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Postprandial lipemia enhances the capacity of large HDL2 particles to mediate free cholesterol efflux via SR-BI and ABCG1 pathways in type IIB hyperlipidemia[S]

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Abstract Lipid and cholesterol metabolism in the postprandial phase is associated with both quantitative and qualitative remodeling of HDL particle subspecies that may influence their anti-atherogenic functions in the reverse cholesterol transport pathway. We evaluated the capacity of whole plasma or isolated HDL particles to mediate cellular free cholesterol (FC) efflux, cholesteryl ester transfer protein (CETP)-mediated cholesteryl ester (CE) transfer, and selective hepatic CE uptake during the postprandial phase in subjects displaying type IIB hyperlipidemia (n = 16). Postprandial, large HDL2 displayed an enhanced capacity to mediate FC efflux via both scavenger receptor class B type I (SR-BI)-dependent (+12%; $P < 0.02$) and ATP binding cassette transporter G1 (ABCG1)-dependent (+31%; $P < 0.008$) pathways in in vitro cell systems. In addition, the capacity of whole postprandial plasma (4 h and 8 h postprandially) to mediate cellular FC efflux via the ABCA1-dependent pathway was significantly increased (+19%; $P < 0.0003$). Concomitantly, postprandial lipemia was associated with elevated endogenous CE transfer rates from HDL2 to apoB lipoproteins and with attenuated capacity (−17%; $P < 0.02$) of total HDL to deliver CE to hepatic cells. Postprandial lipemia enhanced SR-BI and ABCG1-dependent efflux to large HDL2 particles. However, postprandial lipemia is equally associated with deleterious features by enhancing formation of CE-enriched, triglyceride-rich lipoprotein particles through the action of CETP and by reducing the direct return of HDL-CE to the liver.—Julia, Z., E. Duchene, N. Fournier, N. Bellanger, M. J. Chapman, W. Le Goff, and M. Guerin. **Postprandial lipemia enhances the capacity of large HDL2 particles to mediate free cholesterol efflux via SR-BI and ABCG1 pathways in type IIB hyperlipidemia. *J. Lipid Res.* 2010.** 51: **3350–3358.**

Supplementary key words ATP binding cassette transporter G1 • high density lipoprotein • cellular cholesterol efflux • scavenger receptor class B, type I

Elevated postprandial hypertriglyceridemia is a characteristic metabolic disorder associated with increased cardiovascular risk (1). After ingestion of dietary fat, an increase in plasma triglyceridemia is observed, reflecting transient accumulation of plasma triglyceride-rich lipoproteins (TRL) of intestinal and hepatic origin, namely, apoB48-containing chylomicrons (CM), apoB100-containing very low density lipoprotein (VLDL), and their remnants. The systemic accumulation of these particles represents a pro-atherogenic consequence of the postprandial period, as they can penetrate the arterial intima at sites of endothelial dysfunction, with retention by the extracellular matrix, thereby contributing to cholesterol accumulation and plaque formation (2). During the postprandial phase, plasma cholesteryl ester transfer protein (CETP) activity is enhanced as a result of an increase in circulating cholesteryl ester (CE) acceptors and/or CETP concentrations (3, 4). On a quantitative basis, both CM and large VLDL-1 particles represent the preferential acceptors of CE from HDL among TRL during the postprandial phase (5). This process favors CE enrichment of TRL

Abbreviations: ABCG1, ATP binding cassette transporter G1; apo, apolipoprotein; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CM, chylomicron; FC, free cholesterol; LCAT, lecithin cholesterol acyltransferase; SR-BI, scavenger receptor class B, type I; RCT, reverse cholesterol transport; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

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and equally involves enrichment of HDL particles in TG; as such, they become a substrate for hepatic lipase. Concomitantly, during LPL-mediated lipolysis of postprandial TRL, an excess of surface components containing apolipoproteins, unesterified cholesterol, and phospholipids is generated and sequesters to HDL, thereby increasing the total circulating HDL pool and enhancing the remodeling of small HDL3 into large CE-rich HDL2 particles (6).

Plasma HDL particles exert potent anti-atherogenic effects, including cellular cholesterol efflux and antioxidative and anti-inflammatory activities (7). Cellular efflux of cholesterol is a critical component of the reverse cholesterol transport (RCT) pathway, which involves the centripetal movement of free cholesterol from peripheral tissues, including the vessel wall, to the liver (8, 9). This process is frequently cited as the primary mechanism by which HDL protects against atherosclerosis and by which it may induce plaque regression. Cellular cholesterol efflux represents the exit of free cholesterol (FC) from membrane to lipid-free or lipid-poor apoAI via an energy-mediated process through ABCA1, a transporter belonging to the ATP binding cassette family (10). FC efflux from the plasma membrane also occurs toward mature HDL through ABCG1, another member of the ABC family (11), and equally via the scavenger receptor-BI (SR-BI) (12). The contribution of these pathways to overall cholesterol efflux is function of their tissue distribution and acceptor affinity. Passive diffusion also contributes to FC desorption from the plasma membrane; recently new evidence has been presented to support a key role of this process (13, 14). Cholesterol associated with mature α-HDL can be returned to the liver via at least three pathways. First, HDL particles can deliver cholesterol to the liver by direct interaction with specific hepatic receptors, primarily via the SR-BI receptor, by a selective uptake process and to a lesser degree, via the LDL receptor when HDL particles contain apoE (12, 15). Second, it has been proposed that holo-HDL particles might be potentially endocytosed by hepatocytes, although the precise identity of the specific receptor(s) involved remains controversial (7). Third, the CE content of HDL particles can be transferred to apoB-containing lipoproteins through the action of CETP, with ultimate uptake by specific hepatic receptors. This third pathway is responsible for up to 50% of RCT in humans (16).

Postprandial studies are frequently conducted in healthy subjects but rarely in patients displaying elevated cardiovascular risk (17). Transient elevation of triglyceridemia represents a key marker of postprandial response and reflects the occurrence and accumulation of TRL. The impact of postprandial lipoprotein metabolism on the RCT pathway remains to be elucidated. Indeed, postprandial metabolism is associated with an intense intravascular remodeling of all classes of plasma lipoprotein particles. Thus, the postprandial phase is associated with both quantitative and qualitative modification of HDL subspecies that may influence their anti-atherogenic activities. In this context, we have previously observed that cholesterol efflux capacity via SR-BI to serum was increased during the postprandial phase in both normolipidemic and hyperlipidemic subjects (3). Here we report that postprandial lipemia is associated with quantitative and qualitative modifications of large HDL particles that increase the capacity of these particles to mediate cholesterol efflux via both SR-BI and ABCG1 pathways but, conversely, reduce delivery of cholesteryl esters to hepatic cells.

METHODS

Subjects and postprandial time course

Sixteen males between 36 and 59 years of age (mean, 46 ± 7 years), who displayed a combined hyperlipidemia typical of the type IIB lipid phenotype with concomitant elevation of circulating levels of cholesterol (251 ± 7 mg/dl) and triglycerides (179 ± 14 mg/dl), were selected for the study. Subjects were on average overweight (body mass index, 27 ± 2 kg/m^2), but none was obese or displayed the apolipoprotein E2/E2 genotype. Patients were excluded if they presented with diabetes mellitus, secondary causes of hyperlipimia, uncontrolled hypertension, or a history of major cardiovascular events. All patients were nonsmokers. They were without any lipid-lowering therapy, and their diet was stabilized (AHA step one diet or equivalent) during a six-week period before inclusion in the study.

For each subject, a postprandial time course was performed following consumption of a solid mixed meal that provided a total of 1,200 kcal (14% protein, 38% carbohydrate, and 48% fat) as previously described (5). Subjects were asked to abstain from alcohol and vigorous exercise for 24 h before the day of the test. Blood samples were obtained immediately before the test meal and at 2 h, 4 h, and 8 h after ingestion. Blood was collected by venipuncture from the antecubital vein into sterile EDTA-containing tubes (final concentration of EDTA, 1 mg/ml); plasma was separated immediately by low-speed centrifugation (2500 rpm) for 20 min at 4°C.

The study was performed in accordance with the ethical principles set forth in the Helsinki Declaration. The study protocol and amendment were reviewed and approved by an ethics committee and met national institutional requirements. Written informed consent was obtained from all patients.

Lipoprotein fractionation

Chylomicrons (Sf > 400) were isolated by centrifugation at 20,000 rpm for 45 min at 15°C using a SW41 Ti rotor in a Beckman XL70 ultracentrifuge (3). Subfractions of triglyceride-rich lipoproteins [i.e., VLDL1 (Sf 60–400) and VLDL-2 (Sf 20–60)] were isolated from chylomicron-free plasma by nonequilibrium density gradient ultracentrifugation as previously described (18). HDL subfractions were isolated from chylomicron-free plasma by isopycnic density gradient ultracentrifugation (19).

The quantification of preβ-HDL in plasma was performed as previously described (20) The relative abundance of the human apoAI among the α- or preβ-HDL species was determined by scanning reflectance densitometry (Quantity One software; Bio-Rad). The amount of preβ-HDL was expressed as the percentage of total apoAI (% apoAI, relative concentration) and as absolute concentration (mg/l apoAI) by multiplying its percentage by plasma apoAI levels.

Biochemical analysis

The lipid contents of plasma and isolated lipoprotein fractions, total protein, and apoAI were quantified with an Autoanalyzer (Konelab 20). Reagent kits from Roche Diagnostics and ThermoElectron were used for determination of total cholesterol and triglyceride levels, respectively. The levels of unesterified

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cholesterol and phospholipids were determined with commercial reagent kits (Wako Diagnostics). Cholesteryl ester mass, calculated as (TC − FC) × 1.67, represents the sum of the esterified cholesterol and fatty acid moieties (19). The Bicinchoninic acid assay reagent (Pierce) was used for protein quantification. ApoAI was determined using immunoturbidimetric assays (Thermo-Electron reagents and calibrators).

Determination of endogenous CETP activity

Determination of endogenous CE transfer from HDL to apolipoprotein B-containing lipoproteins was assayed by modification of the method of Guerin et al. as previously described (21, 22). Cholesteryl ester transfer was determined after incubation of whole plasma (500 µl) from individual subjects at 37°C or 0°C for 3 h in the presence of radiolabeled HDL (25 µg HDL-CE) and iodoacetamide (final concentration, 1.5 mmol/l) for inhibition of lecithin cholesterol acyltransferase (LCAT). Following incubation, plasma lipoproteins were fractionated by isopycnic density gradient ultracentrifugation, and the radioactive content of each isolated lipoprotein fraction was quantified by liquid scintillation spectrometry with a Trilux 1450 (Perkin Elmer). The CETP-dependent CE transfer was calculated from the difference between the radioactivity transferred at 37°C and 0°C. The rate of CE transfer was calculated from the known specific radioactivity of radiolabeled HDL-CE and expressed as µg CE transferred/h/ml plasma (22).

Free cholesterol efflux assays

Fu5AH cells were maintained in Eagle's MEM containing 5% new-born calf serum, Raw264.7 cells were maintained in DMEM supplemented with 10% fetal bovine serum, and CHO-K1 cells (wild-type and hABCG1, mouse ABCA1 or Cla1 transfected cells) were maintained in Ham's F-12 medium containing 10% fetal bovine serum. All media were supplemented with 1% L-glutamine and 0.75% penicillin-streptomycin.

Lipid efflux assays using Fu5AH, Raw264.7, CHO-K1, and CHO-hABCG1, CHO-mouse ABCA1, or CHO-Cla1 cells were performed as described previously (21, 23). It has been previously demonstrated that Fu5AH cells highly express SR-BI and that such expression correlates with the rate of HDL-mediated cellular free cholesterol efflux (24). We recently confirmed these observations (25). Equally, in Raw264.7 cells, the expression of ABCA1 is induced by up to 70-fold by stimulation with 8Br cAMP and leads to a concomitant induction of cellular free cholesterol efflux to apoAI (26).

Fu5AH cells. After plating, cells were labeled by incubation with [^{3}H]cholesterol (1 µ Ci/ml) in culture medium for 48 h. Subsequently, Fu5AH cells were incubated for 24 h in serum-free medium supplemented with BSA (0.5%). After equilibration, cholesterol acceptors (2.5% diluted plasma or 10 µg phospholipid/ml of isolated HDL subfractions) were added in serum-free medium and incubated with cells for 4 h at 37°C.

Raw264.7 cells. The day after cell plating, cells were loaded and labeled with acetylated LDL (50 µg/ml) and 0.5 µ Ci/ml [^{3}H]cholesterol for 24 h in serum-free DMEM containing glucose (4.5 g/l) and BSA (0.2%) (DGGB, DMEM-Glutamine-Glucose-BSA). After incubation, Raw264.7 cells were incubated with DGGB in the absence or presence of cAMP (0.3 mM) for 16 h to induce ABCA1 expression. Cholesterol acceptors (2.5% diluted plasma) were added to Raw264.7 cells in serum-free DMEM for 4 h at 37°C in the presence or absence of 0.3 mM 8-Br cAMP. The ABCA1-dependent efflux was calculated as the difference between fractional cholesterol efflux to cells in the presence or absence of 8-Br cAMP.

CHO-K1 cells (WT and hABCG1, mouse ABCA1 or Cla1). Two days after plating, cellular cholesterol was labeled by incubation of cells with culture medium and 1 µ Ci/ml [^{3}H]cholesterol for 24 h. Equilibration of the label was performed for 90 min in serum-free medium and BSA (0.1%). After equilibration of labeling, acceptors (2.5% diluted plasma, 2.5% diluted apoB-depleted plasma or 5 µg PL/ml of isolated HDL subfractions) were added to the cells in serum-free medium containing BSA (0.1%) for 4 h at 37°C. The ABCG1-dependent efflux was calculated as the difference between efflux to hABCG1-transfected CHO-K1 cells and efflux to wild-type CHO-K1 cells. The ABCA1-dependent efflux was calculated as the difference between efflux to mouse ABCA1-transfected CHO-K1 cells and efflux to wild-type CHO-K1 cells. The Cla1-dependent efflux was calculated as the difference between efflux to Cla1-transfected CHO-K1 cells and efflux to wild-type CHO-K1 cells.

All efflux experiments were performed in triplicate for each sample. Fractional cholesterol efflux was calculated as the amount of the label recovered in the medium divided by the total label in each well (radioactivity in the medium plus radioactivity in the cells). The background cholesterol efflux obtained in the absence of any acceptor was subtracted from the efflux values obtained with the test samples. The capacity of HDL subfractions, HDL2 or HDL3, to mediate free cholesterol efflux was expressed as % of cholesterol efflux per mole of acceptor particle. Molar concentration of HDL particle was calculated as the sum of molar concentration of individual lipid and protein as previously described (27, 28). The protein moiety was considered to consist of two apolipoproteins, apoAI and apoAII, and the molecular weight of the protein moiety in each HDL subfraction was calculated using mass content of apoAI and apoAII converted to molarity (29, 30).

Optimal plasma dilutions of HDL-PL concentrations were determined on the basis of dose response curves for the release of free cholesterol from each cellular model as previously described (23). In addition, we used the release of labeled cellular cholesterol to quantify efflux; this approach has been shown to accurately reflect net mass transfer of cholesterol from cells to extracellular acceptors under our experimental conditions (23).

In vitro selective hepatic uptake of HDL-CE

In vitro selective HDL-CE uptake was performed as previously described (31). HepG2 cells were maintained in DMEM supplemented with 10% fetal bovine serum, 1% L-glutamine, and 0.75% penicillin-streptomycin. HepG2 or Fu5AH cells were plated in 24-well tissue culture plates (10^6 cells/well). Two days after plating, cells were washed three times with PBS and once with serum-free medium. Cells were subsequently incubated in the presence of ^{3}H-CE labeled HDL (60 µg protein/ml) diluted in serum-free medium at 37°C for 5 h. At the end of incubation, the medium was removed, and cells were washed four times with PBS and incubated in the presence of an excess of unlabeled HDL (100 µg protein/ml) for 30 min. Cells were then washed four times with PBS and solubilized with 200 µl of NaOH 0.2N for 15 min at room temperature with gentle mixing. The protein content in each well was determined. The radioactive content of 100 µl of each cell lysate was measured by liquid scintillation counting. Selective uptake was calculated from the known specific radioactivity of radiolabeled HDL-CE and expressed in µg HDL-CE/µg cell protein.

Statistical analysis

Experimental data were analyzed using the SAS software (SAS/STAT User's Guide, Version 8; SAS Institute Inc., Cary, NC). Postprandial lipemia was quantified by calculating the area under the curve (AUC) and the incremental AUC (iAUC) for plasma triglyceride and lipoprotein subfractions. The incremental

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AUC represents the increase in area in response to the test meal relative to lipid or lipoprotein concentrations determined before meal intake. Repeated-measure ANOVA was performed to assess changes in plasma lipid levels, lipoprotein concentrations, CETP activity, cellular free cholesterol efflux, and in HDL-CE, selective uptake during the postprandial phase. Results were considered statistically significant at $P < 0.05$. Values are given as means ± SEM.

RESULTS

Time course of plasma triglycerides during postprandial phase

Following consumption of the solid mixed meal, postprandial hypertriglyceridemia reached a peak (+90%; $P < 0.0001$) 4 h after meal intake (178.8 ± 14.4 mg/dl and 341.2 ± 26.4 mg/dl before and 4 h after meal intake, respectively) (supplementary Fig. I). Postprandial variations in plasma TG levels reflected transient accumulation of circulating triglyceride-rich lipoproteins, mainly in the form of plasma chylomicrons-TG (32.6 ± 4.8 mg/dl and 162.3 ± 19.0 mg/dl before and 4 h after meal intake, respectively; $P < 0.0001$) and VLDL-1-TG (76.5 ± 7.1 mg/dl and 109.0 ± 8.6 mg/dl before and 4 h after meal intake, respectively; $P < 0.0001$).

Interestingly, 4 h postprandially, we observed a significant reduction (178.5 ± 6.5 mg/dl and 142.1 ± 8.7 mg/dl before and 4 h after meal intake, respectively; $P < 0.0001$) in plasma LDL-C levels compared with baseline, whereas those of HDL-C remained unchanged throughout the postprandial phase.

Effects of postprandial lipemia on plasma HDL subspecies

Variations in plasma concentrations of individual HDL subfractions following meal intake are presented in **Fig. 1.** Postprandial lipemia differentially affects plasma levels of HDL2 and HDL3 particles. The absolute plasma HDL2 concentration increased progressively throughout the postprandial phase (+9%; $P < 0.02$ and +18%; $P < 0.0001$ at 4 h and 8 h after meal intake, respectively, compared with baseline). By contrast, the absolute plasma levels of HDL3 decreased significantly 4 h postprandially (−8%; $P < 0.007$). In addition, we observed a significant increase in postprandial plasma HDL2-apoAI levels (+16%; $P < 0.02$ at 4 h versus baseline and +26%; $P < 0.001$ at 8 h versus baseline) as well as a significant reduction in postprandial plasma HDL3-apoAI levels (−10%; $P < 0.0005$ at 4 h versus baseline and −8%; $P < 0.02$ at 8 h versus baseline).

Quantification of preβ-HDL particles in type IIB patients before and after meal intake revealed that the relative concentration of preβ-HDL particles, expressed in percentage of apoAI, was not significantly influenced by the postprandial phase (19.4 ± 1.2%, 16.9 ± 1.1%, 18.5 ± 1.7%, and 17.7 ± 1.8% before, 2 h, 4 h, and 8 h after meal intake, respectively). Equally, absolute plasma concentrations of preβ-HDL particles remained constant throughout the postprandial phase (148.2 ± 7.8 mg/l, 127.7 ± 8.5 mg/l, 142.0 ± 16.5 mg/l, and 141.8 ± 14.0 mg/l before, 2 h, 4 h, and 8 h after meal intake, respectively). Interestingly, we detected a slight increase in plasma apoAI levels 8 h after meal intake compared with baseline (127.1 ± 3.8 mg/dl and 121.3 ± 4.2 mg/dl at 8 h and 0 h, respectively); however this latter variation did reach significance ($P = 0.056$).

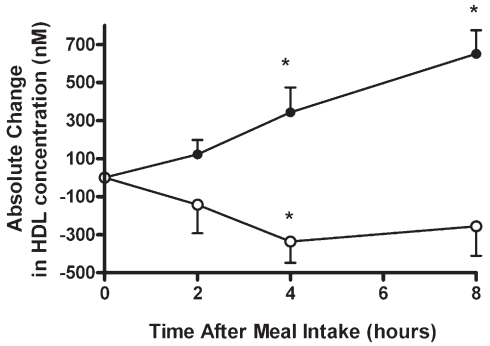

Fig. 1. Line plots showing absolute change in plasma HDL2 (●) and HDL3 (○) levels determined in 16 type IIB hyperlipidemic patients following ingestion of a typical Western meal. Values are mean ± SEM.

The mean weight chemical compositions of native HDL subspecies, expressed as percentage of free cholesterol, esterified cholesterol, triglycerides, phospholipids, and protein contents, determined throughout the postprandial phase are presented in **Table 1**. A significant mean reduction in the CE/TG ratio in HDL2 was observed 4 h (−23%; $P < 0.008$) and 8 h (−27%; $P < 0.0002$) after meal intake. In addition, we detected a significant increase in the relative proportion of phospholipids in HDL2 subspecies 8 h postprandially (+6%; $P < 0.0007$). In consequence, the surface-to-core lipid ratio increased progressively throughout the postprandial phase.

Effects of postprandial lipemia on cellular free cholesterol efflux

The capacity of whole plasma to mediate free cholesterol efflux via the ABCA1 pathway was significantly increased at 4 h (+19%; $P < 0.0003$) and at 8 h (+22%; $P < 0.003$) after meal intake compared with baseline (**Fig. 2**). Equally, the capacity of postprandial plasma from type IIB patients displayed an increased capacity to mediate free cholesterol efflux via the SR-BI pathway compared with baseline (+15%; $P < 0.03$ and +18%; $P < 0.0004$, 4 h and 8 h after meal intake, respectively, relative to baseline). By using apoB-depleted plasma as the cellular cholesterol acceptor, we observed a significant ($P < 0.008$) increment in SR-BI-dependent efflux 4 h and 8 h postprandially (15.6 ± 0.8%, 19.0 ± 0.6%, and 18.5 ± 0.5% before and 4 h and 8 h after meal intake, respectively), thereby suggesting an increase in cellular free cholesterol efflux to postprandial HDL particles via SR-BI. The efflux capacity of whole plasma via the ABCG1 pathway remained unchanged throughout the postprandial phase, whereas that of apoB-depleted plasma increased significantly 4 h (+10%; $P < 0.02$) after meal intake (23.6 ± 1.2% and 26.0 ± 1.3% before and 4 h after meal intake, respectively), suggesting that ABCG1-dependent efflux to HDL particles is equally enhanced during the postprandial phase (supplementary Fig. II).

To demonstrate that postprandial lipemia is associated with an enhanced capacity of postprandial plasma to

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TABLE 1. Chemical composition of plasma HDL subfractions in type IIB patients during postprandial phase

	Hours	%FC	%CE	%TG	%PL	%P	CE/TG	FC+PL / CE+TG
HDL2b	0	6.7 ± 0.5	27.9 ± 1.0	6.1 ± 0.5	22.1 ± 1.1	37.2 ± 0.9	4.57	0.85
	2	6.9 ± 0.5	**26.2 ± 0.8**[a]	6.4 ± 0.7	22.7 ± 1.3	37.8 ± 0.7	4.09	0.91
	4	6.3 ± 0.4	**24.9 ± 1.3**[a]	6.9 ± 0.4	**23.0 ± 1.3**[a]	38.9 ± 1.0	**3.61**[a]	0.92
	8	6.3 ± 0.3	**24.4 ± 1.0**[a]	**7.2 ± 0.4**[a]	**23.5 ± 1.3**[a]	38.6 ± 0.9	**3.39**[a]	**0.94**[a]
HDL2a	0	3.4 ± 0.1	19.5 ± 0.6	4.7 ± 0.4	25.6 ± 1.1	46.9 ± 0.9	4.15	1.20
	2	3.3 ± 0.1	**18.7 ± 0.6**[a]	5.2 ± 0.4	25.1 ± 1.1	47.7 ± 0.8	**3.60**[a]	1.19
	4	3.3 ± 0.1	**17.7 ± 0.6**[a]	**5.6 ± 0.4**[a]	26.1 ± 1.1	47.2 ± 1.0	**3.16**[a]	**1.26**[a]
	8	3.5 ± 0.2	**17.2 ± 0.6**[a]	**5.9 ± 0.3**[a]	**27.1 ± 1.1**[a]	46.4 ± 0.9	**2.92**[a]	**1.32**[a]
HDL3a	0	2.7 ± 0.2	18.5 ± 0.5	4.3 ± 0.3	24.1 ± 1.1	50.5 ± 1.0	4.30	1.18
	2	2.5 ± 0.1	**18.0 ± 0.6**[a]	4.7 ± 0.2	23.4 ± 1.1	51.4 ± 0.7	3.83	1.14
	4	2.5 ± 0.1	**16.8 ± 0.6**[a]	**5.1 ± 0.3**[a]	24.1 ± 1.1	51.6 ± 0.9	**3.29**[a]	1.21
	8	2.8 ± 0.2	**16.0 ± 0.5**[a]	**5.1 ± 0.3**[a]	24.6 ± 1.0	51.5 ± 0.9	**3.14**[a]	**1.30**[a]
HDL3b	0	2.2 ± 0.2	15.0 ± 0.6	4.5 ± 0.3	19.0 ± 1.1	59.2 ± 1.2	3.33	1.09
	2	2.2 ± 0.3	14.4 ± 0.7	4.8 ± 0.5	18.1 ± 1.0	60.5 ± 0.9	3.00	1.06
	4	2.0 ± 0.2	**13.8 ± 0.7**[a]	4.8 ± 0.4	18.4 ± 0.9	61.1 ± 1.0	2.88	1.10
	8	2.3 ± 0.2	**12.8 ± 07**[a]	5.2 ± 0.4	18.8 ± 0.9	60.9 ± 0.8	2.46	1.17
HDL3c	0	1.6 ± 0.3	10.5 ± 0.9	4.3 ± 0.3	12.9 ± 0.9	70.7 ± 1.4	2.44	0.98
	2	1.7 ± 0.4	9.8 ± 0.8	5.3 ± 0.6	11.7 ± 1.0	71.5 ± 1.2	1.85	0.89
	4	1.7 ± 0.2	**8.9 ± 0.7**[a]	4.9 ± 0.4	11.9 ± 0.9	72.5 ± 1.3	1.82	0.99
	8	1.4 ± 0.2	**9.0 ± 0.6**[a]	5.4 ± 0.6	11.8 ± 0.7	72.4 ± 1.0	1.67	0.92

The chemical composition of each HDL subfraction was determined before and after meal intake in 16 type IIB hyperlipidemic patients. Values are mean ± SEM. Boldface type highlights significant changes. CE, cholesteryl ester; FC, free cholesterol; TG, triglyceride; P, protein; PL, phospholipid.

[a] $P < 0.05$ versus before meal intake.

mediate cellular free cholesterol efflux via both SR-BI and ABCA1, we confirmed our observations obtained using Fu5AH and Raw cells $^{+/-}$cAMP by using CHO cells overexpressing human SR-BI homolog (i.e., Cla1 or mouse ABCA1 genes, respectively). Similar conclusions were reached using these cellular models, indicating that both SR-BI/Cla1 and ABCA1 are specifically implicated in the increased efflux capacities of postprandial plasma (data not shown).

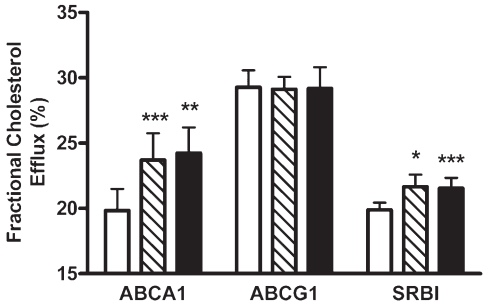

Fig. 2. Bar graph showing fractional free cholesterol efflux determined in 16 type IIB hyperlipidemic patients before meal intake (open bar) and 4 h (hatched bar) and 8 h (closed bar) after ingestion of a typical Western meal. Free cholesterol efflux from cells was determined after 4 h incubation in the presence of 40-fold diluted plasma. The SR-BI-dependent efflux was determined in cultured rat hepatoma Fu5AH cells expressing high levels of SR-BI. The ABCA1-dependent efflux was calculated as the difference between fractional cholesterol efflux to cells in the presence or absence of 8-Br cAMP. The ABCG1-dependent efflux was calculated as the difference between efflux to hABCG1-transfected CHO-K1 cells and efflux to wild-type CHO-K1 cells. Values are mean ± SEM. *$P < 0.05$, **$P < 0.005$, and ***$P < 0.0005$ versus before-meal intake. Abbreviations: ABCG1, ATP binding cassette transporter G1; SR-BI, scavenger receptor class B, type I.

The capacity of isolated HDL2 and HDL3 subfractions to mediate cellular free cholesterol efflux (expressed in terms of HDL-PL concentration or as a function of mole number) via the SR-BI and ABCG1 pathways is shown in **Figs. 3 and 4**, respectively. HDL2 particles isolated from postprandial plasma displayed an increased capacity to mediate cholesterol efflux through SR-BI at 4 h (+12%; $P < 0.02$) and 8 h (+33%; $P < 0.002$) relative to baseline (Fig. 3). By contrast, HDL3 isolated from postprandial plasma displayed reduced capacity to mediate cholesterol efflux via the SR-BI pathway compared with fasting HDL3 particles (−8%; $P < 0.04$ and −20%; $P < 0.0001$ at 4 h and 8 h after meal intake, respectively, compared with baseline). Equally, we observed an increased capacity of postprandial HDL2 particles to mediate cholesterol efflux via the ABCG1-dependent pathway (+31%; $P < 0.003$ and +31%; $P < 0.008$ at 4 h and 8 h, respectively, relative to baseline) compared with HDL2 particles isolated from fasting plasma (Fig. 4).

Effects of postprandial lipemia on cholesteryl ester transfer rates

Plasma CETP activity, expressed as a percentage of CE transferred from HDL to apoB-containing lipoproteins, was significantly increased 8 h (+17%; $P < 0.0002$) postprandially compared with CE transfer activity before meal intake (40.5 ± 3.1%, 46.9 ± 2.8%, and 47.5 ± 2.6% before and 4 h and 8 h after meal intake, respectively). The impact of postprandial lipemia on the capacity of HDL subfractions to act as donors of cholesteryl ester to CETP and apoB-lipoproteins in the indirect pathway of reverse cholesterol transport is presented in **Table 2**. Transfer rates of cholesteryl ester from HDL2 to apoB-lipoproteins in type IIB patients were significantly higher (+30%; $P < 0.03$) 8 h postprandially compared with baseline, reflecting a significantly

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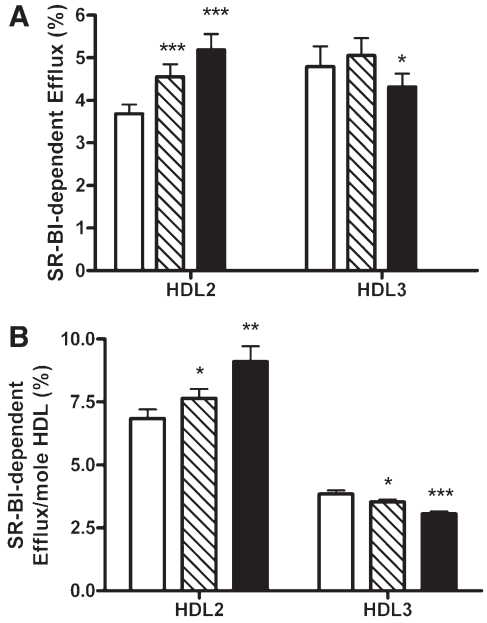

Fig. 3. Bar graph showing the capacity of HDL2 and HDL3 subfractions isolated from 16 type IIB hyperlipidemic patients before meal intake (open bar) and 4 h (hatched bar) and 8 h (closed bar) after ingestion of a typical Western meal to mediate free cholesterol efflux through the SR-BI. A: Fractional cholesterol efflux from cells determined after 4 h incubation in the presence of isolated HDL particles (10 μg PL/ml). B: Fractional cholesterol efflux expressed per moles of HDL particles. Values are mean ± SEM. $*P < 0.05$, $**P < 0.005$, and $***P < 0.0005$ versus before-meal intake. Abbreviation: SR-BI, scavenger receptor class B, type I.

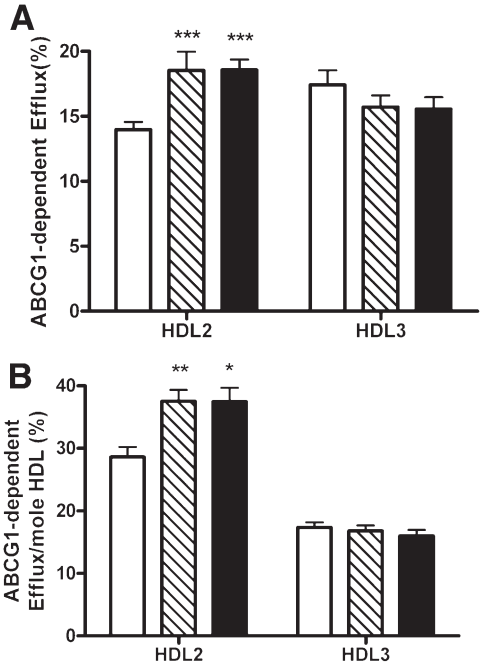

Fig. 4. Bar graph showing the capacity of HDL2 and HDL3 subfractions isolated from 16 type IIB hyperlipidemic patients before meal intake (open bar) and 4 h (hatched bar) and 8 h (closed bar) after ingestion of a typical Western meal to mediate free cholesterol efflux through ABCG1. The ABCG1-dependent efflux was calculated as the difference between efflux to hABCG1-transfected CHO-K1 cells and efflux to wild-type CHO-K1 cells. A: Fractional cholesterol efflux from cells determined after 4 h incubation in the presence of isolated HDL particles (5 μg PL/ml). B: Fractional cholesterol efflux expressed per moles of HDL particles. Values are mean ± SEM. $*P < 0.05$, $**P < 0.005$, and $***P < 0.0005$ versus before-meal intake. Abbreviation: ABCG1, ATP binding cassette transporter G1.

elevated CE transfer from both HDL2b (+49%; $P < 0.05$) and HDL2a (+19%; $P < 0.03$) subfractions. By contrast, the CE transfer rate from HDL3 remained unchanged throughout the postprandial phase. Thus, when individual donor HDL particle subfractions were considered, we observed that the HDL2 subfraction acted as the major CE donor 8 h after meal intake, accounting for 60% of total cholesteryl ester transferred from HDL.

Impact of postprandial lipemia on selective CE uptake in the liver

The impact of the postprandial phase on the capacity of HDL particles to deliver cholesteryl esters to the liver was evaluated using cellular hepatic models of human origin (HepG2) and of murine origin (Fu5AH) (**Fig. 5**). HDL particles isolated from the plasma of type IIB patients during postprandial lipemia displayed a significant reduction in their capacity to deliver CE to hepatic cells at 4 h (−19%; $P < 0.0001$ and −14%; $P < 0.04$ in Fu5AH and HepG2 cells, respectively) and 8 h (−16%; $P < 0.002$ and −17%; $P < 0.02$ in Fu5AH and HepG2 cells, respectively) after meal intake compared with those isolated from fasting plasma.

DISCUSSION

In the present study, we demonstrate that postprandial lipemia is associated with quantitative and qualitative modifications of HDL particles, which significantly influence their function in critical steps of the reverse cholesterol transport pathway (i.e., in cellular cholesterol efflux and selective hepatic CE uptake). Indeed, we observed that postprandial lipemia is associated with significant elevation in plasma HDL2 levels and reduction in plasma HDL3 levels. Such quantitative modifications occurred concomitantly with an increase in the capacity of large HDL2 particles to mediate cellular free cholesterol efflux via both SR-BI and ABCG1 pathways. We equally observed an elevated CETP-mediated CE transfer from HDL2 to apoB-containing lipoproteins and a reduced capacity of HDL particles to deliver cholesteryl esters to the liver.

SR-BI is responsible for selective uptake of CE from HDL mainly in the liver and steroidogenic tissues, but it is equally implicated in cellular cholesterol efflux from peripheral tissues to plasma lipoprotein acceptors (32), primarily large HDL particles, in a phospholipid-dependent manner (33). During postprandial lipemia, SR-BI-mediated CE liver uptake was decreased, whereas SR-BI-dependent efflux was enhanced as a result of both an increase in PL-enriched HDL2 levels and in the capacity of these particles to mediate cholesterol efflux. This finding is entirely consistent with the known mechanism of action of SR-BI. Indeed, it is well established that modification of HDL lipid content can affect selective CE uptake mediated by SR-BI. In particular, TG enrichment of HDL particles re-

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TABLE 2. Cholesteryl ester transfer rates in type IIB patients during postprandial phase

	0 h	2 h	4 h	8 h
HDL2	22.9 ± 2.7	23.9 ± 3.0	21.3 ± 2.6	**29.9 ± 2.3**[a]
HDL2b	8.1 ± 2.3	8.1 ± 2.2	6.9 ± 1.9	**12.1 ± 1.7**[a]
HDL2a	14.9 ± 0.9	15.8 ± 1.3	14.4 ± 1.3	**17.8 ± 1.5**[a]
HDL3	22.1 ± 2.1	21.0 ± 2.2	22.0 ± 2.7	20.3 ± 1.4
HDL3a	15.1 ± 1.1	14.9 ± 1.5	15.4 ± 1.7	14.8 ± 0.8
HDL3b	6.0 ± 0.8	5.0 ± 0.6	5.4 ± 0.9	4.6 ± 0.6
HDL3c	1.0 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.0 ± 0.2

Rates of cholesteryl ester (CE) transfer are expressed in μg CE transferred/h/ml plasma. Values are mean ± SEM.

[a] $P < 0.05$ versus before-meal intake.

duces the CE/TG ratio and decreases CE selective uptake, while HDL-TG depletion increases CE selective uptake (34). Moreover, sphingomyelin (SM) enrichment of HDL also reduces CE uptake in SR-BI-expressing cells while increasing cholesterol efflux (35). Interestingly, it has been demonstrated that plasma remnant lipoprotein particle (RLP)-SM levels increase during postprandial lipemia in a similar manner to those of plasma RLP-TG (36). Because a significant proportion of the surface constituents of HDL are derived from hydrolysis of TRL particles (37), it is likely that transfer of SM from postprandial TRL favors formation of HDL-PL-enriched particles displaying a increased SM/PC ratio during postprandial lipemia. Taken together, these observations strongly suggest that postprandial HDL metabolism is intimately related to SR-BI activity. In this context, it is relevant that SR-BI has been previously shown to modulate postprandial triglyceride response through its action on intravascular chylomicron metabolism (38, 39) and more recently through its action on intestinal cholesterol absorption and TRL secretion (40).

In the present study, despite an increase of ABCG1-dependent efflux to postprandial HDL2 particles, the overall capacity of whole plasma to remove cellular free cholesterol via ABCG1 was not significantly modified throughout the postprandial phase. Note that postprandial lipemia is associated not only with both an increase in HDL2 levels and a decrease in HDL3 levels but also with a reduction in plasma LDL-C levels. ApoB-containing lipoproteins have been shown to represent potential cellular cholesterol acceptors for the ABCG1-mediated cholesterol efflux (11, 41). Efflux experiments performed in the presence of apoB-depleted plasma support the possibility that a reduction of cholesterol efflux to LDL particles during the postprandial phase might counterbalance the increased ABCG1-dependent cellular efflux to HDL2 particles.

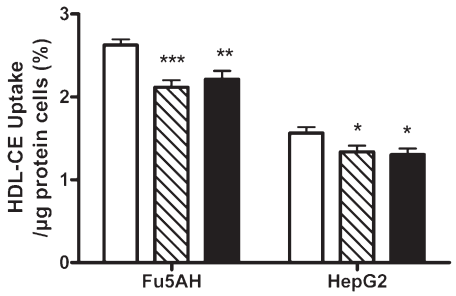

Fig. 5. Bar graph showing in vitro capacity of HDL particles (d:1.063–1.21 g/ml) to deliver cholesteryl esters to hepatic cellular models of murine origin (Fu5AH) and of human origin (HepG2). HDL particles were isolated from 16 type IIB hyperlipidemic patients before meal intake (open bar) and 4 h (hatched bar) and 8 h (closed bar) after ingestion of a typical Western meal. Values are mean ± SEM. *$P < 0.05$, **$P < 0.005$, and ***$P < 0.0005$ versus before meal intake.

We presently observed a significant increase in the capacity of whole postprandial plasma to remove cellular free cholesterol efflux compared with fasting plasma using the mouse macrophage cell line Raw264.7, in which the expression of ABCA1 can be stimulated with 8 Br-cAMP (26). ABCA1 is known to mediate the export of cellular cholesterol and phospholipids to lipid-poor or lipid-free apolipoproteins (mainly apoAI) to form nascent preβ-HDL particles. In addition, it has been previously demonstrated that preβ-HDL levels predict the variance of the ABCA1-dependent efflux (20), suggesting an increase in preβ-HDL levels during postprandial lipemia. However, quantification of preβ-HDL particles in plasma from hyperlipidemic patients failed to reveal any significant variation in both the relative concentration of preβ-HDL particles and in absolute plasma concentrations of preβ-HDL particles throughout the postprandial phase. Since ABCA1 is known to preferentially mediate cholesterol efflux to lipid-free or lipid-poor apoAI, we propose that efficient ABCA1 substrates are generated postprandially. This is entirely consistent with previous studies showing that free apoAI can be detected only in postabsorptive serum. Indeed, during postprandial phase, free apoAI accounted for at least 20% of the total plasma apoAI levels, whereas apoAI associated with HDL remained unaltered, suggesting that free apoAI is liberated from triglyceride-rich lipoproteins during lipolysis (42). Equally, lipid-poor apoAI can be generated within plasma during the remodeling of mature spherical HDL particles by CETP-dependent mechanisms.

Note that in addition to receptor or transporter-mediated cholesterol efflux, the nonspecific release of cholesterol from the cell surface membrane to extracellular acceptors mediated by passive aqueous diffusion occurs in all cell types. The aqueous transfer mechanism has been proposed to be quantitatively as important as cholesterol efflux mediated by specific transporters or receptors (13). Efflux experiments conducted in the present study do not evaluate aqueous diffusion process. Thus, we cannot rule

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out the possibility that the observed quantitative and qualitative modification of HDL particles during the postprandial phase might in part influence the nonspecific release of cholesterol from cells.

In agreement with earlier studies (3, 4), we presently observed that CETP-mediated CE transfer from HDL to apoB-containing lipoproteins was enhanced during the postprandial phase in type IIB subjects as a result of a significant and specific increase in the CE transfer rate from HDL2 particles. Note that this observation is entirely consistent with the observed reduction in the CE/TG ratio in large HDL2 particles and is indicative of a progressive depletion of CE at the expense of TG in their hydrophobic core throughout the postprandial phase. In addition, we have previously reported that the increment in CETP activity during postprandial lipemia is associated with marked elevation in postprandial CE mass transferred from HDL to both chylomicrons and the large VLDL-1 subfraction (3, 18). Considered together, these observations indicate that the formation of atherogenic CE-enriched TRL particles is enhanced during postprandial lipemia in type IIB subjects.

In conclusion, our present observations in subjects displaying mixed (IIB) dyslipidemia demonstrate that postprandial lipemia is associated with structural modifications of HDL2 particles that impact their function in critical steps of the RCT pathway (i.e., cellular cholesterol efflux and hepatic delivery of HDL-CE). Postprandial lipemia enhanced SR-BI- and ABCG1-dependent efflux to large HDL2 particles. However, postprandial lipemia is equally associated with deleterious features by enhancing formation of CE-enriched, triglyceride-rich lipoprotein particles through the action of CETP and by inducing structural changes in HDL particles that reduce the direct return of HDL-CE to the liver.

All contributors participated in aspects of the study, analysis and interpretation of data, and development of the article. The final version of the article was seen and approved by all authors. Z.J. performed in vitro selective HDL-CE uptake and endogenous CETP-mediated CE transfer experiments and contributed to efflux experiments and data interpretation. E.D. performed biochemical analysis on fasting and postprandial plasmas and contributed to lipoprotein characterization. N.F. performed preβ-HDL quantification and contributed to data interpretation and development of the article. N.B. contributed to efflux experiments. M.J.C. contributed to development of the article. W.L.G. contributed to data interpretation and development of the article. M.G. performed lipoprotein fractionation and characterization, statistical analyses, and data interpretation, as well as development of the article. M.G. also contributed to efflux experiments and to the conception and design of the study.

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