4 mmol/L and 7.9 mmol/L were recruited from respondents to a notice requesting volunteers placed in Dunedin Hospital. In addition, men were recruited from a register of individuals with cholesterol levels in this range that was established by the Human Nutrition Department, University of Otago. Men who expressed interest in the study were screened and those with plasma cholesterol levels in the appropriate range and who did not have any serious illnesses were accepted into the study. None of the subjects was taking drugs known to affect lipid metabolism or following a lipid-lowering diet. The study was approved by the Otago ethics committee and all participants gave written and informed consent.

Study Design

The study had a single-blinded, randomized, crossover design. Subjects were randomized to receive a meal (milkshake) containing either olive oil or safflower oil. At least 1 week later, the men received the alternate meal. Blood was taken at baseline in the early morning after an overnight fast and at 4 hours and 6 hours after the meals. Height and body weight were measured immediately prior to the first meal.

Meals

The milkshakes contained olive or safflower oils (90 g), ice cream (100 g), skim milk (200 mL), yogurt (10 g), tinned apricots without syrup (50 g), egg yolk (12 g), egg white (30 g), and chocolate flavoring (15 g, 92% sugar). The milkshakes contained 79% energy from fat, 15% energy from carbohydrate, and 6% energy from protein and 173 mg cholesterol. The polyunsaturated:saturated fat ratio (P:S ratio) was 0.45 for the olive oil meal and 4.02 for the safflower oil meal. Participants consumed a volume of milkshake to give 40 g of fat intake per square meter of body surface area. Subjects were instructed to refrain from eating or drinking beverages except water during the postprandial period after the meals. They were also instructed to maintain their usual diet during the nonintervention days of the study.

Transport of RBC Cholesterol to Plasma

The methods used in this assay were essentially those reported by Chung et al.[5] Briefly, blood was collected in tubes containing disodium EDTA and the tubes were placed in an ice bath immediately after collection. The blood was spun at 1,000 rpm for 10 minutes in a low-speed centrifuge precooled to 4°C. The plasma supernatant was collected and kept briefly at 4°C leaving one third of the plasma trapped within the packed RBCs. Packed cells (8 mL) were transferred to a plastic tube using a Gilson pipette with a wide-bore plastic tip. Plasma (1 mL) was added to the packed cells to give a 2-fold excess of RBCs to plasma. The remaining plasma was divided into 2 aliquots. One aliquot of plasma and the RBC-plasma mixture were incubated at 37°C without shaking for 18 hours while the other aliquot of plasma was maintained at 4°C. At the end of the incubation period, the RBC-plasma mixture was centrifuged at 2,500 rpm and 4°C for 30 minutes and the separated plasma was harvested. An aliquot (2 mL) of this plasma and from the plasma samples incubated at 4°C and 37°C were adjusted to $d = 1.006$ g/mL and ultracentrifuged in a Beckman (Palo Alto, CA) type 50.3-Ti rotor for 18 hours at 40,000 rpm and 10°C. The $d < 1.006$ g/mL fraction containing very-low-density lipoproteins (VLDL) and chylomicrons, and the $d > 1.006$ g/mL fraction were collected quantitatively by tube slicing. HDL were isolated in the supernatant after precipitation of apoB-containing lipoproteins from plasma with dextran sulfate and magnesium chloride.[8] Cholesterol and TG in plasma and plasma fractions were measured using commercial kits and calibrators (Roche, Boehringer Mannheim, Germany). Plasma unesterified cholesterol (UC) was measured enzymatically using reagents with composition similar to the Boehringer Mannheim kit. Cholesterol in the LDL fraction was calculated by subtracting HDL cholesterol from cholesterol in the $d > 1.006$ g/mL fraction. Net accumulation of cell cholesterol in plasma and plasma lipoprotein fractions was calculated as the difference (Tables 1 and 2, C-B) in cholesterol levels between plasma incubated at 37°C in the presence or absence of RBCs.

Statistics

Values are given as the mean ± SD unless stated otherwise. Generalized estimating equations were used to analyze the data.[9] Estimates and tests of their significance were obtained for the effect of meals and the parameters for the comparison between plasma incubated at 37°C in the presence and absence of cells (cell cholesterol in plasma, Table 1, C-B) and between plasma incubated at 37°C and at 4°C (Table 1 B-A). Tests for the effects of time after the meal in B-A and C-B comparisons were also performed. Initially, the interaction between the effects of meals and time, meals and the postprandial effect in control incubations at 4°C (A), and the 3-way interaction were considered, but as they were not statistically significant they were not included in the model. The effects of meal and the postprandial effect were also analyzed separately in the control data. Incremental area under the curve (iAUC) was calculated as described previously.[10] Two-tailed tests of significance were used and a P value less than .05 was considered to be statistically significant.

RESULTS

The mean age of the participants was 47 ± 8 years and their mean body mass index was 27.58 ± 3.91 kg/m^2. Figure 1 shows the time-course of the net increase in cell cholesterol in plasma and its HDL fraction during incubation of nonfasted plasma at 37°C with and without RBCs. Plasma and RBCs were obtained from 3 mildly hypercholesterolemic subjects. Net accumulation of cell cholesterol in plasma increased most rapidly during the initial 9 hours of the incubation and pla-

Table 1. Plasma Lipids in Fasting and Postprandial Plasma Incubated With or Without RBCs and the Concentration of Cell Cholesterol in Plasma: Effect of Meals Rich in Olive and Safflower Oils

	Time (h)	A Control 4°C	B 37°C −RBC	C 37°C +RBC	B-A*	C-B* Cell Cholesterol in Plasma
				Olive		
TG	0	1.79 ± 0.79				
	4	3.38 ± 1.38				
	6	2.39 ± 1.03				
				Safflower		
	0	1.74 ± 0.62				
	4	3.26 ± 1.29				
	6	2.63 ± 1.43				
P time		<.001				
P meal (control)		.88				
				Olive		
TC	0	6.26 ± 0.69	6.29 ± 0.69	7.03 ± 0.74		0.74 ± 0.21
	4	6.41 ± 0.68	6.39 ± 0.65	7.21 ± 0.74		0.82 ± 0.22
	6	6.35 ± 0.67	6.38 ± 0.67	7.14 ± 0.77		0.76 ± 0.28
				Safflower		
	0	6.38 ± 0.72	6.42 ± 0.72	7.16 ± 0.77		0.74 ± 0.15
	4	6.43 ± 0.76	6.44 ± 0.79	7.26 ± 0.79		0.82 ± 0.16
	6	6.41 ± 0.76	6.42 ± 0.77	7.25 ± 0.84		0.83 ± 0.18
P time		<.001				.004
P meal (control)		.50				
P meal (overall)†					.39	
				Olive		
UC	0	1.61 ± 0.16	1.08 ± 0.16	1.35 ± 0.17	−0.53 ± 0.09	0.27 ± 0.06
	4	1.73 ± 0.18	1.13 ± 0.16	1.44 ± 0.16	−0.61 ± 0.12	0.31 ± 0.08
	6	1.70 ± 0.16	1.10 ± 0.16	1.38 ± 0.17	−0.61 ± 0.12	0.28 ± 0.09
				Safflower		
	0	1.64 ± 0.19	1.12 ± 0.16	1.38 ± 0.15	−0.52 ± 0.10	0.26 ± 0.06
	4	1.74 ± 0.22	1.15 ± 0.17	1.46 ± 0.16	−0.58 ± 0.10	0.30 ± 0.07
	6	1.74 ± 0.22	1.12 ± 0.16	1.41 ± 0.17	−0.61 ± 0.10	0.28 ± 0.05
P time		<.001			<.001	<.001
P meal (control)		.27				
P meal (overall)					.15	
				Olive		
CE	0	4.65 ± 0.55	5.21 ± 0.57	5.69 ± 0.61	0.56 ± 0.14	0.48 ± 0.19
	4	4.67 ± 0.52	5.27 ± 0.53	5.77 ± 0.61	0.59 ± 0.14	0.50 ± 0.18
	6	4.64 ± 0.52	5.28 ± 0.58	5.76 ± 0.64	0.64 ± 0.14	0.48 ± 0.24
				Safflower		
	0	4.74 ± 0.56	5.30 ± 0.59	5.78 ± 0.65	0.56 ± 0.14	0.47 ± 0.14
	4	4.69 ± 0.56	5.29 ± 0.65	5.80 ± 0.67	0.59 ± 0.14	0.53 ± 0.16
	6	4.67 ± 0.56	5.29 ± 0.64	5.84 ± 0.70	0.62 ± 0.12	0.55 ± 0.16
P time		.37			<.001	.22
P meal (control)		.60				
P meal (overall)					.54	

NOTE. Values are mean ± SD (n = 21) and are in mmol/L.

Abbreviations: TC, total cholesterol; UC, unesterified cholesterol; CE, cholesteryl esters.

*The overall effect of B-A and C-B were significant at $P < .001$ for all variables.

†Overall effect of meal in the absence of interaction effects and relates to both B-A and C-B contrasts.

teaued thereafter. The amount of cell cholesterol in the plasma HDL fraction increased with time and was between 21% and 39% of the total amount of cell cholesterol in plasma during the incubation period.

Table 1 shows the effect of meals rich in olive and safflower oils on cholesterol content of plasma incubated with or without RBCs. Mean plasma TG levels were 188% of baseline at 4 hours and between 133% and 151% at 6 hours after the meals. The postprandial increase in plasma TG was not significantly different between the meals. Plasma VLDL-TG paralleled plasma TG during the study (olive: 0.99 ± 0.54, 2.50 ± 1.31, 1.63 ± 0.94 mmol/L; safflower: 1.10 ± 0.61, 2.52 ± 1.19, 1.93 ± 1.30 mmol/L) and these concentrations were also not significantly different between the meals. Control data in Table 1 indicate significant increases in plasma UC during the postprandial period after both meals and plasma cholesterol increased significantly after the olive oil meal and these changes were not significantly different between the meals. When fasted

Table 2. Cholesterol Concentration in Lipoprotein Fractions in Fasting and Postprandial Plasma Incubated With or Without RBCs and the Concentration of Cell Cholesterol in the Fractions: Effect of Meals Rich in Olive and Safflower Oils

	Time (h)	A Control 4°C	B 37°C −RBC	C 37°C +RBC	B-A*	C-B* Cell Cholesterol in Plasma
				Olive		
VLDL	0	0.54 ± 0.29	0.70 ± 0.39	0.80 ± 0.44	0.17 ± 0.13	0.10 ± 0.08
	4	0.74 ± 0.31	1.24 ± 0.50	1.41 ± 0.56	0.50 ± 0.23	0.17 ± 0.13
	6	0.68 ± 0.34	0.96 ± 0.51	1.13 ± 0.62	0.28 ± 0.20	0.17 ± 0.13
				Safflower		
	0	0.52 ± 0.25	0.72 ± 0.39	0.83 ± 0.45	0.20 ± 0.17	0.11 ± 0.08
	4	0.72 ± 0.39	1.22 ± 0.51	1.38 ± 0.58	0.48 ± 0.22	0.18 ± 0.11
	6	0.69 ± 0.38	1.02 ± 0.62	1.16 ± 0.71	0.33 ± 0.26	0.15 ± 0.10
P time		<.001			<.001	<.001
P meal (control)		.83				
P meal (overall)†					0.96	
				Olive		
LDL	0	4.37 ± 0.73	4.02 ± 0.75	4.50 ± 0.83	−0.35 ± 0.16	0.47 ± 0.19
	4	4.31 ± 0.66	3.64 ± 0.64	4.04 ± 0.75	−0.67 ± 0.25	0.40 ± 0.21
	6	4.27 ± 0.62	3.84 ± 0.64	4.25 ± 0.73	−0.43 ± 0.23	0.41 ± 0.20
				Safflower		
	0	4.47 ± 0.62	4.13 ± 0.59	4.59 ± 0.65	−0.35 ± 0.17	0.47 ± 0.18
	4	4.32 ± 0.64	3.75 ± 0.58	4.10 ± 0.61	−0.59 ± 0.22	0.34 ± 0.21
	6	4.30 ± 0.59	3.85 ± 0.59	4.34 ± 0.57	−0.45 ± 0.19	0.48 ± 0.16
P time		<.001			<.001	.006
P meal (control)		.68				
P meal (overall)					.55	
				Olive		
HDL	0	1.25 ± 0.24	1.38 ± 0.29	1.53 ± 0.32	0.14 ± 0.08	0.14 ± 0.05
	4	1.24 ± 0.25	1.33 ± 0.29	1.50 ± 0.32	0.09 ± 0.08	0.18 ± 0.10
	6	1.23 ± 0.25	1.38 ± 0.29	1.56 ± 0.32	0.15 ± 0.08	0.18 ± 0.06
				Safflower		
	0	1.28 ± 0.24	1.40 ± 0.30	1.55 ± 0.33	0.12 ± 0.09	0.15 ± 0.07
	4	1.25 ± 0.23	1.33 ± 0.28	1.55 ± 0.28	0.09 ± 0.09	0.21 ± 0.15
	6	1.26 ± 0.25	1.42 ± 0.30	1.56 ± 0.33	0.16 ± 0.09	0.14 ± 0.08
P time		.06			<.001	.05
P meal (control)		.20				
P meal (overall)					.28	

NOTE. Values are mean ± SD (n = 21 for all but the 4-hour point after the safflower meal when n = 20) and are in mmol/L.
Abbreviations: VLDL, very-low-density lipoprotein fraction; LDL, low-density lipoproteins; HDL, high-density lipoproteins.
*The overall effect of B-A and C-B were significant at $P < .001$ for all variables.
†Overall effect of meal in the absence of interaction effects and relates to both B-A and C-B contrasts.

plasma was incubated with RBCs, there was a 12% increase in plasma cholesterol compared with levels in plasma incubated at 37°C (C-B) due to net loss of cholesterol from cell membranes. The accumulation of cell cholesterol in plasma increased significantly when cells were incubated with postprandial compared with fasted plasma. During incubation of plasma at 37°C without RBCs, plasma UC decreased and plasma CE increased by a similar amount due to plasma LCAT activity (B-A). The magnitude of these changes increased significantly during the postprandial period. Plasma UC concentration increased significantly when plasma was incubated at 37°C in the presence compared with absence of RBCs due to replenishment of esterified plasma UC by cellular UC. This accumulation of cellular UC in plasma increased significantly during the postprandial period. Part of the cell cholesterol lost to plasma was subsequently esterified and increased plasma CE content. There were no significant effects of the type of fat in the meal on the changes in plasma cholesterol, UC, and CE during incubation of fasted and postprandial plasma in the presence and absence of RBCs.

Table 2 shows the effect of meals rich in olive and safflower oils on cholesterol content of plasma lipoproteins during incubation of fasted and postprandial plasma in the presence or absence of RBCs. In control plasma maintained at 4°C, cholesterol in the VLDL fraction increased significantly and LDL cholesterol decreased significantly during the meals and these changes were not significantly different between the meals. Plasma HDL cholesterol levels did not change significantly during the meals. Cholesterol in the VLDL and HDL fractions increased and cholesterol in the LDL fraction decreased equivalently compared with control values (B-A) due to the combined activities of LCAT and CE transfer protein in plasma incubated at 37°C in the absence of RBCs. In postprandial plasma incubated at 37°C, there was a 2- to 3-fold larger cholesterol increase in the VLDL fraction and a 1.7- to 2-fold larger cholesterol decrease in the LDL fraction compared with

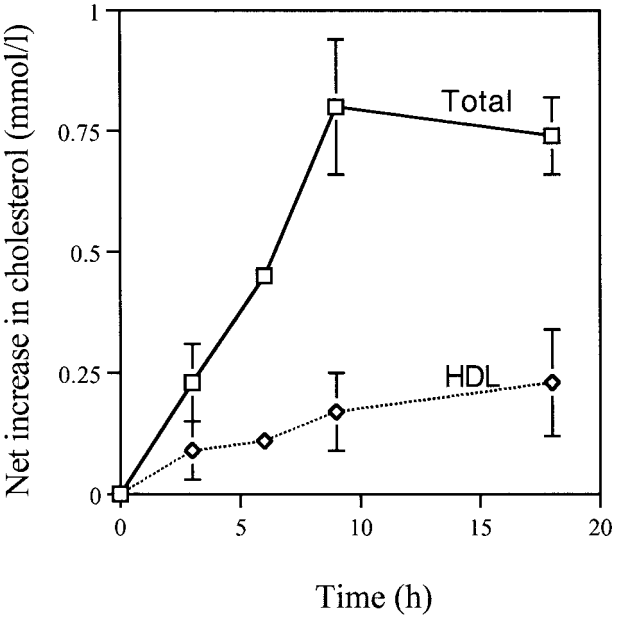

Fig 1. Time-course of the net increase in cell cholesterol in plasma (Total) and its HDL fraction during incubation of hypercholesterolemic plasma with RBCs at 37°C. Net increase in cell cholesterol in plasma was calculated as the difference between values in plasma incubated at 37°C in the presence and absence of RBCs. Nonfasted plasma and cells were obtained from 3 mildly hypercholesterolemic subjects. Values are means ± SD. The SD was smaller than the diameter of 2 of the data points.

fasted plasma. The increase in HDL cholesterol in incubated 4-hour postprandial plasma was significantly smaller compared with the corresponding increase in fasted plasma. The difference in lipoprotein cholesterol content between plasma incubated at 37°C with and without RBC (C-B) indicates the net accumulation of cell cholesterol in plasma lipoproteins. When RBC were incubated with fasted plasma, the majority (46% to 66%) of the cell cholesterol appeared in the plasma LDL fraction and the remainder was approximately equally divided between the HDL and VLDL fractions. Levels of cell cholesterol in the VLDL (0.07 mmol/L, 64%) and HDL (0.06 mmol/L, 40%) fractions increased significantly and levels in the LDL (0.13 mmol/L, 28%) fraction decreased significantly in plasma isolated 4 hours after the safflower oil meal and incubated with RBCs. The type of fat in the meal did not significantly influence changes in lipoprotein cholesterol content in plasma incubated in the presence or absence of RBCs. The iAUC for cell cholesterol in the VLDL (olive: 0.30 ± 0.19 mmol · h/L; safflower: 0.35 ± 0.46 mmol · h/L, $P = 0.39$), LDL (olive: −0.28 ± 0.57 mmol · h/L; safflower: −0.33 ± 0.77 mmol · h/L, $P = .78$), and HDL (olive: 0.13 ± 0.31 mmol · h/L; safflower: 0.17 ± 0.52 mmol · h/L, $P = .82$) fractions and plasma TC (olive: 0.23 ± 0.55 mmol · h/L; safflower: 0.35 ± 0.46 mmol · h/L, $P = .45$) were not significantly different between the meals.

The net accumulation of cell cholesterol was correlated significantly ($r = 0.823$ to 0.937, $P < .001$) with the increase in CE (an estimate of LCAT activity) in plasma incubated with RBCs at all time points and during both meals. The net accumulation of cell cholesterol in plasma was not correlated significantly with fasted and postprandial levels of plasma lipids and lipoproteins during the study.

DISCUSSION

In this study, net loss of cell cholesterol was increased similarly during incubation of RBCs with postprandial plasma isolated from subjects with mild to moderate hypercholesterolemia after liquid meals rich in PUFA or MUFA vegetable oils and low in cholesterol. While much of this extra cell cholesterol was carried by TG-rich lipoproteins, LDL was the main ultimate acceptor of cell membrane cholesterol in both fasted and postprandial plasma.

The mean net accumulation of cell cholesterol in fasted plasma incubated with RBCs in this study was approximately one quarter lower (0.74 mmol/L *v* 1 mmol/L) compared with a previous study that similarly incubated RBCs with plasma from healthy subjects.[5] The lower net cholesterol transport in the present study is almost certainly due mainly to the one third lower ratio of RBCs to plasma (2:1 vol/vol). The 23% higher levels of plasma cholesterol and composition of RBC membranes did not appear to be responsible for this lower accumulation of RBC cholesterol in plasma. Net RBC cholesterol transport to fasted plasma (0.77 ± 0.23 *v* 0.67 ± 0.18 mmol/l) was not clearly different between 13 of the men with baseline cholesterol levels (6.70 ± 0.47 mmol/L) higher than the range of the levels in the previous study and 10 of the men (including 2 who were excluded with cholesterol levels below the inclusion criterion) with baseline cholesterol levels (5.40 ± 0.33 mmol/L) within the range of the previous study.[5] The lipid composition of RBC is normal in subjects with hyperlipidemia.[11]

The magnitude of the increase in cell cholesterol accumulation in 4-hour postprandial plasma incubated with RBCs was 3 to 4 times smaller in our data (0.08 mmol/L) compared with the corresponding increase (0.26 mmol/L) in plasma incubated with RBCs from healthy subjects reported previously.[5] This smaller postprandial increase in cell cholesterol accumulation in plasma can be attributed only in part to the lower ratio of RBCs to plasma and the lower fat intake (40 g/m^2 body area *v* 50 g/m^2 body area) and the resulting 23% lower level of postprandial lipemia in the present study. The main factor underlying the smaller increase in cell cholesterol transport appeared to be the markedly smaller increase in cholesterol esterification (5% *v* 38%) in postprandial plasma that was estimated by the increase in plasma CE levels (Table 1, B-A). Cholesterol esterification in the presence of cells was closely linked to the amount of cell cholesterol lost to plasma in our data and in previous studies.[4,5] The LCAT reaction reduces influx of cholesterol from lipoproteins to RBCs and thereby results in a net loss of cholesterol from cells to plasma.[12] Inhibition of LCAT activity nearly abolishes net transfer of RBC cholesterol to plasma in vitro.[5]

Factors responsible for the smaller magnitude of the postprandial increase in plasma cholesterol esterification during prolonged incubation of plasma in the present study are not entirely clear. This smaller increase did not appear to be due to

the presence of hypercholesterolemia. The postprandial change in the increase in plasma CE during incubation of plasma at 37°C was similar (data not shown) in 13 of the men with more marked hypercholesterolemia and 10 men with lower baseline cholesterol levels that were comparable to those described by Chung et al.[5] The low content of saturated fatty acids and cholesterol in the meal may have limited the postprandial increase in LCAT activity. Wallentin and Vikrot have reported unchanged LCAT activity in postprandial plasma of healthy subjects after a meal rich in PUFA and low in cholesterol and increased activity when saturated fatty acids replaced PUFA in the meal.[13] This increase in LCAT activity was linked to an increase in plasma phospholipids during the meal rich in saturated fatty acids that did not occur during the PUFA meal.[13] Postprandial LCAT activity also tends to be lower after meals low in cholesterol compared with meals rich in cholesterol.[14] Cholesterol content of the meal was markedly lower in the present study compared with that of Chung et al[5] (173 mg *v* 600 mg).

In healthy subjects after a meal rich in fat and cholesterol, chylomicrons (isolated in the VLDL fraction) are recipients of much of the extra cell cholesterol in postprandial plasma during incubation with RBCs.[5] Furthermore, the capacity of postprandial plasma to promote cholesterol loss from RBCs isolated from healthy subjects is correlated closely with plasma TG levels.[5] In the present study, the VLDL fraction was also a major recipient of cell cholesterol in postprandial plasma incubated with RBCs. However, the postprandial increase in cell cholesterol content of the VLDL fraction was approximately 3 times less than in the previous study (0.07 mmol/L *v* 0.23 mmol/L). Also, plasma TG levels were unrelated to the capacity of postprandial plasma to promote cholesterol loss from RBC. This finding may be due to the lesser impact of postprandial lipemia on cell cholesterol transport in our data. The smaller increase in TG-rich lipoprotein cell cholesterol content may be due to the lower postprandial increase in cell cholesterol esterification in plasma leading to transfer of less cell cholesterol-derived CE from HDL to TG-rich lipoproteins. Quantitatively, the 23% lower level of postprandial lipemia does not appear to account for much of this 3-fold lower accumulation of cell cholesterol in TG-rich lipoproteins compared with the previous study.[5] These findings suggest that factors other than the magnitude of postprandial lipemia may have an important influence on the capacity of postprandial plasma to promote loss of cholesterol from cells in subjects with mild to moderate hypercholesterolemia during meals rich in PUFA or MUFA and low in cholesterol.

Postprandial plasma isolated after a meal rich in oleic acid–rich fat enhances efflux of cholesterol from cultured Fu5AH rat hepatoma cells compared with plasma isolated after a meal rich in sunflower oil independently of the level of postprandial lipemia in healthy women.[7] In the present study, the type of fat in the meal did not appreciably alter net transport of cholesterol from RBCs to postprandial plasma in vitro. Thus, any change in the efflux of RBC cholesterol did not differentially influence net transport of cell cholesterol to postprandial plasma isolated during the meals. However, net transport of cell cholesterol to plasma depends on both efflux of cell cholesterol and influx of cholesterol from plasma lipoproteins to cells. Furthermore, influx of plasma cholesterol more than efflux of cell cholesterol determines the magnitude of net loss of cholesterol from RBCs to plasma in vitro.[3,5]

Plasma lipoproteins may act as a "sink" for cell cholesterol.[5,15] The present data appear to support this concept and suggest that LDL may be an important "sink" for cell cholesterol that is transported to plasma in subjects with mild to moderate hypercholesterolemia. Incubation of fasted and postprandial plasma in the presence of RBC attenuated the substantial decrease in LDL cholesterol levels during incubation of plasma at 37°C in the absence of cells. A major proportion (~60%) of RBC cholesterol accumulated in the LDL fraction during incubation of cells with fasted plasma. Cell cholesterol appears to replace cholesterol lost from LDL during cholesterol esterification and transfer of CE to TG-rich lipoproteins. A previous study also suggests that LDL is an important plasma acceptor of cell cholesterol.[16] Huang et al have reported that radiolabeled cellular UC accumulates in LDL during incubation of cultured fibroblasts with human plasma.[16] Cell cholesterol is rapidly taken up by pre β-HDL particles and then transferred to LDL. Cellular UC in LDL is redistributed relatively slowly to α-HDL by an LCAT-dependent pathway.[16] Accumulation of RBC cholesterol in plasma LDL in vitro is also in keeping with its role as the major quantitative acceptor of CE transferred from HDL in fasted and postprandial plasma.[17,18] Newly synthesized radiolabeled CE is largely transferred from HDL into the LDL fraction of incubated fasted plasma.[5] In contrast to the present findings, Chung et al[5] have reported that cell cholesterol mainly accumulates in the VLDL fraction of fasted plasma during incubation with RBCs. However, the amount of cell cholesterol in plasma lipoproteins was calculated as the difference between lipoprotein cholesterol levels in plasma incubated at 37°C in the presence of RBCs and control plasma maintained at 4°C.[5] For VLDL, this difference will include cholesterol that is not derived from cells but has been transferred as preformed CE from HDL and LDL to VLDL during incubation of plasma at 37°C.[5] Furthermore, calculation of the content of cell cholesterol in plasma lipoproteins as the difference between lipoprotein cholesterol levels in plasma incubated at 37°C in the presence and absence of RBCs indicated that the amount of cell cholesterol in LDL (0.51 mmol/L) was approximately twice that in VLDL (0.29 mmol/L) and HDL (0.20 mmol/L).[5] This distribution of cell cholesterol among plasma lipoproteins is comparable with the corresponding distribution in our data.

Epidemiologic studies have established an inverse relationship between plasma HDL cholesterol levels and risk of premature atherosclerotic disease. Glomset has postulated that HDL protects against the development of atherosclerosis by mediating the removal of excess cholesterol from peripheral cells and its transport to the liver.[19] In keeping with this hypothesis, there is evidence that serum HDL concentration may modulate the rate of cell cholesterol release in vitro.[20] A positive relationship between serum HDL cholesterol levels and the unidirectional efflux/exchange of radiolabelled cholesterol from cultured Fu5AH hepatoma cells to whole serum has been reported previously.[20] On the other hand, HDL levels do not appear to determine the net amount of cell membrane cholesterol transported to plasma in vitro. Chung et al have

reported that the capacity of plasma from healthy subjects to accumulate cell membrane cholesterol during prolonged incubation with RBCs is unrelated to plasma HDL cholesterol levels.[5] Furthermore, baseline HDL cholesterol levels did not predict the ability of hypercholesterolemic plasma to promote net efflux of RBC cholesterol to plasma in the present study. These findings suggest that HDL levels may not be rate-limiting in the net transport of cell membrane cholesterol to plasma via the high-capacity, nonspecific pathway in vivo. Factors such as the kinetics of HDL metabolism and the active mobilisation of cell cholesterol for efflux may be more important determinants of RCT and its antiatherogenicity in vivo.[2,21]

This study has limitations. White blood cells were not removed from the RBC fraction. However, we estimate the amount of white blood cell cholesterol was less than 1% of the cholesterol contained in RBC membranes and would not appreciably influence the present findings. Loss of RBC cholesterol during incubations with plasma was not assessed by measurement of RBC cholesterol content. On the other hand, the increase in plasma cholesterol must closely match the amount of cholesterol lost from RBCs during incubation with plasma. RBCs are the only source of this excess plasma cholesterol and they do not internalize plasma lipoproteins.

In conclusion, the present data suggest there is a relatively small increase in the capacity of postprandial plasma and its TG-rich lipoproteins to promote loss of cholesterol from cell membranes after high-fat meals rich in PUFA and MUFA and low in cholesterol in subjects with mild to moderate hypercholesterolemia. Plasma LDL was the main ultimate acceptor of cell cholesterol that diffused to fasted and postprandial plasma and this may tend to maintain plasma LDL cholesterol levels that increase risk of coronary heart disease in subjects with hypercholesterolemia. Low-fat, lipid-lowering diets rich in PUFA and MUFA may reduce this risk by decreasing plasma LDL cholesterol levels but may have minimal effect on the capacity of postprandial plasma to accept cell membrane cholesterol. Further studies to test the effect of the cholesterol content of the meal on cell cholesterol transport to postprandial plasma are warranted.

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