Platelet Function in Patients With Familial Hypertriglyceridemia: Evidence That Platelet Reactivity Is Modulated by Apolipoprotein E Content of Very–Low-Density Lipoprotein Particles

Javier Pedreño, Eva Hurt-Camejo, Olov Wiklund, Lina Badimón, and Lluis Masana

We evaluated platelet function in patients with familial hypertriglyceridemia (FHTG). Compared with healthy gender-matched controls, platelets from patients showed lower aggregation ($P < .01$) and thromboxane formation ($P < .01$) in response to collagen. Very–low-density lipoprotein (VLDL) particles obtained from the patients inhibited collagen-induced aggregation, whereas VLDL particles from controls had opposite effects. The VLDL-induced effect was regulated by its apolipoprotein E (apoE) content. Indeed, apoE-VLDL–rich fractions caused antiaggregative effects, whereas apoE-VLDL–poor fractions produced a strong proaggregative response. Since we have recently demonstrated that VLDL particles may regulate the activity of platelet low-density lipoprotein (LDL) receptor by a phenomenon of downregulation and desensitization, in this study, we have investigated the effect of prolonged exposure to circulating VLDL levels on the activity of platelet LDL receptor by a double-blind controlled study with gemfibrozil (600 mg twice daily) in 18 subjects with FHTG. Platelets from patients exhibited fewer platelet LDL receptors and ^{125}I-LDL binding was saturable at a lower protein concentration. After 6 months, gemfibrozil therapy versus placebo had a marked lipid-lowering effect, significantly decreased triglycerides (61%), VLDL cholesterol (72%), apoB (28%), and apoE (55%), and increased high-density lipoprotein (44%) and apoA-I (18%). Furthermore, gemfibrozil affected the apoprotein composition of VLDL: total protein increased by 28%, the molar ratio of apoE to apoB decreased 64%, and apoE content decreased 55%. However, no differences in phospholipid, triglyceride, or total cholesterol were detected. Moreover, platelet function was markedly altered by gemfibrozil therapy. Collagen-induced aggregation and thromboxane formation were significantly enhanced ($P < .01$). The initial antiaggregative pattern of VLDL particles was changed to a significant proaggregative effect ($P < .01$), and the number of LDL binding sites was markedly upregulated ($P < .001$). Both receptor upregulation and the change in the aggregative effect of VLDL particles were associated with the reduction of apoE content in VLDL particles ($P < .05$). The overall results indicate that in the regulation of platelet reactivity in hypertriglyceridemic patients, apoE content of VLDL particles and their interaction with the platelet LDL receptor are involved.

EMERGING KNOWLEDGE of the multiple interactions between triglyceride-rich lipoproteins and the hemostatic system has influenced both the view of hypertriglyceridemia as a risk factor for coronary atherosclerosis and thrombosis and the policies for intervention.[1,2] The positive, fairly strong, and consistent relationship between serum triglycerides and hypercoagulability and lower fibrinolytic capacity in type IV and IIb patients is well established.[3,4] The evidence therefore shows that hypertriglyceridemia may be considered a prothrombotic state.[5] However, there are only limited and controversial data concerning their association with platelet hypersensitivity. Several groups have reported that in comparison to type II patients, type IV patients present a decrease in platelet function,[6-9] while other reports described a similar increase in platelet responsiveness.[10,11]

On the other hand, it is well known that very–low-density lipoprotein (VLDL) particles from normolipidemic patients enhance platelet aggregation in response to several agonists,[12,13] whereas recent data suggest that VLDL particles from hypertriglyceridemic patients seem to have opposite effects.[14] Therefore, specific changes in the composition of lipoproteins could participate in the aggregative effect of VLDL particles. In fact, the evidence shows that inhibition of platelet aggregation might involve the interaction between apolipoprotein E (apoE)-rich lipoproteins and specific membrane lipoprotein receptors.[15,16] Since triglyceride apoE-rich lipoproteins (VLDL and intermediate-density lipoprotein [IDL]) may compete with low-density lipoprotein (LDL) to occupy platelet LDL receptors[17] and are also capable of inducing receptor desensitization and downregulation,[18] one might speculate that the interaction between apoE-rich VLDL particles and the platelet LDL receptor could play a key role in regulating platelet responsiveness in hypertriglyceridemic patients. It is well known that apoE-rich VLDL particles transform into IDL and are more effective than apoE-poor VLDL in competing with LDL for binding to the classic LDL receptor of nucleated cells.[19] Furthermore, the VLDL receptor only recognizes the apoE-containing lipoproteins,[20] and it has been recently demonstrated that the apoE-poor VLDL subfraction is more resistant to lipolysis by lipoprotein lipase than its apoE-rich counterpart.[21]The overall data confirm that apoE has a central role in the function and metabolic fate of VLDL. Furthermore, multiple studies in hypertriglyceridemic patients have shown that the fibric acid derivative gemfibrozil decreases VLDL-triglyceride synthesis, stimulates its clearance with increased lipoprotein lipase activ-

From the Universidad Rovira i Virgili, Facultad de Medicina, Unitat de Recerca en Lípids i Arteriosclerosi, Reus, Spain; Wallenberg Laboratory, Sahlgrenska University Hospital, Göteborg, Sweden; and Centro de Investigación Cardiovascular, Consejo Superior de Investigaciones Científicas, Hospital de la Santa Cruz y Sant Pablo, Universidad Autonoma de Barcelona, Barcelona, Spain.

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Address reprint requests to Javier Pedreño, MD, PhD, Universidad Rovira i Virgili, Facultad de Medicina, Unitat de Reçerca en Lipids i Arteriosclerosi, Sant Llorenç 21 Reus, Tarragona, Spain.

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ity, and alters the apoprotein composition of VLDL in a way that may favor VLDL receptor–mediated clearance of the apoE-rich VLDL subfractions, because substantial amounts of apoE-poor VLDL particles remain after drug therapy.[22,23] However, there have been recent reports of a nonbeneficial thrombogenic effect of this agent. Platelet function in vivo was profoundly altered: the platelet count, platelet aggregation, and urinary excretion of 11-dehydrothromboxane B2 increased.[24]

Since the apoE content of VLDL regulates the metabolism of particles and triglyceride-rich lipoproteins such as VLDL may compete with LDL to occupy platelet binding sites, we have investigated whether platelet function in type IV patients is modulated by a competitive mechanism involving VLDL particles and LDL receptor. For this purpose, platelet sensitivity was evaluated in 18 patients with familial hypertriglyceridemia (FHTG) in a double-blind controlled study with gemfibrozil (600 mg twice daily) or placebo for 6 months.

SUBJECTS AND METHODS

Patients and Study Design

To investigate whether high circulating lipoprotein levels induce long-term desensitization of the platelet LDL receptor, a double-blind controlled study with gemfibrozil (600 mg twice daily for 6 months) was conducted in 18 men aged between 40 and 61 years (mean, 51) with FHTG. Patients with more than 1 first-degree relative with hypertriglyceridemia were diagnosed with FHTG after excluding endocrine, renal, and hepatic diseases, a history of alcoholism (drug abuse), smokers, and secondary hyperlipidemias. We excluded patients with anticoagulant and antiplatelet treatment and patients with arterial hypertension (>150/90 mm Hg), or those who had a myocardial infarction or unstable angina pectoris in the preceding 12 months. The patients were included after provision of consent for the protocol previously approved by the institutional review board. The study was performed in ambulatory patients, and their recommended dietary habits were maintained. For at least 6 weeks before the first analysis, they did not use any lipid-lowering medication. Patients were randomized in 2 parallel groups of 9 patients to receive 600 mg/d gemfibrozil twice daily in a double-blind fashion or placebo for 6 months, and were examined each month during the study. On each visit, the fulfillment of the dietary and pharmacological treatment was evaluated. Blood samples were obtained to determine lipids, lipoproteins, and apoproteins, as well as platelet LDL receptor activity, platelet aggregation, and thromboxane B_2 (TXB_2) formation before treatment and after 1 and 6 months of treatment. A group of 15 healthy subjects, nonsmokers matched for sex and age to the FHTG patients, were recruited as a control group and to determine interassay variation.

Materials

Bovine serum albumin (BSA) was purchased from Fluke Chemie (Basel, Switzerland), and fibrinogen was obtained from Kabi (Stockholm, Sweden). Sucrose and EDTA were supplied by Merck (Darmstadt, Germany). ^{125}I-Na was from New England Nuclear (Boston, MA), and Iodo-Gen was from Sigma Chemical (St Louis, MO). Sephadex PD-10 columns and Hi-Trap Heparin columns were obtained from Pharmacia (Uppsala, Sweden).

Lipoprotein Isolation

LDL (density = 1.019 to 1.063 g/mL) particles were isolated from the pooled sera of normolipidemic subjects and separated by sequential ultracentrifugation[25] in an ultracentrifuge (Centrikon T 2060; Kontron Instruments, Milan, Italy). Serum was treated with NaN_3 7.5 mmol/L, gentamicin sulfate 0.1 mmol/L, phenylmethylsulfonyl fluoride 1 mmol/L EDTA 3 mmol/L, chloramphenicol 0.25 mmol/L, and aprotinin 0.25 mmol/L. Lipoproteins were washed during flotation with a potassium bromide solution of the respective lipoprotein density containing EDTA (1 mmol/L, NaN_3 3 mmol/L, gentamicin sulfate 0.1 mmol/L, chloramphenicol 0.25 mmol/L, glutathione 0.65 mmol/L, and NaCl 150 mmol/L. LDL was dialyzed for 24 to 48 hours against phosphate-buffered saline, pH 7.4, containing 10 mmol/L EDTA and 0.15 mmol/L chloramphenicol (buffer A). The lipoprotein concentration was expressed in terms of its protein content determined by the Bradford method using BSA as standard.[26] The composition in apolipoproteins was assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The specific mobility of lipoprotein particles was confirmed by agarose-acrylamide gel electrophoresis (AAGE). The degree of oxidation of lipoprotein particles was assessed using several procedures: (1) thiobarbituric acid–reactive substance (TBARS) content, (2) AAGE mobility (expressed as R_F), and (3) antioxidant content (α-tocopherol, β-carotene, and lycopene) separated by high-performance liquid chromatography. Rich and poor fractions of apo E-VLDL were obtained by heparin chromatography using Hi-Trap Heparin columns following the protocol previously reported.[27]

Isotopic Label of Lipoproteins

Freshly isolated LDLs obtained from a pool of healthy individuals were labeled with ^{125}I-Na by the Iodo-Gen method[28] following the procedure previously described.[29] Unbound iodine was eliminated by chromatography using a Sephadex PD-10 column previously equilibrated with buffer A. ^{125}I-LDL was eluted with buffer A and dialyzed against the same buffer for 24 hours and used immediately. Finally, and before each binding assay, ^{125}I-LDL was filtered (0.45 μm; Millipore, Bedford, MA) and the protein concentration was determined. Final specific radioactivity was 2.2 ± 0.3 Bq/ng protein. The purity of ^{125}I-LDL was confirmed by gel filtration in a fast protein liquid chromatographic device (Pharmacia) following the method previously described.[30] The absence of oxidation was assessed by TBARS and AAGE methods and antioxidant content.

Platelet Isolation

Platelets were isolated from 40 mL freshly drawn citrated (sodium citrate 3.8% wt/vol) blood from 15 healthy controls matched for sex and age and 18 FHTG patients using the method previously described.[17] Platelets were isolated for a maximum of 2 hours after the samples were obtained, and all procedures were performed at room temperature. Platelet-rich plasma (PRP) was obtained by centrifugation at 300 × *g* for 10 minutes. The platelet pellet was then washed, and the final volume was adjusted to produce a final platelet count of 10^{12}/L with incubation buffer (20 mmol/L Tris hydrochloride and 0.15 mol/L NaCl, pH 7.45) containing BSA 5 g/L.

Platelet LDL Binding Assays

Platelet ligand-binding assays were performed as previously described.[17,30-32] Washed platelets (0.1 mL) were incubated at room temperature for 25 minutes with ^{125}I-LDL (protein concentration, 0.01 to 1 g/L) in a final volume of incubation buffer of 0.25 mL. After incubation, an aliquot of 0.20 mL was removed and layered gently onto 0.8 mL 20% sucrose solution in buffer A in Eppendorf tubes, and centrifuged at room temperature for 3 minutes at 16,000 × *g*. The platelet pellet was then recovered by cutting the tips of the tubes and analyzed for radioactivity in a gamma counter. Binding determined in the presence of ^{125}I-LDL represents total binding. The bound lipoprotein was calculated on the basis of the specific activity of ^{125}I-LDL, and the results are expressed as nanograms of protein bound to 10^8 platelets. Finally, to determine the number of receptors per platelet (Bmax) and the dissociation constant (K_d), the specific binding was evaluated by Scatchard analysis[33] using the program KINETIC/EBDA/LIGAND.[34]

Laboratory Analysis

Serum concentrations of cholesterol and triglycerides were determined by enzymatic methods (Boehringer, Mannheim, Germany). Lipoproteins were separated and analyzed by the Lipid Research Clinics method.[35] Briefly, a serum sample (density 1.006 g/mL) was centrifuged at 105,000 × *g* for 18 hours at 15°C in a Kontron Centrikon T-2060 ultracentrifuge equipped with a TFT 45.6 rotor (Kontron Instruments, Milan, Italy). Triglyceride and cholesterol levels were then determined in the supernatant (VLDL fraction, d < 1.006 g/mL) by enzymatic methods (Boehringer). Phospholipid content was determined enzymatically by a reagent kit supplied by Wako (Osaka, Japan). The total protein content of VLDL was determined by the Bradford method[26] using BSA as standard. A portion of the infranatant was treated with phosphotungstate-Mg^{2+} to precipitate the apoB-containing lipoproteins, and cholesterol was subsequently determined. The content of apoA-I, apoB-100, and apoE in VLDL particles and total serum was determined by nephelometry using a commercial immunoturbidimetric method (Behring, Marburg, Germany).

Aggregation Studies and Thromboxane Formation

Blood with 3.8% sodium citrate was collected from FHTG patients and from controls who did not use any medications for the previous 10 days. PRP was obtained by centrifugation at 200 × *g* for 10 minutes at room temperature. To prevent initial platelet activation, EDTA (5.6 mmol/L) and prostaglandin E_1 (5.6 μmol/L) were added. PRP was then further centrifuged at 900 × *g* for 6 minutes. The platelet pellet was washed in a volume of buffer (0.15 mol/L NaCl, 20 mmol/L Tris, 5 mmol/L glucose, and 1 mmol/L EDTA, pH 7.4) equal to that of the discarded plasma and centrifuged at 900 × *g* for 6 minutes, and finally resuspended at a concentration of 3 to 5 × 10^{11} platelets/L in Tyrode's buffer (0.14 mol/L NaCl, 2.7 mmol/L KCl, 1 mmol/L $CaCl_2$, 12 mmol/L $NaHCO_3$, 0.4 mmol/L NaH_2PO_4, 5.5 mmol/L glucose, 3.5 mg/mL BSA, and 10 mmol/L HEPES, pH 7.4). Platelets that were washed and resuspended in this manner showed ''swirling'' as a gross indication of maintenance of the discoid shape and were fully responsive to weak agonists in the presence of fibrinogen for at least 2 hours. Platelet aggregation was performed and recorded on a 4-channel light aggregometer (Aggrecorder II model PA 3220; Menarni, Barcelona, Spain) as previously described.[17] Briefly, washed platelets (0.4 mL) with added fibrinogen (600 nmol/L) were stimulated in the aggregometer cuvette at 37°C with the threshold aggregating concentration (TAC) of collagen. The TAC was defined as the lowest concentration of collagen that produced at least a 50% change in light transmittance within 5 minutes. When platelets showed at least a 50% change in light transmittance, TXB_2 formation was measured following a standard radioimmunoassay procedure (Advanced Magnetics, Barcelona, Spain). Briefly, 0.2 mL of the incubation mixture was immediately transferred to Eppendorf tubes containing 1 vol ice-cold ethanol and 1 vol ice-cold Tris. The samples were centrifuged at 12,000 × *g* for 2 minutes, and the supernatant was stored at −40°C. TXB_2 was determined by radioimmunoassay. Data are expressed as picomoles of TXB_2 per 10^8 platelets per 5 minutes.

Thrombogenicity of VLDL lipoproteins from patients, controls, and apoE-rich and -poor fractions was determined with a method previously reported.[17,30,32] VLDL particles at 50 μg protein/mL were preincubated for 10 minutes at room temperature with platelet suspensions before the addition of collagen to trigger aggregation. Blank samples included Tyrode's buffer, fibrinogen, and lipoprotein particles or saline.

Statistical Tests

Results are expressed as the mean ± SEM. To compare healthy matched controls and patient groups, the Mann-Whitney *U* test for independent groups was used, and to investigate differences between FHTG patients, the Wilcoxon paired *t* test was used. ANOVA was applied to evaluate differences between treatments. Correlations between the platelet LDL receptor activity response (as a percentage of Bmax recovery *v* the basal period) and other biochemical and functional parameters were analyzed by suitable regression tests.

RESULTS

Baseline Characteristics of the Subjects

Table 1 shows the characteristics of nontreated FHTG patients and healthy controls matched for sex and age. The plasma lipid patterns of FHTG patients were consistent with the characteristic features of their hyper lipoproteinemia. FHTG patients had higher apoB-100, apoE, triglycerides, and VLDL cholesterol and lower HDL cholesterol and apoA-I levels than the healthy controls. Platelets from FHTG patients exhibited a considerably lower LDL binding capacity than those from healthy controls. Platelet ^{125}I-LDL binding was saturable at a lower concentration of LDL protein ($P < .01$) in FHTG patients (0.15 ± 0.04 g/L) versus healthy subjects (0.28 ± 0.16 g/L). A Scatchard plot of the data yielded a linear correlation coefficient (*r*) of .91 and −.87 (the average value for all controls and patients), respectively. Assuming an apparent molecular weight for apoB-100 of 550 kd, values for the Bmax and K_d (mean ± SEM) were significantly lower ($P < .01$ and $P < .05$, respectively) in FHTG patients (1,218 ± 185 binding sites with a K_d of 37 ± 13 nmol/L) than in the healthy controls (1,906 ± 264 binding sites with a K_d of 53 ± 18 nmol/L). However, platelet aggregation in response to collagen (1 μg/mL), determined as previously reported,[17,30,32] was markedly lower in FHTG patients (78% ± 14% in healthy controls *v* 43% ± 11% in FHTG patients, $P < .01$), and there were significant differences in collagen-TAC values (1.05 ± 0.8 μg/mL in healthy controls *v* 3.1 ± 1.3 μg/mL in FHTG patients,

Table 1. Basal Characteristics of FHTG Patients and Healthy Controls Matched for Sex and Age

Variable	Healthy Controls (n = 15)	FHTG Patients (n = 18)	*P*
Age range (yr)	39-62	40-61	NS
SBP (mm Hg)	135 ± 10	131 ± 11	NS
DBP (mm Hg)	81 ± 6	86 ± 4	NS
TC (mg/dL)	178 ± 56	190 ± 71	NS
VLDL-C (mg/dL)	9 ± 4	53 ± 18	<.001
LDL-C (mg/dL)	118 ± 52	120 ± 42	NS
HDL-C (mg/dL)	45 ± 10	26 ± 8	<.001
Triglycerides (mg/dL)	90 ± 31	298 ± 146	<.001
ApoB-100 (mg/dL)	118 ± 29	138 ± 38	<.05
ApoA-I (mg/dL)	138 ± 25	114 ± 18	<.05
ApoE (mg/dL)	2.01 ± 1.1	9.3 ± 3.5	<.01
Collagen TAC (μg/mL)	1.05 ± 0.8	3.1 ± 1.3	<.01
Collagen-induced TXB_2 formation (pmol/10^8 platelets/5 min)	216 ± 18	156 ± 14	<.01
Bmax (receptors per platelet)	1,906 ± 264	1,218 ± 185	<.01
K_d (μg/mL)	53 ± 18	37 ± 13	<.05
Saturability of receptor (g LDL protein/L)	0.28 ± 0.16	0.15 ± 0.04	<.01
VLDL thrombogenicity (% of control)	+26	−41	<.01

NOTE. Values are the mean ± SD.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; VLDL-C, VLDL cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; NS, nonsignificant.

$P < .01$). This was associated with significant ($P < .01$) decreases in platelet TXB_2 formation (216 ± 18 pmol in healthy controls *v* 156 ± 14 pmol in FHTG patients). Furthermore, VLDL particles from FHTG patients significantly reduced collagen-induced platelet aggregation (51% ± 13% to 21% ± 10%, $P < .01$). In contrast, VLDL particles from healthy controls had opposite effects (50% ± 11% to 73% ± 15%, $P < .01$). When apoE was removed from VLDL particles by heparin chromatography (apoE-VLDL-poor particles), the proaggregative effects were similar to those of VLDL particles from healthy controls (35% ± 8% to 63% ± 11%, $P < .01$). In contrast, apoE-VLDL-rich particles showed opposite effects (65% ± 11% to 32% ± 13%, $P < .01$). Data on VLDL composition are shown for all patients and healthy controls in Table 2. For FHTG patients, VLDL protein ($P < .05$), triglyceride ($P < .01$), and cholesterol ($P < .01$) expressed as a percentage of total particle weight and apoE content ($P < .01$) were significantly higher, whereas phospholipid content was lower ($P < .01$). The measurements for the healthy controls and FHTG patients with placebo treatment allowed calculation of the intraindividual coefficient of variation for each of the blood lipid and platelet function parameters (Table 3).

Effect of Gemfibrozil Treatment on Lipid Metabolism

Sixteen of 18 randomized patients completed the study. The 2 dropouts were due to inadequate compliance with the treatment and the visit appointments. Patients who completed the study did not report any serious adverse clinical or laboratory (hematological and biochemical tests) events during the study. Changes in body weight were not significant, and blood pressure was not affected by gemfibrozil treatment. FHTG patients randomized to gemfibrozil presented similar clinical and demographic characteristics compared with FHTG patients on placebo (Table 4). No statistically significant differences in plasma lipids, VLDL composition (results not shown), or platelet function parameters were found between patients randomized to gemfibrozil and those randomized to placebo. Placebo treatment did not influence the evaluated lipid and lipoprotein parameters. Gemfibrozil markedly reduced plasma triglyceride and VLDL cholesterol levels (Table 5). The mean percent reduction in triglycerides and VLDL cholesterol was 61% to 72%, respectively ($P < .001$). Small but significant ($P < .05$) reductions in mean total (12%) and LDL (8%) cholesterol levels were also detected. There were more pronounced decreases of 28% and 55% in apoB-100 and apoE levels ($P < .05$). HDL cholesterol and apoA-I increased by 44% and 18%, respectively ($P < .01$). Data on VLDL composition after 6 months of therapy are shown in Table 6. Total protein in VLDL was 28% higher in FHTG patients for treatment versus placebo; however, apoE content and the apoE/apoB ratio were 55% and 64% lower in FHTG medication recipients than in FHTG placebo recipients. On the other hand, no significant differences in phospholipid, triglyceride, or total cholesterol content were detected.

Table 2. VLDL Composition in FHTG Patients and Healthy Controls

Variable	Healthy Controls (n = 15)	FHTG Patients (n = 18)	*P*
Protein*	6.5 ± 0.5	8.2 ± 0.9	.05
Phospholipid*	29 ± 3.1	14.3 ± 1.8	.01
Triglycerides*	54 ± 3.5	62.1 ± 4.5	.01
Total cholesterol*	9.3 ± 1.1	15.3 ± 3.2	.01
Apo E (mg/dL)	1.72 ± 0.3	7.5 ± 1.9	.01

NOTE. Values are the mean ± SD.
*Percentage of total particle weight (lipid + protein = 100%).

Table 3. Intraindividual Variability (coefficient of variation, %) for Lipid and Platelet Parameters in Placebo-Treated Patients and Healthy Controls

Variable	Healthy Controls (n = 15)	Placebo-Treated FHTG Patients (n = 9)
TC (mg/dL)	3 ± 1	5 ± 2
VLDL-C (mg/dL)	12 ± 6	18 ± 9
LDL-C (mg/dL)	5 ± 2	8 ± 4
HDL-C (mg/dL)	11 ± 4	16 ± 8
Triglycerides (mg/dL)	21 ± 7	24 ± 14
ApoB-100 (mg/dL)	6 ± 5	13 ± 6
ApoA-I (mg/dL)	9 ± 3	11 ± 5
ApoE (mg/dL)	10 ± 4	10 ± 3
Collagen TAC (μg/mL)	23 ± 11	21 ± 10
Collagen-induced TXB_2 formation (pmol/10^8 platelets/5 min)	19 ± 10	22 ± 14
Bmax (receptors per platelet)	12 ± 8	14 ± 5
K_d (μg/mL)	11 ± 6	12 ± 5
VLDL thrombogenicity (% of control)	28 ± 12	24 ± 9

NOTE. Values are the mean ± SD based on 3 different measurements performed at baseline and after 1 and 6 months of placebo administration. A similar protocol was used with healthy controls. Abbreviations are listed in Table 1.

Effect of Gemfibrozil Treatment on Platelet Function

Placebo treatment did not influence platelet LDL binding characteristics (Table 5). Treatment with gemfibrozil for 1

Table 4. Basal Characteristics of FHTG Patients by Treatment Group

Variable	FHTG Patients: Gemfibrozil-Treated (n = 9)	FHTG Patients: Placebo-Treated (n = 9)	*P*
Age range (yr)	40-62	40-60	NS
SBP (mm Hg)	133 ± 9	132 ± 10	NS
DBP (mm Hg)	84 ± 5	86 ± 3	NS
TC (mg/dL)	189 ± 65	192 ± 73	NS
VLDL-C (mg/dL)	55 ± 14	54 ± 16	NS
LDL-C (mg/dL)	119 ± 41	121 ± 40	NS
HDL-C (mg/dL)	24 ± 7	25 ± 6	NS
Triglycerides (mg/dL)	301 ± 121	296 ± 153	NS
ApoB-100 (mg/dL)	141 ± 34	137 ± 37	NS
ApoA-I (mg/dL)	118 ± 14	115 ± 15	NS
ApoE (mg/dL)	9.7 ± 2.8	10.1 ± 3.4	NS
Collagen TAC (μg/mL)	3.0 ± 1.0	3.2 ± 1.1	NS
Collagen-induced TXB_2 formation (pmol/10^8 platelets/5 min)	165 ± 12	159 ± 17	NS
Bmax (receptors per platelet)	1197 ± 179	1239 ± 197	NS
K_d (μg/mL)	40 ± 15	38 ± 11	NS
Saturability of receptor (g LDL protein/L)	0.19 ± 0.05	0.13 ± 0.03	NS
VLDL thrombogenicity (% of control)	−38	−44	NS

NOTE. Values are the mean ± SD. Abbreviations are listed in Table 1.

Table 5. Effect of Gemfibrozil and Placebo Treatment on Lipids and Parameters of Platelet Function

Variable	Basal		1 Month		6 Months	
	Placebo	Gemfibrozil	Placebo	Gemfibrozil	Placebo	Gemfibrozil
TC (mg/dL)	192 ± 73	189 ± 65	188 ± 64	172 ± 64*	196 ± 71	169 ± 57*
VLDL-C (mg/dL)	54 ± 16	55 ± 14	59 ± 11	30 ± 12*	51 ± 15	15 ± 8†
LDL-C (mg/dL)	121 ± 40	119 ± 41	126 ± 38	113 ± 41*	128 ± 48	112 ± 44*
HDL-C (mg/dL)	25 ± 6	24 ± 7	26 ± 9	31 ± 8	22 ± 12	36 ± 12*
Triglycerides (mg/dL)	296 ± 153	301 ± 121	324 ± 109	220 ± 98*	305 ± 118	116 ± 31†
ApoB-100 (mg/dL)	137 ± 37	141 ± 34	136 ± 31	107 ± 21*	134 ± 40	97 ± 19*
ApoA-I (mg/dL)	115 ± 15	118 ± 14	121 ± 12	110 ± 12	116 ± 12	136 ± 21*
ApoE (mg/dL)	10.1 ± 3.4	9.7 ± 2.8	9.5 ± 1.9	7.6 ± 1.8*	9.3 ± 2.1	4.1 ± 1.4*
Collagen TAC (μg/mL)	3.2 ± 1.1	3.0 ± 1.0	3.1 ± 0.9	2.7 ± 0.5*	2.9 ± 1.1	1.6 ± 0.3*
Collagen-induced TXB_2 formation (pmol/10^8 platelets/5 min)	159 ± 17	165 ± 12	151 ± 10	189 ± 20*	159 ± 18	221 ± 21*
Bmax (receptors per platelet)	1,239 ± 197	1,197 ± 179	1,221 ± 188	1,608 ± 224*	1,287 ± 201	1,810 ± 188†
K_d (μg/mL)	38 ± 11	40 ± 15	41 ± 9	43 ± 11	39 ± 11	47 ± 21*
VLDL thrombogenicity (% of control)	−44	−38	−44	+15*	−40	+27†

NOTE. Values are the mean ± SD. Abbreviations are listed in Table 1.

*$P < .05$ *v* basal.

†$P < .001$ *v* basal.

month significantly increased Bmax and K_d values by 30% ($P < .05$) and 15% (nonsignificant), respectively. However, the differences versus healthy subjects were still significant ($P < .05$). After 6 months of therapy, Bmax ($P < .001$) and K_d ($P < .05$) values were fully normalized. The upregulation of platelet LDL receptors induced by gemfibrozil therapy correlated with all of the changes in triglyceride-rich lipoprotein metabolism, especially the reduction in total triglycerides ($r = .43$, $P < .05$), VLDL cholesterol ($r = .58$, $P < .05$), apoB-100 ($r = .65$, $P < .05$), and apoE ($r = .61$, $P < .05$). However, the decrease in total cholesterol or LDL cholesterol did not correlate with platelet LDL receptor activity. Gemfibrozil treatment was also associated with a statistically significant ($P < .01$) decrease in collagen TAC determined after 1 or 6 months of therapy (2.7 ± 0.5 and 1.6 ± 0.3 mg/mL, respectively). Moreover, platelets from patients treated with gemfibrozil synthesized significantly ($P < .01$) higher levels of TXB_2 than platelets from placebo-treated patients from the first month of treatment. Finally, after 6 months of therapy, gemfibrozil changed the thrombogenicity of VLDL particles from an initial antiaggregative effect to a slight but significant ($P < .001$) proaggregative effect. This change in the aggregative pattern was related to the composition of VLDL particles, specifically the apoE content ($P < .01$). Gemfibrozil therapy significantly increased the protein content of VLDL. However, the apoE content significantly decreased, which indicates that the main subfractions after treatment are apoE-poor VLDL particles. Finally, a significant association between the upregulation of platelet LDL receptor activity and the reduction in apoE content of VLDL particles was also detected ($r = .32$, $P < .05$).

DISCUSSION

It is widely accepted that statins influence both plasma lipids and platelet function in hypercholesterolemic patients and thus are beneficial from an antithrombotic and antiatherosclerotic point of view.[36-39] However, in familial combined hyperlipidemic patients, although gemfibrozil therapy decreases plasma lipoprotein levels, platelet function in vivo seems to be profoundly altered.[24] These results may suggest that both VLDL and LDL particles play a key role in the regulation of the antithrombotic effects mediated by lipid-lowering agents. Multiple lines of evidence show that when platelets are separated from the plasma environment and incubated with physiological and supraphysiological concentrations of isolated LDL, they become rapidly sensitized.[6,12,17] However, there are limited and controversial data concerning the effect of VLDL particles on platelet function. Some investigators have reported that VLDL obtained from normolipidemic patients enhances platelet aggregation,[12,13] whereas VLDL obtained from hypertriglyceridemic patients has opposite effects.[14,24] These results indicate that specific changes in the composition of VLDL may participate in the aggregative effects of particles.

Table 6. VLDL Composition in FHTG Patients by Treatment Group

Variable	Basal		1 Month		6 Months	
	Placebo	Gemfibrozil	Placebo	Gemfibrozil	Placebo	Gemfibrozil
Protein†	8.0 ± 1.1	8.3 ± 0.9	8.3 ± 1.3	9.8 ± 0.8*	8.0 ± 0.8	10.3 ± 1.1*
Phospholipid†	13.8 ± 1.3	14.6 ± 1.9	14.4 ± 2.1	14.1 ± 2.0	13.2 ± 3.0	14.8 ± 1.2
Triglycerides†	63.3 ± 4.4	62.2 ± 4.1	62.8 ± 5.1	64.5 ± 3.6	61.8 ± 4.3	60.5 ± 3.8
Total cholesterol†	16.1 ± 3.1	15.1 ± 3.3	15.0 ± 3.2	15.4 ± 2.5	17.0 ± 3.2	14.5 ± 2.9
ApoE (mg/dL)	7.6 ± 1.3	7.3 ± 1.7	7.5 ± 1.1	5.1 ± 0.7*	7.3 ± 0.8	3.3 ± 0.7*
ApoE/ApoB ratio	5.0 ± 2.6	4.89 ± 2.7	4.93 ± 2.1	3.26 ± 1.8*	4.85 ± 2.2	2.5 ± 0.8*

*$P < .05$ *v* basal.

†Percentage of total particle weight (lipid + protein = 100%).

The aim of the present study was to investigate the effects of VLDL particles on platelet function. For this purpose, we determined the collagen-induced aggregation, thromboxane formation, and binding characteristics (Bmax and K_d) of platelet LDL binding in platelets from nontreated and gemfibrozil-treated FHTG patients. Furthermore, specific changes in the composition of VLDL particles and their effect on platelet function were investigated. Finally, we also determined the thrombogenicity of VLDL particles, evaluating the effect of VLDL particles from both groups of patients on platelet collagen-induced aggregation.

Our study shows that FHTG patients present a platelet hypoaggregative pattern characterized by a significant decrease in both platelet aggregation and TXB_2 formation (45% and 28%, respectively). This agrees with the results of previous studies.[14,24] For the first time, we present evidence that apoE-VLDL-rich particles are able to decrease platelet aggregation, whereas apoE-VLDL-poor fractions seem to enhance the platelet response. These results suggest that the apoE content of VLDL may have a key role in the effect of these particles on platelet aggregation.

Two complementary mechanisms could be considered responsible for platelet reactivity induced by atherogenic lipoproteins: a non–receptor-dependent mechanism characterized by changes in platelet membrane lipid composition,[7] and a receptor-dependent mechanism that involves interaction between LDL particles and specific receptors located on platelet membranes.[40-42] Early studies showed that platelet membranes contain several hundred LDL receptors per cell with nanomolar affinity, and they are believed to interact with phospholipase C via guanine nucleotide–binding proteins or G proteins.[43-47] Our group previously demonstrated that the platelet LDL receptor is different from the classic LDL receptor in nucleated cells—it does not mediate an endocytotic response and it recognizes both native and oxidized LDL particles with the same apparent affinity.[17,30] Furthermore, the possibility that $\alpha_{IIb}\beta_3$-integrin or glycoprotein IIb-IIIa complex[48] may be related has been recently excluded,[31,32,49,50] and we have demonstrated recently that platelet CD36 functions as the specific high-affinity receptor for both native and oxidized LDL particles.[51] A striking result arising from our previous studies was that VLDL particles are able to interact with the platelet LDL receptor and compete with LDL particles for specific binding sites.[17] We have recently demonstrated that with in vitro conditions, atherogenic lipoproteins such as native and oxidized LDL, IDL, and VLDL particles are able to regulate the activity of platelet LDL receptor by a phenomenon of down regulation and desensitization.[18]

In this study, we have demonstrated that a downregulation of the number of platelet LDL receptors (40% decrease) with changes in the K_d (31% decrease) occurs in FHTG patients. To investigate whether prolonged exposure to high circulating triglyceride-rich lipoprotein levels induces a long-term desensitization mechanism with a loss of receptors and platelet responsiveness to lipoproteins, we performed this double-blind controlled study with gemfibrozil in 18 men with FHTG. It is well known that in hypertriglyceridemic patients, gemfibrozil decreases VLDL-triglyceride synthesis, stimulates their clearance with increased lipase activity, and decreases apoE content in VLDL particles in a way that may also favor the clearance of apoE-rich VLDL particles by lipoprotein receptors (VLDL and LDL receptor). Since the apoE content of lipoproteins has a central role in the function and metabolic fate of triglyceride-rich lipoproteins[19-21] and is related to the inhibition of platelet aggregation,[15,16] one might speculate that gemfibrozil therapy may have potential thrombogenic side effects. In fact, previous studies indicated that gemfibrozil treatment in familial combined hyperlipidemic patients induced proaggregative effects: platelet function in vivo was altered and the platelet count, platelet aggregation, and urinary excretion of 11-dehydrothromboxane B_2 increased.[24] In our double-blind controlled study, placebo treatment did not alter platelet function. On the other hand, we confirm that although gemfibrozil had a profound lipid-lowering effect, it enhanced platelet aggregation and thromboxane formation and changed the pattern of VLDL particles from antiaggregative to proaggregative.

Although the effects of lipid-lowering agents on both the composition of the platelet membrane and platelet aggregation have been widely investigated,[36-38,52] little is known about the effects of lipid-lowering agents on the regulation of platelet LDL receptor. Two open-uncontrolled studies in hypercholesterolemic patients found that gemfibrozil (6 months)[53] and etofibrate (6 weeks)[54] significantly upregulated platelet LDL receptors, but there were significant hypoaggregative effects. However, neither the effects of VLDL on platelet function nor the regulation of VLDL-LDL receptor interaction were investigated. In our study, gemfibrozil induced a marked upregulation in the number of platelet LDL receptors. After 1 month of therapy, the number of platelet LDL receptors was approximately 70% of the normal level, and the levels were completely normal after 6 months of treatment. Furthermore, parallel changes in platelet aggregation and thromboxane formation were detected. There was a correlation between the upregulation of platelet LDL receptor and the reduction in triglyceride, VLDL cholesterol, apoB, and apoE levels. Furthermore, the changes in both the VLDL-induced aggregative patterna nd receptor upregulation were associated with a lower apoE content in VLDL particles. These results, together with our previous in vitro data showing that apoE-VLDL-rich and -poor particles cause opposite effects on platelet aggregation, allow us to postulate that the different effects of VLDL in normolipidemic and hypertriglyceridemic subjects depend on the apoE content. However, further studies are needed to investigate whether changes in the composition of the platelet membrane from an apoE-rich to apoE-poor state could also induce greater aggregation. Furthermore, our results demonstrate for the first time that modulating the activity of the platelet LDL receptor by a desensitization mechanism with upregulation of the receptor number may also be an important pathway for regulating the platelet aggregation response in type IV patients. The initial downregulation of the platelet LDL receptor observed in FHTG patients may be caused by prolonged exposure to circulating apoE-rich VLDL levels, and the recovery in the number of receptors by gemfibrozil therapy may be directly associated with lower apoE-rich VLDL levels. We postulate that combining the recovery of platelet LDL binding capacity (by reducing VLDL levels) and maintenance of unaltered LDL levels (in our

study, gemfibrozil increased Bmax to 46%, but only decreased LDL cholesterol to 7.5%) with a decrease in the apoE-rich VLDL subpopulation may lead to an increase of platelet aggregation in type IV patients. In contrast, the recovery of platelet LDL binding capacity with a concomitant significant reduction in LDL levels (in type II patients)[53,54] may lead to a potential antithrombotic situation, even in the presence of lower apoE-rich VLDL levels. Therefore, specific dyslipidemic disorders and lipid-lowering agents with differential effects on lipoprotein particles may cause the activity of platelet LDL receptors to be regulated differently.

We conclude that gemfibrozil increases platelet activity and upregulates LDL receptors. These changes may be considered prothrombotic and thus adverse for the FHTG patient. It is tempting to speculate that the favorable effect on cardiovascular mortality observed in several clinical trials[55] may be more pronounced if a platelet inhibitor such as aspirin is administered with gemfibrozil.

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