Cholesteryl ester flux from HDL to VLDL-1 is preferentially enhanced in type IIB hyperlipidemia in the postprandial state

Maryse Guerin,[1],* Pascal Egger,* Céline Soudant,* Wilfried Le Goff,* Arie van Tol,[†] Reynald Dupuis,[§] and M. John Chapman*

Institut National de la Santé et de la Recherche Médicale,* INSERM Unité 551, Dyslipoproteinemia and Atherosclerosis, Hôpital de la Pitié, 75651 Paris, France; Department of Biochemistry,[†] Erasmus University, Rotterdam, The Netherlands; and Pfizer,[§] Paris, France

Abstract Postprandial triglyceride-rich lipoproteins (TRL) exert proatherogenic effects at the arterial wall, including lipid deposition. Following consumption of a mixed meal (1,200 kcal), plasma-mediated cellular free cholesterol (FC) efflux, lecithin:cholesterol acyltransferase (LCAT), and cholesteryl ester transfer protein (CETP) activities were determined in subjects (n = 12) displaying type IIB hyperlipidemia and compared with those in a normolipidemic control group (n = 14). The relative capacity of plasma to induce FC efflux from Fu5AH cells via the SR-BI receptor was significantly increased 4 h postprandially (+23%; $P <$ 0.005) in the type IIB group, whereas it remained unchanged for postprandial plasma from normolipidemic subjects. LCAT activity was significantly elevated 2 h postprandially in both the IIB and control groups, (+46% and +36%, respectively; $P <$ 0.005 vs. respective baseline value). In type IIB subjects, total cholesteryl ester (CE) mass transfer from HDL to total TRL [chylomicrons (CMs) + VLDL-1 + VLDL-2 + IDL] increased progressively from 15 ± 2 µg CE/h/ml at baseline to 28 ± 2 µg CE transferred/h/ml (+87%; $P =$ 0.0004) at 4 h postprandially. CE transfer to CMs and VLDL-1 was preferentially stimulated (2.6-fold and 2.3-fold respectively) at 4 h in IIB subjects and occurred concomitantly with elevation in mass and particle number of both CMs (2.3-fold) and VLDL-1 (1.3-fold). Furthermore, in type IIB subjects, CETP-mediated total CE flux over the 8 h postprandial period from HDL to potentially atherogenic TRL was significantly enhanced, and notably to VLDL-1 (32-fold elevation; $P <$ 0.005), relative to control subjects. Such CE transfer flux was reflected in a significant postprandial increase in CE-TG ratio in both CMs and VLDL-1 in type IIB plasmas. In conclusion, HDL-CE is preferentially targeted to VLDL-1 via the action of CETP during alimentary lipemia, thereby favoring formation and accumulation of atherogenic CE-rich remnant particles. —Guerin, M., P. Egger, C. Soudant, W. Le Goff, A. van Tol, R. Dupuis, and M. J. Chapman. **Cholesteryl ester flux from HDL to VLDL-1 is preferentially enhanced in type IIB hy-** perlipidemia in the postprandial state. *J. Lipid Res.* **2002.** 43: 1652–1660.

In order to maintain cholesterol homeostasis in peripheral tissues, excess cellular cholesterol is returned to the liver for excretion via a multistep process termed "reverse cholesterol transport" (RCT) (1). A key component of this process involves the transfer of a significant portion of the cholesteryl ester (CE) pool in HDL to apoB-containing lipoproteins (VLDL, IDL, and LDL) via the action of the cholesteryl ester transfer protein (CETP) (2).

Hyperlipidemia of phenotype IIB is associated with an increased risk of premature coronary artery disease and is characterized by concomitant elevation of circulating levels of atherogenic apoB-containing, triglyceride-rich (VLDL) and cholesterol-rich lipoproteins (VLDL remnants, IDL, and LDL including small, dense LDL) (3). In type IIB hyperlipidemia during the fasting state, CETP is implicated in the intravascular formation of atherogenic small, dense LDL through an indirect mechanism involving an elevated rate of CE transfer from HDL to VLDL, and more specifically, to large VLDL-1 particles (4, 5). Indeed, elevated levels of CE-enriched VLDL-1 are associated with enhanced formation of atherogenic small, dense LDL in hypertriglyceridemic states and notably in hyperlipidemias of phenotypes IIB and IV and in the dyslipidemias of type 2 diabetes and metabolic syndrome (6).

Postprandial lipoprotein metabolism is characterized by

Abbreviations: CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; TG, triglyceride; TRL, triglyceride-rich lipoprotein; CM, chylomicron; RCT, reverse cholesterol transport.

[1] To whom correspondence should be addressed.
    e-mail: mguerin@infobiogen.fr

the transient circulation and accumulation in plasma of potentially atherogenic particles of intestinal and hepatic origin, notably CMs, VLDL, and their remnants, that may infiltrate and undergo retention in the vessel wall (7). In patients with CAD, postprandial triglyceride (TG) levels are typically elevated and remain so over a prolonged period in comparison with patients without CAD (8). During the lipolytic process, surface components (free cholesterol, phospholipids, and non-apoB apolipoproteins) of CMs and VLDL are sequestered to HDL (9). Furthermore, during alimentary lipemia, the CETP-mediated transfer of neutral lipids (CEs and TGs) between plasma lipoprotein particles is accelerated (10, 11, 12) favoring CE enrichment of triglyceride-rich lipoproteins (TRL) on the one hand, and on the other, allowing transformation of CE-enriched HDL into TG-rich HDL particles which become a substrate for hepatic lipase. Intravascular remodelling of HDL thus results in the formation of lipid-poor HDL particles (including preβ-HDL) possessing elevated capacity to accept cellular cholesterol (13). Such particles are a substrate for LCAT, and thus postprandial lipemia is characterized by activation of plasma LCAT activity (14–17), a finding which led to the proposal that the postprandial phase is associated with activation of RCT (14).

In earlier studies, the capacity of postprandial plasma lipoproteins from healthy normolipidemic subjects to promote receptor-independent cholesterol efflux from cell membranes was evaluated (18). Using red blood cells as donors of membrane cholesterol via passive diffusion in the aqueous phase, CMs were shown to represent the most potent acceptors of free cholesterol among postprandial lipoproteins. CMs may therefore contribute to RCT in vivo during the postprandial phase. In contrast however, accumulation of CMs and their remnants in the postprandial phase, as occurs in atherogenic dyslipidemia of type IIB (19), is potentially pro-atherogenic.

During the process of RCT, cellular cholesterol efflux can occur by passive diffusion or may involve receptors for HDL or apoA-I such as the scavenger receptor BI (SR-BI) or ATP binding cassette transporter 1 (ABCA1) (20, 21). Specific HDL particle populations such as preβ-HDL play a determinant role in mediating free cholesterol efflux from peripheral cells (13). During lipolysis, nascent HDL equally acquires additional phospholipids and free cholesterol as a result of transfer from TRL particles by the phospholipid transfer protein (PLTP) (22). HDL cholesterol (HDL-C) is then esterified by lecithin:cholesterol acyltransferase (LCAT) forming larger, CE-rich HDL particles. The return of HDL CEs to the liver may involve at least three distinct metabolic pathways (23). First, CEs may be taken up by the liver via selective uptake from HDL without concomitant degradation of HDL protein. This selective uptake pathway involves the action of SRBI (24). Second, the pathway involves the cellular uptake of intact HDL particles and the degradation of HDL-associated lipids and proteins (23). Finally, a major part of HDL CEs may be transferred to TRL and LDL particles by CETP, which are subsequently catabolized upon uptake by specific hepatic receptors (25).

In the present study, we evaluated the effect of a typical Western meal on key steps of RCT, including cellular free cholesterol efflux to postprandial plasma, on plasma LCAT-mediated cholesterol esterification and on CETP-mediated lipid transfer to apo-B-containing lipoproteins in subjects displaying type IIB hyperlipidemia and compared them to the corresponding processes in a normolipidemic control group. Our data indicate that during the postprandial phase in type IIB dyslipidemia, cellular cholesterol is preferentially targeted to elevated levels of TRL, and notably to VLDL-1, via the action of CETP, thereby favoring formation and accumulation of CE-rich remnant particles of elevated atherogenicity.

MATERIALS AND METHODS

Patients

Twelve male patients aged between 35 and 66 years (mean: 52 ± 3 years) and displaying a combined dyslipidemia typical of the type IIB lipid phenotype, i.e., with fasting plasma levels of cholesterol >250 mg/dl, TGs >150 mg/dl, and apoB >140 mg/dl, were selected for the study (Table 1). In this context, it is relevant that the European Atherosclerosis Society meeting on familial combined forms of dyslipidemia (Helsinki, Finland, 1998) reached a consensus that a plasma level of apoB ≥ 140 mg/dl is a key biological feature of the familial form of this disorder and indicative of a dense LDL phenotype. Patients had ceased taking lipid-lowering drugs 6 weeks before the day of the test. This 6 week period corresponded to a dietary stabilization period (AHA step one diet or equivalent). Patients were excluded if they displayed dysbetalipoproteinemia, diabetes mellitus, secondary causes of hyperlipidemia such as uncontrolled hypothyroidism, renal impairment or nephrotic syndrome, or known liver or muscle disease. Other exclusion criteria included uncontrolled hypertension or any major cardiovascular event (myocardial infarction, severe or unstable angina pectoris, angioplasty, or cardiovascular surgery). None of the subjects was obese (body mass index < 30 kg/m^2) or consumed more than 18 g alcohol/day. Ten patients displayed the apoE3/E3 phenotype, one the apoE3/E4 heterozygous phenotype, and one the apoE4/E4 phenotype. Fourteen healthy normolipidemic men age matched served as control subjects (Table 1).

The study was performed in accordance with the ethical principles set forth in the Declaration of Helsinki. 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.

TABLE 1. Plasma lipid and apolipoprotein profile in type IIB patients and normolipidemic control subjects

	Control Group (n = 14)	Type IIB Group (n = 12)
Total cholesterol (mg/dl)	170 ± 6	287 ± 11^a
Triglycerides (mg/dl)	70 ± 6	201 ± 16^a
LDL-cholesterol (mg/dl)	101 ± 14	199 ± 18^a
HDL-cholesterol (mg/dl)	53 ± 2	34 ± 2^a
Apolipoprotein B (mg/dl)	80 ± 4	161 ± 6^a
Apolipoprotein AI (mg/dl)	160 ± 5	136 ± 9^a
Lp(a) (mg/dl)	16 ± 4	14 ± 3

[a] $P < 0.0005$ versus normolipidemic control subjects.

Test meal and blood samples

The test meal consisted of freshly prepared commercially available foods: instant mashed potatoes mixed with oil (2/3 sunflower oil and 1/3 rapeseed oil), beef steak, cheese, bread, and apple. This meal represented a typical Western meal of a total of 1,200 kcal and consisted of 14% protein, 38% carbohydrate, and 48% fat, providing 66 g fat and 142 mg cholesterol (26). The fatty acid composition of the meal was as follows: 16:0, 13%; 18:1, 36.4%; 18:2, 31.8%; and 18:3, 2.2%.

Subjects were requested to abstain from alcohol and vigorous exercise for 24 h before the day of the test. For each subject, a baseline blood sample was collected at 8 AM after an overnight fasting period. After a standardized breakfast (300 kcal, containing 12% protein, 70% carbohydrate, and 18% fat) at 8:30 AM, the subjects consumed the test meal at 11:30 AM. Plasma samples were obtained before the test meal (0 h) and at 2, 4, and 8 h after ingestion of the meal. Blood was collected into sterile EDTA-containing tubes (final concentration of EDTA, 1 mg/ml) and plasma separated immediately by low-speed centrifugation (2,500 rpm) for 20 min at 4°C.

Lipid and protein analyses

The lipid content of plasma and isolated lipoprotein fractions was quantified enzymatically by using Boehringer Mannheim kits (Meylan, France) for total cholesterol (TC) and free cholesterol (FC). CE mass was calculated as (TC−FC) × 1.67 and thus represents the sum of the esterified cholesterol and fatty acid moieties (27). Bio-Mérieux kits (Marcy-l'Etoile, France) were used for determination of TG and phospholipids (PL). Bicinchoninic acid assay reagent (Pierce Rockford, IL) was utilized for protein quantification. Lipoprotein mass was calculated as the sum of the mass of the individual lipids and protein components for each lipoprotein fraction. Fasting plasma LDL-C was calculated using the Friedewald formula.

Isolation of plasma TRL subfractions

CMs (CM; 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. Each plasma sample (3 ml) was overlayered with 4 ml of a d = 1.006 g/ml solution. After flotation, CM were collected in one fraction of 2 ml. Subfractions of TRL, i. e. VLDL-1 (Sf 60–400), VLDL2 (Sf 20–60), and IDL (Sf 12–20), were isolated from CM free-plasma (2 ml) by nonequilibrium density gradient ultracentrifugation as previously described (28).

Cellular free cholesterol efflux

The capacity of each baseline and postprandial plasma to facilitate cellular cholesterol efflux was determined by use of rat Fu5AH hepatoma cells according to the method of de la Llera Moya (29). This experimental system has been validated as an in vitro model for cellular efflux via the SR-BI receptor (30). Briefly, cells were maintained in EMEM containing 5% calf serum and plated on 2.4 cm multiwell plates using 2 ml/well, i.e., 40,000 Fu5AH cells/well. Two days after plating, cellular cholesterol was labeled by incubation for 48 h with [³H]cholesterol (1 μCi/well). To allow equilibration of the label, the cells were then washed with phosphate-buffered saline and incubated for 24 h in EMEM containing 0.5% BSA. Plated cells were subsequently incubated at 37°C with 2.5% plasma diluted with EMEM, or with a 2.5% dilution of a d > 1.063 g/ml fraction depleted in apoB-containing lipoproteins and isolated from postprandial plasma after centrifugation at 40,000 rpm for 24 h at 15°C. After 4 h of incubation, the medium was removed, and the cell monolayer washed three times with PBS. Cholesterol was extracted from cells with isopropanol. [³H]cholesterol radioactivity was measured in both medium and cells. Cholesterol efflux (expressed as

a percentage), was calculated as the amount of the label recovered in the medium divided by the total label in each well (radioactivity in medium + radioactivity in cells).

Measurement of LCAT activity

Endogenous LCAT activity was measured by the nonradioactive endogenous cholesterol esterification method (31) in which the decrease in plasma free cholesterol content with time of incubation is measured enzymatically. Briefly, aliquots of plasma (40 μl) were incubated at 37°C for 2 h in the presence or absence of iodoacetamide (150 mmol/l). Following incubation, free cholesterol content was measured enzymatically. The cholesterol esterification rate was determined from the decrease in plasma free cholesterol content following incubation and is expressed as nanomoles of CE formed/h/ml.

Determination of CE transfer from HDL to TRL subfractions

Determination of CE transfer from HDL to TRL was assayed by modification of the method of Guérin, Dolphin, and Chapman (32), which exclusively involves physiological CE mass transfer between endogenous lipoprotein donor and acceptor particles in plasma from each patient. HDLs containing radiolabeled CEs were prepared from the plasma as previously described (32). Briefly, HDLs were isolated from the d > 1.063 g/ml fraction of each patients' plasma by ultracentrifugation at 100,000 rpm for 3.5 h at 15°C with a Beckman TL100 centrifuge. The d > 1.063 g/ml fraction was incubated overnight at 37°C in the presence of 4 μCi of [1,2,6,7-³H]cholesterol (specific activity 71 Ci/mmol) to allow endogenous LCAT to esterify radioactive free cholesterol. After incubation, the density of the d > 1.063 g/ml was increased to d = 1.21 g/ml by adding dry solid KBr. HDL-containing radioactive esterified cholesterol was then isolated by centrifugation at 100,000 rpm for 5.5 h at 15°C. Radiolabeled HDL preparations displayed a specific radioactivity that ranged from 5,000 to 14,500 cpm/μg CE. CE transfer was determined after incubation of whole plasma (3 ml) from individual subjects at 37°C or 0°C for 3 h in the presence of radiolabeled HDL (equivalent to 1% of the total HDL-CE mass present in 1 ml of subject's plasma) and iodoacetamide (final concentration 1.5 mmol/l). After incubation, CMs were isolated by ultracentrifugation at 20,000 rpm for 45 min. The plasma sample (3 ml) was overlayered with 5 ml of d = 1.006 g/ml solution. After centrifugation, CMs were collected in one fraction of 2 ml. CM free-plasma (2 ml) was used to isolate VLDL1, VLDL2, and IDL as described above. The total HDL fraction (d = 1.063–1.21 g/ml) was isolated by sequential ultracentrifugation at 45,000 rpm for 48 h. The radioactive CE content of each isolated lipoprotein fraction was quantified by liquid scintillation spectrometry with a Rack Beta 1209. The rate of CE transfer was calculated from the known specific radioactivity of radiolabeled HDL-CE after its addition to plasma and is expressed in micrograms CE transferred/h/ml plasma (32).

Statistical analysis

The data were analyzed using SAS software. Postprandial lipemia was quantified by calculating the incremental area under the curve (iAUC) for plasma TG and TRL (CMs and VLDL-1). The iAUC represents the increase in area following the response of the test meal above baseline concentrations. Repeated-measure ANOVA was performed to assess changes in plasma TG levels, in lipoprotein concentrations, and in CE transfer from HDL to TRL during the postprandial phase. Results were considered statistically significant at $P < 0.05$. Values are given as mean ± SEM.

RESULTS

Time course of plasma TG profile during postprandial lipemia

The effect of the typical Western meal on plasma TG levels was determined in 12 subjects displaying the type IIB phenotype and in 14 normolipidemic subjects (**Fig. 1**). Two hours after meal intake, plasma TG concentration was increased by 2-fold ($P < 0.0001$) in the normolipidemic group and by 1.6-fold ($P < 0.0001$) in the type IIB group as compared with the baseline level which was normalized to 0 (Fig. 1). In the type IIB group, maximal elevation in plasma TG levels ($+80\%$; $P < 0.0001$) was observed 4 h after meal intake; thereafter, a progressive decrease occurred from 4 to 8 h. Plasma TG concentrations remained significantly elevated, however, 8 h postprandially in comparison with baseline ($+40\%$; $P = 0.008$). In addition, plasma TG accumulation during the postprandial response in type IIB patients was significantly increased as compared with that of a normolipidemic population after ingestion of the same typical Western meal (Fig. 1). Indeed, the incremental area under the curve for TG was 4-fold elevated ($P < 0.0005$) in type IIB patients as compared with normolipidemic subjects (818 ± 115 and 196 ± 20 iAUC for TG in type IIB patients and in controls respectively).

Time course of the mass profile of plasma lipoprotein subspecies during postprandial lipemia

The effect of the typical Western meal on postprandial plasma CMs (Sf > 400) and VLDL-1 (Sf 60–400) concentrations in the type IIB patient group and in the normolipidemic control group is shown in **Fig. 2**. The iAUC for plasma CMs from 0 to 8 h was significantly increased ($P < 0.0001$) in type IIB patients as compared with the normolipidemic subject group (620 ± 11 and 249 ± 7 iAUC for CMs in type IIB patients and in controls respectively). Interestingly, the iAUC for plasma CMs from 0 to 2 h was not significantly different between the two groups. Finally, the absolute concentration of CMs remained elevated 8 h postprandially in the type IIB patient group, thereby indi-

cating that the fasting state was not completely restored at this time point. As shown in **Fig. 3**, the iAUC for plasma VLDL-1 from 0 to 8 h was 10-fold increased ($P < 0.0001$) in type IIB patients as compared with the normolipidemic group (231 ± 70 and 23 ± 3 iAUC for VLDL-1 in type IIB patients and in controls respectively).

Moreover, minor but nonetheless significant reductions in the levels of VLDL-2 (-12%; $P < 0.05$) were observed at 2 h and 4 h postprandially in type IIB patients. In addition, subtle changes in HDL2 mass ($+6\%$) were noted in this group, although such variations did not attain significance. By contrast, we detected a significant increase in plasma levels of HDL2 mass ($+13\%$; $P < 0.005$) 4 h postprandially in the normolipidemic control group.

Effects of postprandial lipemia on cellular free cholesterol efflux

Four hours after meal intake, we observed a significant elevation ($+23\%$; $P = 0.005$; **Fig. 4**) in the capacity of postprandial plasma (40-fold dilution) from type IIB subjects to mediate free cholesterol efflux from cells as compared with the baseline value obtained before meal intake ($18.6 \pm 2.0\%$; Fig. 4); as a consequence, efflux rates to IIB and control plasmas were similar at 2, 4, and 8 h postprandially. The overall capacity of normolipidemic plasma remained unchanged over the postprandial phase. Interestingly, fasting plasma from normolipidemic subjects displayed a significantly higher capacity ($+16\%$, $P < 0.0001$) to mediate cellular free cholesterol efflux as compared with that from type IIB patients, a finding that may reflect the significantly lower mean HDL-C level in type IIB group (Table 1).

In parallel, we determined the capacity of the d > 1.063 g/ml fraction deficient in apoB-containing lipoproteins from type IIB plasmas to mediate cholesterol efflux from Fu5AH cells. As shown in Fig. 4, both postprandial apoB-containing lipoproteins and non-apoB-containing lipoproteins contributed to the observed elevation in the total capacity of postprandial plasma from type IIB patients to mediate free cholesterol efflux. However, cholesterol efflux to apoB-containing lipoproteins may be indirect and
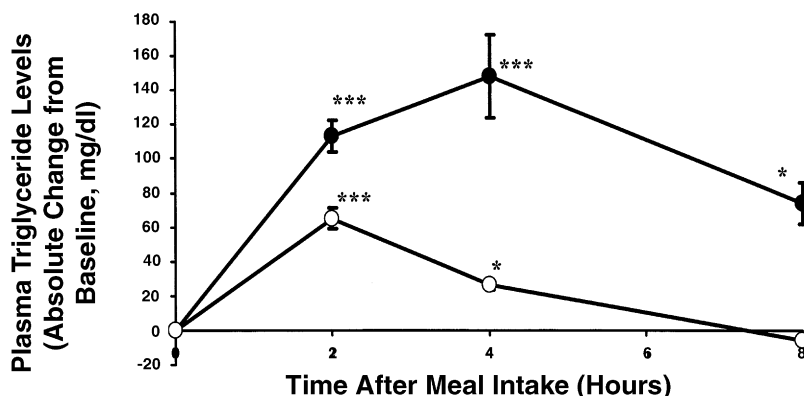

Fig. 1. Line plots showing the absolute change in plasma triglyceride (TG) concentrations following meal intake in type IIB hyperlipidemic subjects (n = 12) (closed circles) and in normolipidemic control subjects (n = 14) (open circles). Values are means ± SEM. ***$P <$ 0.0005 and *$P <$ 0.05 versus before meal intake.
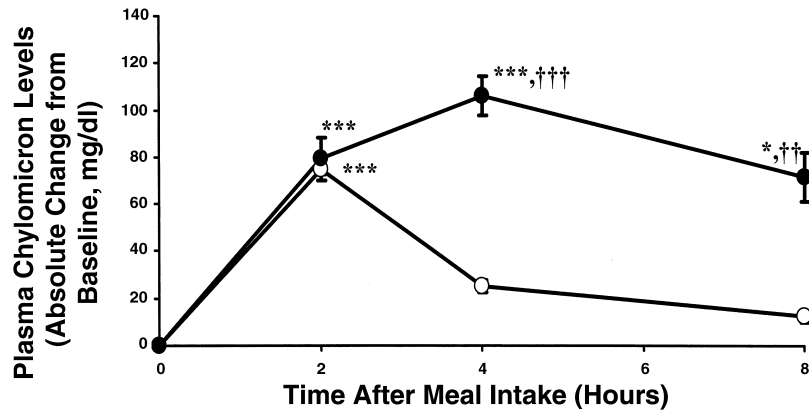
Fig. 2. Line plots showing the absolute change in plasma concentrations of chylomicrons (CMs) following meal intake in type IIB hyperlipidemic subjects (n = 12) (closed circles) and in normolipidemic control subjects (n = 14) (open circles). ***$P < 0.0005$ and *$P < 0.05$ versus before meal intake. †††$P < 0.0005$ and ††$P < 0.005$ versus normolipidemic control subjects.

may result from cellular free cholesterol efflux to apoA-I-containing lipoproteins and subsequent equilibration and transfer of cholesterol to apoB-containing lipoproteins.

Effects of postprandial lipemia on plasma LCAT activity

The cholesterol esterification rate, which reflects plasma LCAT activity, was measured on plasma samples taken throughout the postprandial phase and revealed a significant 1.4-fold increase 2 h ($P < 0.001$) postprandially in both type IIB and normolipidemic groups in comparison with baseline (144 ± 10 and 106 ± 3 nmol CE formed/h/ml 2 h postprandially and before meal intake respectively, in type IIB patients and 142 ± 11 and 97 ± 9 nmol CE formed/h/ml 2 h postprandially and before meal intake respectively, in normolipidemic control subjects); subsequently, enzyme activity progressively decreased to 8 h attaining baseline levels (104 ± 9 and 106 ± 7 nmol CE formed/h/ml 8 h postprandially in type IIB patients and in normolipidemic subjects, respectively). In addition, the increase in LCAT activity in response to meal intake was significantly correlated with the increase in plasma TG levels ($r = 0.39$; $P = 0.016$) in both

groups of patients, and equally with the appearance of CMs in plasma ($r = 0.38$; $P = 0.019$).

Postprandial CE mass transfer from HDL to TRL subfractions

As shown in **Fig. 5**, the transfer rate of CE from HDL to total TRL (CM + VLDL-1 + VLDL-2 + IDL) in type IIB hyperlipidemic plasma was significantly increased at 4 ($+87\%$; $P = 0.0004$) and 8 h ($+63\%$; $P < 0.007$) after meal intake, as compared with the baseline value. A similar increase in total CE transferred from HDL to total TRL subfractions was observed 2 h postprandially in the normolipidemic control group ($+83\%$; $P < 0.005$). Moreover, we detected a progressive but differential increase in CE mass transferred from HDL to individual TRL subfractions over the 8 h time course after meal intake (**Table 2**). Indeed, in type IIB patients, similar levels of CE transfer to VLDL-1 and IDL were observed 2 h postprandially, whereas transfer rates to both CMs and VLDL-2 were lower (-25%). At 4 h however, CE transfer to VLDL-1 was maximal and some 1.4-, 1.7-, and 2-fold greater than that to IDL, CMs, and VLDL-2,
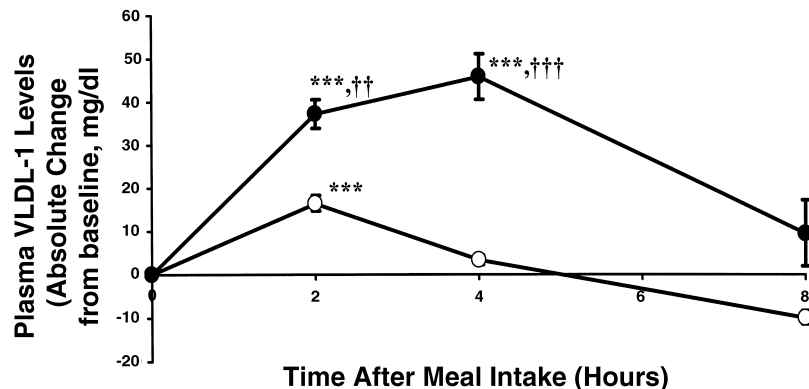

Fig. 3. Line plots showing the absolute change in plasma concentrations of VLDL-1 following meal intake in type IIB hyperlipidemic subjects (n = 12) (closed circles) and in normolipidemic control subjects (n = 14) (open circles). ***$P < 0.0005$ versus before meal intake. †††$P < 0.0005$ and ††$P < 0.005$ versus normolipidemic control subjects.
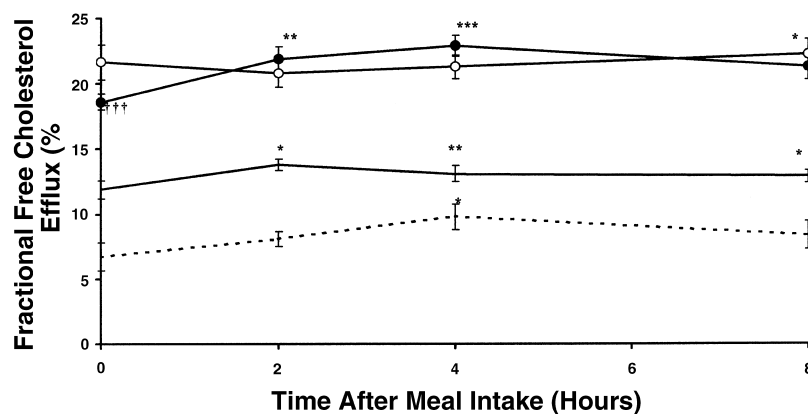
Fig. 4. Effect of meal intake on plasma-mediated cellular free cholesterol efflux over the postprandial period (0–8 h). 40-fold diluted plasma from type IIB hyperlipidemic subjects (n = 12) (closed circles), 40-fold diluted plasma from normolipidemic control subjects (n = 14) (open circles), apoB-free plasma (d > 1.063 g/ml fraction) isolated from plasmas from type IIB subjects (n = 12) (solid lines) and apoB-containing lipoprotein fraction (d < 1.063 g/ml) from plasmas from type IIB subjects (n = 12) (dotted line). The capacity of the apoB-containing lipoproteins to mediate free cholesterol efflux was obtained by calculation of the difference between total efflux mediated by plasma minus the efflux mediated by the d > 1.063 g/ml fraction. Values are mean ± SEM. ***$P < 0.0005$, **$P < 0.005$, and *$P < 0.05$ versus before meal intake. †††$P < 0.0005$ versus normolipidemic control subjects.

respectively. Interestingly, such transfer rates were maintained at the 8 h time point in postprandial type IIB plasmas. These findings are entirely consistent with the evolution of the CE-TG ratio in the CM and VLDL-1 subfractions over the postprandial time course (CM CE-TG ratio: 0.09 ± 0.01, 0.15 ± 0.02, and 0.20 ± 0.04 at 2, 4, and 8h, respectively; $P < 0.05$ for 4 h versus 2 h and $P < 0.01$ for 8 h versus 2 h and VLDL-1 CE-TG ratio: 0.26 ± 0.03, 0.28 ± 0.03, and 0.36 ± 0.04 at 2, 4, and 8h, respectively; $P < 0.001$ for 8 h versus 2 h) which are indicative of progressive enrichment of the hydrophobic core of these particles in CE at the expense of TG. By contrast, in normolipidemic subjects, we observed a large and significant increase (5.8-fold; $P < 0.0001$) in the rate of CE transfer from HDL to CMs following meal intake, whereas CE transfer to other TG-rich subspecies (VLDL-1, VLDL-2, and IDL) remained unchanged over the postprandial phase.

When total CE mass transferred from HDL to individual TRL subspecies was expressed relative to plasma lipoprotein mass, the capacity of each lipoprotein subfraction per unit mass to accept CE from HDL can be estimated. Four hours postprandially, the ability of type IIB VLDL-1, VLDL-2, and IDL to accept CE on a per particle basis from HDL was significantly increased by 80% (3.1 ± 0.6 and 5.6 ± 1.3 µg CE/h/mg lipoprotein mass before and 4 h after meal intake; $P < 0.05$ for VLDL-1), 70% (4.7 ± 0.9 and 8.0 ± 0.9 µg CE/h/mg before and after 4 h meal intake; $P < 0.005$ for VLDL-2), and 47% (6.2 ± 1.2 and 9.1 ± 1.2 µg CE/h/mg before and after 4 h meal intake; $P < 0.05$ for IDL). As apoB (B-48 + B-100) content in VLDL-1 fractions represented ≥80% of the total protein moiety by SDS-PAGE, we estimated relative particle numbers in postprandial IIB as compared with normal plasmas on this basis. Thus, plasma apoB in type IIB VLDL-1 at 2 h postprandial was 2-fold greater than that in control plasma (14.8 mg/dl vs. 7.3 mg/dl) and 3-fold greater at 4 h (15.3 mg/dl
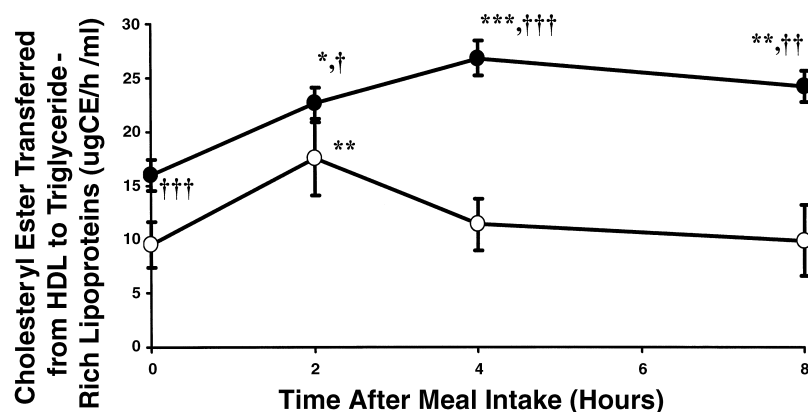

Fig. 5. Effect of meal intake on plasma cholesteryl ester transfer protein (CETP)-mediated cholesteryl ester (CE) mass transferred from HDL to total triglyceride-rich lipoproteins (TRL) expressed as micrograms CE transferred/h/ml in type IIB hyperlipidemic subjects (n = 12) (closed circles) and in normolipidemic control subjects (n = 14) (open circles). Values are mean ± SEM. ***$P < 0.0005$, **$P < 0.005$, and *$P < 0.05$ versus before meal intake. †††$P < 0.0005$, ††$P < 0.005$, and †$P < 0.05$ versus normolipidemic control subjects.

TABLE 2. Cholesteryl ester transfer rates from HDL to individual triglyceride-rich lipoprotein subfractions during the postprandial phase in type IIB patients and normolipidemic control subjects

Lipoprotein Subfraction	Postprandial Time Point	Control Group (n = 14)		Type IIB Group (n = 14)	
		CE	Change from 0 h	CE	Change from 0 h
	h	$\mu g/h/ml$	%	$\mu g/h/ml$	%
Chylomicrons					
	0	1.6 ± 0.3		2.3 ± 0.5[d]	
	2	9.3 ± 1.2[a]	+480	5.5 ± 1.1[c,d]	+140
	4	3.2 ± 0.5[c]	+100	6.1 ± 1.0[b,d]	+165
	8	2.3 ± 0.6	+44	5.1 ± 1.0[c,d]	+122
VLDL-1					
	0	3.0 ± 0.5		4.5 ± 0.8[d]	
	2	3.1 ± 0.5	+3	6.6 ± 1.1[b,d]	+47
	4	2.8 ± 0.6	−7	10.2 ± 1.6[b,d]	+127
	8	2.9 ± 0.4	−3	9.5 ± 1.8[b,d]	+111
VLDL-2					
	0	2.2 ± 0.2		3.2 ± 0.5[d]	
	2	2.1 ± 0.2	−5	4.2 ± 0.7[d]	+31
	4	2.4 ± 0.2	+9	4.7 ± 0.4[c,d]	+47
	8	2.1 ± 0.1	−5	4.2 ± 0.6[d]	+31
IDL					
	0	2.7 ± 0.4		4.9 ± 0.7[d]	
	2	2.9 ± 0.3	+7	6.3 ± 1.1[d]	+28
	4	2.9 ± 0.3	+7	7.0 ± 1.0[c,d]	+43
	8	2.5 ± 0.6	−7	5.5 ± 0.9[d]	+12

Values are mean ± SEM.
[a] $P < 0.0005$.
[b] $P < 0.005$.
[c] $P < 0.05$ versus before meal intake.
[d] $P < 0.05$ versus normolipidemic control subjects.

vs. 5.3 mg/dl) in type IIB as compared with control VLDL-1. Clearly then, VLDL-1 particles numbers were markedly elevated in IIB plasma at 2 and 4 h postprandial as compared with those in the corresponding control samples.

DISCUSSION

The present study has revealed that elevated rates of CETP-mediated CE transfer from HDL to CMs, VLDL-1, VLDL-2, and IDL are intimately associated with the enhanced formation and accumulation of atherogenic CE-enriched, lipoprotein remnant-like particles in type IIB hyperlipidemic subjects during the postprandial phase. Specifically, we observed a marked postprandial elevation in the mass of both CMs (2.3-fold) and VLDL-1 (1.3-fold), and thus in numbers of potential CE acceptor particles, in addition to a concomitant increment in VLDL-1 particle acceptor capacity for CE (1.8-fold). Indeed, a highly significant 3-fold increase in total net CE flux over the 8 h postprandial period from HDL to TRL was detected relative to that in control subjects. Furthermore, among the circulating postprandial TRL pool, specific CE targeting to VLDL-1 increased some 32-fold relative to CE transfer rates in control plasmas.

In order to determine whether postprandial changes in the quantitative and qualitative features of atherogenic apoB-containing lipoproteins and of anti-atherogenic HDL may influence cellular free cholesterol efflux, we measured the capacity of postprandial plasma to induce cholesterol efflux from Fu5AH rat hepatoma cells which express high levels of the SR-BI receptor (30). Indeed, the SR-BI receptor pathway represents a critical component of the RCT process, as this receptor is expressed at high levels in human monocyte-macrophages and lipid-loaded foam cells; indeed, the latter are major cellular components of the lipid-rich, atherosclerotic plaque (33). Postprandial lipemia observed in type IIB subjects was significantly associated with increase (+23%; $P < 0.005$) at 2 h in the capacity of whole plasma to efflux cellular free cholesterol. This finding is consistent with a previous report (18) in which postprandial as compared with fasting plasma was more potent in promoting free cholesterol efflux from red blood cells; the mechanism of such efflux remains indeterminate however, but may primarily involve efflux of cholesterol by receptor-independent, passive diffusion. Earlier studies (13, 34) have demonstrated that preβ-HDL constitute preferential acceptors of cellular free cholesterol and thus increased levels of preβ HDL particles in plasma may enhance cholesterol efflux. The efficiency of a given plasma to promote cellular cholesterol efflux can also be enhanced by addition of phospholipid (35). Equally, it has been proposed that cholesterol removal from cells induced by phospholipid-enriched serum results from the action of PLTP and requires the formation of preβ-HDL particles (35). Indeed, PLTP is responsible for the interconversion of HDL$_3$ into both larger and smaller HDL particles, the latter resembling preβ-HDL (36). Moreover, the production of preβ-HDL is dependent upon phospholipid concentration in plasma. Indeed, a significant amount of preβ-HDL can be generated by addition of a minimum of 0.25 mg PL/ml serum (35). Moreover, a marked increase in cellular free cholesterol efflux occurred when 0.50 mg PL was added to plasma. We presently observed a maximal increment of 0.48 mg PL/ml plasma (+22%; $P < 0.0005$) following meal intake. Taken together, these observations strongly suggest that HDL particles that are active in facilitating cholesterol efflux are formed during the early postprandial phase (0–2 h) in type IIB hyperlipidemic plasma, possibly as a result of the release of surface fragments from TRL during lipolysis.

Maximal increase (+36% and 46%) in LCAT activity was observed 2 h after consumption of a typical Western mixed meal in both type IIB subjects and controls. A similar observation was reported earlier by others (14–17). The half-life of LCAT in plasma has been estimated as 6–10 days in familial LCAT-deficient patients transfused with LCAT (37). It appears unlikely therefore that the activation of plasma LCAT observed during the postprandial phase resulted from an increase in LCAT protein mass, because enzyme activity progressively returned to baseline 8 h after meal intake. It is more probable that elevation in LCAT activity resulted from an increase in enzyme substrate availability. Indeed, we observed a significant increment in both plasma free cholesterol (+34%) and phospholipid (+20%) levels simultaneous with LCAT activation. Interestingly, significant elevation in cholesterol esterification rate during postprandial lipemia was not associated with
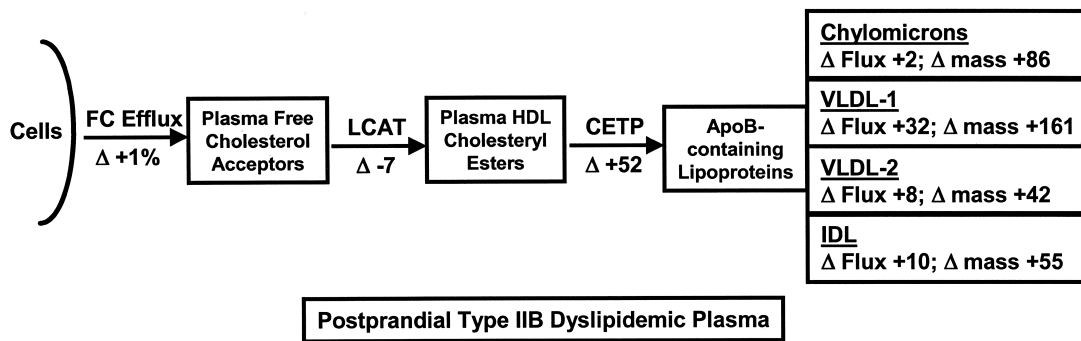
Fig. 6. Enhanced CETP-mediated CE transfer occurs in type IIB dyslipidemia during the postprandial phase as compared with that in normolipidemic subjects, thereby enhancing formation and accumulation of atherogenic CE-rich remnant particles. During postprandial lipemia, plasmas from type IIB and normolipidemic subjects exhibit similar capacities to efflux free cholesterol from Fu5AH cells and equally display similar rates of LCAT-mediated cholesterol esterification. However, CETP-mediated net CE transfer flux from HDL to potentially atherogenic TRL (VLDL-1 > IDL > VLDL-2 > CMs) is significantly enhanced in type IIB plasmas; such enhanced CE transfer occurs concomitantly with elevated concentrations of these particles in type IIB subjects. Δ values represent the differences between incremental area under the curve (iAUC) values for the total 8 h postprandial phase for each component process of reverse cholesterol transport (RCT) in type IIB versus normolipidemic plasmas. Efflux is expressed as a percentage, LCAT activity as nanomole of CE formed/h/ml and CETP-mediated CE transfer flux from HDL to TRL as microgram CE transferred/h/ml. Δ values for total postprandial concentrations of TRL (expressed as mass in mg/dl) represent the mean differences between type IIB and normolipidemic plasmas over the 8 h period.

an increase in CE levels in plasma but rather with preferential enrichment of the hydrophobic core of CM and VLDL-1 particles with CE transferred from HDL. It is of interest that postprandial LCAT activation was positively correlated ($P = 0.0016$) with increase in plasma TG levels. A similar correlation ($P = 0.019$) was found between LCAT activity and levels of CM particles. These results indicate that LCAT activity is closely related to the metabolism of postprandial TRL particles. Indeed, as previously suggested (14), enhanced postprandial LCAT activity may reflect liberation of surface lipid fragments from TRL that sequester to HDL and act as LCAT substrates.

During postprandial lipemia in type IIB subjects, CE transfer from HDL to TRL was accelerated (+87% at 4 h) as a result of increase in postprandial lipoprotein concentrations and thus particle numbers, as well as from a higher relative capacity of these particles (specifically VLDL-1) to act as CE acceptors. Nonesterified fatty acids may equally stimulate the ability of CETP to promote transfer of CEs from HDL to apoB-containing acceptor particles (38). Nonesterified fatty acids present at the surface of lipolyzed TRL increase the negative surface charge, thereby inducing increased electrostatic interaction between these lipoproteins and CETP. In agreement with a recent study (39), we presently observed a 3-fold increase in plasma free fatty acid levels 8 h after meal intake in type IIB hyperlipidemia (data not shown). Clearly we cannot exclude the possibility that free fatty acids liberated by lipolysis at the surface of TG-rich particles may contribute to their enhanced activity as acceptors of CE via the action of CETP.

Given our experimental procedure for the isolation of VLDL-1 (Sf 60–400), part of the mass of this fraction may correspond to CM remnants of intestinal origin. However, Karpe et al. (40) demonstrated a major contribution of liver-derived TRL to postprandial triglyceridemia. Indeed, these authors observed that the intestinally derived apoB-48-containing Sf 60–400 fraction accounted for less than 20% of the total postprandial Sf 60–400 fraction, and that at least 80% of lipoprotein particles isolated in the Sf 60–400 fraction at the postprandial peak corresponded to liver-derived apoB-100-containing lipoprotein particles in both normotriglyceridemic and hypertriglyceridemic subjects (40). Such relative proportions of apoB-48 and apoB-100 in the postprandial VLDL-1 fraction were confirmed by SDS-PAGE (data not shown).

Considered together, our data support the contention that activation of a key step in the RCT pathway, and specifically of CE transfer to TRL subfractions from HDL, occurs during postprandial lipemia in type IIB hyperlipidemia, in comparison to the same process in normolipidemic subjects following ingestion of a typical Western meal (**Fig. 6**). Indeed, we presently observed that cellular free cholesterol efflux and LCAT-mediated cholesterol esterification were similar in postprandial plasmas from type IIB and control subjects, in contrast to CETP-mediated CE transfer to TRL particles, which is specifically accelerated in type IIB hyperlipidemia as indicated by the significant increment in net CE flux over the 8 h period relative to controls (+52 μg CE transferred/h/ml plasma); indeed, more than half of this increment was accounted for by VLDL-1. Such enhanced CE transfer results from an elevation in mass and particle number of both VLDL-1 and CMs, but equally from an increase in VLDL-1 particle acceptor capacity for CE. By this mechanism, the formation and accumulation of potentially atherogenic CE-enriched, lipoprotein remnant-like particles, which primarily include CMs and VLDL-1, are enhanced in the postprandial phase in type IIB hyperlipidemia, a dyslipidemia associated with premature atherosclerosis.

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