PLTP activity in premenopausal women: relationship with lipoprotein lipase, HDL, LDL, body fat, and insulin resistance

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Abstract **Plasma phospholipid transfer protein (PLTP) is thought to play a major role in the facilitated transfer of phospholipids between lipoproteins and in the modulation of high density lipoprotein (HDL) particle size and composition. However, little has been reported concerning the relationships of PLTP with plasma lipoprotein parameters, lipolytic enzymes, body fat distribution, insulin, and glucose in normolipidemic individuals, particularly females. In the present study, 50 normolipidemic healthy premenopausal females were investigated. The relationships between the plasma PLTP activity and selected variables were assessed. PLTP activity was significantly and positively correlated with low density lipoprotein (LDL) cholesterol (r_s = 0.53), apoB (r_s = 0.44), glucose (r_s = 0.40), HDL cholesterol (r_s = 0.38), HDL_3 cholesterol (r_s = 0.37), lipoprotein lipase activity (r_s = 0.36), insulin (r_s = 0.33), subcutaneous abdominal fat (r_s = 0.36), intra-abdominal fat (r_s = 0.29), and body mass index (r_s = 0.29). HDL_2 cholesterol, triglyceride, and hepatic lipase were not significantly related to PLTP activity. As HDL_2 can be decreased by hepatic lipase and hepatic lipase is increased in obesity with increasing intra-abdominal fat, the participants were divided into sub-groups of non-obese (n = 35) and obese (n = 15) individuals and the correlation of PLTP with HDL_2 cholesterol was re-examined. In the non-obese subjects, HDL_2 cholesterol was found to be significantly and positively related to PLTP activity (r_s = 0.44). Adjustment of the HDL_2 values for the effect of hepatic lipase activity resulted in a significant positive correlation between PLTP and HDL_2 (r_s = 0.41), indicating that the strength of the relationship between PLTP activity and HDL_2 can be reduced by the opposing effect of hepatic lipase on HDL_2 concentrations. We conclude that PLTP-facilitated lipid transfer activity is related to HDL and LDL metabolism, as well as lipoprotein lipase activity, adiposity, and insulin resistance.**—Murdoch, S. J., M. C. Carr, J. E. Hokanson, J. D. Brunzell, and J. J. Albers. **PLTP activity in premenopausal women: relationship with lipoprotein lipase, HDL, LDL, body fat, and insulin resistance.** ***J. Lipid Res.*** **2000.** 41: **237–244.**

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Phospholipid transfer protein (PLTP) is considered to be responsible for the majority of the facilitated transfer and exchange of phospholipids occurring between lipoproteins in plasma (1). As a result of this transfer activity, PLTP is thought to play a role in the augmentation of high density lipoprotein (HDL) mass (2) as well as modification of the HDL particle composition and size (3, 4). Consequently, PLTP may dictate the distribution of HDL subpopulations (3, 5, 6).

PLTP has been suggested to affect HDL mass and distribution by promoting the transfer to HDL of the redundant surface of very low density lipoprotein (VLDL) or chylomicrons that are undergoing core shrinkage due to lipoprotein lipase-induced triglyceride hydrolysis (7, 8). The transfer of redundant surface, which would include the reported PLTP-induced facilitated transfer of unesterified cholesterol (9) as well as phospholipid, would augment HDL mass, particularly that of HDL_2 (8) and may minimize vesicle formation (8, 10), particularly in conditions of elevated plasma free fatty acids. Vesicles have been identified in atherosclerotic lesions and may be involved in the development of atherosclerosis (11). From these observations, we postulate that PLTP-facilitated lipid transfer would be positively related to HDL, particularly HDL_2 concentrations, as well as to components of lipolysis such as lipoprotein lipase and plasma triglyceride.

PLTP has also been reported to modify HDL by another mechanism which involves particle fusion (3, 4) resulting in the remodeling of HDL primarily into larger particles with loss of apoA-I as well as into smaller particles that are rich in apoA-I (4). The smaller lipid-poor particles are similar to pre-beta HDL, which has been suggested to be

Abbreviations: PLTP, phospholipid transfer protein; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; apo, apolipoprotein; BMI, body mass index; NIDDM, non-insulin-dependent diabetes mellitus; LPL, lipoprotein lipase; HL, hepatic lipase; IAF, intra-abdominal fat; SQAF, subcutaneous abdominal fat; CETP, cholesteryl ester transfer protein.

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the initial acceptor of membrane cholesterol. Thus the lipid-poor HDL particles formed by PLTP-induced remodeling of HDL may play a role in promoting cellular cholesterol efflux.

Despite these in vitro observations, the specific role of PLTP in human metabolism has not been thoroughly investigated. The few studies carried out on small numbers of normolipidemic subjects found no relationship between PLTP activity (12, 13) or PLTP mass (14) and plasma lipids or lipoprotein parameters. However, PLTP activity was found to be positively related to body mass index (BMI) in a study of obese and non-obese males (15). PLTP activity tended to be higher in male Type II diabetics (16) and was highest when male Type II diabetics were also obese (17). PLTP mass was also increased in Type II diabetics (14). In healthy males subjected to a hyperglycemic clamp, PLTP activity decreased as did plasma triglyceride and free fatty acids, and PLTP activity was found to be negatively correlated with insulin sensitivity (18). These observations suggest that PLTP activity may be regulated by or coordinated with free fatty acid metabolism. On the other hand, in a study of male and female subjects with NIDDM, PLTP mass was positively correlated with glycemia but not with insulin or plasma lipids (14) and the authors suggested that PLTP was related more to glucose metabolism than to lipid metabolism. The relationships of PLTP with plasma lipids may vary with gender as PLTP activity has been reported to be slightly higher in male mice as compared to female mice (19) and no human studies to date have specifically focused on females.

We therefore undertook an investigation of the relationship of PLTP with selected plasma lipoprotein parameters, enzymes involved in plasma lipid metabolism, and a variety of measurements of body fat and insulin resistance in a cohort of normal healthy premenopausal women. Females were specifically studied to compare or confirm the previously reported observations in men of the relationship of PLTP with plasma lipids, insulin, and obesity.

METHODS

Subjects

Fifty healthy premenopausal women (ages 42–54 years) were recruited at random. The study group was comprised of 47 Caucasians and 3 non-Caucasians and was part of a larger group previously described (20). Characteristics of the subjects are listed in Table 1. 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.

The women were deemed premenopausal through the use of daily menstrual diaries, with the requirement of at least one menstrual cycle occurring in the previous 6-month period.

None of the subjects were taking medications affecting lipid metabolism including oral contraceptives. They did not demonstrate any lipid disorders, diabetes, or liver disease nor were they pregnant. Subjects were excluded if they had total triglyceride or low density lipoprotein (LDL) cholesterol concentrations above the 95th percentile for their age, small dense LDL as determined by polyacrylamide gradient gel electrophoresis (21) or a BMI greater than 40 kg/m^2.

This study was approved by the University of Washington Human Subjects Review Committee, and informed consent was given by each each subject prior to participation.

Sample collection

After a 12–14-h fast, blood was collected and immediately placed on ice. For lipid measurements and phospholipid transfer protein, lipoprotein lipase and hepatic lipase activity assays as well as density gradient ultracentrifugation, blood was collected in 0.1% EDTA. For determination of insulin and glucose, blood was collected in sodium heparin from supine subjects at 15, 20, and 25 min after placement of an intravenous line. 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, glucose, and insulin

Total cholesterol, triglyceride, LDL-cholesterol, apoB, HDL, HDL_2 and HDL_3 cholesterol were determined by standardized methodologies at the Northwest Lipid Research Laboratories (22). HDL and HDL_3 cholesterol were determined in the plasma supernatant after precipitation with dextran sulfate and magnesium chloride (23, 24). A mean value for glucose and insulin was obtained from the three sequential samplings. Insulin was determined by RIA and glucose was measured by the glucose oxidase method (25).

PLTP activity

PLTP activity was determined by measuring the transfer of labeled phosphatidylcholine from vesicles to HDL_3 (lacking apoE) as previously described (12) but without the use of plasma as a carrier. This method reflects the phospholipid transfer activity of PLTP but not that of cholesteryl ester transfer protein (CETP) (12). 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) was 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 250 μl of TSEA buffer (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 samples were incubated for 15 min at 37°C. This incubation time and plasma volume has been shown to measure PLTP activity in the linear part of the PLTP activity curve (12). The same batch of vesicles and HDL was used for all PLTP assays to maintain consistency. After the 15-min incubation, the samples were placed on ice. When cool, 500 μl TSEA and 100 μl of a 1% dextran sulfate/0.5 m $MgCl_2$ solution were added to precipitate the vesicles. After 30 min on ice, the samples were centrifuged at 4°C for 30 min at 3,000 rpm and the radioactivity in 600 μl of the supernatant was counted to determine the amount of radioactivity transferred from the vesicles to HDL during the incubation period. The activity is expressed in micromoles of phospholipid transferred per ml of plasma per h. The carrier plasma used in previous assays was found to be unnecessary for vesicle precipitation and was thus excluded as the radioactivity that remained in the supernatant in the absence of a carrier was equivalent to <0.3% transfer activity. Furthermore, the exclusion of the carrier eliminated a variable that may vary from batch to batch. For each assay the background transfer to HDL in the absence of plasma was subtracted from the activity measured in the presence of plasma. Three human control plasmas, flash frozen and stored at −70°C until use, were included in quadru-

plicate 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 (26). 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 7.2, 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 (LPL) allowed for the calculation of the lipoprotein lipase activity. Hepatic lipase (HL) 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 10% for LPL and 14% for HL.

Determination of body composition

Total body fat and total abdominal fat were determined using a single X-ray DEXA (dual-energy X-ray absorptiometry) scan (Hologic QDR 1500). The inter-assay coefficient of variation for fat mass, lean mass, and % body fat were 1.6%, 1.3%, and 1.3%, respectively.

Intra-abdominal fat and subcutaneous abdominal fat were measured by CT scanning (GE high speed Advantage) at the level of the umbilicus. A single image was analyzed by a single blinded observer for a cross-sectional area of fat using a density contour program as previously described (27).

Statistical analyses

Pearson and Spearman rank order correlation coefficients were calculated to determine correlations between PLTP and the variables listed in Table 2. Spearman correlations were used to reduce the possible contribution of any outliers to the correlation. Results are reported as the mean and the standard deviation. Nonparametric Mann-Whitney U Test was used to determine significant differences between the parameters of the non-obese as compared to the obese group. Multiple Linear Regression was used to analyze the relationship between selected variables using Sigma Stat and Statview software.

RESULTS

The characteristics of the subjects are listed in **Table 1**. The subjects were normolipidemic by selection with an average total cholesterol and triglyceride concentration of 182 ± 30 mg/dl and 73 ± 32 mg/dl, respectively. The clinical characteristics of the participants were in the normal range with respect to glucose, insulin, lipoprotein lipase (LPL), and hepatic lipase (HL) activity. Fifteen subjects were classified as obese, having a BMI greater than 27 kg/m^2.

The correlations of plasma PLTP activity with various plasma components for all the subjects are listed in **Table 2**. Pearson and Spearman rank order correlation coefficients resulted in similar correlations in most cases. Only Spearman correlations are reported. PLTP activity showed the strongest positive correlation with LDL cholesterol (r_s = 0.53) followed by total apoB (r_s = 0.44). PLTP activity was significantly and positively correlated with HDL cholesterol (r_s = 0.38) and HDL_3 cholesterol (r_s = 0.37) but the relationship with HDL_2 cholesterol did not attain significance. PLTP activity was positively correlated with LPL activity (r_s = 0.36) but was not related to HL activity. Measurements of body fat such as intra-abdominal fat (IAF) (r_s = 0.29), subcutaneous abdominal fat (SQAF) (r_s = 0.36) as well as body mass index (BMI) (r_s = 0.29) were positively correlated with PLTP activity but the correlation with total body fat did not reach significance. Insulin (r_s = 0.33) and glucose concentrations (r_s = 0.40) were also positively related to PLTP activity. The variables that were found to be significantly correlated with PLTP activity in univariate analysis (Table 2) were included in multiple linear regression analysis to determine the extent of PLTP activity that could be predicted by the combined variables. Using stepwise linear regression analysis, the variables that resulted in the strongest relationship and remained significant were HDL (partial r = 0.493, P = <0.001), LDL (partial r = 0.452, P = <0.001), and insulin (partial r = 0.365, P = 0.003) resulting in a combined r of 0.716 (P = <0.001), predicting 51% of the variance in PLTP activity. The results were similar when apoB was substituted for LDL or HDL_3 was substituted for HDL.

Although the participants were considered representative of a normal population, 15 (30%) of the subjects had a BMI greater than 27 kg/m^2 and would be considered obese. To determine whether the correlations observed between PLTP activity and the various parameters measured were due to the presence of obese individuals in the study, the participants were divided into a non-obese and an obese group, based on their BMI. The characteristics of the two groups are listed in **Table 3**. The obese group

TABLE 1. Characteristics of the subjects (n = 50)

Variable	Mean	Range
Age (yr)	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/h)	14.9 ± 2.2	10.5–20.3
LPL activity (μmol/ml/h)	12.2 ± 6.2	3.6–35.7
HL activity(μmol/ml/h)	7.9 ± 3.2	2.7–16.9
Insulin (μU/ml)	11 ± 6	4–33
Glucose (mg/dl)	75 ± 6	63–89
Weight (kg)	70.9 ± 15.3	49.2–112.0
BMI (kg/m^2)	25.6 ± 4.7	18–37.9
IAF (cm^2)	63.3 ± 40.4	16.4–172.5
SQAF (cm^2)	235.4 ± 132.7	63.7–612.2
Total body fat (%)	37.3 ± 7.4	26–52

Values are means ± SD; PLTP, phospholipid transfer protein; LPL, lipoprotein lipase; HL, hepatic lipase; BMI, body mass index; IAF, intraabdominal fat (CT scan); SQAF, subcutaneous abdominal fat (CT scan); total body fat by DEXA scan.

TABLE 2. Correlations between PLTP and variables

PLTP Activity and	Spearman Correlation Coefficients: All Subjects (n = 50)	P	BMI <27 kg/cm² (n = 35)	P	BMI ≥27 kg/cm² (n = 15)	P
HDL cholesterol	0.38[b]	0.007	0.52[c]	0.001	0.119	0.672
HDL_2 cholesterol	0.27	0.056	0.44[b]	0.009	0.240	0.389
HDL_3 cholesterol	0.37[b]	0.008	0.51[b]	0.002	0.087	0.757
LDL cholesterol	0.53[c]	0.000	0.51[b]	0.002	0.472	0.075
ApoB	0.44[b]	0.002	0.40[a]	0.018	0.375	0.168
Triglyceride	0.05	0.713	−0.01	0.963	−0.032	0.909
LPL	0.36[a]	0.011	0.34[a]	0.045	0.351	0.200
HL	0.21	0.154	0.21	0.219	−0.315	0.253
Insulin	0.33[a]	0.020	0.31	0.069	0.244	0.382
Glucose	0.40[b]	0.004	0.36[a]	0.035	0.282	0.308
BMI	0.29[a]	0.044	0.16	0.345	0.154	0.584
IAF	0.29[a]	0.043	0.11	0.534	0.157	0.575
SQAF	0.36[a]	0.011	0.29	0.097	0.100	0.722
Total body fat	0.23	0.104	0.09	0.609	0.169	0.547

PLTP, phospholipid transfer protein; LPL, lipoprotein lipase; HL, hepatic lipase; BMI, body mass index; IAF, intraabdominal fat (CT Scan); SQAF, subcutaneous abdominal fat (CT scan).

[a] $P \leq 0.05$; [b] $P \leq 0.01$; [c] $P \leq 0.001$.

demonstrated higher triglyceride, apoB, HL activity, insulin, glucose, IAF, SQAF, and total body fat and lower HDL_2 cholesterol as compared to the non-obese group. The difference between PLTP activity in the non-obese as compared to the obese group did not reach significance. In the non-obese group (BMI < 27 kg/m^2) (n = 35) (Table 2), the majority of the correlations with PLTP activity were similar to those previously determined for all the subjects (Table 2). However, the positive relationship between PLTP activity and HDL_2 was significant in the non-obese group and the positive correlations with HDL, HDL_2, and HDL_3 cholesterol were stronger and similar to correlations between PLTP activity and LDL or apoB. The positive correlation between PLTP activity and LPL or glucose remained but the correlation of PLTP activity with insulin did not reach significance. After the exclusion of the obese participants, the measurements of body fat were not significantly related to PLTP activity. In the obese group (BMI ≥ 27 kg/m^2) (n = 15), none of the variables listed were significantly correlated with PLTP activity (Table 2).

Initially, the reason for the lack of a significant relationship between PLTP activity and HDL_2 in the entire group of subjects was unclear if one accepts the premise that PLTP mediates the facilitated transfer of redundant surface to HDL during VLDL and chylomicron lipolysis, in vivo. Previous in vitro studies have demonstrated an increase in HDL_2 as a result of a shift in lipid from VLDL to HDL during LPL-induced VLDL lipolysis (28–30) which

TABLE 3. Characteristics of the subjects grouped by BMI

Variable	Mean BMI < 27 kg/m² n = 35	Mean BMI ≥ 27 kg/m² n = 15	P
Age (yr)	46.4 ± 3.3	46.7 ± 3.4	0.848
Total cholesterol (mg/dl)	180 ± 31	188 ± 27	0.403
Triglyceride (mg/dl)	65 ± 24	92 ± 40	0.022[a]
ApoB (mg/dl)	78 ± 14	89 ± 20	0.030[a]
LDL cholesterol (mg/dl)	106 ± 24	116 ± 24	0.223
HDL cholesterol (mg/dl)	60 ± 15	54 ± 13	0.096
HDL_2 cholesterol (mg/dl)	14 ± 7	11 ± 6	0.030[a]
HDL_3 cholesterol (mg/dl)	46 ± 9	43 ± 7	0.165
PLTP activity (μmol/ml/hr)	14.6 ± 2.5	15.5 ± 1.5	0.105
LPL activity(μmol/ml/hr)	12.2 ± 7.1	12.4 ± 4	0.374
HL activity(μmol/ml/hr)	7.1 ± 2.8	9.6 ± 3.3	0.007[b]
Insulin (μU/ml)	8.6 ± 3.1	15 ± 8.6	0.002[b]
Glucose (mg/dl)	74 ± 6	79 ± 5.1	0.008[b]
Weight (kg)	63.1 ± 7.4	89.1 ± 13.4	<0.001[c]
BMI (kg/m²)	23.3 ± 1.9	31.7 ± 3.7	<0.001[c]
IAF (cm²)	43.4 ± 20	109.6 ± 38.1	<0.001[c]
SQAF (cm²)	172.6 ± 65	382 ± 135.6	<0.001[c]
Total body fat (%)	34.1 ± 5.5	44.8 ± 5.5	<0.001[c]

Values are means ± SD; PLTP, phospholipid transfer protein; LPL, lipoprotein lipase; HL, hepatic lipase; BMI, body mass index; IAF, intraabdominal fat (CT scan); SQAF, subcutaneous abdominal fat (CT scan); total body fat by DEXA scan.

[a] $P \leq 0.05$; [b] $P \leq 0.01$; [c] $P \leq 0.001$.

TABLE 4: Multiple linear regression analysis to determine independent associations with HDL_2

	Independent Variable									
	PLTP Activity		IAF		HL		LPL		Combined	
	r_p	P	r_p	P	r_p	P	r_p	P	r_c	P
Dependent variable										
HDL_2	0.38	0.006	−0.44	0.001	—	—	—	—	0.51	0.001
HDL_2	0.34	0.009	—	—	−0.49	<0.001	—	—	0.55	<0.001
HDL_2	—		—	—	−0.41	<0.001	0.51	<0.001	0.67	<0.001
HDL_2	0.06	0.687	—	—	—	—	0.51	<0.001	0.54	<0.001

Table 4 contains the multiple linear regression analyses that were used to assess whether an independent variable (PLTP activity) was significantly related to the dependent variable (HDL_2) after controlling for a second independent variable (IAF or HL) or to determine whether an independent variable (PLTP activity or HL) remained significantly related to the dependent variable (HDL_2) when a second independent variable (LPL) was included in the regression analysis; r_p, partial r; r_c, combined r; PLTP, phospholipid transfer protein; IAF, intra-abdominal fat; HL, hepatic lipase; LPL, lipoprotein lipase.

was augmented when a preparation containing PLTP and CETP activity was added to the lipolysis incubation (8). In the present study, when the subjects were separated into the two groups based on their BMI, the correlation of PLTP activity with HDL_2 became significant in the non-obese subjects. Thus it is possible that adiposity affects the HDL_2 concentration in such a way that the positive relationship of HDL_2 with PLTP activity cannot be detected. Because HDL_2 is reduced as intra-abdominal fat increases (20, 31), we questioned whether this effect masked the positive relationship of HDL_2 with PLTP activity. Using multiple linear regression analysis with HDL_2 as the dependent variable and IAF and PLTP activity as the independent variables (**Table 4**), it was confirmed that PLTP activity was significantly and positively associated with HDL_2 (partial $r = 0.38$) while IAF was negatively related (partial $r = -0.44$) and both variables contributed to the prediction of the HDL_2 concentration (combined $r = 0.51$).

Intra-abdominal fat can regulate HL activity (20, 32). Increased HL activity is considered to be responsible for the decrease in HDL_2 that occurs with an increase in intra-abdominal fat. HL was correlated with IAF ($r = 0.51$, $P = 0.001$) in these individuals. In univariate analysis, HL was also negatively correlated with HDL_2 ($r_s = -0.50$, $P < 0.001$) (demonstrating a weaker negative correlation with HDL_3, $r_s = -0.31$, $P = 0.029$). Thus, the negative association of IAF with HDL_2 may reflect the activity of HL in the present study. Using multiple linear regression analysis (Table 4), HL demonstrated a negative association with HDL_2 (partial $r = -0.49$) similar to that of IAF whereas PLTP activity demonstrated a significant positive relationship with HDL_2 (partial $r = 0.34$) (combined $r = 0.55$). When the HDL_2 concentrations were adjusted for the effect of HL activity, the univariate correlation between PLTP activity and the adjusted HDL_2 became significant ($r_s = 0.41$, $P = 0.004$) increasing from an r_s of 0.27 ($P = 0.056$) using the unadjusted HDL_2 values (Table 2), confirming the results of the multivariate analysis.

LPL has been found to influence HDL_2 concentrations in a manner that is distinct from that of HL (33). This was confirmed in the present study. In multivariate analysis (Table 4), LPL was positively associated with HDL_2 (partial $r = 0.51$) and HL was negatively related (partial $r = -0.41$) with a combined r of 0.67. To determine whether the relationship between PLTP activity and HDL_2 was independent of LPL activity, the separate contribution of PLTP activity to the prediction of HDL_2 concentrations was determined by adjusting the HDL_2 values for LPL as well as HL activity. The HDL_2 concentrations adjusted for the contribution of both LPL and HL were not related to PLTP activity in univariate analysis suggesting that the positive relationship of PLTP activity with HDL_2 is dependent upon LPL activity. This observation was confirmed using multivariate analysis (Table 4) which demonstrated that LPL was positively and significantly related to HDL_2 concentration (partial r = 0.51) but the association of PLTP activity with HDL_2 (or HDL_2 adjusted for HL) was no longer significant. This was also the case for HDL and HDL_3 in multivariate analysis. LPL activity was significantly related to HDL (partial $r = 0.56$, $P < 0.001$) and HDL_3 (partial $r = 0.54$, $P < 0.001$) but PLTP was not significantly related to HDL and HDL_3.

To ensure that obesity, which can increase the mass of adipose LPL per cell (34) while decreasing skeletal muscle LPL (35), is not responsible for the observed relationship between LPL and PLTP activity, the association of LPL with various measures of body fat was determined. LPL was not correlated with IAF, SQAF, and percent body fat in univariate analysis. Furthermore, the significant positive correlation between LPL and PLTP activity was maintained in the participants after the exclusion of the obese subjects (Table 2), suggesting that obesity does not contribute significantly to the observed association between post-heparin LPL activity and PLTP activity. LPL activity may also be affected by insulin but LPL was not correlated with insulin in these individuals.

LDL-cholesterol and apoB showed the highest positive correlation with PLTP activity ($r_s = 0.53$, $P < 0.001$) in the entire group but was similar to the positive correlation between HDL and PLTP in the non-obese group ($r_s = 0.51$, $P = 0.002$). LDL was also positively correlated with VLDL-cholesterol ($r_s = 0.54$, $P < 0.001$), triglyceride ($r_s = 0.53$, $P < 0.001$), glucose ($r_s = 0.48$, $P < 0.001$) and IAF ($r_s = 0.32$, $P = 0.023$) but in multivariate analysis, PLTP (partial

$r = 0.44$, $P < 0.001$) and triglycerides (partial $r = 0.42$, $P = 0.001$) remained significantly and positively related to LDL cholesterol (combined $r = 0.70$, $P < 0.001$) while glucose and IAF no longer demonstrated a significant association.

DISCUSSION

The results of the present study indicate that PLTP activity is positively related to HDL and LDL/apoB metabolism as to well as to LPL, insulin, glucose, and adiposity in healthy normolipidemic premenopausal women.

The positive correlation of HDL_2 and HDL_3 with PLTP has not been previously observed in healthy normolipidemic females and males (12, 14) whether obese or non-obese (15). The only previous report of a relationship of PLTP activity with HDL was in a study of individuals having low HDL and cardiovascular disease (6). The HL-induced reduction of HDL concentrations may have concealed the relationship between PLTP and HDL (HDL_2 in particular) in the study of obese subjects. The relationship of PLTP activity with HDL_2 was shown to be independent of and opposite to HL activity in the present study. The results suggest that as HL reduces HDL_2 with increasing IAF, as previously reported in these women (20), the PLTP-associated augmentation of HDL_2 is masked. Furthermore, although PLTP activity may increase with IAF potentially increasing HDL, the counteracting decrease of HDL due to HL activity may overshadow this effect. In contrast to the lack of association with HL activity, the effect of PLTP activity on HDL, HDL_2 and HDL_3 appears to be dependent upon LPL activity. This may reflect the interrelationship between LPL and PLTP in the regulation of HDL concentrations in that LPL-induced VLDL and chylomicron triglyceride hydrolysis is required before PLTP can maximally increase HDL mass by the facilitated transfer of the surface lipids.

The relationship between PLTP activity and HDL has been reported in several animal studies. PLTP activity was highly positively correlated with HDL cholesterol and phospholipid and HDL size in multiple inbred mouse strains and among (SMxNZB) F1xSM backcross animals (19). When the various strains of mice were placed on a high fat, high cholesterol diet, the change in PLTP activity was positively correlated with the change in HDL cholesterol, phospholipid and HDL peak size. In a study of transgenic mice, a decrease in PLTP activity correlated with a decrease in HDL cholesterol and HDL size (36), providing further support to the concept that PLTP activity appears to be a determinant of HDL concentration and size.

The positive association of HDL, HDL_2 and HDL_3 concentrations with PLTP activity is consistent with the suggested role of PLTP in transferring excess surface to HDL during VLDL lipolysis. However, because the transfer of surface, in vitro, has been found to augment HDL_2 mass with a concurrent shift in the HDL_3 mass to the HDL_2 density, a stronger correlation with HDL_2 than with HDL_3 would have been expected if the transfer of redundant surface is the sole cause of the relationship between PLTP activity and the HDL fractions. Therefore, the correlation with both HDL_2 and HDL_3 fractions may also reflect the role of PLTP in HDL modification in that HDL particles are converted to both larger (HDL_2) and smaller (HDL_3) particles through the action of PLTP.

The relationship of LPL with PLTP activity has not been previously reported. The positive relationship observed between LPL and PLTP activities may reflect the role of PLTP in the transfer of redundant surface during VLDL and chylomicron metabolism. Possibly coordinate regulation exists for PLTP and LPL synthesis or secretion to ensure adequate surface transfer with an increased rate of lipolysis. LPL is synthesized mainly in adipose tissue and skeletal muscle and PLTP mRNA has been identified in substantial amounts in human adipose tissue (37) as well as in skeletal muscle (5).

Previous studies have reported that PLTP activity and mass tend to be higher in subjects with NIDDM (14, 16), particularly when combined with obesity (17), and are correlated with glucose and glycated hemoglobin. However, glucose was not found to be related to PLTP mass in normolipidemic non-diabetic subjects, nor was insulin related in subjects with NIDDM (14). In the present study, the positive correlation of insulin and glucose with PLTP activity in normolipidemic, normoglycemic women suggests that these relationships with PLTP exist even in a non-diabetic population. Possibly, the fewer subjects and inclusion of both men and women in the previous study (14) compared to the present study may be responsible for the differing results. Furthermore, the basal insulin level, determined from three sequential blood samplings in the present study, is a more accurate indicator of insulin resistance as compared to basal insulin determined from a single sampling as there are inherent fluctuations in plasma insulin concentrations.

BMI has been reported to be positively related to PLTP activity in men (15, 17) and this relationship was confirmed in premenopausal women in the present study. PLTP activity was positively correlated with most of the measures of adiposity and the correlations fell in a similar range ($r_s = 0.29–0.38$). The relationship of PLTP activity with IAF and subcutaneous abdominal fat has not been previously reported. PLTP activity appears to be positively related to both types of fat deposition. When the subjects were divided into non-obese and obese groups, the measurements of body fat in the non-obese group were no longer significantly related to PLTP. This may have occurred because the subjects were divided into the two groups based on their relative body mass (BMI), which reflects body fat in most cases, restricting the range of these variables and thus the detection of a significant correlation. In the obese group, none of the correlations between PLTP and the various parameters were significant, possibly due to the small number of subjects which limited the ability to detect a relationship. The previous study that reported increased PLTP activity in obese males (15) found that the obese subjects also had increased triglyceride and insulin concentrations as compared to the non-obese subjects. An

increase in triglyceride and insulin concentration was observed in the obese group in the present study along with an increase in apoB, HL activity, glucose and body fat measurements. The difference in PLTP activity between the obese and non-obese subjects did not reach significance in the present study. This observation may suggest that the relationship of PLTP activity with BMI and other body fat measurements, IAF in particular, primarily reflects the association of PLTP activity with insulin resistance that can accompany an increase in body fat.

The positive association of PLTP activity with LDL cholesterol and apoB and lack of a correlation with triglyceride was somewhat unexpected in the context of the role of PLTP in the transfer of redundant surface. However, in normolipidemic individuals where triglyceride is relatively low and uptake of LDL is not rate limiting, the correlation between LDL or apoB and PLTP activity may occur because the LDL cholesterol or apoB concentration represents the end product of VLDL lipolysis and therefore reflects the mass of excess surface that has been transferred to HDL. This relationship may not exist in hypertriglyceridemic individuals (particularly in those with large VLDL particles where the amount of surface would not be reflected by the amount of apoB or the cholesterol content) or in individuals with problems in remnant removal where the particles become enriched in cholesteryl ester by CETP as a result of their long plasma half-life. The majority of studies did not observe a positive relationship between PLTP activity or mass and non-HDL cholesterol (12–14) except in a group that included both obese and non-obese males (15). Triglyceride was also positively correlated with PLTP in this study (15).

Several of the positive correlations with PLTP activity were not observed in other studies of healthy normolipidemic individuals. The findings of a positive relationship between PLTP activity and LDL-cholesterol, apoB, HDL_2 and HDL_3 cholesterol may be due to the larger number of subjects investigated and the selection of premenopausal women. The fact that the correlations were observed in women rather than in men may be related to hormonal differences.

In conclusion, PLTP activity appears to be positively related to HDL as well as LDL/apoB metabolism in agreement with its proposed role in lipoprotein metabolism. The positive relationship between PLTP activity and LPL, insulin, glucose and obesity points to a role in fatty acid and/or glucose metabolism. Possibly, under conditions of increased free fatty acid flux that can occur with insulin resistance, obesity and impaired glucose tolerance, PLTP synthesis is increased. PLTP mRNA has been identified in both the liver and the small intestine (5), sources of VLDL and chylomicron production. An increased amount of PLTP under conditions of elevated plasma free fatty acids may prevent vesicle formation. Vesicles have been observed in PLTP knockout mice on a high fat diet (10), suggesting a role for PLTP in shuttling excess surface lipid to HDL as a means of avoiding vesicle formation. Elevated PLTP may be required in situations of insulin resistance to facilitate the transfer of an augmented amount of VLDL surface mass present as a result of elevated VLDL production in the liver (38). The role of PLTP in glucose metabolism may be intertwined with that of free fatty acid metabolism under conditions of insulin resistance. The answer awaits the results of future studies focusing on NIDDM and insulin resistance.

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