PLTP activity decreases with weight loss: changes in PLTP are associated with changes in subcutaneous fat and FFA but not IAF or insulin sensitivity

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Abstract Phospholipid transfer protein (PLTP) activity is elevated in obese and diabetic subjects. No prospective studies have examined the effect of weight loss on PLTP activity and assessed whether the resultant changes in activity are related to changes in body weight, insulin resistance, or both. PLTP activity was measured at baseline in 46 subjects (body mass index = 19–64 kg/m^2) and after diet-induced weight loss in 19 of the obese subjects. Total body fat mass (FM) by dual-energy X-ray absorptiometry, intraabdominal fat (IAF), and abdominal subcutaneous fat (SQF) by CT scan, insulin sensitivity (S_I) by frequently sampled intravenous glucose tolerance test, leptin, and lipids were determined. At baseline, PLTP activity correlated with FM (r = 0.36, P = 0.02) and SQF (r = 0.31, P = 0.045), but not with IAF (r = 0.16, P = 0.32) or S_I (r = 0.10, P = 0.52). With diet-induced weight loss (16 ± 7.3 kg), PLTP activity significantly decreased 9.1% (P = 0.002). The change in PLTP activity correlated with the change in SQF (r = 0.55, P = 0.014) (33.6% decrease), but not with IAF (r = 0.09, P = 0.73) or S_I (r = 0.18, P = 0.44), and was highly correlated with the change in nonesterified fatty acid (NEFA) (r = 0.71, $P < 0.001$). In conclusion, elevated PLTP activity in obese subjects is likely a result of increased body fat, reflected by SQF, and is influenced by NEFAs but is not directly related to insulin resistance.—Murdoch, S. J., S. E. Kahn, J. J. Albers, J. D. Brunzell, and J. Q. Purnell. **PLTP activity decreases with weight loss: changes in PLTP are associated with changes in subcutaneous fat and FFA but not IAF or insulin sensitivity. *J. Lipid Res.* 2003.** 44: **1705–1712.**

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Phospholipid transfer protein (PLTP) is a plasma protein that has been reported to have significant effects on both lipid and lipoprotein metabolism (1–4). The initial role suggested for PLTP was one of transfer of surface from VLDL to HDL, augmenting HDL mass and buoyancy (2, 5, 6). PLTP can mediate HDL particle fusion (4, 7) and facilitate cellular cholesterol and phospholipid efflux (3). PLTP has also been found to be related to LDL metabolism (8, 9), and is specifically positively associated with buoyant LDL mass (10, 11).

PLTP has been reported to be elevated in obese individuals (8, 11–13) and in diabetic subjects (14–16) and has been associated with insulin resistance (14, 17, 18). However, no perturbation studies have been carried out to date to confirm the selective effect of either weight loss or change in diabetic status/insulin resistance on PLTP activity. The separation of the effect of obesity from that of diabetes and insulin resistance on PLTP activity is complex, because insulin resistance tends to increase with weight gain (12, 13), and type 2 diabetic subjects, who are insulin resistant, are also frequently obese (14, 15). Thus, it is difficult to determine whether the reported increase in PLTP with obesity is due to an increase in fat mass per se or due to the specific metabolic consequences associated with diabetes/insulin resistance. As weight gain occurs, both intraabdominal fat (IAF) and subcutaneous fat (SQF) increase, and the relative increase in each fat depot varies with the individual. It is the amount of IAF as compared with SQF fat that has been found to be more strongly associated with insulin resistance (19) in both obese as well as lean individuals. Because PLTP mRNA has been identified in both IAF and SQF (20), the elevated PLTP associated with diabetes or obesity may be the result of an increased production of

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Abbreviations: FM, total body fat mass; HL, hepatic lipase; IAF, intraabdominal fat; LPL, lipoprotein lipase; PLTP, phospholipid transfer protein; S_I, insulin sensitivity; SQF, subcutaneous fat.

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PLTP in IAF (related to insulin resistance) and/or in SQF (related to total body fat). Therefore, the investigation of the relationship of changes in PLTP with changes in SQF and weight loss in comparison to changes in IAF and insulin sensitivity (S_I) may elucidate the possible mechanisms responsible for the effects of obesity and diabetes on PLTP activity, leading to further hypotheses and testing.

In the following study, we initially investigated the relationship of PLTP activity with variables related to body fat and insulin resistance in a cross-sectional study involving a large group of both obese and nonobese subjects. These subjects exhibited a wide range of body mass index (BMI) values and were thus an ideal group in which to study the reported positive associations of PLTP activity with obesity, insulin resistance, SQF, and IAF. From this baseline group, a subset of the subjects comprised of obese individuals was then studied before and after weight loss, a perturbation that affects both body fat and insulin resistance. In this group, we investigated the influence of weight loss on plasma PLTP activity to determine if PLTP activity in fact decreased with weight loss and whether the change in PLTP activity was selectively related to changes in variables associated with fat mass or S_I.

METHODS

Subject selection

Subjects (n = 46) were recruited with a BMI ranging from 19 to 64 kg/m^2 for the cross-sectional study (**Table 1**). Of these subjects, 19 obese subjects participated in the weight loss study (**Table 2**). The subjects were otherwise normal, healthy individuals. In the baseline group, there were 26 females and 20 males (12 of the females were postmenopausal, six were taking estrogen). In the subjects who participated in the weight loss study, there were eight males and 11 females. Of the females, four were postmenopausal and two were taking estrogen. Exclusion criteria consisted of endocrine causes of obesity such as hypothyroidism or Cushing's syndrome, diabetes, excessive alcohol intake, smoking, or use of any medications. This study was approved by the University of Washington Human Subjects Review Committee, and informed consent was given by each subject prior to participation.

Sample collection

After a 12 h fast, an intravenous line was inserted. After a 15 min rest period, blood was sampled in 0.1% EDTA for PLTP activity, leptin, fasting lipid profile, and nonesterified fatty acid (NEFA) determination. NEFAs were sampled in 0.1% EDTA plus tetrahydrolipstatin to inhibit lipase activities. Three samples for NEFA and insulin analyses were taken 5 min apart. Using these methods of assaying NEFA and insulin, we are able to obtain values that we consider a valid measure of mean fasting NEFA and fasting insulin, because the values are less likely to be affected by a rise in catecholamines and in vitro hydrolysis in the case of NEFA, and the pulsatile variation in the case of plasma insulin. 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. Subsequently, a tolbutamide-modified frequently sampled intravenous glucose tolerance test (FSIVGTT) was performed on the subjects. These procedures were repeated after the 2 to 3 week weight-stabilization period after weight loss.

TABLE 1. Demographic and phenotypic characteristics of subjects at baseline

Variable	Baseline	Range
PLTP (μM/ml/h)	15.9 ± 2.4	11.7–24.5
HL (μM/ml/h)	10.4 ± 4.9	2.8–23
LPL (μM/ml/h)	10.6 ± 4.3	4.1–21.7
Weight (kg)	96.7 ± 33.9	49.8–225
BMI (kg/m^2)	33.2 ± 9.2	19–64
FM (kg)	33.6 ± 15.1	4.8–65.2
% FM	38.1 ± 13.3	7.2–58.4
SQF (cm^2)	378 ± 225	16.5–1,037
IAF (cm^2)	118 ± 68.5	6.8–295
$S_I \times 10^{-5}$ min^{-1}·[pmol/l]$^{-1}$	2.6 ± 2.34	0.5–11
Insulin (μU/ml)	14.6 ± 8.8	2.3–36.7
Glucose (mg/dl)	86 ± 10.8	58–106
Leptin (ng/ml)	22.4 ± 16.2	0.94–58.2
NEFA (mEq/l)	0.527 ± 0.23	0.13–1.16
TC (mg/dl)	179 ± 39	103–275
TG (mg/dl)	113 ± 108	21–548

BMI, body mass index; FM, total body fat mass; HL, hepatic lipase; IAF, intraabdominal fat; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; PLTP, phospholipid transfer protein; S_I, insulin sensitivity; SQF, subcutaneous fat; TC, total cholesterol; TG, triglyceride. All subjects (n = 46).

Weight loss protocol

After obtaining baseline plasma samples and determining body composition, obese subjects who participated in the weight loss study (n = 19) were placed on a ~1,000 kcal (41% protein, 56% carbohydrate, 2.4% fat) liquid formula diet with supplements to meet 100% RDA for vitamins and electrolytes. This hypocaloric diet continued until 15 kg to 20 kg weight loss was achieved (which occurred over a 3 to 6 month time period). There were 11 females and eight males. Of the females, four were postmenopausal and two were taking estrogen. At the end of the weight loss period, the subjects were restabilized for at least 2 weeks at a steady weight before plasma sampling and body composition was determined.

Analysis of plasma lipids

Total cholesterol (TC) and triglycerides (TGs) were determined by standardized methodologies at the Northwest Lipid Research Laboratories (21).

PLTP activity assay

PLTP activity was determined by measuring the transfer of labeled phosphatidylcholine from vesicles to HDL_3 (8, 22) without the use of plasma as a carrier, as previously described (8). This method reflects the phospholipid transfer activity of PLTP but not that of cholesteryl ester transfer protein. 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 with HDL_3 (150 nM phospholipid), 50 μl of diluted plasma (diluted 1/50, resulting in a 1 ml plasma equivalent), and 300 ml 10 mM Tris, 150 mM NaCl, 1 mM EDTA, and 0.1% sodium azide. 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, the vesicles were precipitated, and the radioactivity in the supernatant was used to determine the transfer of phospholipid from the vesicles to HDL. Three human control plasmas 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.

TABLE 2. Subjects in weight loss study (n = 19): changes with weight loss

Variable	Baseline	Range	After Weight Loss	Range	Change	Range	*P*	% Change
PLTP (uM/ml/h)	16.5 ± 2.7	11.7–24.5	15.0 ± 2.5	11.4–21.4	−1.5 ± 1.7	−1.9–0.64	0.002	−9.1
HL (uM/ml/h)	12.9 ± 5.6	5.3–23	8.3 ± 4.1	2.6–16.2	−4.6 ± 3.2	−12.7–0.4	<0.001	−35.7
LPL (uM/ml/h)	10.4 ± 3.7	5.5–18.4	8.9 ± 3.7	3.1–16.9	−1.5 ± 5.2	−9.7–10.4	0.2	−14.4
Weight (kg)	104 ± 28	71–184	88 ± 25	57–159	−16 ± 7.3	−30–−4	<0.001	−15.4
BMI (kg/m^2)	36.1 ± 6.6	29–54	30.4 ± 6.2	23–47	−5.7 ± 2.4	−9.7–−1.5	<0.001	−15.8
FM (kg)	42.8 ± 10.6	27–65	29.1 ± 10	13–45.8	−13.7 ± 7.1	−31–−2.3	<0.001	−32.0
% FM	44.6 ± 7.4	31.5–58.4	36.0 ± 10	18.6–49.3	−8.6 ± 5	−21–−0.1	<0.001	−19.3
SQF (cm^2)	467 ± 156	224–723	310 ± 141	81–534	−157 ± 83	−325–−51	<0.001	−33.6
IAF (cm^2)	140 ± 66	31–295	78 ± 49	14.6–208	−62 ± 41	−145–−7	<0.001	−44.3
$S_I \times 10^{-5}\,min^{-1} \cdot [pmol/l]^{-1}$	1.90 ± 1.6	0.5–7	2.82 ± 2.2	1.0–10.7	+0.92 ± 1.3	−1.92–3.7	0.007	+48.4
Insulin (μU/ml)	16.7 ± 8.4	4.4–32.7	11.1 ± 5.6	3.3–20.3	−5.6 ± 5.1	−13.4–2.4	0.04	−33.5
Glucose (mg/dl)	82 ± 12	65–99	90 ± 9.8	65–108	+ 8 ± 14.9	−22–29	0.03	+9.8
Leptin (ng/ml)	26.5 ± 14	8–57	17.6 ± 12	2–39	−8.9 ± 8.8	−25–10.7	0.04	−33.6
NEFA (mEq/l)	0.531 ± 0.262	0.13–1.16	0.607 ± 0.267	0.25–1.4	+0.076 ± 0.3	−0.42–0.59	0.2	+14.3
TC (mg/dl)	194 ± 34	131–275	174 ± 47	112–29	−20 ± 27	−68–38	0.005	−10.3
TG (mg/dl)	126 ± 128	21–548	112 ± 100	30–429	−14 ± 54	−119–79	0.28	−11.1

Lipoprotein lipase and hepatic lipase activities

A heparin bolus was administered intravenously and samples were attained for determination of plasma TG lipase activities, as previously described (23). In brief, postheparin plasma, diluted 1:10 with KRP buffer, pH 7.4, was incubated with a triolein-phosphatidylcholine-albumin emulsion, trace labeled with glycerol tri-[1-^{14}C]oleate (Amersham, Arlington Heights, IL) in 0.178 M Tris-HCl, 0.11 M NaCl buffer, pH 8.5, containing 55 mg/ml albumin and .01 mg/ml heparin, resulting in a final incubation pH of 8.2. The incubation was carried out for 60 min at 37°C and the resultant free fatty acids were extracted and ^{14}C content determined. The inclusion of a monoclonal antibody specific for lipoprotein lipase (LPL) allowed for the determination of LPL activity and hepatic lipase (HL) activity. A bovine skim milk LPL standard was included in each assay and used to correct for inter-assay variation. A human postheparin control plasma was assayed to monitor inter-assay variation. The intra-assay coefficient of variation was 7% for human LPL and 6% for human HL. Inter-assay coefficient of variation was 8% for LPL and 10% for HL.

Nonesterified fatty acid determination

NEFAs were determined by a colorimetric kit (Wako) using plasma collected in 0.1% EDTA and tetrahydrolipstatin (to inhibit lipolysis) to achieve a final concentration of 1 μg/ml. The average NEFA of the three plasma samplings was used as a measurement of plasma NEFA concentration.

S_I, insulin, and glucose measurements

The tolbutamide-modified FSIVGTT was performed as previously described (24). Three basal samples were drawn 15 min, 20 min, and 25 min after the placement of an intravenous line for glucose and insulin determination. Glucose (11.4 g/m^2) was injected at time zero as a bolus over a 60 s period. Twenty minutes after the glucose injection, tolbutamide (125 mg/m^2) was injected over a 30 s period. Blood samples for glucose and insulin were drawn at 32 time points over a 4 h period. Plasma glucose concentrations were measured in triplicate using the glucose oxidase method. Plasma insulin was measured in duplicate using a modification of a double antibody RIA (25). S_I was quantified as the S_I index (S_I) from the glucose and insulin data using Bergman's minimal model of glucose kinetics (26). This measure has been demonstrated to be equivalent to that quantified using the euglycemic glucose clamp technique (24, 27).

Leptin determination

Plasma leptin levels were measured by radioimmunoassay (28) (Linco Research, St. Charles, MO).

Body fat measurements

Dual-energy X-ray absorptiometry scan. Total body mass and total body fat mass (FM) were measured following a 12 h fast by dual-energy X-ray absorptiometry scan (Hologic QDR 1500) and used to determine percent body fat (% body fat).

CT scan. IAF and SQF 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 (29). The amount of IAF and SQF measured at the level of the umbilicus has been shown to be highly correlated with total visceral and subcutaneous abdominal fat, respectively, determined from multiple abdominal images (19, 30, 31).

Statistics

Statistical analyses were performed using Sigma Stat (Jandel Scientific). Data are reported as the mean (in the Results section) or the mean ± SD (in the Tables). Correlations of selected variables were determined to assess the relationship of PLTP activity with parameters related to body composition and insulin resistance, and with measurements of lipids and lipase activities. Spearman Rank Order correlations were calculated to reduce the possible contribution of outliers. A paired *t*-test was used to determine the significance of the changes in the variables with weight loss unless the data were not normally distributed, in which case the Mann-Whitney Rank Sum test was used. Multivariate linear regression analyses and forward stepwise regression analyses were carried out to determine the independent effect of SQF, S_I, and sex on the change in PLTP.

RESULTS

The group of 46 subjects studied at baseline was comprised of both obese and nonobese individuals with BMIs ranging from 19 to 64 kg/m^2 (Table 1). In this group of 46 subjects having varied BMI, PLTP activity was positively correlated with FM ($r = 0.36$, $P = 0.02$) and SQF ($r = 0.31$, $P = 0.045$), but was not related to S_I ($r = 0.10$, $P = 0.52$) or IAF ($r = 0.16$, $P = 0.32$) (**Table 3**). PLTP activity was also positively correlated with TG and TC but was not correlated with insulin, glucose, leptin, or NEFA. In multivariate analysis with PLTP as the dependent variable and S_I and FM as independent variables, S_I did not contribute

TABLE 3. Correlations with PLTP activity at baseline

Variable	All Subjects (n = 46)		Subjects in Weight Loss Study (n = 19)	
	r	*P*	*r*	*P*
BMI	0.27	0.07	0.10	0.69
FM	0.36	0.02	0.31	0.22
% FM	0.29	.07	0.30	0.23
SQF	0.31	.045	0.33	0.18
IAF	0.16	0.32	−0.07	0.79
S_I	0.10	0.52	0.25	0.29
Insulin	0.15	0.33	0.28	0.25
Glucose	0.19	0.2	0.25	0.29
Leptin	0.21	0.15	0.37	0.12
HL	−0.12	0.2	−0.50	0.03
LPL	0.19	0.2	0.49	0.03
NEFA	0.18	0.24	0.48	0.04
TC	0.29	.05	0.35	0.14
TG	0.36	.014	0.20	0.41

to the variance in PLTP while FM was significantly associated. Because of the inclusion of both men and women in this study, gender was introduced as a variable in the multivariate analysis and did not affect the results, showing no association with PLTP activity.

The 19 subjects selected for the weight loss study from the baseline group, having BMIs ranging from 29 to 54 kg/m^2, demonstrated correlations similar to those reported for the larger group of 46 subjects (Table 3). Also, a positive correlation of PLTP activity with NEFA ($r = 0.48$, $P = 0.04$) and with LPL activity ($r = 0.49$, $P = 0.03$) and a negative correlation with HL activity ($r = -0.50$, $P = 0.03$) were observed.

Because the 19 subjects that participated in the weight loss study are the major focus of the present investigation, the baseline associations of SQF with FM, and IAF with S_I, were investigated to confirm previous reports (19) that SQF is more strongly associated with total body fat than is IAF, and that IAF is more strongly associated with S_I than is SQF. In agreement with previous observations, SQF was highly correlated with FM ($r = 0.91$, $P = 0.001$), but IAF was not ($r = 0.29$, $P = 0.25$). In contrast, IAF was negatively correlated with S_I ($r = -0.52$, $P = 0.026$), but SQF was not significantly related ($r = -0.25$, $P = 0.31$). S_I was also found to be negatively correlated with fasting insulin ($r = -0.72$, $P < 0.001$), positively correlated with NEFA ($r = 0.58$, $P = 0.01$), and was not significantly related to plasma glucose ($r = 0.125$, $P = 0.6$). These observations were also observed in all 46 subjects.

The effect of the hypocaloric diet-induced weight loss on changes in the variables under investigation was then assessed in the 19 subjects who were studied in a weight-stable period after weight loss. Weight loss resulted in significant decreases in all parameters related to body fat measurements when compared with baseline, including an average decrease of −16 kg body weight (range −4 kg to −30 kg), −13.7 kg FM, and −8.6% body fat. There was a substantial decrease in SQF (−157 cm^2) (−33.6%) as well as IAF (−62 cm^2) (−44.3%). All changes were significant ($P < 0.001$) (Table 2).

PLTP activity significantly decreased with weight loss, demonstrating a mean reduction of −1.5 nM/ml/h (−9.1%) compared with baseline (Table 2). The change in PLTP activity with weight loss ranged from −1.9 to 0.64 nM/ml/h (−25.3% to 12.3%) with 15 of the 19 subjects investigated demonstrating a decrease in activity.

HL activity also decreased with weight loss (Table 2). The change observed for LPL activity was not found to be significant (Table 2).

Fasting insulin decreased −5.6 μU/ml and S_I increased substantially $+0.92 \times 10^{-5}$ $min^{-1} \cdot [pmol/l]^{-1}$ (48.4%) with weight loss, but NEFA did not change significantly (Table 2). Leptin decreased −33.6%. TC decreased significantly, while the change in TG was not significant.

The relationships between the changes in PLTP activity and the changes in selected variables with weight loss (**Table 4**) were then investigated, because the major interest of the study was to determine whether the changes observed in PLTP activity were correlated with the changes in SQF and total body fat or with the changes in IAF and insulin resistance. The results demonstrated that the change in PLTP activity was significantly and positively correlated with the change in SQF ($r = 0.55$, $P = 0.018$) (Table 4, **Fig. 1A**). The correlation with FM did not reach significance ($r = 0.38$, $P = 0.13$) (Table 4, Fig. 1B). In contrast, no significant relationship was determined between the change in PLTP activity and the change in IAF ($r = 0.09$, $P = 0.73$) or S_I ($r = 0.18$, $P = 0.44$) (Table 4). Because obesity and insulin resistance are related, we carried out multivariate analysis to separate the effects of S_I (reflecting insulin resistance) from that of SQF (reflecting obesity). With PLTP as the dependent variable and SQF and S_I as the independent variables, S_I was not associated with PLTP ($P = 0.108$), whereas SQF was significantly related ($P = 0.004$) (multiple $r = 0.68$). Because there were males and females in the weight loss group, we added gender to the multivariate analysis, and no significant effect of gender was observed (multiple $r = 0.72$). In agreement with the multivariate analysis, forward stepwise regression analysis that sets up a model to predict the change in PLTP based on the variables entered demonstrated that only SQF re-

TABLE 4. Correlations of changes in PLTP activity with changes in selected variables (n = 19)

Variable	Correlation of Change in Variable with Change in PLTP Activity	
	r	*P*
BMI	0.31	0.20
FM	0.38	0.13
% FM	0.15	0.55
SQF	0.55	0.018
IAF	0.09	0.73
S_I	0.18	0.44
Insulin	−0.17	0.49
Glucose	0.55	0.014
Leptin	0.15	0.54
HL	0.14	0.55
LPL	0.28	0.23
NEFA	0.71	<0.001
TC	0.63	0.004
TG	0.14	0.56

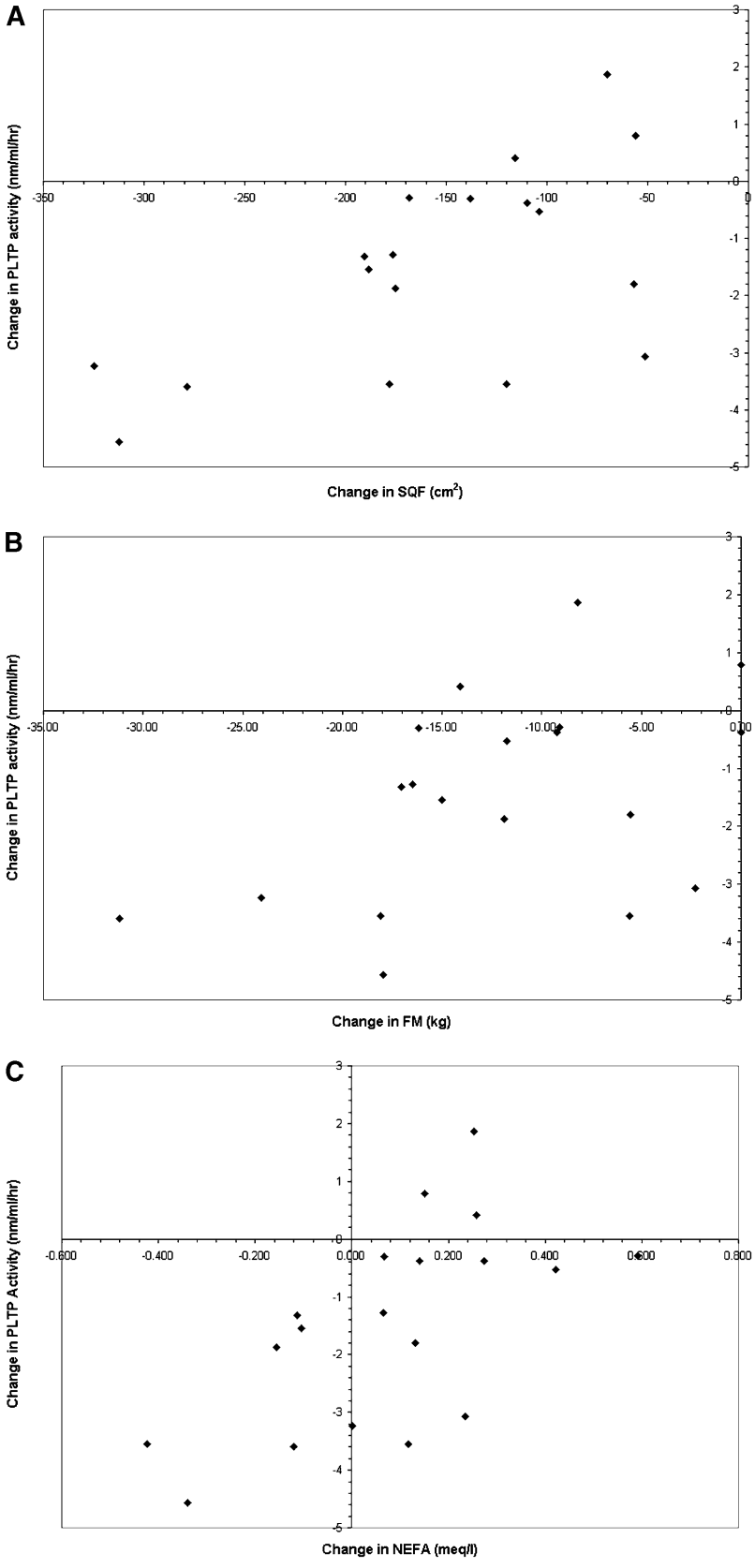

Fig. 1. A: The relationship between the change in plasma phospholipid transfer protein (PLTP) activity and the change in abdominal subcutaneous fat (SQF) as a result of weight loss (n = 19). PLTP activity was determined by measuring the transfer of labeled phosphatidylcholine from vesicles to HDL_3. SQF was measured by CT scan at the level of the umbilicus. B: The relationship between the change in plasma PLTP activity and the change in total body fat mass (FM) as a result of weight loss (n = 19). PLTP activity was determined by measuring the transfer of labeled phosphatidylcholine from vesicles to HDL_3. FM was determined by dual-energy X-ray absorptiometry scan. C: The relationship between the change in plasma PLTP activity and the change in plasma nonesterified fatty acid (NEFA) as a result of weight loss (n = 19). PLTP activity was determined by measuring the transfer of labeled phosphatidylcholine from vesicles to HDL_3. The concentration of NEFA is the mean of three plasma samples taken 5 min apart after insertion of an intravenous line and a 15 min rest period. The plasma was collected in tetrahydrolipstatin to inhibit lipolysis.

mained in the model ($r = 0.6$, $P = 0.008$), whereas S_I and sex were excluded as they did not significantly add to the ability to predict the change in PLTP activity. We also looked at the correlations by dividing the groups by gender. The associations of the change in PLTP with the changes in variables measured were similar to those observed in the weight loss group as a whole. The change in PLTP remained significantly correlated with NEFA in females ($r = 0.76$, $P = 0.006$), was highly correlated with the change in SQF in males ($r = 0.93$, $P < 0.001$), but was not significantly related in females ($r = 0.29$, $P = 0.37$) and was not significantly related to the change in S_I or IAF in either group. Because of the reduced numbers when the groups are divided by gender (11 females and eight males), we consider these correlations less definitive than those of the whole group.

We also investigated whether the change in PLTP activity was associated with changes in other variables related to glucose and lipid metabolism that were measured in the present study (Table 4). Of these variables, the change in PLTP activity was most highly, positively, and significantly associated with the change in NEFA ($r = 0.71$, $P < 0.001$) (Table 4, Fig. 1C). PLTP activity was also positively correlated with the change in TC ($r = 0.63$, $P = 0.004$) and glucose ($r = 0.55$, $P = 0.014$). The change in PLTP was not correlated with the change in HL, LPL, TG, or fasting insulin. The relationship with leptin was also investigated, because leptin is produced mainly in SQF (20) and has been reported to be correlated with PLTP in humans (13) or to be related to a decrease in PLTP mRNA in HepG2 cells (32). In the present study, the change in leptin was not associated with the change in PLTP activity, despite the fact that leptin demonstrated associations similar to those observed for PLTP, being positively correlated with the change in SQF ($r = 0.67$, $P = 0.002$) and change in FM ($r = 0.51$, $P = 0.036$), but not associated with the change in IAF ($r = 0.23$, $P = 0.35$) or S_I ($r = -0.27$, $P = 0.26$).

Because the change in NEFA was so strongly correlated with the change in PLTP, we investigated what other variables correlated with the change in NEFA. NEFA was found to be significantly correlated with SQF($r = 0.64$, $P = 0.004$) and glucose ($r = 0.5$, $P = 0.03$), but was not related to S_I ($r = 0.27$, $P = 0.26$), IAF($r = 0.19$, $P = 0.46$), or insulin (-0.28, $P = 0.26$), indicating that in these subjects, with weight loss, the change in FFA appears to be related to variables associated with obesity rather than insulin resistance.

DISCUSSION

In the present study, we have investigated how weight loss impacts PLTP activity and assessed whether the perturbation in PLTP is associated with a change in body fat or insulin resistance. We have demonstrated that PLTP activity decreases significantly with weight loss in obese subjects and that this decrease is related to the change in body fat, as reflected by SQF. In contrast to several previous reports in cross-sectional studies, the change in PLTP activity as a result of weight loss was not related to the change in insulin resistance or IAF despite significant and substantial changes in both these variables.

These data confirm that PLTP activity is influenced directly or indirectly by body weight, in agreement with previous cross-sectional studies that reported a positive association of PLTP with obesity (8, 11–13). In our study of premenopausal women (8), although a significant difference was not observed in the obese compared with the lean group, PLTP activity was significantly higher in the quartile with the highest BMI (Q4 = 15.61 nM/ml/h vs. Q2 = 13.17 nM/ml/h, $P < 0.05$) (unpublished observations). Previous studies have also found PLTP to be correlated with IAF (8, 11), waist-hip ratio (12, 13), and fasting insulin (8, 13), variables often related to insulin resistance. In the study by Dullaart et al. (12), the quartile with the highest BMI and highest PLTP activity also demonstrated the highest degree of insulin resistance. The data from our cross-sectional study, however, which included specific measures of fat mass and abdominal fat depots and not just BMI, are in agreement with the results of the study by Kaser et al. (13). In that study, the increase in PLTP with obesity was specifically associated with body fat, in that no association between PLTP activity and S_I was observed in the quartile with the highest BMI when subsequently divided into quartiles according to degree of S_I.

In the present study, the decrease in PLTP activity with weight loss may be due to reduced secretion from smaller fat cells or a reduced fat mass, as reflected by the positive association with the change in SQF. The change in PLTP may also be due to a change in a metabolic parameter associated with body fat that is common to obesity, diabetes, and insulin resistance. We observed a highly positive correlation between the change in PLTP activity and the change in NEFA levels in the present study. Because obese individuals and those with type 1 and type 2 diabetes, who have also been shown to have elevated PLTP activity levels, demonstrate an increased concentration or increased flux of NEFA, NEFA may be the common metabolite affecting PLTP activity. An increase in NEFA is also a characteristic of other conditions associated with an increase in PLTP. PLTP activity is elevated in alcoholics, who demonstrate increased NEFA concentrations, and PLTP decreases upon withdrawal (33, 34). PLTP activity decreases with the administration of Acipimox, which is thought to inhibit hormone-sensitive lipase and thus decrease NEFA release from adipose tissue (35). The decrease in PLTP activity during a hyperinsulinemic euglycemic clamp in healthy and type 2 diabetic men (14) was also correlated with the decrease in NEFA levels. In view of the fact that the present study investigates correlations between variables, a cause and effect cannot be determined. Given this limitation, if NEFAs are in fact exerting a direct effect on PLTP activity, the underlying mechanism remains unclear. Candidate mechanisms include a NEFA effect mediated by a nuclear receptor response element altering PLTP transcription (36–38), although the target nuclear receptor is not entirely evident from present knowledge of PLTP regulation

in humans (38, 39). Alternatively, the NEFA-mediated stabilization of apolipoprotein B in the liver, reported to effect an increase in VLDL secretion, may result in a compensatory increase in hepatic PLTP production necessary for the facilitated transfer of the augmented surface mass of VLDL to HDL during lipolysis.

It is possible that body fat (as reflected by measured SQF) and NEFA may have independent effects upon the levels of plasma PLTP activity. This hypothesis is supported by our findings that PLTP activity decreased in association with a substantial decrease in levels of both SQF and NEFA but was unchanged or increased when the decrease in SQF was less extensive and/or NEFA levels increased (Fig. 1A, C). Thus, the resultant PLTP activity in the plasma may reflect the balance between the influences of SQF and NEFA levels. Therefore, with weight loss, as SQF decreases, the production of PLTP from this depot may decrease. A decrease in levels of NEFA would also mediate a decrease in PLTP, but if NEFA levels increase then PLTP activity increases. These observations suggest that NEFA may exert its effect on PLTP production from a depot separate from SQF, such as the liver (40).

The change in PLTP activity was not associated with the change in HL activity, LPL activity, or leptin levels. Both leptin and PLTP mRNA have been identified in SQF at substantial levels (20). In the present study, the change in leptin was associated with the change in SQF and FM but not with IAF and S_I, similar to the findings for PLTP activity. However, the lack of an association of the change in leptin with the change in PLTP in the present study suggests different mechanisms controlling the levels of these parameters in plasma.

A limitation of the present study is the relatively small number of subjects who underwent weight loss and the inclusion of both males and females. However, we included detailed and reproducible measures of body composition and S_I and carried out a tightly controlled perturbation study that achieved a significant weight loss. Furthermore, the subjects were studied under weight-stable condition before and after weight loss, which is an important requirement for the attainment of accurate results.

Although reported by others, the present study does not investigate the change in PLTP with diabetic status. Thus, the increase in PLTP reported in diabetic subjects (14–16) or in subjects with increased insulin resistance (18) may be mediated by a mechanism distinct from that responsible for the increased PLTP observed with obesity. However, it is possible that the increase in PLTP is related to an increase in body fat mass in some of these studies, inasmuch as the subjects with type 2 diabetes, who demonstrated a significant elevation in PLTP activity, also had elevated BMI values (14, 15).

Although we have previously determined that the majority of PLTP in plasma is active, PLTP is also present in an inactive form (41, 42). In the present study, we were interested in the change in functional (active) PLTP, insofar as our ultimate goal is to determine how changes in PLTP activity impact lipoprotein metabolism. It is possible that the decrease in plasma PLTP activity observed with weight loss reflects an increase in the amount of inactive relative to active PLTP rather than a decrease in production. Although not the aim of the present investigation, it would be interesting to address this question in future studies. However, the strong association of the change in PLTP activity with the change in SQF as well as NEFA suggests that the change in PLTP, whether due to a decrease in protein mass or to a reduction in the active form, is most likely related to changes in these parameters.

In summary, the change in PLTP activity with weight loss is related to a decrease in SQF rather than IAF or to an increase in S_I. The results suggest that SQF and NEFA may be major predictors of PLTP activity levels in plasma and may account for a substantive part of the increased PLTP activity observed in obese subjects. With respect to SQF, the decrease in PLTP with weight loss may be a result of reduced production from the diminished SQF stores. Alternatively or concomitantly, PLTP may be decreased because its role in lipid metabolism is less essential as the metabolic consequences of increased body fat, such as elevated VLDL TGs, are reduced. With a decrease in plasma VLDL, for example, PLTP production would be reduced because the requirement for PLTP-mediated intravascular lipid transfer is diminished. NEFA may be involved in this mechanism, and the investigation of the potential mediating role of NEFA in PLTP regulation is an important direction for future studies.

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