Selective and independent associations of phospholipid transfer protein and hepatic lipase with the LDL subfraction distribution

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Abstract **Phospholipid transfer protein (PLTP), hepatic lipase (HL), and lipoprotein lipase (LPL) have all been reported to be intricately involved in HDL metabolism but the effect of PLTP on the apolipoprotein B-containing lipoproteins relative to that of HL and LPL has not been established. Due to our previous observation of a positive correlation of PLTP activity with plasma apoB and LDL cholesterol, the relationship of PLTP with the LDL subfractions was investigated and compared with that of HL and LPL. Plasma lipoproteins from 50 premenopausal women were fractionated by density gradient ultracentrifugation. Correlations were calculated between the cholesterol concentration of each fraction and plasma PLTP, HL, and LPL activity. Plasma PLTP activity was highly, positively, and selectively correlated with the cholesterol concentration of the buoyant LDL/dense IDL fractions, yet demonstrated a complete absence of an association with the dense LDL fractions. In contrast, HL was positively correlated with the dense LDL fractions but showed no association with buoyant LDL. LPL was also positively correlated with several buoyant LDL fractions; however, the correlations were weaker than those of PLTP. PLTP and LPL were positively correlated and HL was negatively correlated with HDL fractions. The results suggest that PLTP and HL may be important and independent determinants of the LDL subpopulation density distributions.**—Murdoch, S. J., M. C. Carr, H. Kennedy, J. D. Brunzell, and J. J. Albers. **Selective and independent associations of phospholipid transfer protein and hepatic lipase with the LDL subfraction distribution.** ***J. Lipid Res.*** **2002.** 43: **1256–1263.**

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Phospholipid transfer protein (PLTP), hepatic lipase (HL), and lipoprotein lipase (LPL) are intricately involved in HDL metabolism, yet their inter-relationships with respect to apolipoprotein (apo)B metabolism have not previously been investigated.

PLTP has been suggested to be active in lipoprotein metabolism due to its ability to facilitate lipid transfer between lipoproteins or between lipoproteins and cell membranes. The initial role determined for PLTP was that of facilitated transfer of the redundant surface formed on VLDL during lipoprotein lipase-mediated lipolysis (1, 2). This process involves the transfer of phospholipid, unesterified cholesterol (1–3), and possibly apoE to HDL as originally suggested in an in vitro study (1) and later in an investigation of PLTP knockout mice (3). Consequently, HDL mass is augmented, favoring conversion of small HDL particles to larger particles (1, 2). PLTP can also mediate HDL inter-conversion (4, 5) and facilitate lipid efflux (6). PLTP appears to be a major determinant of HDL concentrations since knockout mice show a marked reduction in HDL lipid and apoA-I (3) and increased catabolism. A paradoxical decrease in HDL due to increased catabolism has been observed in PLTP transgenic mice (7–9), which may be dependent upon the relative gene dose/activity of PLTP relative to apoA-I (8, 9).

Investigations into the role of PLTP in human lipoprotein metabolism have focused on the effect of PLTP on HDL with studies of normolipidemics finding a positive association (10) or no association (11–13). In our previous study of normolipidemic premenopausal women (10), PLTP activity was not only positively correlated with HDL_2, HDL_3, and LPL, but also with LDL cholesterol (LDL-C) and the apoB concentration. The correlations with HDL were consistent with the postulated role of

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Abbreviations: HL, hepatic lipase; PLTP, phospholipid transfer protein.

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PLTP in lipoprotein metabolism, but the strong positive relationship with LDL-C and apoB was puzzling. It is this relationship of PLTP with the apoB-containing lipoproteins relative to that of HL and LPL that is addressed in the present paper. Recently, it has been reported that in PLTP knockout mice, apoB secretion was reduced, as was plasma apoB in apoB transgenic and apoE knockout mice but not in LDL receptor knockout mice (14). The decrease in apoB was associated with a decrease in atherosclerosis, raising further interest in the role of PLTP in apoB and LDL metabolism.

Hepatic lipase (HL) also has a major influence on LDL and HDL subfractions and concentrations in plasma. Hepatic lipase has been found to be negatively correlated with HDL_2 concentrations (15, 16) and positively correlated with the presence of small dense LDL (17). By hydrolysis of core triglyceride and phospholipid surface, HL is thought to be responsible for the formation of small dense LDL from larger more buoyant LDL and the conversion of HDL_2 to HDL_3. The predominance of small dense LDL in the LDL distribution has been associated with an increased risk of CHD (18–21), increased LDL oxidation (22), and increased proteoglycan binding (23, 24), potentially enhancing the entrapment of LDL in arterial tissues. A separate non-catalytic role for HL is that of a ligand, enhancing hepatic recognition of IDL and facilitating the interaction of HDL_2 with a putative SRB1-like receptor in the liver (25–27).

LPL is an enzyme that is also intricately involved in HDL as well as VLDL/LDL metabolism. In brief, LPL leads to the conversion of VLDL to IDL through hydrolysis of core triglyceride accompanied, to a lesser extent, by phospholipid hydrolysis. The apoC proteins are rapidly transferred from VLDL to HDL with subsequent transfer of apoE, phospholipids, and unesterified cholesterol, resulting in the augmentation of HDL mass (28). In vitro, it has been observed that, as a result of LPL-induced lipolysis of VLDL, large IDL-like particles were formed that were rich in surface material and required further transfer of apoE and lipid in order to approach LDL size and composition (1). In this study, when a preparation containing PLTP (as well as CETP) was added, the transfer of phospholipid, unesterified cholesterol, and apoE doubled and particles approaching the size of large LDL were formed, suggesting that PLTP acts in conjunction with LPL in the conversion of VLDL to LDL. Since CETP does not transfer unesterified cholesterol and appears not to promote net transfer of phospholipid in vivo (29), the results imply that LPL and PLTP work in concert to promote the conversion of VLDL to LDL.

The following study was specifically carried out to clarify the relationship of PLTP with the apoB-containing lipoprotein distribution and compare the associations to those determined for HL and LPL. The lipoproteins were separated by density gradient ultracentrifugation and correlations between the cholesterol concentration of each fraction and plasma PLTP, HL, and LPL activity were obtained to determine if specific and independent associations with selective LDL subfractions were present.

METHODS

Subjects

Fifty healthy premenopausal women (ages 42–54) were recruited at random as has been previously reported (10) (**Table 1**). The study group was comprised of 47 Caucasian and three non-Caucasians. Their mean age was 46.5 ± 3.3 years and weight was 71 ± 15 kg. Their BMI, percent body fat, and body fat distribution were as expected for females of their age. None of the subjects were smokers, or taking medications affecting lipid metabolism, or demonstrating any lipid disorders, diabetes, or liver disease. None were pregnant. The subjects were deemed premenopausal as they were all menstruating. The exclusion criteria were a total triglyceride or LDL-C concentrations above the 95th percentile for their age, small LDL as determined by polyacrylamide gradient gel electrophoresis (30), or a BMI greater than 40 kg/m^2. Since the presence of small dense LDL as the predominant form of LDL is uncommon in premenopausal women comprising 5–10% of the population (31) and is associated with insulin resistance, type 2 diabetes (32), and polycystic ovarian syndrome (33), these women were excluded in order to obtain a population sample representative of normal premenopausal women. The Human Subjects Review Committee of the University of Washington approved the study protocol. Informed consent was obtained from all participants.

Sample collection

After a 12–14 h fast, blood was collected in 0.1% EDTA and immediately placed on ice for lipid measurements and the PLTP activity assay as well as density gradient ultracentrifugation. To determine lipase activities, a bolus of 60 U/kg of heparin was given and blood was sampled after 10 min in lithium heparin tubes. Plasma was obtained by centrifugation at 3,000 rpm for 15 min at 4°C. Fresh plasma was used for lipid determinations. Otherwise, plasma was immediately flash frozen and kept at −70°C until use.

Analysis of plasma lipids

Total, LDL, HDL, HDL_2, HDL_3-C, apoB, and triglyceride were determined by standardized methodologies at the Northwest Lipid Research Laboratories (34). HDL and HDL_3-C were determined in the plasma supernatant after precipitation with dextran sulfate and magnesium chloride (35, 36). The inter-assay coefficient of variation for apoB is 2.5%.

TABLE 1. Characteristics of the subjects[a]

n = 50	Mean	Range
Age	46.5 ± 3.3	42–54
Total Cholesterol (mg/dl)	182 ± 30	111–240
Triglyceride (mg/dl)	73 ± 32	34–170
ApoB (mg/dl)	81 ± 17	48–120
LDL cholesterol (mg/dl)	109 ± 24	50–158
HDL cholesterol (mg/dl)	58 ± 14	35–98
HDL_2 cholesterol (mg/dl)	13 ± 7	5–31
HDL_3 cholesterol (mg/dl)	45 ± 9	29–67
PLTP activity (μmol/ml/hr)	14.9 ± 2.2	10.5–20.3
LPL activity (μmol/ml/hr)	12.2 ± 6.2	3.6–35.7
HL activity (μmol/ml/hr)	7.9 ± 3.2	2.7–16.9
Weight (kg)	70.9 ±15.3	49.2–112.0
BMI (kg/m^2)	25.6 ± 4.7	18–37.9

Values are mean ± SD. PLTP, phospholipid transfer protein; LPL, lipoprotein lipase; HL, hepatic lipase; BMI, body mass index.

[a] This data has previously been reported (10) and is included for completeness.

Density gradient ultracentrifugation

A discontinuous salt density gradient was created in an ultracentrifuge tube (37). One milliliter of plasma was combined with 1.5 ml of a d = 1.006 g/ml NaCl solution (containing 0.01% EDTA, pH 7.4) and 1.5 ml of a d = 1.21 g/ml solution (d = 1.006 g/ml NaCl adjusted to d = 1.21 g/ml with solid KBr, 0.01% EDTA, pH 7.4) and mixed, resulting in a final density of 1.0825 g/ml. A solution (8.5 ml) of d = 1.006 g/ml NaCl (0.01% EDTA, pH 7.4) was placed in a Beckman ultracentrifuge tube #344322 and underlayered with the 4 ml of the d = 1.0825 g/ml solution, containing the plasma. Samples were centrifuged at 65,000 rpm for 70 min (total $w^2t = 1.95 \times 10^{11}$) in a Beckman VTi 65.1 vertical rotor. Thirty-eight 0.34 ml fractions were collected from the bottom of the tube and the cholesterol concentration was determined for each fraction.

PLTP activity assay

PLTP activity was determined by measuring the transfer of labeled phosphatidylcholine from vesicles to HDL_3 (lacking apoE) as previously described (10, 11) but without the use of plasma as a carrier (10). This method reflects the phospholipid transfer activity of PLTP but not that of CETP (11). Briefly, 50 μl of liposomes containing phosphatidylcholine (50 nM), phosphatidylserine (12 nM), and trace labeled with [^{14}C]1-palmitoyl-2-linoleoyl phosphatidylcholine labeled in the linoleoyl-1-C position (New England Nuclear) (specific activity of 170 cpm/nM phospholipid) were combined on ice with HDL_3 (150 nM phospholipid), 50 μl of diluted plasma (diluted 1:50, resulting in a 1 ml plasma equivalent), and 300 μl of Tris saline EDTA buffer (TSEA) (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.1% sodium azide). The plasmas were assayed in triplicate with separate dilutions performed for each. The same batch of vesicles and HDL were used for all PLTP assays to maintain consistency. The samples were incubated for 15 min at 37°C, and 400 μl of TSEA buffer, and 100 ul of a 1% dextran sulfate/0.5M $MgCl_2$ solution were added to precipitate the vesicles. The radioactivity in 600 μl of the supernatant was counted to determine the amount transferred from the vesicles to HDL. Three human control plasmas, flash frozen and stored at −70°C until use, were included in triplicate in each assay and used to correct for inter-assay variation. The intra-assay and inter-assay coefficients of variation were 7.6% and 2.2%, respectively.

Lipoprotein lipase and hepatic lipase activities

After administration of a heparin bolus, the total plasma triglyceride lipase activity was determined as previously described (38). In brief, post-heparin plasma (diluted 1:10) was incubated with a triolein/phosphatidylcholine/albumin emulsion, trace labeled with glycerol tri[1-^{14}C]oleate (Ampersham, Arlington Heights, IL) in 0.178 M Tris-HCl, 0.11 M NaCl buffer (pH 8.5) containing 55 mg/ml albumin and 0.01 mg/ml heparin for 60 min at 37°C. The resultant free fatty acids were extracted and ^{14}C content was determined by liquid scintillation counting. The decrease in activity resulting from the addition of a monoclonal antibody specific for lipoprotein lipase allowed for the calculation of the LPL activity. Hepatic lipase activity was estimated as the activity remaining after the addition of the LPL antibody. The activity of a bovine skim milk LPL standard was determined with each assay and used to correct the results for inter-assay variation. A human post-heparin control plasma was assayed as well to monitor inter-assay variation. The intra-assay coefficient of variation was 7% for LPL and 6% for HL. Inter-assay coefficient of variation was 8% for LPL and 10% for HL.

Statistics

Statistical analyses were performed using Sigma Stat (Jandel Scientific). Results are reported as the mean and the standard deviation. Spearman Rank Order correlations were used to determine the correlation of PLTP, HL, or LPL activity, or apoB with the selected fraction. Spearman Rank Order correlations were used to reduce the possible contribution of any outliers to the correlation. Forward stepwise linear regression was used to determine the independent contribution of variables that were correlated in univariate analysis with the variable of interest.

RESULTS

The density gradient used to study the lipoprotein cholesterol distributions was selected because the gradient design maximizes the resolution of the apoB containing lipoproteins. Thus, the gradient was chosen as the relationships of the LDL subfraction distributions and the apoB-containing lipoprotein distribution with PLTP, HL, and LPL activity are the major foci of the study.

The correlation of plasma PLTP activity with the cholesterol concentration of each fraction was determined (**Fig. 1A**) and the level of significance of each correlation is indicated (Fig. 1B). In these subjects the total plasma LDL-C had demonstrated a substantial positive correlation with PLTP activity ($r = 0.53$, $P < 0.001$) (10). When the cholesterol concentration of the individual fractions of the LDL region of the gradient were correlated with the plasma PLTP activity (Fig. 1A), PLTP activity was found to be highly, positively, and specifically correlated with the cholesterol concentration of the buoyant LDL fractions (fractions 13–18, $r = 0.47$ to 0.57, $P < 0.001$) and the peak LDL fraction (fraction 12, $r = 0.56$, $P < 0.001$), the highest correlations being with fractions 12–15. These correlations are similar to or greater in magnitude than the previously observed correlation between the total plasma LDL and PLTP activity($r = 0.53$) or apoB and PLTP activity ($r = 0.44$) (10). PLTP activity was also positively correlated with fractions corresponding to the dense IDL fractions (fraction 19–22, $r = 0.29$ to 0.41, $P = 0.003$ to 0.04) but the correlations were not as strong as those determined for the buoyant LDL fractions. In contrast to the highly significant correlations observed with the buoyant LDL fractions, there was a complete absence of a significant correlation of PLTP with any of the dense LDL fractions (Fractions 8–11) (Fig. 1A)($r = -0.06$ to 0.20).

PLTP was also positively correlated with the cholesterol of the HDL fractions 1–4 ($r = 0.28$ to 0.35, $P = 0.014$ to 0.047). Because the gradient emphasizes the LDL and IDL region, HDL_2 is not completely separated from HDL_3. Thus, an analysis of the relationship of PLTP with these HDL subfractions is not possible in this study.

To compare the relationship of PLTP and HL, as well as LPL, with the buoyant and dense LDL and the HDL fractions, correlations were calculated for the cholesterol concentrations of the individual fractions with plasma HL and LPL activities. The significance of the correlations between PLTP, LPL or HL, and the cholesterol content of the fractions are indicated (Fig. 1B).

LPL was positively correlated with three of the buoyant LDL fractions (fraction 13 to 15, $r = 0.30$ to 0.36, $P = 0.01$

to 0.034) that were also correlated with PLTP activity (Fig. 1B). However, the strength of the correlations was less than that observed for PLTP. For example, for fraction 13, PLTP demonstrated a positive correlation of 0.57 ($P < 0.001$), whereas the correlation with LPL was 0.36 ($P = 0.01$). LPL was not significantly correlated with the IDL fractions that were correlated with PLTP. LPL was not related to any of the dense LDL fractions. LPL was positively associated with the majority of the HDL fractions (fractions 2–6, $r = 0.349$ to 0.456, $P < 0.001$ to 0.013), showing the strongest correlations with fractions 3–5 ($r = 0.422$ to 0.456, $P < 0.001$–0.003).

Since the activity of LPL and PLTP appear to act in concert in the conversion of VLDL to LDL with consequent augmentation of HDL mass, and PLTP activity has been found to be positively correlated with LPL activity in these subjects ($r = 0.36$, $P = 0.011$) (10), the independence of relationship of PLTP with the LDL or HDL fractions from that of LPL was examined. Using multivariate analysis, with PLTP and LPL as the independent variables and the cholesterol concentration of the fraction as the dependent variable, LPL was no longer significantly correlated with the buoyant LDL fractions while the association with PLTP remained. When the same analysis was carried out for the HDL fractions, LPL remained strongly and positively correlated while PLTP was no longer significantly related.

The correlations observed for HL activity with the LDL and HDL fractions are in contrast to those observed for PLTP and LPL activity (Fig. 1B). While PLTP and LPL demonstrated positive correlations with similar fractions of buoyant LDL and with HDL, HL demonstrated no relationship with any of the buoyant LDL fractions but was positively correlated with the fractions corresponding to dense LDL (fractions 9–11, $r = 0.38$ to 0.43, $P = 0.002$ to 0.006). The relationship of HL with the HDL fractions also differed in that HL was strongly negatively correlated with the HDL fractions (fractions 3–6, $r = -0.33$ to -0.46, $P < 0.001$ to 0.02).

PLTP and HL activity both increase with obesity (16, 39, 40) yet appear to have opposing effects on HDL-C concentrations. As a result, the positive relationship of PLTP with HDL, particularly that of HDL_2, can be masked by

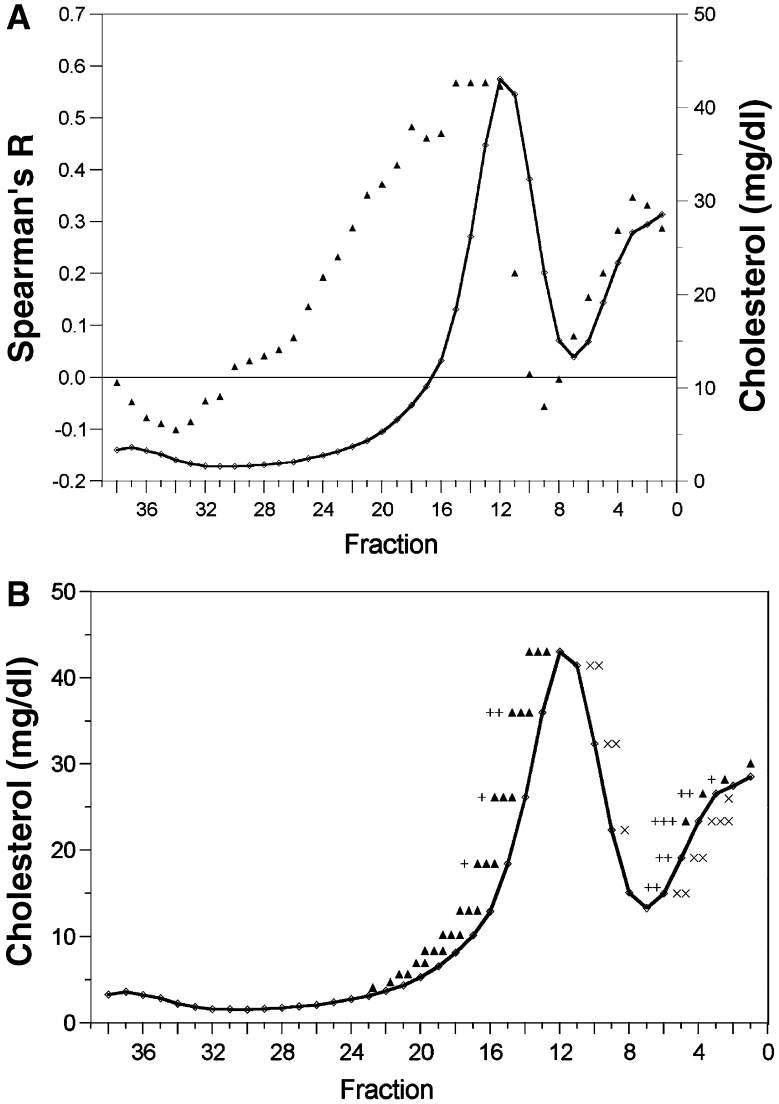

Fig. 1. A: Fractionation of the plasma lipoproteins by density gradient ultracentrifugation and the correlations of plasma PLTP activity with the fractions. The plasma lipoproteins of the 50 women were fractionated by density gradient ultracentrifugation. The mean cholesterol concentration of each fraction was determined (diamond). The correlation of the plasma PLTP activity with the cholesterol concentration of each fraction is shown (triangle). The correlations are significant at the following levels: $r = 0.28$, $P = 0.05$; $r = 0.36$, $P = 0.01$; $r = 0.45$, $P = 0.001$. The distribution of the lipoproteins first separated by sequential ultracentrifugation and then subjected to the density gradient ultracentrifugation results in VLDL isolating in fractions 28–38, IDL in fractions 17–30 with tailing into fractions 31–34, LDL isolating in fractions 7–18, and HDL in fractions 1–8 (45). B: Fractionation of the plasma lipoproteins by density gradient ultracentrifugation and the significance of the correlation of the fractions with PLTP, LPL, and HL activity. The plasma lipoproteins of the 50 women were fractionated by density gradient ultracentrifugation. The mean cholesterol concentration of each fraction is indicated (diamond). The significance of the correlation of the cholesterol concentration of each fraction with plasma PLTP activity (triangle), LPL activity (+), and HL activity (×) are indicated. The level of significance is represented by the number of corresponding symbols shown next to the fraction such that one symbol indicates a significance level of $P < 0.05$, two symbols represent $P < 0.01$, and three symbols indicate $P < 0.001$. Symbols above the line indicate a positive correlation and those below the line represent a negative correlation.

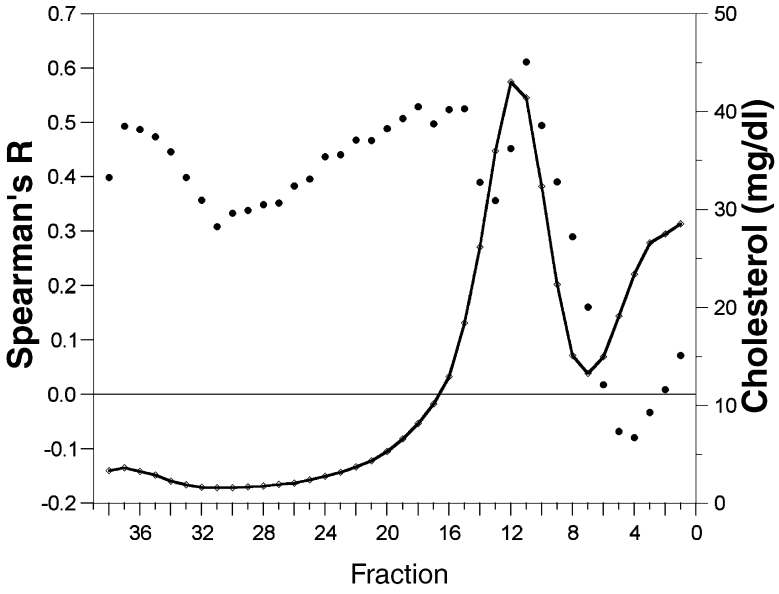

Fig. 2. Fractionation of the plasma lipoproteins by density gradient ultracentrifugation and the correlations of plasma apoB with the fractions. The plasma lipoproteins of the 50 women were fractionated by density gradient ultracentrifugation. The mean cholesterol concentration of each fraction is indicated (diamond). The correlation of the plasma apoB with the cholesterol concentration of each fraction is shown (circle). The significance of the correlations are as follows: $r = 0.28$, $P = 0.05$; $r = 0.36$, $P = 0.01$; $r = 0.45$, $P = 0.001$.

the HL-mediated reduction of HDL (10). Thus, we included both PLTP and HL in multivariate analysis to determine the strength of the correlation of PLTP with the HDL fractions when the contribution of HL was removed, and consequently determine the independent contributions of PLTP and HL to the relationship with the HDL fractions. With multivariate analysis, both PLTP and HL remained independently related to the HDL fractions, but the partial correlations of PLTP with the HDL fractions (fractions 2–4) were strengthened considerably. For example, the correlation of fraction 4 was increased from 0.29 ($P = 0.04$) to 0.36 ($P = 0.007$). PLTP also became significantly and positively correlated with fraction 5 (partial $r = 0.28$, $P = 0.035$).

Since plasma apoB was found to be positively correlated with PLTP activity in these subjects, though less strongly than that of LDL-C (10), and it has been reported in transgenic mice that PLTP may play a role in increasing apoB secretion (14), it is possible that the relationship of PLTP with the LDL fractions merely reflects the correlation of the fractions with apoB. When the correlations of plasma apoB with the cholesterol of the individual fractions were determined, it was found that apoB was significantly correlated with all of the VLDL, IDL, and LDL fractions rather than just the buoyant LDL fractions (**Fig. 2**), but the correlations with apoB were weaker in the buoyant fractions that demonstrated the strongest correlations with PLTP activity (fraction 12–14). In multivariate analysis with apoB and PLTP as the independent variables and the cholesterol concentrations of the fractions in the buoyant region of LDL as the dependent variable, PLTP remained highly and significantly correlated with the cholesterol concentration of the fraction while apoB was no longer associated. It is also possible that HL may have masked a positive relationship of PLTP with the dense fractions of LDL as was observed for HDL. If this were the case then PLTP may be positively associated with all LDL fractions and the correlation with apoB and LDL-C is simply due to increased apoB production resulting in an increase in total LDL. A masking of the association was not found since in multivariate analysis only HL was significantly related to the dense LDL fractions and an independent association with PLTP was not revealed, providing evidence that the association of PLTP with LDL is specific to the buoyant fractions only.

As the number of correlations calculated increase so does the chance of reporting a false positive correlation. Thus a correlation with a significance level of $P = 0.05$ indicates that the chance of a false positive is 1 in 20 while a $P = 0.01$ indicates that the chance is 1 in 100. Considering the number of correlations carried out in the study, if the significance level is set at $P \leqslant 0.01$, PLTP remains significantly correlated with the peak (fraction 12) and buoyant LDL fractions (13–18), the densest IDL fractions (19–20) and HDL fraction 4, adjusted for the effect of HL, but not with the unadjusted HDL values. LPL continues to be correlated with HDL fractions 3–6 and the buoyant LDL fraction 13, but not with fractions 14 and 15. HL remains correlated with the dense LDL fractions and negatively correlated with the HDL fractions 4–5, but not with fraction 3.

DISCUSSION

This is the first study that has reported a comparison of the associations of PLTP, HL, and LPL activity with LDL- and HDL-C. Furthermore, by resolving the LDL distribution by density gradient ultracentrifugation, a comparison of the relationships of PLTP, HL, and LPL with the LDL subfraction distribution was possible. The present study has elucidated that, in this group of premenopausal women, PLTP activity is highly, positively, and specifically associated with the buoyant subfraction of the LDL-C distribution showing no association with dense LDL. This observation is similar though less consistent for LPL, yet in

direct contrast to HL, which was selectively associated with dense LDL. The association of PLTP with the buoyant LDL fractions is consistent with the findings in another population of women (ages 32–74) (41). The results of the present study contribute significantly to the understanding of the basis of the previously reported relationship between PLTP and LDL-C or apoB (10) and demonstrate markedly different associations from those of HL with the LDL subfractions.

When reporting associations, the variability of the assay may affect the strength of the correlation of a variable. The intra-assay coefficients of variation for PLTP, HL, and LPL activity were 7.6%, 6%, and 7%, respectively, while the inter-assay coefficients of variation for PLTP, HL, LPL, and apoB were 2.2%, 10%, 8%, and 2.5%, respectively. Thus, based on the intra-assay coefficients of variation, the strength of the correlations determined should not be affected by a discrepancy in the variability between the PLTP, HL, and LPL assays. For the inter-assay comparison, the coefficient of variation in PLTP is similar to that of apoB but is less than that of LPL. Thus, the correlations with LPL may be an underestimate relative to PLTP for the buoyant LDL fractions, although the correlations were markedly weaker for LPL ($r = 0.36$) than for PLTP ($r = 0.57$). With respect to the remaining comparisons of the correlations, this limitation does not appear to apply since most correlations are either *1*) identified in different fractions as is the case for PLTP correlating positively with buoyant LDL and HL with dense LDL; *2*) opposite to one another, as observed for the positive association of PLTP but negative association of HL with HDL; *3*) stronger for one variable for certain fractions (the correlations of PLTP with the buoyant LDL fractions as compared to those of apoB) and weaker or absent for other fractions (the correlations of apoB with dense LDL and absence of correlations for PLTP).

Since a correlation indicates an association rather than cause and effect, the results may be interpreted several ways. The positive association with the mass of buoyant LDL may indicate that as buoyant LDL or factors contributing to the mass of LDL increase, so too does the synthesis or plasma activity of PLTP. However, based on the present understanding of the role of PLTP in lipoprotein metabolism, and specifically during VLDL lipolysis, a more likely hypothesis is that PLTP may be significantly involved in determining the mass of buoyant LDL. The lack of association with the dense LDL fractions also suggests that the action of PLTP may specifically favor the formation of (large) buoyant LDL as compared to (small) dense LDL. The results are in agreement with the in vitro data (1), which suggested that PLTP is required for conversion of VLDL to LDL since the addition of PLTP substantially increased the transfer of surface lipid and apoE to HDL, and enabled the formation of large LDL-sized particles/dense IDL particles. The present results indicate that, in this group of subjects, PLTP is the variable most strongly associated with buoyant LDL as compared with LPL and HL, predicting 22–33% of the variance in the cholesterol concentration of the buoyant LDL fractions. However, this does not imply that physiologically LPL and HL, as a ligand, are not critical for buoyant LDL formation.

The highly positive relationship of PLTP with the buoyant LDL fractions is in contrast to the positive correlation of HL with the dense LDL fractions. The correlation of hepatic lipase confirms previous reports of a positive association with small dense LDL (17) and is consistent with the concept that HL may be the major determinant of the mass of dense LDL as compared with LPL and PLTP in subjects whose plasma lipids are in the normal range. It is interesting that there was no overlap of PLTP and HL activity with respect to the relationship with the LDL fractions. These observations may indicate that the two LDL subgroups represent two distinct metabolic pools of LDL rather than a continuum of LDL conversion (42).

Because of the concerted activity of PLTP and LPL during VLDL lipolysis, we reasoned that the buoyant LDL fractions may also be positively correlated with LPL. This was the case in some fractions, but the relationship between LPL and the LDL fractions dropped out when PLTP was included in multivariate analysis. The converse was the case for HDL associations. Thus, one interpretation of the data may be that PLTP has a stronger contributory effect on the concentration of buoyant LDL while LPL has a greater effect on HDL. However, since the inter-assay variability is greater for LPL, this may hold true for HDL but is inconclusive for LDL. Furthermore, PLTP and LPL activity are correlated making interpretation of the results complex.

The apoB correlations with the LDL-C fractions did not correspond to those of PLTP activity but, in fact, were weaker in the LDL fractions that demonstrated the strongest correlations with PLTP. This finding suggests that the relationship of PLTP with the buoyant LDL fractions is more complex than being solely due to the correlation of PLTP with apoB, related to a possible role in increased apoB secretion (14). The results strengthen the hypothesis that a large part of the reported relationship of PLTP with LDL-C and apoB is due to the strong positive association with buoyant LDL, reflecting the action of PLTP in the transfer of redundant surface from IDL "remnants" and leading to the formation of large buoyant LDL (1). Thus, apoB-containing particles may increase with increasing PLTP, but the increase appears to be related to a selective augmentation of buoyant LDL particles in these subjects. The converse may also occur such that as VLDL apoB increases, PLTP increases in order to transfer the augmented redundant surface produced during lipolysis, enabling maturation to buoyant LDL.

In the HDL region, PLTP and LPL activity were both found to be positively correlated with the cholesterol concentrations of the fractions, while HL activity was negatively correlated. These correlations are consistent with the literature and are in agreement with the postulated roles of these lipases in HDL metabolism (28, 43, 44) and the role of PLTP in augmenting HDL mass during LPL-induced lipolysis (1, 2). It is notable that the positive correlation of PLTP with the HDL fractions became stronger when the contribution of HL was removed by multivariate

analysis, which is an important point when assessing the relationship of PLTP with HDL, particularly in obese subjects or subjects with insulin resistance.

Since subjects with a predominance of large buoyant LDL as compared with small dense LDL demonstrate a decreased incidence of coronary heart disease (18–21) at equivalent LDL-C concentrations, the observation of the strong positive selective relationship of PLTP with the concentration of buoyant LDL is extremely pertinent. However, since the results of the PLTP knockout in apoB transgenic or apoE null mice suggest that PLTP is proatherogenic (14), this raises the question of the role of PLTP in atherosclerosis and whether the response is the same or different in humans as compared to the mouse model. Therefore, the relationship of PLTP with the development of atherosclerosis in humans requires investigation, particularly as it relates to apoB and the relative abundance of buoyant LDL as compared to that of dense LDL.

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REFERENCES

1. Murdoch, S. J., and W. C. Breckenridge. 1996. Effect of lipid transfer proteins on lipoprotein lipase induced transformation of VLDL and HDL. *Biochim. Biophys. Acta.* **1303:** 222–232.
2. Tall, A. R., S. Krumholz, T. Olivecrona, and R. J. Deckelbaum. 1985. Plasma phospholipid transfer protein enhances transfer and exchange of phospholipids between very low density lipoproteins and high density lipoproteins during lipolysis. *J. Lipid Res.* **26:** 842–851.
3. Qin S., K. Kawano, C. Bruce, M. Lin, C. Bisgaier, A. R. Tall, and X. Jiang. 2000. Phospholipid transfer protein gene knock-out mice have low high density lipoprotein levels, due to hypercatabolism, and accumulate apoA-IV-rich lamellar lipoproteins. *J. Lipid Res.* **41:** 269–276.
4. Tu, A. Y., H. I. Nishida, and T. Nishida. 1993. High density lipoprotein conversion mediated by human plasma phospholipid transfer protein. *J. Biol. Chem.* **268:** 23098–23105.
5. Jauhiainen, M., J. Metso, R. Pahlman, S. Blomqvist, A. van Tol, and C. Ehnholm. 1993. Human plasma phospholipid transfer protein causes high density lipoprotein conversion. *J. Biol. Chem.* **268:** 4032–4036.
6. Wolfbauer, G., J. J. Albers, and J. F. Oram. 1999. Phospholipid transfer protein enhances removal of cellular cholesterol and phospholipids by high-density lipoprotein apolipoproteins. *Biochim. Biophys. Acta.* **1439:** 65–76.
7. Foger B., S. Santimarina-Fojo, R. D. Shamburek, C. L. Parrot, G. D. Talley, and H. B. Brewer, Jr. 1997. Plasma phospholipid transfer protein: adenovirus-mediated over-expression in mice leads to decreased plasma high density lipoprotein (HDL) and enhanced hepatic uptake of phospholipids and cholesteryl esters from HDL. *J. Biol. Chem.* **272:** 27393–27400.
8. Jiang, X–C., O. L. Francone, C. Bruce, R. Milne, J. Mar, A. Walsh, J. L. Breslow, and A. Tall. 1996. Increased prebeta-high density lipoprotein, apoprotein AI, and phospholipid in mice expressing the human phospholipid transfer protein and human apolipoprotein AI transgenes. *J. Clin. Invest.* **96:** 2373–2380.
9. Ehnholm, S., K. Willems van Dijk, B. van't Hof, A. van der zee, V. M. Olkkonen, M. Jauhianen, M. Hofker, L. Havekes, and C. Ehnholm. 1998. Adenovirus mediated over-expression of human phospholipid transfer protein alters plasma HDL levels in mice. *J. Lipid Res.* **39:** 1248–1253.
10. Murdoch, S. J., M. C. Carr, J. E. Hokanson, J. D. Brunzell, and J. J. Albers. 2000. PLTP activity in premenopausal women. Relationship with lipoprotein lipase, HDL, LDL, body fat, and insulin resistance. *J. Lipid Res.* **41:** 237–244.
11. Cheung M. C., G. Wolfbauer, and J. J. Albers. 1996. Plasma phospholipid mass transfer rate: relationship to plasma phospholipid and cholesteryl ester transfer activities and lipid parameters. *Biochim. Biophys. Acta.* **1303:** 103–110.
12. Desrumaux, C., A. Athias, G. Bessede, B. Verges, M. Farnier, L. Persegol, P. Gambert, and L. Lagrost. 1999. Mass concentration of plasma phospholipid transfer protein in normolipidemic, type IIa hyperlipidemic, type IIb hyperlipidemic, and non-insulin-dependent diabetic subjects as measured by a specific ELISA. *Arterioscler. Thromb. Vasc. Biol.* **19:** 266–275.
13. Mero, N., A. Van Tol, L. M. Scheek, T. Van Gent, C. Labeur, M. Rosseneu, and M. R. Taskinen. 1998. Decreased postprandial high density lipoprotein cholesterol and apolipoproteins A-I and E in normolipidemic smoking men: relations with lipid transfer proteins and LCAT activities. *J. Lipid Res.* **39:** 1493–1502.
14. Jiang X. C., S. Qin, C. Qiao, K. Kawano, M. Lin, A. Skold, X. Xiao, and A. R. Tall. 2001. Apolipoprotein B secretion and atherosclerosis are decreased in mice with phospholipid-transfer protein deficiency. *Nat. Med.* **7:** 847–852.
15. Kuusi, T., P. Saarinen, and E. A. Nikkila. 1980. Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoprotein 2 in man. *Atherosclerosis.* **36:** 589–593.
16. Carr, M. C., J. E. Hokanson, S. S. Deeb, J. Q. Purnell, E. S. Mitchell, and J. D. Brunzell. 1999. A hepatic lipase gene promoter polymorphism attenuates the increase in hepatic lipase activity with increasing intra-abdominal fat in women. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2701–2707.
17. Zambon, A., M. A. Austin, B. G. Brown, J. E. Hokanson, and J. D. Brunzell. 1993. Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. *Arterioscler. Thromb.* **13:** 147–153.
18. Gardner, C. D., S. P. Fortmann, and R. M. Krauss. 1996. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. *JAMA.* **276:** 875–881.
19. Stampfer, M. J., R. M. Krauss, J. Ma, P. J. Blanche, L. G. Holl, F. M. Sacks, and C. H. Hennekens. 1996. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *JAMA.* **276:** 882–888.
20. Lamarche, B., A. Tchernof, S. Moorjani, B. Cantin, G. R. Dagenais, P. J. Lupien, and J. P. Despres. 1997. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Circulation.* **95:** 69–75.
21. Zambon, A., J. E. Hokanson, B. G. Brown, and J. D. Brunzell. 1999. Evidence for a new pathophysiological mechanism for coronary artery disease regression: hepatic lipase-mediated changes in LDL density. *Circulation.* **99:** 1959–1964.
22. de Graf, J., H. L. Hak-Lemmers, M. P. Hectors, P. N. Demacker, J. C. Hendriks, and A. F. Stalenhoef. 1991. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arterioscler. Thromb.* **11:** 298–306.
23. Camejo, G., E. Hurt-Camejo, O. Wiklund, and G. Bonders. 1998. Association of apoB lipoproteins with arterial proteoglycans: pathological significance and molecular basis. *Atherosclerosis.* **139:** 205–222.
24. Hurt-Camejo, E., G. Camejo, and P. Sartipy. 2000. Phospholipase A2 and small, dense low-density lipoprotein. *Curr. Opin. Lipidol.* **11:** 465–471.
25. Dichek, H. L., W. Brecht, J. Fan, Z. S. Ji, S. P. McCormick, H. Akeefe, L. Conzo, D. A. Sanan, K. H. Weisgraber, S. G. Young, J. M. Taylor, and R. W. Mahley. 1998. Overexpression of hepatic lipase in transgenic mice decreases apolipoprotein B-containing and high density lipoproteins. Evidence that hepatic lipase acts as a ligand for lipoprotein uptake. *J. Biol. Chem.* **273:** 1896–1903.

26. Amar, M. J., K. A. Dugi, C. C. Haudenschild, R. D. Shamburek, B. Foger, M. Chase, A. Bensadoun, R. F. Hoyt, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1998. Hepatic lipase facilitates the selective uptake of cholesteryl esters from remnant lipoproteins in apoE-deficient mice. *J. Lipid Res.* **39:** 2436–2442.
27. Zambon, A., S. S. Deeb, A. Bensadoun, K. E. Foster, and J. D. Brunzell. 2000. In vivo evidence of a role for hepatic lipase in human apoB-containing lipoprotein metabolism, independent of its lipolytic activity. *J. Lipid Res.* **41:** 2094–2099.
28. Murdoch, S. J., and W. C. Breckenridge. 1995. Influence of lipoprotein lipase and hepatic lipase on the transformation of VLDL and HDL during lipolysis of VLDL. *Atherosclerosis.* **118:** 193–212.
29. Kawano K., S. C. Qin, M. Lin, A. R. Tall, and X. C. Jiang. 2000. Cholesteryl ester transfer protein and phospholipid transfer protein have nonoverlapping functions in vivo. *J. Biol. Chem.* **275:** 29477–29481.
30. Krauss, R. M., and D. J. Burke. 1982. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J. Lipid Res.* **23:** 97–104.
31. Campos, H., E. Blijlevens, J. R. McNamara, J. M. Ordovos, B. M. Posner, P. W. Wilson, W. P. Castelli, and E. J. Shaefer. 1992. LDL particle size distribution. Results from the Framingham Offspring Study. *Arterioscler. Thromb.* **12:** 1410–1419.
32. Selby, J. V., M. A. Austin, B. Newman, D. Zang, C. P. Quesenberry, Jr., E. J. Mayer, and R. M. Krauss. 1993. LDL subclass phenotypes and the insulin resistance syndrome in women. *Circulation.* **88:** 381–387.
33. Pirwany, I. R., R. Fleming, I. A. Greer, C. J. Packard, and N. Sattar. 2001. Lipids and lipoprotein subfractions in women with PCOS: relationship to metabolic and endocrine parameters. *Clin. Endocrinol.* **54:** 447–453.
34. Warnick, G. R. 1986. Enzymatic methods for quantification of lipoprotein lipids. *Methods Enzymol.* **129:** 101–123.
35. Warnick, G. R., J. Benderson, and J. J. Albers. 1982. Dextran sulfate-$Mg2^{+}$ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin. Chem.* **28:** 1379–1388.
36. Bachorik, P. S., and J. J. Albers. 1986. Precipitation methods for quantification of lipoproteins. *In* Methods in Enzymology. J. J. Albers and J. P. Segrest, editors. Vol. 129. Academic Press Inc., Orlando, FL. 78–100.
37. Hokanson, J. E., M. A. Austin, and J. D. Brunzell. 1997. Measurement and clinical significance of low-density lipoprotein subclasses. *In* Handbook of Lipoprotein Testing. N. Rifai, G. R. Warnick, and M. H. Dominiczak, editors. American Association of Clinical Chemistry Press, Washington, D.C. 267–282.
38. Iverius, P. H., and J. D. Brunzell. 1985. Human adipose tissue lipoprotein lipase: changes with feeding and relation to postheparin plasma enzyme. *Am. J. Physiol.* **249:** E107–E114.
39. Dullaart, R. P., W. J. Sluiter, L. D. Dikkeschei, K. Hoogenberg, and A. Van Tol. 1994. Effect of adiposity on plasma lipid transfer protein activities: a possible link between insulin resistance and high density lipoprotein metabolism. *Eur. J. Clin. Invest.* **24:** 188–194.
40. Despres, J. P., M. Ferland, S. Moorjani, A. Nadeau, A. Tremblay, P. J. Lupien, G. Theriault, and C. Bouchard. 1989. Role of hepatic-triglyceride lipase activity in the association between intra-abdominal fat and plasma HDL cholesterol in obese women. *Arteriosclerosis.* **9:** 485–492.
41. Cheung, M. C., R. H. Knopp, B. Retzlaff, H. Kennedy, G. Wolfbauer, and J. J. Albers. 2002. Association of plasma phospholipid transfer protein activity with IDL and buoyant LDL: impact of gender and adiposity. *Biochem. Biophys. Acta.* **1587:** 53–59.
42. Shen, M. M., R. M. Krauss, F. T. Lindgren, and T. M. Forte. 1981. Heterogeneity of serum low density lipoproteins in normal human subjects. *J. Lipid Res.* **22:** 236–244.
43. Santamarina-Fojo, S., and K. A. Dugi. 1994. Structure, function and role of lipoprotein lipase in lipoprotein metabolism. *Curr. Opin. Lipidol.* **5:** 117–125.
44. Thuren, T. 2000. Hepatic lipase and HDL metabolism. *Curr. Opin. Lipidol.* **11:** 277–283.
45. Capell, W. H., A. Zambon, M. A. Austin, J. D. Brunzell, and J. E. Hokanson. 1996. Compositional differences of LDL particles in normal subjects with LDL subclass phenotype A and LDL subclass phenotype B. *Arterioscler. Thromb. Vasc. Biol.* **16:** 1040–1046.