Differences in reactivity of antibodies to active versus inactive PLTP significantly impacts PLTP measurement

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Abstract **Due to conflicting reports concerning the relationship between phospholipid transfer protein (PLTP) activity and mass in plasma, the protein concentration and activity of PLTP were assessed in fractions isolated by fast protein liquid chromatography from the plasma of healthy normolipidemic individuals. Using both polyclonal and monoclonal antibodies, PLTP was identified by Western blot analysis after both SDS and non-denaturing gradient gel electrophoresis, and quantitated by dot blot. PLTP activity was determined using a labeled vesicle/HDL assay. PLTP mass corresponded substantially with the activity distribution using the polyclonal antibody on dot blot with some inactive PLTP being present. However, the monoclonal antibody preferentially reacted with inactive PLTP, primarily associated with LDL and large HDL, overestimating inactive PLTP. Western blot analysis of non-denaturing gradient gels, using the polyclonal antibody, indicated that active PLTP was associated with numerous discrete HDL subpopulations (7.6–12.0 nm) with the major portion being 9–12 nm. Inactive PLTP was associated with particles of 12 to >17 nm. The monoclonal antibody demonstrated a different pattern of reactivity on gradient gels, showing strong reactivity with the inactive PLTP in particles of 12 to >17 nm, but less reactivity with particles of 7.6–12 nm. The differences in reactivities of antibodies for active versus inactive PLTP can account for some of the discrepancies reported in the literature regarding the relationship between PLTP mass and activity.**—Murdoch, S. J., G. Wolfbauer, H. Kennedy, S. M. Marcovina, M. C. Carr, and J. J. Albers. **Differences in reactivity of antibodies to active versus inactive PLTP significantly impacts PLTP measurement.** ***J. Lipid Res.*** **2002.** 43: **281–289.**

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Recent studies of the relationship between phospholipid transfer protein (PLTP) mass and activity in plasma have reported conflicting results. Determination of PLTP mass initially was reported by Desrumaux et al. (1) using a polyclonal-based competitive ELISA. As expected, PLTP mass was highly positively correlated with phospholipid transfer activity ($r = 0.79$, $P < 0.001$) in a group of 30 normolipidemic males and females. In a later study, no correlation between PLTP mass and activity was found using a monoclonal/polyclonal sandwich ELISA in a cohort of 159 non-fasted, randomly selected, Finnish individuals (2). However when the activity and mass were adjusted for the TG concentration, a weak positive correlation was observed ($r = 0.31$, $P < 0.001$). Oka et al. (3) carried out another study using a double monoclonal sandwich ELISA and also reported no correlation between PLTP mass and activity in a group of 132 Japanese subjects. Oka et al. (4), using the double monoclonal sandwich ELISA system for detection of PLTP mass, went on to analyze the mass and activity of PLTP in lipoprotein fractions separated by fast protein liquid chromatography (FPLC). They reported a large discrepancy between the distribution of mass as compared with that of PLTP activity, with substantial mass being present in the LDL and very large HDL region and the majority of the activity being found in the region of small HDL. Some fractions contained mass and no activity whereas other fractions contained activity and no detectable mass. They concluded that PLTP exists in two forms, a catalytically inactive one and an active one.

Since PLTP appears to be intricately involved in HDL metabolism through its ability to augment HDL mass by transferring phospholipids, unesterified cholesterol and possibly apoE and apoA-IV from VLDL during lipolysis (5–8), remodel HDL (9, 10), and increase HDL-mediated cholesterol and phospholipid efflux (11), the accurate measurement of PLTP is important. In the PLTP knockout mouse, HDL concentrations were drastically reduced

Abbreviations: BMI, body mass index; FPLC, fast protein liquid chromatography; MAb, monoclonal antibody; PLTP, phospholipid transfer protein; rPLTP, recombinant PLTP.

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(8). In humans, PLTP activity has been shown to be positively correlated with total HDL as well as HDL2 and HDL3 (12). Due to the inverse association of the HDL concentration and particle size with coronary heart disease (13), the effect of PLTP on HDL concentration, size, and subpopulation distributions is of significant interest. Furthermore, the delineation of the factors that control PLTP activity is crucial to the thorough understanding of the basis for the increased PLTP activity observed in such conditions as obesity (14) and Type II diabetes (15). However, until the reason for the disparity between the measurement of PLTP mass and activity is understood, it will not be possible to use mass as a reflection of plasma PLTP activity, nor to infer relationships between mass and plasma lipoprotein concentrations.

The present study was carried out to investigate and possibly clarify the cause of the discrepancy reported in previous studies between PLTP protein mass and activity and to further characterize the lipoprotein particles associated with active PLTP and inactive PLTP. We have also compared the distributions of PLTP mass and activity to the distributions of apoA-I and apoE in the plasma lipoprotein fractions to determine if there is any relationship between these distributions and that of inactive or active PLTP.

MATERIALS AND METHODS

Subjects

The human subjects investigated (n = 8, 7 premenopausal females and 1 male) were healthy normolipidemic adults. None of the subjects were taking oral contraceptives or medications that affect lipid metabolism. They did not demonstrate any lipid disorders, diabetes, or liver disease, nor were they pregnant. The exclusion criteria were BMI >40kg/m^2, TG, or LDL cholesterol above the 95th percentile for their age, or fasting glucose greater than 110 mg/dl. The Human Subjects Review Committee of the University of Washington approved the study protocol. Informed consent was obtained from all participants.

Analysis of plasma proteins and lipids

ApoA-I, apoE, and albumin concentrations were determined by a nephelometric method using Dade-Behring reagents and instrumentation (BNII). Total cholesterol and TG were analyzed by standardized enzymatic methods at the Northwest Lipid Research Laboratories (16).

Separation of plasma by FPLC

Blood from fasting subjects was collected in 0.1% EDTA on ice and centrifuged for 20 min at 4°C. Plasma was passed through a 0.4 μ filter and 1 ml was immediately applied to a single Superose 6 HR 10/30 column (10 × 300 mm, Pharmacia Fine Chemicals, Piscataway, NJ). The fractions were separated at 4°C by elution with 0.05 M phosphate, 0.15 M NaCl, 0.01% EDTA, 0.02% NaN_3, pH 7.4, pumped at a rate of 0.2 ml/min. Sixty fractions of 500 μl were collected and either analyzed immediately for activity and mass or frozen at −70°C and analyzed for activity at a later time. No discrepancy in the characteristics of the PLTP distribution was observed when frozen samples were analyzed compared with fresh ones. The column was standardized using the following molecular weight markers: thyroglobulin, ferritin, catalase, albumin, and LDL.

PLTP activity assay

PLTP activity was assessed by determining the transfer of labeled phosphatidylcholine from vesicles to HDL3 (lacking apoE) (17), as described (12). This method reflects the phospholipid transfer activity of PLTP but not that of cholesteryl ester transfer protein (CETP) (17). Briefly, 50 μl of liposomes, trace labeled with [^{14}C]1-palmitoyl-2-linoleoyl phosphatidylcholine (New England Nuclear) to a specific activity of 170 cpm/nM phospholipid, containing phosphatidylcholine (50 nM) and phosphatidylserine (12 nM), were combined on ice with HDL3 (150 nM), Tris buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.1% NaN_3) and the FPLC fraction. The volume of the fraction that was selected for measurement of PLTP activity was based on the results of previous analyses of plasma fractions isolated by FPLC. The volume of each fraction added varied from 6.7 to 100 μl to provide activity in the linear, working range of the assay. For fractions containing HDL, 6.7 μl was used in most cases, which approximates HDL in 0.9 μl or less of plasma. For fractions containing LDL, up to 100 μl was used, which approximates LDL in 15 μl or less of plasma. The use of smaller volumes of the LDL fractions yielded no detectable activity. These amounts of endogenous lipoproteins have been shown not to interfere with the PLTP activity assay (17). The assay mixture was incubated for 45 min at 37°C. Under these conditions, the assay was determined to be linear with time and concentration. The same batch of vesicles and HDL was used for all PLTP assays to maintain consistency. Three human quality control plasma samples, flash frozen and stored at −70°C, were included in triplicate in each assay. The intra-assay and inter-assay coefficient of variation was 7.6% and 2.2%, respectively.

Antibodies

Polyclonal antiserum. Polyclonal antibodies were raised in New Zealand white rabbits against recombinant PLTP. Recombinant PLTP was isolated by Ni-NTA agarose (Qiagen) column chromatography from serum-free conditioned media collected from BHK-570 cells that had been transfected with human PLTP-His tag cDNA (18). The anti-PLTP containing IgG fraction was isolated by a caprylic acid method (19) and its purity verified by 10% SDS-PAGE. Aliquots of the polyclonal anti-PLTP IgG were stored at −70°C. The polyclonal antibody reacted against one 81 kDa band in SDS gels corresponding to the mature form of plasma PLTP and has been shown previously to neutralize phospholipid transfer activity mediated by PLTP (17).

Monoclonal antibody (MAb4). Three Balb/c mice were immunized by each receiving intraperitoneal injections of 100 μg of full-length recombinant PLTP (rPLTP) (18) in Freund's incomplete adjuvant. The same dose was repeated 2 weeks later in Freund's complete adjuvant. The mouse with the highest antibody titer against the rPLTP, as determined by ELISA, was boosted with intravenous injections of 50 μg of rPLTP for three consecutive days before the fusion. Splenocytes from each mouse were fused with mouse hybridoma cell line SP2/0-Ag14 as previously described (20). The cells from the most positive wells were cloned by limiting dilutions and subcloned until proof of monoclonality was achieved. The immunoglobulin class and subclass were determined by immunoblotting (Mouse monoclonal antibodies isotyping kit; Amersham, Arlington Heights, IL). To obtain a large amount of the monoclonal antibody with the highest titer to PLTP (MAb4), the hybridoma cells were injected into the peritoneal cavity of pristine primed Balb/c mice. The monoclonal antibody was then purified from ascite fluids by a non-chromatographic procedure performed as described (19) and stored at −70°C in aliquots of 1 mg/ml solutions.

Immunoblotting

Dot blot/PLTP mass assays. Plasma FPLC fractions of each subject were diluted 1:5 with Tris buffer (10 mM Tris, 1 mM EDTA,

150 mM NaCl, 0.01% NaN_3, pH 7.4) and 25 μl were spotted on supported nitrocellulose (Schleicher and Schuell) in a dot blot apparatus (BioRad), followed by rinsing with TBS (20 mM Tris, 137 mM NaCl, pH 7.6). Blotting was carried out using a chemiluminescent substrate (Pierce) with fat free milk and Tween 20 as blocking agents. The primary antibody utilized was either the anti-PLTP polyclonal or the anti-PLTP monoclonal antibody (MAb4) optimized with species-appropriate horseradish peroxidase-conjugated secondary antibody from Kirkegaard and Perry or Vector, respectively. The dot blot procedure was linear with concentration within the working range.

Western blot/PAGE. PAGE of selected plasma FPLC fractions, followed by transfer to nitrocellulose and blotting with polyclonal or monoclonal antibodies was carried out to establish: *1*) size of the PLTP molecule and the specificity of the PLTP antibodies using 4–20% SDS-PAGE (BioRad) under reducing conditions, and *2*) the size of the particles that PLTP was associated with using non-denaturing 4–30% PAGE (Alamo Gels). SDS gels were run at 200 V (constant) at room temperature for 45 min and non-denaturing gels were run at 120 V (constant) for 20 h in a cooled system. For the SDS-gels, molecular weight markers from either BioRad or Gibco were used, whereas for the non-denaturing gels, the high molecular weight markers from Pharmacia were used as standards. The proteins were transferred electrophoretically at 4°C to 0.2 μm supported nitrocellulose membranes at 26 V overnight. The membranes were then subjected to the same blotting procedures as described for dot blots.

RESULTS

Western blot analysis of fresh plasma applied to SDS gels using either polyclonal or monoclonal antibody yielded a single band of 81 kDa (**Fig. 1**). Freshly prepared rPLTP also yielded an 81 kDa band (lane 1).

The distributions of the PLTP and phospholipid transfer activity were determined in eight subjects whose PLTP activity varied over a range of values (11.4–18.7 nmol/ml/h).

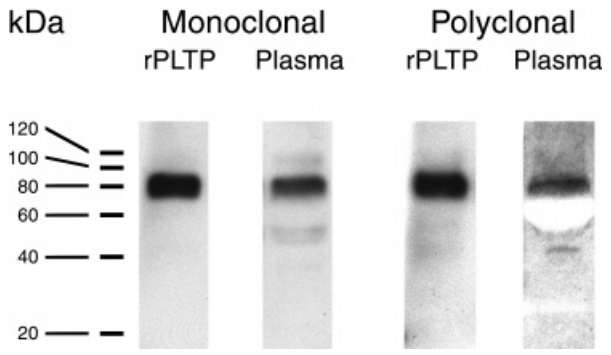

Fig. 1. Western blot analysis of recombinant phospholipid transfer protein (rPLTP) and plasma. Plasma and rPLTP were subjected to SDS-PAGE on 4–20% gels under reducing conditions, transferred to nitrocellulose and immunoblotted. Lane 1 and 2: Western blot of rPLTP and plasma, respectively, using monoclonal antibody (MAb4). Lane 3 and 4: Western blot of rPLTP and plasma, respectively, using polyclonal antibody. Thirty-five nanograms of rPLTP were added to lanes 1 and 3, and 0.5 μl of plasma were added to lanes 2 and 4. The samples were added to sample buffer containing dithiothreitol and briefly incubated in a boiling water bath prior to electrophoresis.

The distributions of five subjects are given as examples in **Fig. 2**. The phospholipid transfer activity distribution was similar to the distribution of apoA-I and HDL cholesterol in all subjects with a portion of active PLTP preferentially associating with large HDL (since the activity peak rose slightly ahead of the apoA-I and HDL cholesterol distribution). Figure 2 demonstrates that the peak of PLTP mass, as detected by dot blot using polyclonal antibodies, eluted in fractions from 32 to 33 or the large HDL region, corresponding to or slightly ahead of the phospholipid transfer activity peak (fractions 33–35). Comparing the distribution of apoE and apoA-I, it appears that the PLTP mass distribution by dot blot using polyclonal antibody bears more similarity to the HDL-apoE distribution (**Fig. 3**) than to the apoA-I distribution (Fig. 2). A trace of inactive PLTP appeared to be present in the IDL/large LDL region (fractions 21–25) in subjects A through D by dot blot using the polyclonal antibody (Fig. 2). For subject E, PLTP was clearly evident in this region. The results using the monoclonal antibody were strikingly different from those of the polyclonal antibody in that the monoclonal antibody selectively reacted with inactive PLTP, and moreover detected a greater proportion of the total protein mass in fractions containing inactive PLTP as compared with the polyclonal antibody. This observation of an increased amount of PLTP detected by the monoclonal antibody as compared with the polyclonal antibody in fractions containing inactive PLTP was consistently observed in all subjects investigated, whereas the amount detected varied. The monoclonal antibody also detected less PLTP mass in the active fractions as compared with the polyclonal antibody on dot blot.

This differential immunoreactivity between the polyclonal and monoclonal antibodies was also clearly evident by Western blot analysis of these fractions when applied to SDS gels (**Fig. 4**). With the polyclonal antibody, immunoreactive PLTP was barely detectable in the large LDL region (fraction 24). In contrast, a considerably greater amount of PLTP was clearly evident in this region using the monoclonal antibody. These observations are in direct agreement with the results of the dot blot assays showing a selective reactivity of the monoclonal antibody with inactive PLTP.

The presence of PLTP without phospholipid transfer activity, observed in fractions corresponding to small LDL/large HDL or IDL/large LDL (Fig. 2), may be the result of the presence of an inhibitor in these fractions as has been demonstrated for CETP (21). To test for the presence of an inhibitor, the phospholipid transfer activity of rPLTP was assayed with and without the addition of fractions that demonstrated immunoreactive PLTP protein but no activity. None of the fractions inhibited the activity of rPLTP, supporting the concept that an inhibitor was not the cause of the lack of transfer activity.

In the region containing small HDL and plasma proteins (fractions 41–45, Fig. 2), phospholipid transfer activity was present, but little or no PLTP mass was detected based on the dot blot assay, which may suggest the presence of another protein that transfers phospholipid from

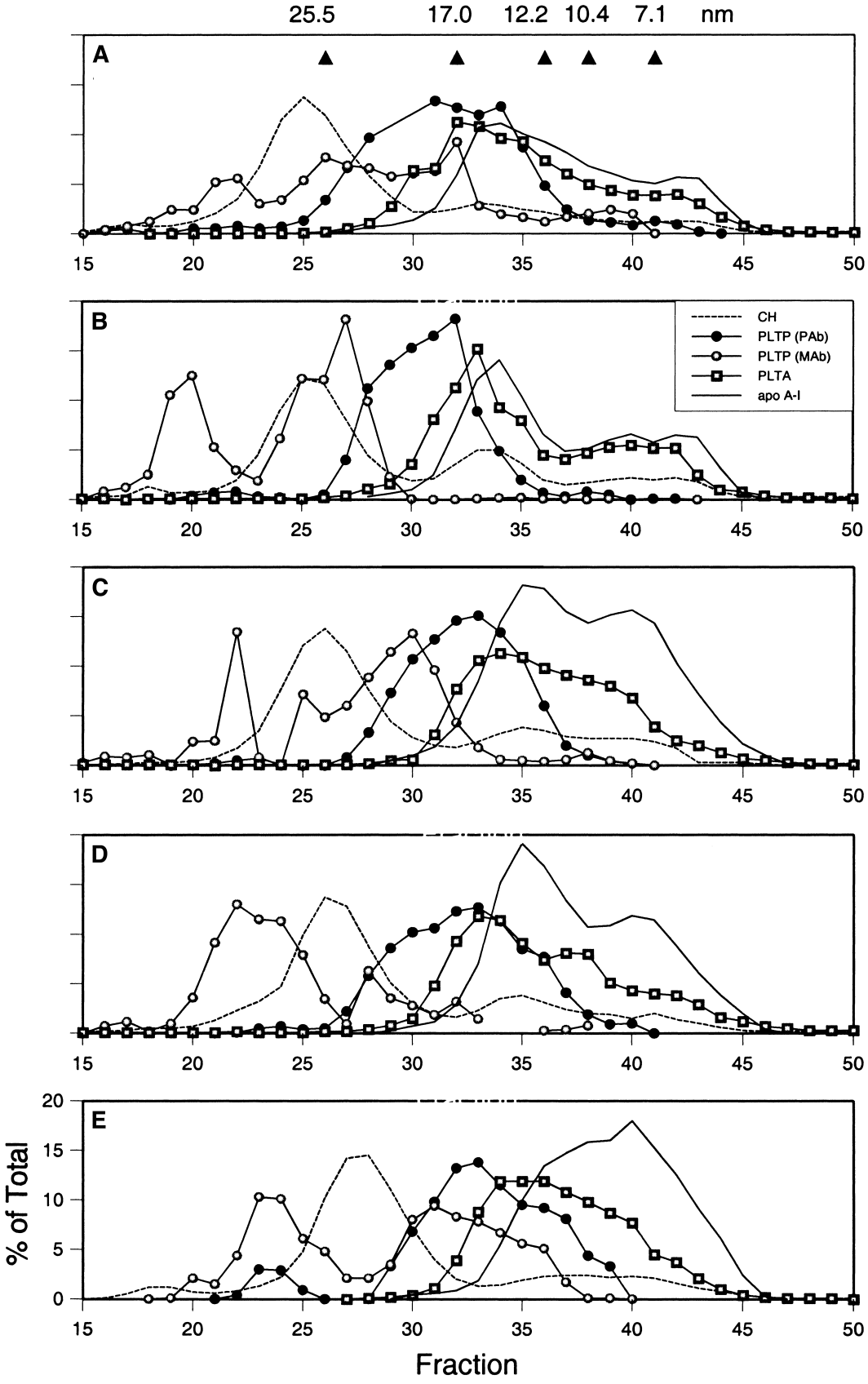

Fig. 2. Elution profiles of plasma fractionated by fast protein liquid chromatography (FPLC); fractions of five subjects, A, B, C, D, and E. Cholesterol distribution (even broken line); phospholipid transfer protein (PLTP) distribution as detected by polyclonal antibody using a dot blot procedure (solid line with closed circle); PLTP distribution as detected by monoclonal antibody (MAb4) (solid line with open circle); PLTA distribution (solid line with closed square); apoA-I distribution assayed by nephelometry (solid line).

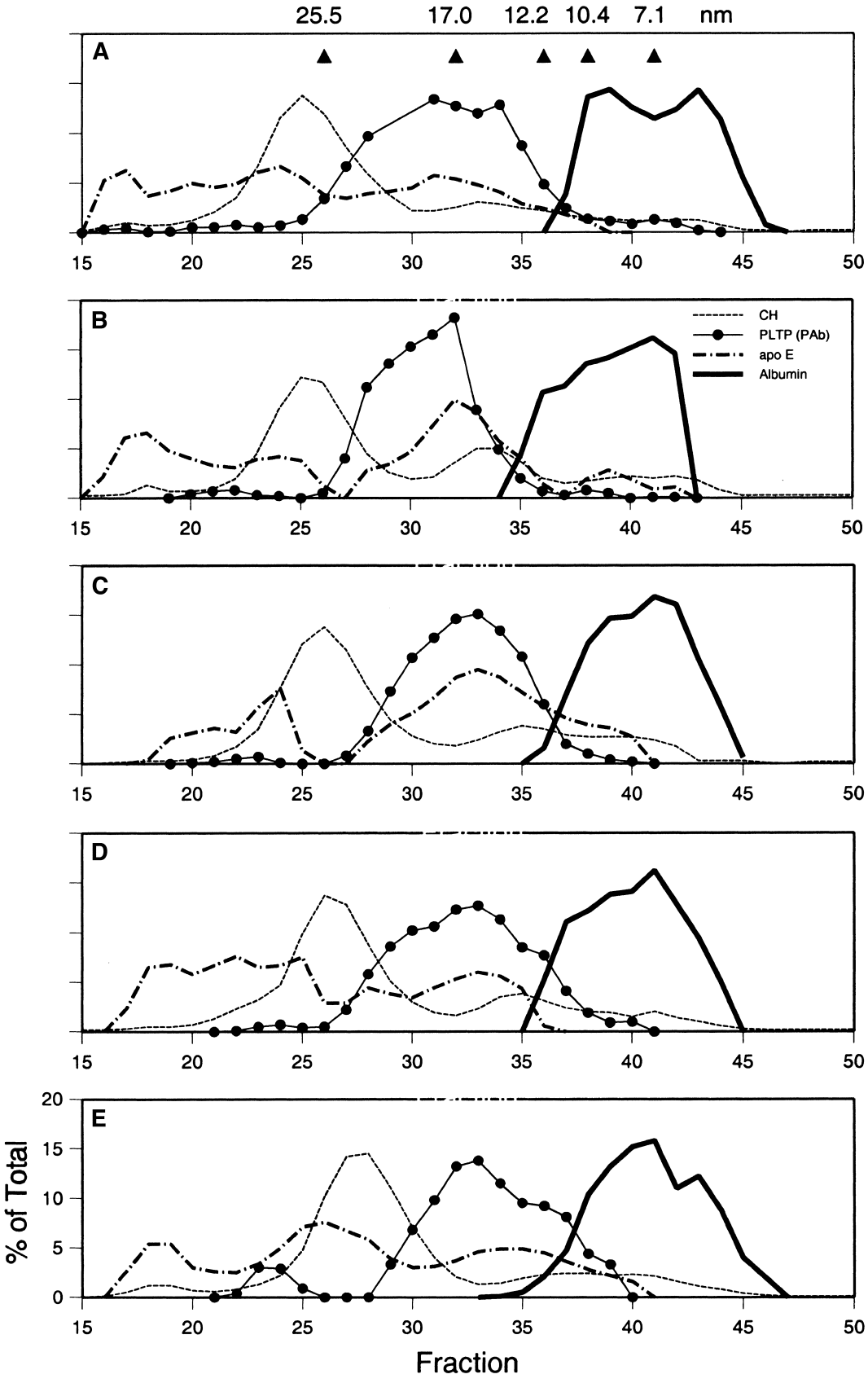

Fig. 3. Elution profile of plasma fractionated by FPLC; fractions of five subjects, A, B, C, D, and E. Cholesterol distribution (even broken line); PLTP distribution as detected by polyclonal antibody using a dot blot procedure (solid line with closed circle); apoE distribution assayed by nephelometry (uneven broken line); albumin distribution measured by nephelometry (solid line).

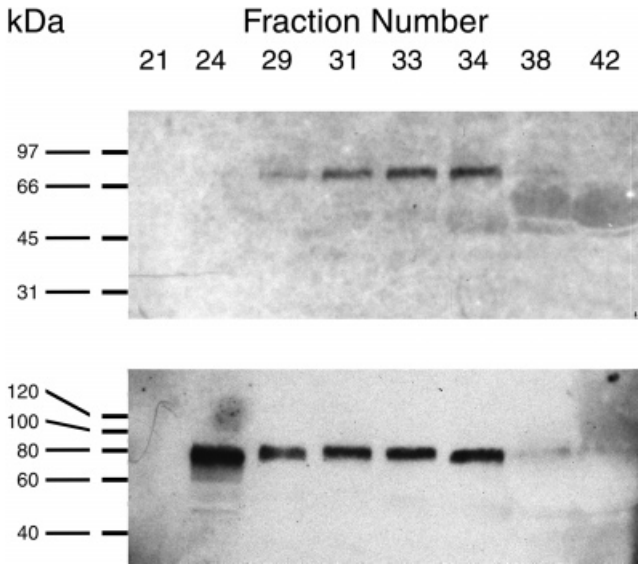

Fig. 4. Western blot analysis of PLTP in selected fractions (subject E) from plasma separated by FPLC. The fractions were subjected to SDS-PAGE on 4–20% gels under reducing conditions, transferred to nitrocellulose and immunoblotted. Top: Western blot analysis of FPLC fractions using polyclonal antibody (rb72). Bottom: Western blot analysis of FPLC fractions using MAb4.

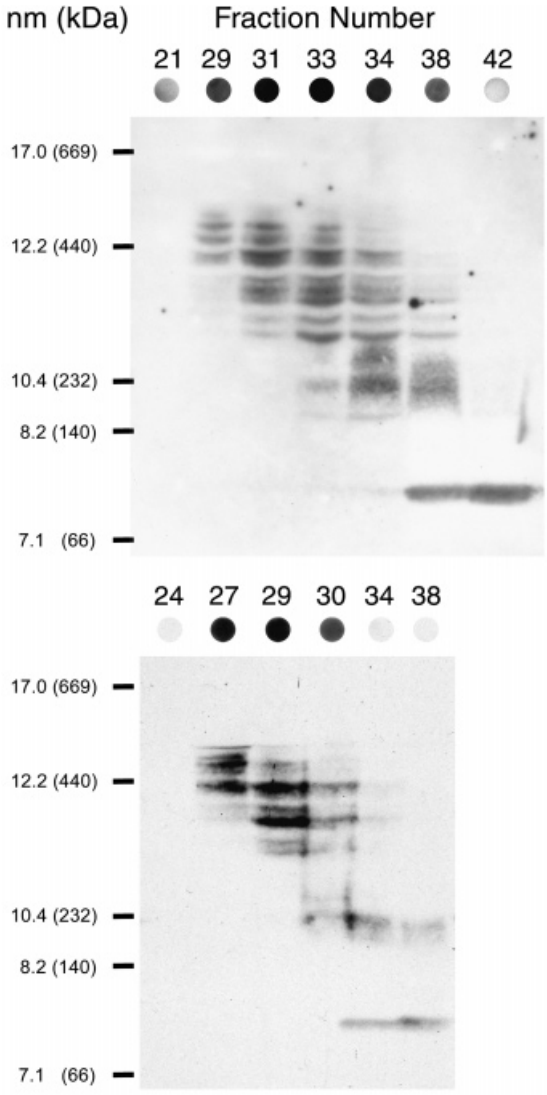

Fig. 5. Dot blot and Western blot analysis of selected fractions of plasma separated by FPLC. The fractions were subjected to either the dot blot procedure or non-denaturing PAGE on 4–30% gels and immunoblotted with polyclonal antibody. Top: Fractions from subject E. Bottom: Fractions from subject F. The circles below the fraction numbers are the results of the dot blot. The dot blots of fractions 21 and 42 from subject E top and fractions 24, 34, and 38 from subject F bottom were not significantly different from the background.

vesicles to HDL. However, full-length PLTP was evident in this region by Western blot on SDS gels, even though albumin appeared to mask PLTP (Fig. 4).

In order to further assess the particle distribution of PLTP, selected FPLC fractions were evaluated by non-denaturing gradient gel electrophoresis and Western blotting using the polyclonal antibody (**Figs. 5** and **6**). Using the results of subject E as an example, PLTP was associated with a large number of discrete size particle subpopulations ranging in size from 14.0 to 7.6 nm (approximating 518–100 kDa) (Fig. 5, top), the proportion varying from fraction to fraction. On examination of the size of the particles associated with PLTP compared with the presence of phospholipid transfer activity in the fractions we found the following patterns. In the small LDL region (fraction 29), where immunoreactive PLTP was detected but no apoA-I or phospholipid transfer activity was measurable, PLTP was associated with four major particle subpopulations ranging from 13.2 to 12.0 nm (486–415 kDa) (Fig. 5, top). In fraction 31, the region intermediate between LDL and HDL, where PLTP specific activity was low but present, PLTP was associated with the large particles detected in fraction 29 but with three additional subpopulations somewhat smaller in size (11.8–11.5 nm or 391–355 kDa), and a small amount was associated with particles from 11.3 to 11.1 nm (329–302 kDa). Interestingly, in fractions where PLTP activity became substantial (fraction 33), PLTP was still found to be associated with the large HDL subpopulations, but the proportion of particles in the 11.1 and 11.3 nm range was noticeably increased, and particles of 10.4 nm appeared. In fraction 34 containing peak PLTP activity, smaller particles of 11.1, 10.8, and 10.4 nm (302, 269, and 227 kDa, respectively) predominated. In fraction 38, where the large particles were absent but particles less than 11.2 nm predominated (11.1–7.6 nm), PLTP activity was still present. An important observation was noted that PLTP mass was clearly present in the fractions from the region of small HDL/plasma proteins on the Western blot of the non-denaturing PAGE (Fig. 5), a region where both the polyclonal and monoclonal antibodies failed to detect PLTP on dot blot analysis. This is in agreement with the results of the Western blot of SDS gels (Fig. 4) where a faint band was present in the small HDL/plasma protein region. For example, in fraction 42 (Fig. 5, top), which contained significant phospholipid transfer activity, PLTP was associated with small particles with an

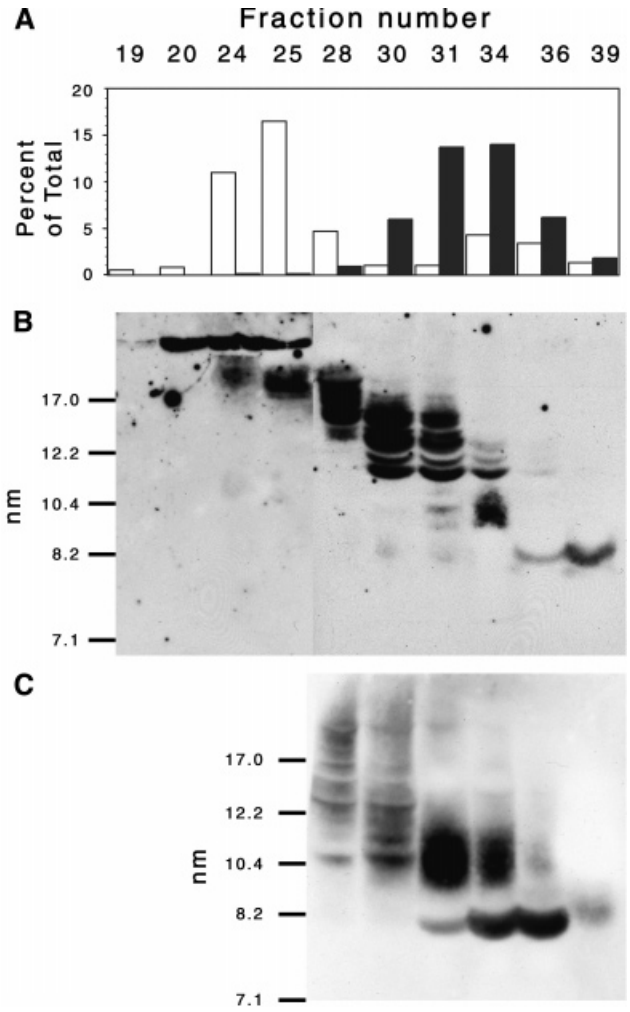

Fig. 6. Western blot analysis of selected plasma fractions separated by FLPC from subject G. A: Relative amounts of phospholipid transfer activity (solid bars) and cholesterol in the fractions (black and white bars). Because only selected fractions are shown, the percent recovery does not add up to 100%. B: Western blot analysis using MAb4 after subjecting the fraction to non-denaturing PAGE on 4–30% gels. C: Western blot analysis using polyclonal antibody after subjecting the fractions to non-denaturing PAGE on 4–30% gels.

estimated size of 7.6 nm (100 kDa), approximating the apparent size of PLTP complexed with lipid but unassociated with apolipoproteins (6). These fractions contained large amounts of albumin as demonstrated in Fig. 3.

The size of the particles bound to PLTP detected by the monoclonal antibody compared with that of the polyclonal antibody is demonstrated in Fig. 6. The Western blot of the FPLC fractions applied to non-denaturing gradient gels are shown as an example in Fig. 6 for subject G. The distribution of PLTP activity and cholesterol of the corresponding fractions is also illustrated. PLTP, as detected by the polyclonal antibody, was associated with a broad range of particle sizes qualitatively similar to that shown in Fig. 5. Fractions containing active PLTP showed a predominance of particles in the 7.8–11.5 nm range. PLTP was again detected in fractions containing very small HDL and plasma proteins confirming its presence in these fractions. The monoclonal antibody demonstrated a different pattern of reactivity as compared with that of the polyclonal antibody. The monoclonal antibody showed strong reactivity in the fractions from the LDL and large HDL region. Additionally, very large particles (>19 nm) were detected by the monoclonal antibody in fractions from the LDL region. The reactivity of the monoclonal antibody was much less than that of the polyclonal antibody with particles in the 7.9–11.5 size range, which were predominant in fractions containing active PLTP when assayed with polyclonal antibody. These observations again indicate the selective reactivity of the monoclonal antibody with inactive PLTP.

DISCUSSION

Contrary to previous reports, we have found that the distribution of mass of PLTP in plasma fractionated by FPLC corresponds substantially with the phospholipid transfer activity when detected by a polyclonal antibody. A comparison of the distribution of PLTP mass determined by dot blot using the polyclonal antibody with the distribution of PLTP activity suggests that the majority of plasma PLTP is active. However, the estimate from these distributions assumes that the polyclonal antibody recognizes equally the two forms of PLTP, and little or no inactive PLTP is found in the size range of particles containing active PLTP. Because neither of these assumptions can be proven from the data available, we have chosen not to provide an estimate of the average amount of active/inactive PLTP present in the plasma samples. A more exact estimation must await the application of quantitative methods that selectively and accurately measure active and inactive PLTP.

We found that the PLTP activity distribution was similar to that of apoA-I and all fractions demonstrating transfer activity also contained apoA-I. There was a preference for a portion of PLTP to be associated with larger HDL since the activity peaked before the apoA-I on the elution profile. Since the fractions from the FPLC isolation contain particles of overlapping size, we consider the size estimate of the particles associated with PLTP on gradient gels to be the more accurate measurement. PLTP is found in lipoprotein particles covering a very broad size range on non-denaturing gradient gels, with the majority being associated with particles of HDL size. PLTP is found in at least 14 distinct subpopulations ranging in size from 7.6 to greater than 17 nm, but significant activity appears to correspond with the appearance of PLTP associated with particles in the 9 to 11.3 nm range. PLTP associated with particles greater than 12 nm were found in fractions containing no activity. The polyclonal antibody detected immunoreactive PLTP on non-denaturing gels primarily associated with particles of HDL size. In contrast, the monoclonal antibody showed poor reactivity with PLTP associated with HDL less than 12 nm in size but appeared to preferentially react with particles greater than 12 nm and those isolating in the IDL/LDL size range containing no phospholipid

transfer activity. Thus, as compared with the polyclonal antibody, the monoclonal antibody appears to have a higher reactivity for inactive PLTP observed on both dot blot, SDS gel, and on non-denaturing gradient gel, resulting in an overestimate of inactive PLTP and an underestimate of the active form.

Oka et al. (4) have recently described the mass and activity distribution of PLTP in plasma isolated by FPLC. These authors concluded that approximately 70% of the total PLTP protein is catalytically inactive in contrast to the present findings. In agreement with their work, we did find some inactive PLTP to be present between HDL and LDL after separation by size exclusion chromatography but the overall amount of inactive PLTP was much less when assessed by the polyclonal antibody. We also found inactive PLTP in the size range of IDL/large LDL particularly when detected by the monoclonal antibody. Both Oka et al. and the present study found that the peak of PLTP mass elutes before that of PLTP activity, but we observed a greater degree of correspondence between the two peaks. One major difference observed in the present study was that the PLTP activity generally followed the apoA-I and HDL cholesterol distribution. In the study of Oka et al. (4), the activity was primarily located in the region corresponding to the size of very small HDL and apolipoprotein-free PLTP based upon the cholesterol and apoA-I distribution. They reported that the peak of phospholipid transfer activity corresponded to an average molecular mass of 160 kDa. We found that the maximum phospholipid transfer activity was associated with particles ranging from 9.0 to 12.1 nm (167–429 kDa), with the predominant particles being in the 10.4 to 11.1 nm size range (227–302 kDa).

Although it initially appeared that there was phospholipid transfer activity in some fractions in the absence of mass, we have determined that PLTP is always present in fractions that demonstrate activity. We have found that the detection of PLTP is blocked in fractions containing small HDL where albumin co-elutes when PLTP is assayed with the dot blot procedure, is faint on Western blot of SDS gels, but is clearly seen on Western blot of non-denaturing PAGE gels. Thus, it appears that a component in these fractions interferes with the binding of PLTP with either the monoclonal or polyclonal antibody under the conditions used. The concurrent sharp rise in albumin with the rapid decrease in detectable PLTP in the FPLC fractions of all individuals examined suggests that albumin may be the interfering component. PLTP was readily detected in the albumin region by Western blot after non-denaturing gel electrophoresis, and to some extent after SDS-gel electrophoresis, presumably because electrophoresis separates the interfering component from PLTP. It should be noted that Oka et al. (4), using a monoclonal antibody-based ELISA, also found PLTP activity but failed to detect PLTP mass in FPLC fractions corresponding to the region of small HDL/albumin.

Thus, the inability to detect or measure PLTP under certain assay conditions is important with respect to estimating PLTP mass and relating the mass to PLTP activity. It is possible that the failure to form an immunocomplex as well as the masking of the PLTP epitopes could occur when measuring PLTP mass by ELISA under defined conditions. Thus, the inability to quantitatively detect all PLTP potentially accounts for some of the discrepancies between PLTP mass and activity reported in the literature. Furthermore, a significant contribution to the discrepancy appears to be related to the observation that the monoclonal antibody reacted to a greater extent with inactive PLTP and less with active PLTP than the polyclonal antibody. Therefore, we speculate that the lack of correlation between PLTP mass and activity reported by other investigators (2–4) is due to the fact that these assays overestimate the inactive PLTP and underestimate the active PLTP. As a consequence, inactive PLTP represents a major portion of the total PLTP measured. In these reports (2–4), a monoclonal antibody was used in the sandwich ELISA, whereas in the report of Desrumaux et al. (1), in which a high correlation between PLTP mass and activity was found, a polyclonal antibody was used. It appears that the monoclonal antibodies against PLTP thus far reported, including the present study, have a stronger reactivity with inactive PLTP than polyclonal antibodies. For each PLTP immunoassay, it would be important to clearly define the relative reactivity of the antibodies to inactive versus active PLTP.

It is unclear why PLTP is apparently inactive when associated with particles in the very large HDL/small LDL and IDL/LDL region. One plausible explanation is that PLTP isolated in the very large HDL/small LDL region is in the process of transferring lipids, gained from the redundant surface of VLDL and IDL during lipolysis, to plasma HDL. PLTP, being saturated with lipids, is unable to further bind and thus transfer phospholipid in the PLTP activity assay. HDL containing apoE was found in the size range of particles that are associated with inactive PLTP, and the PLTP distribution was often similar to that of the apoE distribution in HDL, suggesting that inactive PTLP may be associated with apoE-containing particles (Fig. 3). There is evidence that PLTP may play a role in the transfer of apoE from VLDL to HDL (7, 8). Thus it is possible that PLTP in these very large particles may form a large, surface-rich, transient HDL in the process of transferring apoE along with phospholipid and unesterified cholesterol as a unit derived from VLDL and IDL/remnants. It is also possible, though less likely, that HDL with apoE may be acting as an acceptor of redundant surface or that its interaction with PLTP prevents the latter from transferring lipid in vitro. Inactive PLTP in the IDL/LDL region may reflect a similar process, where PLTP is associated with the surface of VLDL/IDL remnants that are undergoing lipolysis by lipoprotein lipase, and PLTP cannot transfer phospholipid because the lipid binding sites of PLTP are saturated with the redundant surface lipids. It is also possible that inactive PLTP is associating with specific plasma proteins such as alpha-2 macroglobulin, which isolate in the region of large HDL/small LDL rather than being directly bound to lipoprotein particles. Such an association could potentially prevent PLTP from interacting with the vesi-

cles or the HDL-3 in the PLTP activity assay. However, PLTP in the inactive form appears to be associated with many different-sized particles. Thus, it is unlikely that inactive PLTP is bound to a particular protein of a distinct mass, as our findings are more consistent with an association with lipoproteins of varying lipid and protein composition.

An alternative explanation for the presence of inactive and active PLTP may be that the conformation of PLTP may differ when it is associated with larger particles as compared with smaller ones, resulting in a loss of transfer activity as suggested by Oka et al. (4). The binding of PLTP to particles with a certain radius of curvature may affect the conformation of PLTP in such a way that binding or efficient transfer of lipid is impeded. However, other factors besides particle size may be important determinants of PLTP conformation and activity. For example, an association of PLTP with apoA-I-containing particles may be required for PLTP activity because the activity rose as apoA-I increased, and the PLTP activity distribution was similar to that of apoA-I distribution.

In conclusion, we have found good concordance between the distribution of phospholipid transfer protein and phospholipid transfer activity. PLTP is always present when phospholipid activity is evident. Our monoclonal antibody against PLTP had greater reactivity with inactive PLTP and less with active PLTP than our polyclonal antibody, yet both were specific for PLTP. The nature and strength of the relationship between PLTP mass and activity will depend upon the degree to which the antibodies detect inactive PLTP versus active PLTP and likely contribute to the differences reported in previous studies (1–4). These observations must be taken into consideration in future investigations of the relationship between PLTP mass and activity and their respective association with lipoprotein concentrations and metabolic parameters.

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