Influence of insulin sensitivity and the TaqIB cholesteryl ester transfer protein gene polymorphism on plasma lecithin:cholesterol acyltransferase and lipid transfer protein activities and their response to hyperinsulinemia in non-diabetic men

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Abstract **Lecithin:cholesteryl acyl transferase (LCAT), cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP), and lipoprotein lipases are involved in high density lipoprotein (HDL) metabolism. We evaluated the influence of insulin sensitivity and of the TaqIB CETP gene polymorphism (B1B2) on plasma LCAT, CETP, and PLTP activities (measured with exogenous substrates) and their responses to hyperinsulinemia. Thirty-two non-diabetic men without hyperlipidemia were divided in quartiles of high (Q_1) to low (Q_4) insulin sensitivity. Plasma total cholesterol, very low + low density lipoprotein cholesterol, triglycerides, and apolipoprotein (apo) B were higher in Q_4 compared to Q_1 ($P < 0.05$ for all), whereas HDL cholesterol and apoA-I were lowest in Q_4 ($P < 0.05$ for both). Plasma LCAT activity was higher in Q_4 than in Q_1 ($P < 0.05$) and PLTP activity was higher in Q_4 than in Q_2 ($P < 0.05$). Insulin sensitivity did not influence plasma CETP activity. Postheparin plasma lipoprotein lipase activity was highest and hepatic lipase activity was lowest in Q_1. Insulin infusion decreased PLTP activity ($P < 0.05$), irrespective of the degree of insulin sensitivity. The CETP genotype exerted no consistent effects on baseline plasma lipoproteins and LCAT, CETP, and PLTP activities. The decrease in plasma PLTP activity after insulin was larger in B1B1 than in B2B2 homozygotes ($P < 0.05$). These data suggest that insulin sensitivity influences plasma LCAT, PLTP, lipoprotein lipase, and hepatic lipase activities in men. As PLTP, LCAT, and hepatic lipase may enhance reverse cholesterol transport, it is tempting to speculate that high levels of these factors in association with insulin resistance could be involved in an antiatherogenic mechanism. A possible relationship between the CETP genotype and PLTP lowering by insulin warrants further study.**—Riemens, S. C., A. Van Tol, B. K. Stulp, and R. P. F. Dullaart. **Influence of insulin sensitivity and the TaqIB cholesteryl ester transfer protein gene polymorphism on plasma lecithin:cholesterol acyltransferase and lipid transfer protein activities and their response to hyperinsulinemia in non-diabetic men.** *J. Lipid Res.* **1999.** 40: **1467–1474.**

Supplementary key words insulin sensitivity • CETP gene • LCAT • CETP • PLTP • HDL cholesterol

The negative relationship between the level of high density lipoprotein (HDL) cholesterol and the risk of cardiovascular disease is commonly explained by the crucial role of HDL in reverse cholesterol transport (1–4). By this process cholesterol from peripheral cells is taken up by HDL and transported to the liver where it is metabolized and excreted in the bile (2–4).

Several plasma proteins are involved in HDL metabolism and remodeling. Phospholipid transfer protein (PLTP) facilitates the transfer and exchange of phospholipids between lipoproteins (2, 5, 6). This lipid transfer has the ability to convert small-sized HDL (HDL_3) in smaller and larger particles (7, 8). During this process preβ-HDL particles originate that can act as initial acceptors of free cholesterol from cell surfaces (2, 6, 9). Subsequently, free cholesterol is esterified within the HDL fraction by lecithin:cholesterol acyl transferase (LCAT) (10). Cholesteryl ester transfer protein (CETP) has the ability

Abbreviations: apo, apolipoprotein; BMI, body mass index; CETP, cholesteryl ester transfer protein; FFA, free fatty acids; HL, hepatic lipase; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; LPL, lipoprotein lipase; PCR, polymerase chain reaction; PLTP, phospholipid transfer protein; VLDL, very low density lipoproteins; Q, quartile.

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to transfer cholesteryl ester from HDL towards very low and low density lipoproteins (VLDL + LDL) (2, 3, 10, 11). In addition, HDL metabolism is governed by lipoprotein lipase (LPL) and hepatic lipase (HL) in such a way that LPL-mediated hydrolysis of triglyceride-rich lipoproteins enhances the incorporation of lipoprotein surface constituents in HDL (10, 12) and that HL stimulates HDL triglyceride hydrolysis (12) and preβ-HDL generation (13).

A low level of HDL cholesterol and high plasma triglycerides are prominent features of the insulin resistance syndrome (14). Insulin resistance in non-diabetic and Type 2 diabetic subjects is associated with a tendency for lower LPL activity and higher HL activity in post-heparin plasma, alterations which may contribute to a low HDL cholesterol (15–17). Little is known about the effect of insulin resistance on the plasma activity levels of PLTP, CETP, and LCAT and their possible responses to insulin in non-diabetic individuals. Plasma CETP activity is higher in obese than in lean subjects (18, 19). Moreover, a positive correlation between body mass index (BMI), the waist/hip ratio, and plasma PLTP activity has been found (19), whereas plasma LCAT activity is not related to obesity (19). Acute endogenous and exogenous hyperinsulinemia lowers plasma PLTP activity (20, 21), and this effect is diminished in obese Type 2 diabetic patients (21). Equivocal effects of insulin on plasma CETP activity have been reported, including a lack of effect in both healthy and Type 2 diabetic subjects (20, 21), a more pronounced decrease in Type 2 diabetic compared to control subjects (22) and vice versa (23).

HDL cholesterol metabolism is also under genetic control (24). Among other factors, the B1B2 polymorphism of intron 1 of the CETP gene (presence or absence of a TaqIB restriction site) was found to be a determinant of HDL cholesterol concentration in some (25–27), but not in all studies (28, 29). Interestingly, the CETP gene polymorphism may be implicated in atherosclerosis development (27, 29).

In the present study, the associations between insulin resistance and lipoprotein lipases as well as plasma PLTP, LCAT, and CETP activities and their responses to acute hyperinsulinemia were evaluated in men without diabetes mellitus. The TaqIB CETP genotype was documented to preliminary assess its effect on the possible LCAT and lipid transfer protein responses to insulin.

SUBJECTS AND METHODS

Subjects

The study was approved by the local medical ethics committee and all participants provided written informed consent. Only non-smoking men participated to avoid effects of smoking and of the menstrual cycle on lipid levels (30, 31). Maximal alcohol allowance was 2 beverages per day. None of the participants had a fasting plasma total cholesterol >6.5 mmol/l and/or triglycerides >2.3 mmol/l on screening, clinically manifest cardiovascular disease, hypertension (defined as systolic blood pressure ⩾160 mmHg and/or diastolic blood pressure ⩾95 mmHg, or a family history of hyperlipidemia. None of the subjects used any medication. The thyreotropin level, liver function tests, and the serum creatinine level were within the normal range. Diabetes was excluded by a 75 gram oral glucose tolerance test with a fasting venous blood glucose level of 6.1 mmol/l and a 2 h postglucose level of 10.0 mmol/l as cut-off values (32). Body mass index (BMI) was calculated as weight divided by height squared, and severe obesity, defined as a BMI >30 kg/m^2, was an exclusion criterion. The waist/hip ratio was measured as the ratio of the smallest circumference between rib cage and iliac crest and the largest circumference between waist and thigh (33). The participants did not change their dietary habits at least 2 weeks prior to the study and they did not consume alcohol on the day before the study. They were fasting from 20.00 h onwards. On the study day they remained supine after 8.00 h. One hand vein was cannulated and the intravenous catheter was kept patent with a NaCl drip (154 mmol/l, 30 ml/h). This hand was placed in a thermoregulated box with an ambient temperature of 55°C in order to obtain arterialized venous blood. A forearm vein of the contralateral arm was cannulated for administration of dextrose and insulin. Baseline blood samples were taken after 1 h of rest. A euglycemic clamp was then started with insulin administered at a rate of 30 mU/kg/h during 3 h. An insulin bolus of 5 mU/kg was given directly before the start of the clamp. Blood glucose was measured at 5–10-min intervals and its target level during the clamp was approximately 0.4 mmol/l below the fasting level. A variable dextrose infusion (20% w/w), to which KCl (10 mmol per 500 ml of dextrose) was added, was infused to maintain the blood glucose level. A subsequent blood sample was taken at the end of the clamp. The mean glucose infusion rate (M value in μmol/kg/min) during the third hour of the clamp was calculated as a measure of insulin sensitivity. Thus, the higher the M value, the higher was the insulin sensitivity.

Laboratory measurements

Blood was collected into ethylene diaminetetracetic acid (1.5 mg/ml)-containing tubes and placed on ice immediately. Plasma was obtained within 30 min by centrifugation at 3000 rpm for 15 min at 4°C. Plasma samples were frozen at −70°C until analysis. Lipids were assayed in whole plasma and in the HDL fraction after precipitation of apolipoprotein (apo) B-containing lipoproteins with polyethylene glycol-6000 (34). VLDL + LDL lipids were calculated as the difference between plasma and the HDL lipids. Cholesterol and triglycerides were measured enzymatically. ApoA-I and apoB were measured by immunoturbidimetry (Boehringer Mannheim, Almere, The Netherlands, cat. no. 726478 and 726494, respectively). Plasma free fatty acids (FFA) were determined enzymatically (Wako, Germany, cat. no. 994-754-75409). Plasma LCAT activity was measured using excess exogenous substrate containing [^{3}H]cholesterol as described (19, 35). The plasma LCAT activity levels vary linearly with the amount of plasma used in the incubation. The plasma CETP activity level was determined after removal of VLDL + LDL from each sample, using an isotope assay that measures the transfer of [1-^{1}4C-oleate]-cholesteryl ester from labeled LDL to an excess of unlabeled pooled normal HDL (19, 36). To inhibit LCAT, dithiobis-2-nitrobenzoic acid was added to the incubation mixture. CETP activity was calculated as the bidirectional transfer between labeled LDL and HDL. The LCAT and CETP activity levels so measured correlate strongly with the plasma mass concentrations of these proteins (37, 38). Plasma PLTP activity was determined using a liposome vesicles-HDL system according to a previously described procedure (19, 39). Plasma samples were incubated with [^{3}H]phosphatidylcholine-labeled liposomes and an excess of pooled normal HDL, with subsequent precipitation of the liposomes using a mixture of NaCl, $MgCl_2$, and heparin at final concentrations of 230 mmol/l, 92 mmol/l, and 200 U/ml,

respectively. The plasma PLTP activity level is linearly correlated with the amount of plasma added to the assay system. The method is not influenced by the phospholipid transfer promoting properties of CETP (39). Plasma LCAT, CETP, and PLTP activity levels were related to the activities measured in human pool plasma and are expressed in arbitrary units (AU, corresponding to the percentages of the activities in the pool plasma). Plasma for LPL and HL activities was obtained 20 min after intravenous administration of heparin, 50 IU/kg. This test was performed within 2 weeks before the glucose clamp. LPL and HL were assayed as reported previously (40).

Blood glucose was analyzed with an APEC glucose analyzer (APEC Inc., Danvers, MA). Plasma insulin was measured by radioimmunoassay. Thyreotropin was assayed by immunochemiluminescence. Serum creatinine and transaminases, γ-glutamyltransferase, and alkaline phosphatase were measured with a SMA-C autoanalyzer (Technicon Instruments Inc., Tarrytown, NY).

The B1B2 polymorphism of intron 1 of the CETP gene was analyzed as described (29, 41). Peripheral blood was used for DNA extraction. Erythrocytes were lysed by ammonium chloride treatment. Phenol/chloroform extraction was performed to extract genomic DNA from leukocytes. Polymerase chain reaction (PCR) mixtures contained template DNA (500 ng), primers (50 pmol), deoxynucleotide triphosphates (200 μmol/l each), Taq polymerase (2.5 U, Pharmacia Inc., Sweden), and Taq pol buffer (5 μl, Pharmacia Inc., Sweden) in a total volume of 50 μl. PCR was carried out using a Perkin-Elmer Gene Amp PCR system 2400 (Perkin-Elmer Inc., Foster City, CA). The primers encompassed a 535 base pair DNA fragment that contains a polymorphic site in intron 1 of the CETP gene (29). Thirty cycles of 30 sec at 94°C, 30 sec at 60°C, and 60 sec at 73°C were used, and aliquots of the PCR products (15 μl) were then digested with 8 U of TaqIB (Pharmacia Inc., Sweden) for 2 h at 65°C. Restriction fragments were analyzed by agarose gel electrophoresis, ethidium bromide staining, and visualization on an ultraviolet transilluminator. The B1 allele contains the TaqIB restriction site and is split into 361 and 174 base pair products upon digestion. The B2 allele is unaffected by TaqIB digestion.

Statistical analysis

Parameters are given as mean ± SD unless stated otherwise. Data were divided according to quartiles of the M value (insulin sensitivity) and according to the CETP genotype. Between-group differences in baseline parameters and in changes in parameters were evaluated by Kruskal-Wallis analysis of variance or by the unpaired Wilcoxon test. Within-group changes in parameters were analyzed by the paired Wilcoxon test. Duncan's method was applied to correct for multiple comparisons. Multiple stepwise regression analysis was performed to disclose which parameters independently contributed to HDL cholesterol. A two-sided P value <0.05 was considered significant.

RESULTS

Thirty-two men participated in the study, of whom 24 had normal glucose tolerance and 8 were categorized as having impaired glucose tolerance according to the newly proposed WHO criteria (32). This number of participants was chosen to be able to divide them into quartiles of insulin sensitivity (**Table 1**). There were no differences in age among the quartiles. BMI tended to be higher ($P < 0.10$) and the waist/hip ratio was significantly higher in the quartile of subjects with the lowest insulin sensitivity (Q_4) compared to the quartile of subjects with the highest insulin sensitivity (Q_1). No differences in fasting blood glucose were found among the quartiles, whereas plasma insulin levels at baseline and at the end of the clamp were higher in Q_4 than in Q_1. Mean alcohol consumption in the entire group was 0.6 ± 0.7 drinks per day. In the whole group, the coefficient of variation of blood glucose, reflecting the stability of the clamp was 6.1 ± 2.4%.

Plasma total cholesterol, VLDL + LDL cholesterol, plasma triglycerides, and apoB levels were higher in Q_4 than in Q_1, whereas HDL cholesterol and apoA-I levels were lower in Q_4 compared to Q_1 (**Table 2**). Baseline plasma FFA were not significantly different among the quartiles of insulin sensitivity, but at the end of the clamp plasma FFA were higher in Q_4 than in Q_1 and Q_2. **Figure 1** shows that LPL activity was lower in Q_3 than in Q_1. In the combined quartiles with the lowest insulin sensitivity, LPL activity was lower than in the combined quartiles with the highest insulin sensitivity (118 ± 48 vs. 143 ± 39 U/l, $P < 0.05$). HL activity was higher in Q_2, Q_3, and Q_4 than in Q_1. Baseline plasma LCAT activity was higher in Q_4 than in Q_1. Baseline plasma PLTP activity was higher in Q_4 than in Q_2 and the difference between Q_4 and Q_1 was close to significance ($P < 0.07$). In the combined quartiles with the lowest insulin sensitivity, baseline plasma PLTP activity was higher than in the combined quartiles with the highest sensitivity (89 ± 12 vs. 80 ± 12 AU, respectively, $P < 0.05$). In contrast, no effect of insulin sensitivity on baseline

TABLE 1. Clinical characteristics, fasting blood glucose, and plasma insulin according to quartiles of insulin sensitivity (M value)

Variable	Q_1 (n = 8)	Q_2 (n = 8)	Q_3 (n = 8)	Q_4 (n = 8)
M value (μmol/kg/min)	43.7 ± 9.8 (32.7–62.1)	28.6 ± 2.1 (26.3–31.4)	23.2 ± 2.3 (19.1–26.1)	12.7 ± 4.0 (7.9–18.9)
Age (years)	50 ± 8	54 ± 8	51 ± 8	55 ± 8
BMI (kg/m^2)	23.6 ± 2.6	25.3 ± 4.0	25.9 ± 3.1	26.1 ± 1.8
Waist/hip ratio	0.88 ± 0.06	0.94 ± 0.07	0.94 ± 0.06	0.95 ± 0.06[a]
Fasting blood glucose (mmol/l)	4.7 ± 0.4	4.8 ± 0.7	4.7 ± 0.4	4.6 ± 0.4
Plasma insulin (mU/l)				
Baseline	9.3 ± 3.3	10.4 ± 5.2	9.4 ± 4.0	14.7 ± 6.4[a]
End of clamp	26.9 ± 5.2	32.6 ± 9.5	35.6 ± 7.2	39.8 ± 13.3[a]

Data given as mean ± SD and range. M value, insulin sensitivity index; BMI, body mass index.
[a] $P < 0.05$ from quartile with highest insulin sensitivity (Q_1).

TABLE 2. Fasting plasma lipid parameters according to quartiles of insulin sensitivity (M value)

Variable	Q_1 (n = 8)	Q_2 (n = 8)	Q_3 (n = 8)	Q_4 (n = 8)
Plasma total cholesterol (mmol/l)	4.42 ± 0.53	4.78 ± 0.68	4.84 ± 0.53	5.06 ± 0.75[a]
VLDL + LDL cholesterol (mmol/l)	3.02 ± 0.49	3.59 ± 0.85[c]	3.70 ± 0.61	4.12 ± 0.72[b]
HDL cholesterol (mmol/l)	1.40 ± 0.32	1.19 ± 0.21	1.14 ± 0.15[a]	0.94 ± 0.18[b]
Plasma triglycerides (mmol/l)	0.72 ± 0.17	0.96 ± 0.38	0.85 ± 0.20[c]	1.45 ± 0.51[b]
Plasma FFA (μmol/l)				
Baseline	489 ± 48	481 ± 245	510 ± 233	607 ± 351
End of clamp	41 ± 17	36 ± 28[c]	95 ± 57	132 ± 85[a]
Apolipoprotein A-I (g/l)	1.35 ± 0.18	1.19 ± 0.13	1.18 ± 0.07[a]	1.12 ± 0.13[a]
Apolipoprotein B (g/l)	0.68 ± 0.11	0.80 ± 0.15[a]	0.83 ± 0.16[a]	0.88 ± 0.18[b]

Data given as mean ± SD.
[a] $P \leq 0.05$, [b] $P < 0.01$ from quartile with highest insulin sensitivity (Q_1).
[c] $P < 0.05$ from quartile with lowest insulin sensitivity (Q_4).

plasma CETP activity was observed (Fig. 1). Multiple regression analysis showed that HDL cholesterol was positively related to plasma LCAT activity ($P = 0.01$) and negatively to plasma triglycerides ($P < 0.001$), HL activity ($P < 0.01$), and plasma CETP activity ($P = 0.05$, multiple $r = 0.74$). In this model, no significant relationships of HDL cholesterol with plasma PLTP activity ($P = 0.15$) and LPL activity ($P = 0.54$) were present. As illustrated in **Fig. 2**, no significant changes in plasma LCAT and CETP activity were found in any of the quartiles of insulin sensitivity after insulin infusion. Plasma PLTP activity, in contrast, was decreased by insulin in all quartiles. There was no effect of insulin sensitivity on the % decrement in PLTP activity.

Eight subjects had B2B2, 13 had B1B2, and 11 had B1B1 CETP genotype. The B2 allele frequency was 45%. The CETP genotype distribution was in Hardy-Weinberg equilibrium ($\chi^2_{df2} = 0.52$, n.s.). There were no significant differences in age, BMI, waist/hip ratio, M value, fasting blood glucose, as well as in plasma insulin at baseline and at the end of the clamp among the three CETP genotype groups (data not shown). No effects of the CETP genotype on HDL cholesterol, plasma apoA-I levels, plasma LCAT activity, and CETP activity were observed (data not

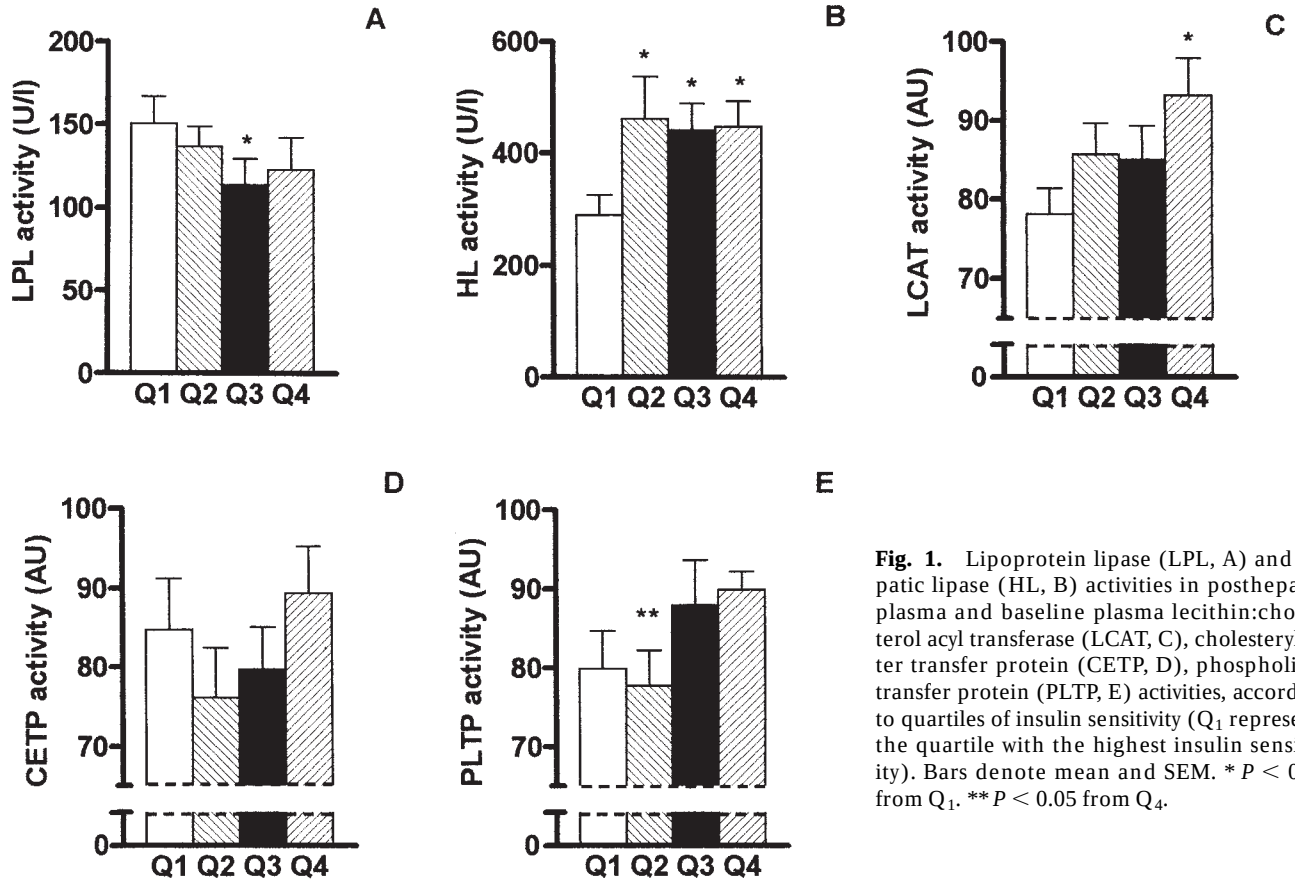

Fig. 1. Lipoprotein lipase (LPL, A) and hepatic lipase (HL, B) activities in postheparin plasma and baseline plasma lecithin:cholesterol acyl transferase (LCAT, C), cholesteryl ester transfer protein (CETP, D), phospholipid transfer protein (PLTP, E) activities, according to quartiles of insulin sensitivity (Q_1 represents the quartile with the highest insulin sensitivity). Bars denote mean and SEM. * $P < 0.05$ from Q_1. ** $P < 0.05$ from Q_4.

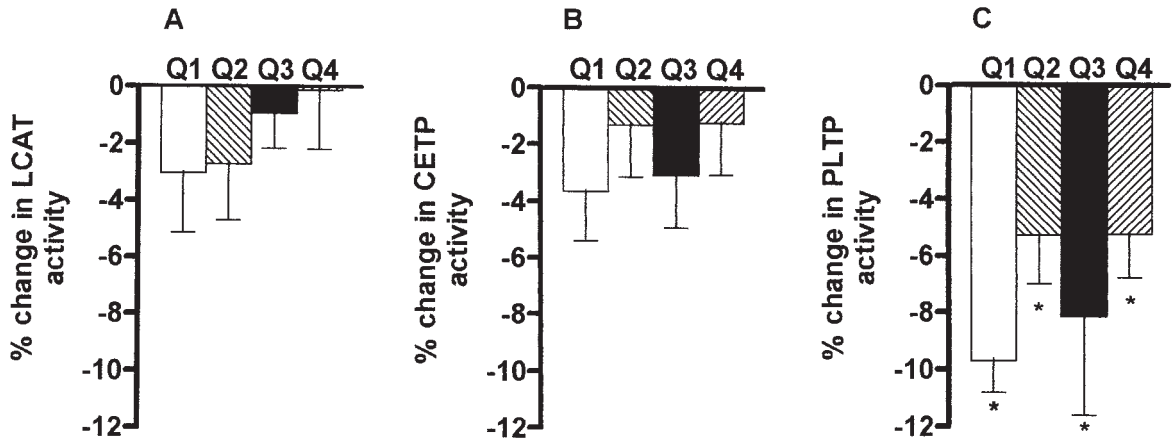

Fig. 2. Changes in plasma lecithin:cholesterol acyl transferase (LCAT, A), cholesteryl ester transfer protein (CETP, B), and phospholipid transfer protein (PLTP, C) activities in response to insulin infusion, according to quartiles of insulin sensitivity (Q_1 represents the quartile with the highest insulin sensitivity). Bars denote mean ± SEM; *$P < 0.05$ from baseline.

shown). There was also no effect on HDL cholesterol, when HDL was measured in unfrozen samples by sodium phosphotungstate and $MgCl_2$ precipitation. Plasma PLTP activity was lower ($P < 0.05$) in B1B1 homozygotes (76 ± 10 AU) than in B1B2 heterozygotes (91 ± 13 AU), but the difference between B1B1 and B2B2 homozygotes (84 ± 12 AU) was not significant. Plasma LCAT activity did not change during insulin infusion in any of the 3 CETP genotype groups. Plasma CETP activity fell slightly in B1B1 homozygotes (−3.5 ± 4.7%, $P < 0.05$), and the difference in change with B2B2 homozygotes (0.6 ± 5.1%) was significant ($P < 0.05$). Plasma PLTP activity decreased in all CETP genotype groups ($P < 0.05$ to $P < 0.01$). The decrease in PLTP activity was larger ($P < 0.05$) in B1B1 (−8.7 ± 7.7%) than in B2B2 homozygotes (−4.8 ± 4.4%). The decrease in the B1B2 heterozygotes was 7.1 ± 5.3%.

DISCUSSION

This study suggests that insulin sensitivity influences LPL and HL activities in postheparin plasma, as well as plasma LCAT and PLTP, but not CETP activity in nondiabetic men. Thus, the well-documented higher plasma triglyceride levels found in insulin-resistant individuals (42, 43) do not only cluster with a lower plasma LPL activity but also with higher plasma LCAT and PLTP activity levels.

The higher plasma LPL and lower HL activities in the most insulin-sensitive individuals compared to more insulin-resistant subjects extend recent observations in nondiabetic men (17). In that study, insulin resistance was indirectly assessed by documentation of fasting hyperinsulinemia, and the difference in plasma HL activity between subjects with low and high fasting serum insulin levels was not significant (17). Another report has demonstrated relationships between low plasma LPL activity, high HL activity, and low HDL cholesterol, either as an isolated abnormality or in conjunction with high plasma triglycerides (16), although such associations have not always been observed (44). When multiple regression analysis was performed in the present study, HDL cholesterol was found to be negatively related to HL activity, whereas there was no independent effect of LPL, in agreement with our earlier data (45). This analysis furthermore showed that HDL cholesterol was positively related to plasma LCAT activity and negatively to plasma CETP activity, in keeping with the actions of these factors to generate cholesteryl ester in the HDL fraction and to promote cholesteryl ester transfer from HDL to triglyceride-rich lipoproteins (2, 10, 11). No independent effect of plasma PLTP activity on the cholesterol concentration in the total HDL fraction was observed. In vitro studies emphasized the role of PLTP in HDL remodeling (6–9).

Novel observations of our study include the associations of insulin sensitivity with plasma LCAT as well as PLTP activity. The plasma LCAT and lipid transfer protein activity assays used in this study may be regarded as a measure of their mass concentrations in plasma (37, 38). Using the same assay, it was previously found that plasma LCAT activity is positively correlated with plasma triglycerides (19), and that plasma LCAT activity is higher in Type 2 diabetic patients in conjunction with higher plasma triglyceride levels (45). Although it is well established that insulin resistance and plasma triglyceride levels are increased in obesity (14, 46, 47), plasma LCAT activity is not related to body mass index and waist/hip ratio (19). Therefore, adipose tissue is unlikely to represent a primary site influencing the association between insulin resistance and high plasma LCAT activity. Rather, it seems plausible that the liver, which is considered to be an important source of LCAT (48), is metabolically involved in the interrelation between insulin resistance, high plasma triglycerides, and LCAT activity. In support of this possibility, in vitro studies have demonstrated that triglycerides and LCAT are cosecreted by rat hepatocytes in culture when incubated with FFA (49). A high level of plasma PLTP activity has been documented in several situations associated with insulin resistance, such as obesity (19, 21), smoking (30), and in

some (21, 45) but not all studies (50, 51) in Type 2 diabetes mellitus. The present data suggest an association between insulin resistance and plasma PLTP activity in non-diabetic, non-smoking men without severe obesity. High plasma PLTP activity in obese Type 2 diabetic patients coincides with elevated plasma triglycerides and a diminished lowering of plasma PLTP, triglycerides, and FFA in response to exogenous insulin (21). In the current study, baseline plasma triglycerides as well as the plasma FFA levels attained after insulin were highest in the most insulin-resistant subjects, but no effect of the degree of insulin resistance on the plasma PLTP response to insulin was found. This observation suggests that a high basal plasma PLTP level can be dissociated from a blunted decrease by acute hyperinsulinemia in non-diabetic men. Adipose tissue is likely to provide an important source of CETP in human plasma (18, 19, 52, 53). Nonetheless, the plasma level of CETP activity was not related to insulin sensitivity, nor was there a clear response to insulin in the present and our previous studies (20, 21). Other reports showed a minor decrease in plasma CETP after insulin administration to non-diabetic patients (22, 23). Taken together, the associations of insulin sensitivity with plasma LCAT and PLTP, but not CETP activity and the lowering of PLTP, but not LCAT and CETP by insulin provide support for the notion that these plasma protein factors are affected differently by the degree of insulin resistance and acute insulin administration.

Several studies have shown that the B2B2 CETP genotype is associated with a higher HDL cholesterol and a lower plasma CETP level (25–27). However, such associations have not been unequivocally reported (28, 29), and appear to be modified by metabolic and environmental factors (29, 41, 54). In the present study with a rather limited number of participants, no consistent effects of the CETP genotype on plasma lipoproteins as well as on baseline plasma CETP, PLTP, and LCAT activity levels were observed. Our observation that the decrease in plasma PLTP activity after insulin was smaller in B2B2 than in B1B1 homozygotes is of potential interest. In view of the possible protective effect of the B2B2 genotype on atherosclerosis (27, 29), and the considerable homology between cDNA encoding for CETP and PLTP (55), this preliminary finding warrants further evaluation.

Despite being implicated in low HDL cholesterol levels (15–17, 56) (present study), in vitro studies suggest that a high HL activity may promote reverse cholesterol transport (13, 57). In accordance with an antiatherogenic function of HL, overexpression of HL in mice retards diet-induced aortic cholesterol accumulation (58), and HL deficiency is characterized by premature atherosclerosis (59). Overexpression of LCAT in rabbits inhibits atherosclerosis development as well (60), but the effect of this manipulation is strongly dependent on the species involved (61). The plasma concentration of preβ-HDL particles, which are considered to be antiatherogenic (9, 50), is enhanced in human PLTP transgenic mice (62, 63). Taken together, these experimental data support the hypothesis that HL, LCAT, and PLTP activities may be involved in a defense mechanism against atherosclerosis. Although such evidence cannot be extrapolated to the human situation, it is tempting to speculate that high levels of HL, LCAT, and PLTP activities in association with insulin resistance could imply an antiatherogenic phenomenon.

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