Decreased PLTP mass but elevated PLTP activity linked to insulin resistance in HTG: effects of bezafibrate therapy

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Abstract **Hypertriglyceridemia (HTG) is associated with insulin resistance, increased cholesteryl ester transfer (CET), and low HDL cholesterol. Phospholipid transfer protein (PLTP) may be involved in these relationships. Associations between CET, lipids, insulin resistance, CETP and PLTP activities, and PLTP mass were investigated in 18 HTG patients and 20 controls. Effects of 6 weeks of bezafibrate treatment were studied in HTG patients. HTG patients had higher serum triglycerides, insulin resistance, free fatty acid (FFA), and CET, lower levels of HDL cholesterol (−44%) and PLTP mass (−54%), and higher CETP (+20%) and PLTP activity (+48%) than controls. Bezafibrate reduced triglycerides, CET (−37%), insulin resistance (−53%), FFA (−48%), CETP activity (−12%), PLTP activity (−8%), and increased HDL cholesterol (+27%), whereas PLTP mass remained unchanged. Regression analysis showed a positive contribution of PLTP mass ($P = 0.001$) but not of PLTP activity to HDL cholesterol, whereas insulin resistance positively contributed to PLTP activity ($P < 0.01$). Bezafibrate-induced change in CET and HDL cholesterol correlated with changes in CETP activity and FFAs, but not with change in PLTP activity. Bezafibrate-induced change in PLTP activity correlated with change in FFAs ($r = 0.455$, $P = 0.058$). We propose that elevated PLTP activity in HTG is related to insulin resistance and not to increased PLTP mass.** **Bezafibrate-induced diminished insulin resistance is associated with a reduction of CET and PLTP activity.**—Jonkers, I. J. A. M., A. H. M. Smelt, H. Hattori, L. M. Scheek, T. van Gent, F. H. A. F. de Man, A. van der Laarse, and A. van Tol. **Decreased PLTP mass but elevated PLTP activity linked to insulin resistance in HTG: effects of bezafibrate therapy.** ***J. Lipid Res.*** **2003.** 44: **1462–1469.**

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Over the past years, it has been demonstrated that hypertriglyceridemia (HTG) must be faced as an independent risk factor for cardiovascular disease (CVD) (1). However, this increased CVD risk of patients with HTG cannot solely be attributed to the increased levels of serum triglycerides (TGs). About half of this increased risk is due to the frequently occurring low levels of HDL cholesterol in HTG (1).

HDL plays a crucial role in the process of reverse cholesterol transport, in which excess cholesterol is transported from peripheral cells to the liver, where it is taken up for excretion as cholesterol and bile acids in the feces (2). Several factors are involved in the regulation of HDL metabolism and remodeling, including cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP) (3, 4). CETP enables the transfer of cholesteryl ester from HDL toward VLDL and LDL. PLTP is an HDL-associated transfer protein that facilitates the transfer and exchange of phospholipids between lipoproteins (3). In addition, PLTP is involved in the generation of pre-β-HDL particles (5) and has been demonstrated to enhance CETP-mediated transfer of cholesteryl ester between isolated HDL and apolipoprotein B (apoB)-containing lipoproteins in vitro (6). Recently, it has been shown that overexpression of PLTP in transgenic mice results in a decrease in HDL cholesterol and an increase in pre-β-HDL formation (7). In addition, PLTP appears to be involved in VLDL secretion (8, 9) and promotes diet-induced atherosclerosis in transgenic mice (8, 10).

Interestingly, Riemens et al. demonstrated that PLTP activity is related to insulin resistance (11). Since HTG is frequently accompanied by insulin resistance (12), we hypothesized that PLTP activity might be increased in HTG

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Abbreviations: CET, cholesteryl ester transfer; CETP, cholesteryl ester transfer protein; CVD, cardiovascular disease; FFA, free fatty acid; HTG, hypertriglyceridemia; PLTP, phospholipid transfer protein; PPAR, peroxisome proliferator-activated receptor.

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and contribute to HDL cholesterol and cholesteryl ester transfer (CET) in HTG. However, PLTP is known to circulate in plasma in two forms, one active and one inactive (13–15). Recently, a sandwich ELISA for measurement of PLTP mass was developed by Oka et al. (16). To date, there are no reports of PLTP mass in HTG, and thus it is unclear whether PLTP activity in HTG is affected by PLTP mass. Therefore, the aim of the present study was to investigate the contribution of PLTP and CETP activities and PLTP mass to plasma HDL cholesterol levels and CET in patients with HTG. In addition, we studied whether the known HDL cholesterol-increasing effect of lipid-lowering therapy by bezafibrate is related to changes in CET, plasma CETP activity, PLTP activity, and PLTP mass in HTG patients.

METHODS

Patients and control subjects

The study population consisted of 18 unrelated patients with endogenous HTG who were recruited from the lipid clinic of the Leiden University Medical Center. All patients received personal dietary advice. The diagnosis of endogenous HTG was based on the means of two fasting blood samples obtained after at least 8 weeks of a prudent diet. The diagnostic criteria for endogenous HTG were: total serum TG >4.0 mmol/l, VLDL cholesterol >1.0 mmol/l, and LDL cholesterol <4.5 mmol/l. Exclusion criteria were a history of CVD, homozygosity for apoE2, secondary hyperlipidemia (renal, liver, or thyroid disease, fasting glucose >7.0 mmol/l, and/or alcohol consumption of more than 40 g/day), and the use of lipid-lowering drugs. Twenty normolipidemic control subjects were recruited in response to a local newspaper advertisement.

Study design

The patients were randomized in a double-blind cross-over fashion to receive bezafibrate (400 mg once daily) or placebo for 6 weeks. The two treatment periods were separated by a 6 week wash-out period. Before and at the end of each treatment period, fasting venous blood samples were obtained to determine lipids, insulin, glucose, free fatty acids (FFAs), CET, CETP activity, PLTP activity, hepatic lipase (HL) activity, and PLTP mass. Insulin resistance was calculated using the homeostasis model approximation index, which correlates well with the results from both hyperinsulinemic euglycemic clamp and the intravenous glucose tolerance test by the following formula: insulin resistance = insulin/($22.5e^{-lnglucose}$) (17). From the control subjects, fasting blood samples were obtained at baseline. Informed consent was obtained from each participant, and the protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center.

Laboratory measurements

Serum was obtained after centrifugation at 1,500 *g* for 15 min at room temperature. Three milliliters of fresh serum was ultracentrifuged for 15 h at 232,000 *g* at 15°C in a TL-100 tabletop ultracentrifuge using a TLA-100.3 fixed angle rotor (Beckman, Palo Alto, CA). The ultracentrifugate was divided in a d < 1.006 and d 1.006–1.25 g/ml fraction, designated as the VLDL and LDL-HDL fraction, respectively. HDL cholesterol was measured in the LDL-HDL fraction after precipitation of LDL with phosphotungstic acid and $MgCl_2$. Triglycerides, total cholesterol, and FFA were measured enzymatically using commercially available kits. Insulin was measured with a conventional radioimmuno assay (Medgenix, Brussels, Belgium). Glucose was measured with a Hitachi 747 analyzer according to standard procedures (Roche Diagnostics, Mannheim, Germany).

Plasma CET was assayed as described (18), with minor modifications. In brief, [^{3}H]cholesterol was incorporated in an albumin emulsion and was equilibrated overnight with plasma-free cholesterol at 4°C, followed by incubation of the sample at 37°C for 3 h. Subsequently, VLDL+LDL were precipitated by addition of phosphotungstate/$MgCl_2$. Lipids were extracted from the precipitate, and the radioactive cholesteryl esters were isolated on silica columns and counted. This assay system is not influenced by cholesterol esterification in LDL (18). Since it has been demonstrated that CET is strongly correlated with net cholesteryl ester mass transfer from HDL toward VLDL and LDL in the absence of active LCAT, CET can be regarded as an accurate estimate of cholesteryl ester mass transfer in plasma (19). CETP activity was determined after removal of VLDL+LDL from each sample (20). The isotope assay measures the transfer of [1-^{14}C-oleate]cholesteryl ester from labeled LDL to an excess of unlabeled HDL in the presence of the LCAT inhibitor dithiobis-2-nitrobenzoic acid. CETP activity was calculated as the bidirectional transfer between labeled LDL and HDL. The CETP activity levels obtained by this method are strongly correlated with CETP mass concentrations in plasma (21). Plasma PLTP activity level was measured in a liposome vesicles-HDL system as described (22). Plasma samples were incubated with [^{3}H]phosphatidylcholine-labeled liposomes and an excess of pooled normal HDL, followed by precipitation of the liposomes with a mixture of NaCl, $MgCl_2$, and heparin (final concentrations 230 mmol/l, 92 mmol/l, and 200 U/ml, respectively). The measured PLTP activities are linearly correlated with the amount of plasma used in the incubations. The method is not influenced by the phospholipid transfer-promoting capacities of CETP (22). All these assays were performed using the same batch of substrates. CET is expressed in nmol/ml/h. Serum CETP and PLTP activity levels were related to a human plasma pool and are expressed in percentages of the activities in this reference plasma. The activity levels in the reference plasma were 198 nmol/ml/h and 21.2 μmol/ml/h for CETP and PLTP, respectively. The within-assay coefficients of variation of CET, CETP, and PLTP are 7.1%, 2.7%, and 3.5%, respectively. Plasma for HL activity assays was obtained 20 min after intravenous injection of heparin (50 IU/kg body weight). HL activity was assayed as described previously (23). Plasma PLTP mass was measured using a sandwich ELISA with intra- and interassay coefficients of variation of less than 5% (16).

Data analysis: comparison between HTG patients and controls

All data are expressed as means ± SD, except for triglycerides, which is expressed as median interquartile ranges because of its skewed distribution. Differences between patients on placebo therapy and controls were calculated using the Mann-Whitney U test. Differences in sex, use of β-blocking agents, and smoking habits between patients and controls were tested with Fisher's exact test.

To identify determinants of CET, HDL cholesterol, PLTP activity, and PLTP mass, the following strategy was performed. First, univariate linear regression analysis was performed in the combined group of controls and HTG patients during placebo therapy with CET, HDL cholesterol, PLTP activity, or PLTP mass as dependent variable and the determinant of interest as independent variable. The level of serum triglycerides was entered into these models after logarithmic transformation to obtain a normal distribution of the data. Then, to adjust for group-associated confounding, linear regression analysis was performed with the outcome parameter (i.e., CET, HDL cholesterol, PLTP activity, or

PLTP mass) as the dependent variable, and both the study parameter (i.e., the significant determinant as identified in univariate analysis) and the presence of HTG as independent variables.

Then five different linear regression analyses were performed with CET, HDL cholesterol, PLTP mass, PLTP activity, and CETP activity as dependent variables, and the presence of HTG, smoking, use of β-blocking agents, and body mass index as independent variables. These analyses were performed to investigate whether the group-associated differences in the outcome variables could be explained by these other factors.

Data analysis: effects of bezafibrate therapy in HTG patients

Differences between placebo and bezafibrate therapy were evaluated pairwise using Wilcoxon's paired signed ranks test. For each HTG patient, the bezafibrate-induced difference in study parameter (i.e., HDL cholesterol, CET, PLTP activity, CETP activity, triglycerides, FFA, and insulin resistance) was calculated by subtracting the value of the study parameter on placebo from that obtained upon bezafibrate therapy. Interrelationships between these paired differences in study parameters were evaluated using Spearman's rank correlation analysis. $P < 0.05$ was considered significant.

RESULTS

Baseline comparison between HTG patients and controls

Age and sex were similar in HTG patients and controls, whereas the proportion of smokers, the proportion of participants using β-blocking agents, and body mass index were higher in the HTG group than in the control group (**Table 1**).

Treatment with a placebo had no effect on serum lipid levels (data not shown). Therefore, only the values obtained at the end of both treatment periods were compared. As expected, HTG patients showed a higher serum total cholesterol and total triglycerides compared with controls. The increase in total cholesterol and total triglycerides in HTG was caused by significantly higher VLDL cholesterol and VLDL-triglycerides respectively, whereas both LDL cholesterol and HDL cholesterol were significantly lower than in the control group (**Table 2**).

In addition, HTG patients had higher serum glucose, insulin, insulin resistance, and FFA levels than the normolipidemic controls (Table 2).

CET in HTG patients more than doubled the average of the control group. PLTP activity levels in HTG patients were about 50% higher than those in controls, whereas PLTP mass was about 50% lower in HTG patients than in controls. Plasma CETP activity and HL activity levels were higher in HTG patients than in controls (Table 2).

TABLE 1. Baseline characteristics

	Controls (n = 20)	Hypertriglyceridemic Patients (n = 18)	*P*
Age (years)[a]	47.9 ± 7.4	48.5 ± 8.8	ns
Gender (M/F)	18/2	16/2	ns
Smoking (yes/no)	2/18	7/11	ns
Use of β-blocking agents (yes/no)	0/20	5/13	0.017
Body mass index (kg/m^2)[a]	24.2 ± 3.3	28.0 ± 2.8	0.001

[a] Data are expressed as mean ± SD.

Determinants of CET, HDL cholesterol, PLTP activity and PLTP mass

CET. Body mass index, serum triglycerides, glucose, insulin, insulin resistance, FFA, PLTP and CETP activity, and PLTP mass could significantly predict CET in the combined group of controls and HTG patients using univariate regression analysis (all $P \leq 0.05$). However, after adjustment for HTG, body mass index, insulin resistance, PLTP activity, and PLTP mass disappeared as significant contributors to CET, whereas the other factors (TG, insulin, FFA, and CETP activity) still significantly contributed to CET (**Table 3**).

HDL cholesterol. Body mass index, serum triglycerides, glucose, insulin, insulin resistance, FFA, PLTP activity, CETP activity, and PLTP mass could all significantly predict HDL cholesterol in the combined group of controls and HTG patients on placebo using univariate regression analysis (all $P < 0.01$). However, after adjustment for HTG, only PLTP mass ($\beta = 0.601$, $P = 0.001$) remained a significant contributor to HDL cholesterol (**Fig. 1**). The fact that none of the other determinants contributed to HDL cholesterol may be explained by the divergent levels of HDL cholesterol between the control and HTG group (range 0.86–2.00 mmol/l vs. 0.50–0.94 mmol/l for controls and HTG patients, respectively). Therefore, linear regression analysis was also performed in the separate groups. With the exception of PLTP mass ($\beta = 0.529$, $P = 0.017$), none of the other variables contributed to HDL cholesterol levels in the control group (all $P > 0.18$). In contrast, HDL cholesterol levels in the HTG group could significantly be predicted by both PLTP mass ($\beta = -0.535$, $P = 0.027$) and serum triglycerides ($\beta = -0.487$, $P = 0.04$), whereas the other variables that were associated with HDL cholesterol levels in the combined group did not significantly contribute to HDL cholesterol (all $P > 0.12$). The relationship between PLTP activity and HDL cholesterol was not masked by HL activity, since, after adjustment for HL activity, regression analysis did not reveal a significant contribution of PLTP activity to HDL cholesterol in either the HTG group or in controls (both $P > 0.53$).

PLTP activity. Body mass index, serum triglycerides, glucose, insulin, insulin resistance, FFA, CETP activity, and PLTP mass all significantly predict PLTP activity in the combined group of controls and HTG patients on placebo using univariate regression analysis (all $P \leq 0.05$). However, after adjustment for the disorder HTG, triglycerides, FFA, CETP activity, and PLTP mass disappeared as significant contributors to PLTP activity, whereas body mass index, serum glucose, serum insulin, and insulin resistance remained significant contributors to PLTP activity (Table 3, **Fig. 2**). Moreover, in the controls a significant relationship was found between PLTP activity and insulin resistance ($r = 0.58$; $P = 0.007$).

In addition, CET, PLTP activity, and CETP activity all remained significantly higher, whereas HDL cholesterol and

TABLE 2. Activity in controls and hypertriglyceridemic patients on placebo and bezafibrate therapy

	Controls (n = 20)	Hypertriglyceridemic Patients (n = 18)	
		Placebo	Bezafibrate
Total cholesterol (mmol/l)	5.47 ± 0.78	8.21 ± 2.46[a]	5.91 ± 1.24[c]
Total triglycerides (mmol/l)	0.92 (0.59–1.09)	10.81 (6.28–12.88)	4.05 (3.12–4.88)[c]
VLDL cholesterol (mmol/l)	0.26 ± 0.18	4.37 ± 2.02[a]	1.43 ± 0.62[c]
VLDL triglycerides (mmol/l)	0.55 ± 0.36	10.03 ± 6.11[a]	2.89 ± 1.07[c]
LDL cholesterol (mmol/l)	3.52 ± 0.87	2.67 ± 0.64[a]	3.58 ± 0.84[c]
HDL cholesterol (mmol/l)	1.32 ± 0.29	0.72 ± 0.13[a]	0.91 ± 0.13[c]
Glucose (mmol/l)	5.17 ± 0.33	5.93 ± 0.76[a]	5.79 ± 0.59
Insulin (mU/l)	11.1 ± 6.8	55.6 ± 26.3[a]	25.5 ± 15.7[c]
Insulin resistance (HOMA index)	2.57 ± 1.70	14.63 ± 7.02[a]	6.76 ± 4.69[c]
Free fatty acids (mmol/l)	0.57 ± 0.17	1.07 ± 0.63[a]	0.56 ± 0.25[c]
CET (nmol/ml/h)	36.4 ± 10.9	86.1 ± 23.5[a]	53.9 ± 19.8[c]
CETP activity (%)	90.6 ± 16.9	108.8 ± 26.3[b]	95.3 ± 21.3[c]
HL activity (AU)	306 ± 120	496 ± 111[a]	488 ± 152
PLTP activity (%)	82.2 ± 12.6	122.1 ± 23.2[a]	111.9 ± 21.1[c]
PLTP mass (mg/l)	12.4 ± 2.3	5.7 ± 1.4[a]	5.4 ± 1.6

CET, cholesteryl ester transfer; CETP, cholesteryl ester transfer protein; PLTP, phospholipid transfer protein; HL, hepatic lipase; HOMA, homeostasis model approximation. Serum lipids and lipoproteins, glucose, insulin, insulin resistance, free fatty acids, HL activity, CET, PLTP, and CETP activity in controls and hypertriglyceridemic patients on placebo and bezafibrate therapy. Data are expressed as mean ± SD, except for total triglycerides, which is expressed as median (interquartile range).

[a] $P < 0.01$ versus controls.

[b] $P = 0.05$ versus controls.

[c] $P < 0.01$ versus hypertriglyceridemia (HTG) patients on placebo.

PLTP mass remained significantly lower in the HTG group compared with controls after adjustment for smoking, use of β-blocking agents, and body mass index in regression analysis.

Effects of bezafibrate therapy. Body mass index of HTG patients remained stable during the study (28.37 ± 2.70 kg/m^2 vs. 28.03 ± 2.80 kg/m^2 for bezafibrate and placebo, respectively). Bezafibrate therapy changed the lipid profile of the HTG patients toward that observed in the control group: total cholesterol and total triglycerides were significantly lowered because of reductions in VLDL cholesterol and triglycerides, whereas both LDL and HDL cholesterol significantly increased (Table 2).

Insulin, insulin resistance, and FFA levels significantly decreased upon bezafibrate therapy (all $P < 0.01$), whereas the bezafibrate-induced reduction in glucose levels failed to achieve statistical significance ($P = 0.091$; Table 2).

Bezafibrate therapy caused significant reductions in CET, PLTP activity, and CETP activity, whereas PLTP mass and HL activity remained unaffected (Table 2).

TABLE 3. Standardized coefficients calculated with linear regression analysis

	Standardized Coefficient (β) Univariate Analysis	*P*	Standardized Coefficient (β) after Adjustment for HTG	*P*
Contribution to CET				
Body mass index	0.436	0.006	−0.012	0.916
Log triglycerides	0.911	<0.001	0.529	<0.001
Serum glucose	0.316	0.053	−0.209	0.071
Serum insulin	0.755	<0.001	0.305	0.042
Insulin resistance	0.706	<0.001	0.175	0.259
Serum FFA	0.727	<0.001	0.427	<0.001
PLTP activity	0.620	<0.001	0.029	0.844
CETP activity	0.565	<0.001	0.288	0.004
PLTP mass	−0.789	<0.001	−0.340	0.070
Contribution to PLTP activity				
Body mass index	0.591	<0.001	0.266	0.044
Log triglycerides	0.688	<0.001	−0.100	0.543
Serum glucose	0.622	<0.001	0.298	0.025
Serum insulin	0.722	<0.001	0.366	0.035
Insulin resistance	0.757	<0.001	0.453	0.009
Serum FFA	0.448	0.005	0.106	0.415
CETP activity	0.328	0.044	0.043	0.726
PLTP mass	−0.494	0.002	0.438	0.080

Standardized coefficients calculated with linear regression analysis in the combined subjects (controls and hypertriglyceridemic patients on placebo, n = 38) using both univariate analysis and after adjustment for the disorder HTG.

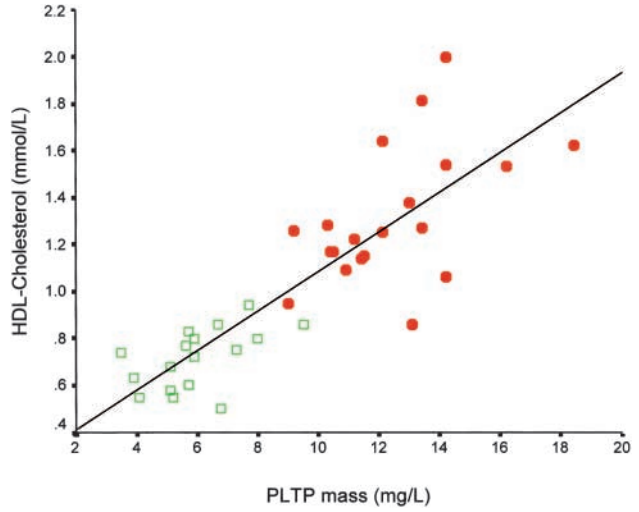

Fig. 1. Relationship between plasma phospholipid transfer protein (PLTP) mass and HDL cholesterol in controls (closed dots) and hypertriglyceridemia (HTG) patients on placebo (open squares). $\beta = 0.601$ ($P = 0.001$) after adjustment for the presence of the disorder HTG.

Interrelationships of paired differences in study parameters upon bezafibrate therapy. Bezafibrate-induced changes in CETP and FFA correlated positively with changes in CET and inversely with the changes in HDL cholesterol (all $P < 0.02$). In contrast, changes in PLTP activity did not correlate with change in serum TG, changes in CET, changes in CETP activity, or changes in HDL cholesterol (all $P > 0.27$), whereas changes in PLTP activity tended to correlate with changes in FFA levels ($r = 0.455$, $P = 0.058$). A significant inverse correlation was observed between the bezafibrate-induced change in CET and the change in HDL cholesterol. In addition, change in serum TG correlated significantly with change in HDL cholesterol, but not with change in CET ($r = -0.408$, $P = 0.09$; **Table 4**).

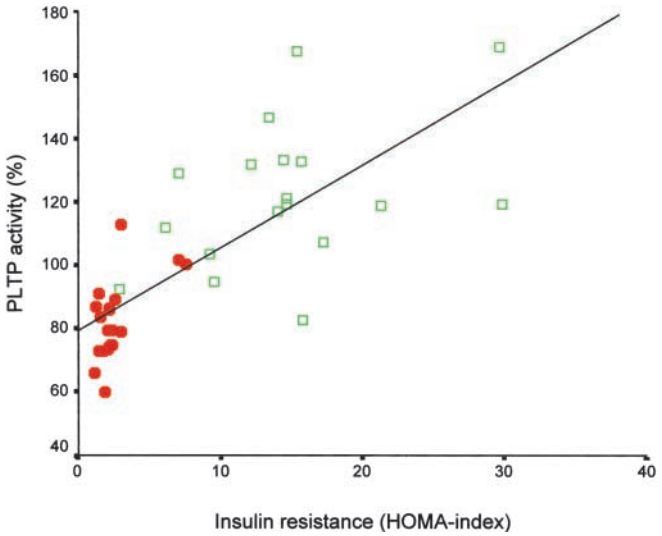

Fig. 2. Relationship between plasma PLTP activity and insulin resistance in controls (closed dots) and HTG patients on placebo (open squares). $\beta = 0.453$ ($P = 0.009$) after adjustment for the presence of the disorder HTG. PLTP activity is expressed as percentage of the activity in reference plasma.

DISCUSSION

The present study shows that HTG patients have higher CET, PLTP activity, and CETP activity, as well as lower levels of both HDL cholesterol and PLTP mass in comparison to normolipidemic controls. Regression analysis showed that PLTP mass but not PLTP activity significantly contributed to HDL cholesterol levels, whereas insulin resistance contributed to PLTP activity levels. Bezafibrate therapy reduced PLTP activity and CET, and increased HDL cholesterol levels. However, this reduction in PLTP activity did not correlate with the reduction in CET or with increase in HDL cholesterol.

In line with earlier observations (24, 25), a higher CET was observed in HTG patients than in normolipidemic controls. Higher serum triglycerides and higher CETP and PLTP activity levels accompanied the increase in CET in HTG. It is known that the presence of both triglyceride-rich lipoproteins that accept cholesteryl ester from HDL and the plasma activity levels of CETP govern CET. Regression analysis shows that, in contrast to PLTP activity, serum triglycerides, CETP activity, FFA, and insulin contributed to CET in normolipidemic controls as well as in HTG patients. Since HTG patients have significantly higher FFA levels than controls [most likely a result of the presence of insulin resistance in HTG (26)] FFA levels may contribute to the higher CET in HTG patients than in controls. This observation is supported by an earlier report by Lagrost et al. (27), who demonstrated that FFAs are able to modulate CETP activity.

A novel finding of our study is the elevated PLTP activity in HTG patients. Regression analysis showed no significant contribution of serum triglycerides to PLTP activity. However, insulin resistance did positively contribute to PLTP activity, as in normolipidemic women and patients with non-insulin-dependent diabetes mellitus (NIDDM) (11, 15, 28). In line with earlier reports (11, 15, 20), regression analysis showed a significant positive contribution of body mass index to PLTP activity. Although the HTG patients had a significantly higher body mass index than controls, statistical analysis persisted in showing a significantly higher PLTP activity in HTG patients compared with controls after adjustment for body mass index. This

TABLE 4. Spearman correlation coefficients of paired differences upon bezafibrate therapy in hypertriglyceridemic patients

	Δ HDL Cholesterol	Δ CET	Δ CETP Activity	Δ PLTP Activity	Δ FFA
Δ TG	−0.548[a]	0.408	0.251	0.212	0.657[a]
Δ FFA	−0.619[a]	0.550[a]	0.395	0.455	
Δ PLTP activity	0.250	0.276	0.421		
Δ CETP activity	−0.669[a]	0.635[a]			
Δ CET	−0.574[a]				

[a] $P < 0.05$.

observation indicates that, although body mass index contributes to PLTP activity, other factors associated with HTG, such as insulin resistance, determine PLTP activity as well. The relatively low contribution of body mass index to PLTP activity is further emphasized by the observed significant reduction in PLTP activity without concurrent changes in body mass index upon bezafibrate. Based on these data, we hypothesize that the increased PLTP activity in HTG is due to the presence of insulin resistance.

The main aim of this study was to investigate whether PLTP activity contributes to increased CET or to low HDL cholesterol levels in HTG. Although the higher PLTP activity in HTG patients was accompanied by higher CET and lower HDL cholesterol levels, regression analysis showed that PLTP activity did not contribute significantly to CET or HDL cholesterol. Murdoch et al. demonstrated a positive correlation between PLTP activity and both HDL cholesterol and LDL cholesterol in normolipidemic women (15). Colhoun et al. studied normolipidemic Type 1 diabetes mellitus patients who have increased PLTP activity, and found that PLTP activity not only correlates with HDL cholesterol and LDL cholesterol, but also independently with plasma apoA-I, apoA-II, and apoB, and with HDL size: positively with large HDL particles and negatively with small HDL particles (29). In contrast to the situation in normolipidemic individuals, HTG is associated with disturbances in lipolytic enzymes: HL activity may be increased in HTG as shown in the present study. This could mask the relationships between PLTP activity and lipid parameters. However, after adjustment for HL activity in both controls and HTG patients, no significant relationship was found between PLTP activity and lipid parameters.

This is the first report describing the presence of decreased PLTP mass in hypertriglyceridemic patients, thereby extending earlier the report of Oka et al. (30) in patients with hypoalphalipoproteinemia. In line with the earlier report, we observed a significant positive relationship between plasma PLTP mass and HDL cholesterol, both in normolipidemic men and in hypertriglyceridemic patients (Fig. 2). However, upon bezafibrate therapy, we observed a significant rise in HDL cholesterol without any effects on PLTP mass. It should be emphasized that, although HDL cholesterol levels rose by 26%, they were still below the normal range. In spite of the observed decreased PLTP mass in HTG, we observed increased PLTP activity in HTG patients compared with controls. Statistical analysis showed no association between PLTP mass and PLTP activity, which confirmed earlier reports (15, 16, 31) and is in line with the occurrence of both active and inactive forms of PLTP in human plasma. Animal studies showed that elevation of hepatic PLTP expression results in increased VLDL secretion (9, 10). It is hypothesized that PLTP is secreted into plasma in the active form, and that VLDL and PLTP secretion are both increased in insulin-resistant states. These processes are both up-regulated by high FFA concentrations, explaining both the elevation of PLTP activity and the HTG. During its lifetime in plasma, PLTP is probably inactivated and degraded while bound to HDL (14), explaining the close correlation between plasma HDL and PLTP concentration. There is evidence that PLTP has a ligand function between HDL and putative hepatic HDL receptors (32). It remains to be explained how the decreased level of plasma PLTP in HTG gives rise to increased PLTP activity. HTG could be a condition with increased specific activity of the active form of PLTP.

The regulation of PLTP is complex and involves the nuclear receptor subfamilies of LXR and FXR (33), which influence the expression of many genes involved in lipid metabolism. Moreover, at least in mice, peroxisome proliferator-activated receptor (PPAR)-retinoid X receptor heterodimers are involved in expression of the PLTP gene, and fenofibrate treatment increases PLTP activity by up-regulation of PLTP expression via a PPARα-dependent mechanism (34). FFAs are also able to up-regulate gene expression via PPARα, a mechanism that may be involved in the elevation of PLTP activity in HTG (see above). In the present study, the effect of bezafibrate on PLTP activity is relatively small. Treatment with bezafibrate may have little effect on PLTP expression in HTG, as the expression of PLTP is already high to start with. As bezafibrate treatment results in a complete normalization of plasma FFA levels, the overall result will be a relatively small effect on PLTP activity. In fact, an 8% decrease in PLTP activity is observed.

Bezafibrate therapy in HTG patients caused a significant reduction in CET and a significant increase in HDL cholesterol, as earlier reported by Mann et al. (25). The bezafibrate-induced reduction in CET may increase (anti-atherogenic) HDL and reduce the amount of atherogenic, cholesteryl ester-enriched VLDL and LDL, thus reducing the risk of CVD in patients with HTG. In addition to the observed bezafibrate-induced decrease in CET and increase in HDL cholesterol, bezafibrate also resulted in decreases of insulin resistance, CETP activity, and PLTP activity.

Bezafibrate therapy of patients with HTG significantly improved insulin sensitivity, as it did in hyperlipidemic heart transplant patients (35) and in patients with NIDDM (36). In addition to the observed concurrent reduction in CET and insulin resistance upon bezafibrate, a significant correlation was observed between the bezafibrate-induced change in FFA and change in CET, demonstrating the importance of FFA levels in determination of CET, as described above.

The reduction in CETP activity upon fibrate therapy has been reported before (37, 38), whereas one study with gemfibrozil reported an increase of plasma CETP activity (39). It must be noted that in the latter study, the patients only had very mild HTG (mean TG 3.4 ± 1.1 mM) in comparison to our patients.

In the present study, significant correlations were observed between the bezafibrate-induced decrease in CETP and both the reduction in CET and the increase in HDL cholesterol, underlining the important role of CETP in the regulation of lipid metabolism.

Bezafibrate therapy was associated with a significant 8% reduction in PLTP activity in HTG patients. To our knowl-

edge, only one report has described the effects of fibrates on PLTP activity in humans and observed a nonsignificant 7% decrease in PLTP activity upon gemfibrozil treatment (39). Since only six HTG patients participated in that study, gemfibrozil-induced reduction in PLTP activity may have failed to achieve statistical significance due to the limited number of studied patients. Furthermore, we observed a concurrent reduction in PLTP activity and insulin resistance upon bezafibrate therapy in HTG patients, as well as a positive relationship between PLTP activity and insulin resistance in the combined group of controls and HTG patients (Fig. 2), suggesting that in patients with HTG, PLTP activity is directly related to glucose and/or FFA metabolism. This hypothesis is supported by recent in vitro observations showing that high glucose increases both PLTP mRNA and functional activity, possibly through the involvement of PPARs (40).

Interestingly, it has been reported that intravenous infusion of a triglyceride emulsion, giving rise to increased FFA as well as triglycerides concentrations, increases plasma PLTP activity levels (41), suggesting that PLTP is up-regulated under a condition of excess turnover of triglycerides and FFA. FFA flux is increased in HTG, presumably as a result of insulin resistance in adipose tissue (26). Thus, elevated PLTP activity could be viewed as a compensatory mechanism to facilitate transfer of elevated concentrations of lipolytic surface fragments formed during increased turnover of VLDL. This is an important function of PLTP, as demonstrated in PLTP knock-out mice (42). Consistent with this view, bezafibrate-induced reduction in FFA levels tended to correlate with the reduction in PLTP activity. However, no significant correlations were observed between changes in lipid parameters and PLTP activity upon bezafibrate therapy. This may be due to the fact that bezafibrate affects not only PLTP activity but also other parameters affecting lipid metabolism, such as lipases and synthesis of apoA-I and apoA-II (43).

In conclusion, our study shows that both PLTP activity and CET are increased in HTG patients compared with controls, probably as a result of the presence of insulin resistance in HTG, but PLTP activity does not contribute to HDL cholesterol levels. In contrast, HTG is associated with decreased PLTP mass that contributes to low HDL cholesterol in HTG. Bezafibrate therapy improves insulin resistance and concomitantly decreases PLTP activity and CET. We hypothesize that the increased PLTP activity in HTG patients is a compensatory mechanism for the transfer of excess lipids in HTG.

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