Relationship between phospholipid transfer protein activity and HDL level and size among inbred mouse strains

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Abstract Because of the paucity of data on phospholipid transfer protein (PLTP) activity and lipoprotein phospholipid in mouse strains, plasma PLTP activity (PLTA), plasma phospholipid and cholesterol, HDL phospholipid and cholesterol, and HDL size distribution were determined in 15 inbred mouse strains. The 15 inbred mouse strains differed in their relatedness to one another and consisted of six largely unrelated groups: Castaneus, Swiss, C57BL, AKR, DBA, and NZB. Lipid and PLTA analyses were performed on plasma pools from male and female mice that had fasted for 4 h prior to blood draw. Among the representative unrelated strains fed the chow diet, there was a highly significant relationship between PLTA and plasma phospholipid (r_s = 0.727, $P < 0.01$), HDL phospholipid (r_s = 0.762, $P < 0.01$), HDL cholesterol (r_s = 0.699, $P < 0.02$), percentage of large HDL particles (r_s = 0.699, $P < 0.02$), and HDL peak size (r_s = 0.776, $P < 0.01$). Similar results were obtained among these strains fed a high fat, high cholesterol diet. PLTA increased in all strains fed the high fat diet ($\bar{X}$ = 94%, range 6 to 221%). Strain SM having relatively low PLTA and HDL was crossed with strain NZB having high PLTA and HDL. The F1 progeny from this cross were backcrossed to strain SM and 41 male backcross progeny collected. Among these individual backcrossed animals, PLTA was highly correlated with plasma phospholipid (r_s = 0.508, P = 0.001), HDL phospholipid (r_s = 0.566, $P < 0.001$), HDL cholesterol (r_s = 0.532, $P < 0.001$), and percentage of large HDL particles (r_s = 0.446, P = 0.020). Therefore, we conclude that PLTP is a determinant of HDL level and size in mice.—Albers, J. J., W. Pitman, G. Wolfbauer, M. C. Cheung, H. Kennedy, A-Y. Tu, S. M. Marcovina, and B. Paigen. **Relationship between phospholipid transfer protein activity and HDL level and size among inbred mouse strains. *J. Lipid Res.* 1999.** 40: **295–301.**

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The plasma phospholipid transfer protein (PLTP) promotes the mass transfer of phospholipids between circulating plasma lipoproteins (1, 2). In vitro evidence suggests that PLTP plays an important role in high density lipoprotein (HDL) remodeling by mediating the conversion of HDL to larger and to smaller particles (3), facilitating the transfer of phospholipids between very low density lipoprotein (VLDL) remnants and HDL (4), and modulating the activity of cholesteryl ester transfer protein (CETP) (1).

The mouse has become a widely accepted model of human diseases including atherosclerosis, cardiovascular disease, and hyperlipidemia (5–7). The existence of inbred, recombinant inbred (RI), and mutant strains (8) and the development of molecular techniques allowing for the creation of transgenic (9) and knockout mice (10) have led to numerous insights into the role of apolipoproteins, lipolytic enzymes, and lipid transport proteins in lipoprotein metabolism and atherosclerosis (5, 7, 11, 12). Inbred strains are particularly useful models because of their well-defined genetic backgrounds and the strain-specific differences in phenotypic expression for traits related to lipid metabolism and atherosclerosis (13–17). There have been several publications examining the genetic differences in plasma lipids among inbred strains of mice (18–24). However, none have measured HDL phospholipid or examined the relationships between phospholipid transfer protein activity (PLTA) and HDL phospholipid or cholesterol, or between PLTA and HDL size. In order to assess potential relationships between PLTA and HDL among mouse strains, we have conducted a comprehensive survey of plasma lipids, PLTP activity, HDL levels, and HDL size in both male and female mice fed a chow diet and then a high fat, high cholesterol diet.

Abbreviations: HDL, high density lipoproteins; PLTP, phospholipid transfer protein; PLTA, phospholipid transfer protein activity; VLDL, very low density lipoproteins; CETP, cholesteryl ester transfer protein.

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MATERIALS AND METHODS

Mice and diets

Male and female mice from 14 inbred strains (129/SvJ, A/HeJ, A/J, AKR/J, BALB/cJ, C3H/HeSnJ, C57BL/6J, C57BLKS/J, C57L/J, DBA/2J, NZB/BINJ, SJL/J, SM/J, and SWR/J), and one wild-derived strain (Cast/Ei) were obtained from The Jackson Laboratory, Bar Harbor, ME. Mice were housed in a climate-controlled facility with a 14-h light and 10-h dark cycle. After weaning at 21 days, mice were maintained on a chow diet (Old Guilford 234A, Guilford, CT). Mice were offered free access to food and water throughout the experiment, except when fasting conditions were required. All experiments were approved by the Institution's Animal Care and Use Committee.

After reaching 6–8 weeks of age, mice were fed an atherogenic diet for 6 weeks. The source of chemicals and the diet have been described previously (25, 26). The atherogenic diet contained (w/w) 15% dairy fat, 50% sucrose, 20% casein, 0.5% cholic acid, 1.0% cholesterol, cellulose, vitamins, and minerals.

For the breeding experiment, SM/J(SM) females were mated to NZB/BINJ(NZB) males. Then F1 females were mated to SM males to produce the (SM × NZB) F1 × SM backcross. The backcross animals were maintained on the chow diet until bled at 14 ± 2 weeks of age.

Lipid measurements

Mice were fasted for 4 h before blood was collected for lipid determination. Blood was collected in the morning by retro-orbital bleed into EDTA-coated tubes and plasma was separated by centrifugation at 1500 rpm, 5 min at 4°C. Standard lipid and lipoprotein analyses were performed in duplicate as described (27) on pooled plasma samples. Each pool was obtained by combining an equal amount of plasma from eight mice. Lipids and lipoprotein analysis was also performed in individual plasma from strains SM and NZB, the F1, and the (SM × NZB) F1 × SM backcross.

Phospholipid transfer protein activity

The plasma phospholipid transfer activity mediated by PLTP was determined by measuring the transfer of [^{14}C]phosphatidylcholine from phospholipid liposomes to HDL_3 (2). In short, 50 μl [^{14}C]phosphatidylcholine tracer-labeled liposomes containing 50 nmol of phosphatidylcholine and 12.5 nmol phosphatidylserine, 50 μl HDL_3 containing 157 nmol phospholipid, 50 μl of diluted plasma, and Tris buffer up to a final volume of 400 μl were incubated with shaking at 37°C for 15 min. After cooling on ice, 500 μl of carrier plasma protein (heat-inactivated at 56°C for 30 min and diluted 2:5 in 10 mm Tris buffer, pH 7.4) was added, and the liposomes were precipitated with 50 μl of 2% dextran sulfate combined with 50 μl of 1 m $MgCl_2$. The radioactivity transferred to HDL was counted in a 650-μl aliquot. As phospholipid transfer activity in mouse plasma is considerably higher than in human plasma (27), only half the amount of plasma (0.5 μl) was assayed compared to human plasma. Three different aliquots of each plasma pool were diluted at 1:100 and 50 μl was assayed for phospholipid transfer activity. We included in each assay 50-μl aliquots of three different 1:50 diluted, human control plasma samples, in quadruplicate. The human control samples were stored in small aliquots at −70°C and thawed only once to insure reproducibility of phospholipid transfer activity. Furthermore, only one preparation each of ^{14}C-labeled liposomes and HDL_3 was used throughout the study to minimize inter-assay variation. The amount of phospholipid transferred by plasma PLTP was calculated as percent of total radioactivity per assay tube transferred to HDL minus background transfer (tubes without PLTP source).

Non-denaturing gradient gel electrophoresis

Non-denaturing polyacrylamide gradient gel electrophoresis was used to analyze the HDL particle size distribution. Electrophoresis was performed on 20 μl of mouse plasma and 10 μl of high molecular weight standards (Pharmacia Biotech, Inc., Piscataway, NJ), using 4–30% preformed polyacrylamide gel (Alamo Gels, Inc., San Antonio, TX) in 0.09 m Tris, 0.08 m boric acid, 0.003 m EDTA, pH 8.35, at 200 volts for 20 hours at 4°C. Lipoproteins and the molecular weight standards were visualized with Sudan Black B and Coomassie G250, respectively, and scanned with a densitometer (LKB Ultrascan XL Laser Densitometer) as described (28).

Data analysis

Both Pearson and Spearman correlations were computed and generally provided similar results. However, in the case of the correlations among strains fed the high fat, high cholesterol diet, spuriously high correlations were sometimes obtained due to an outlier. Therefore, Spearman correlations and significant tests are reported. To test gender differences, the Wilcoxon Matched Pairs Test was used. Statistical procedures were performed using the software package STATISTICA/w (StatSoft, Tulsa, OK).

RESULTS

Relationship among PLTP activity, lipid and lipoprotein levels and HDL size

A survey of plasma phospholipid transfer activity (PLTA) and plasma and lipoprotein lipids, was performed on 15 mouse strains fed a chow diet and again after 6 weeks on a high fat, high cholesterol diet (Tables 1–3). In addition, the HDL size distribution was assessed in males and females from each strain. When mice were fed the chow diet, there was considerable variation among strains in PLTP activity (14–37 μmol/ml per h), plasma cholesterol (1.12–3.26 mmol/L), HDL cholesterol (0.73–2.51 mmol/L) (**Table 1**); plasma phospholipid (1.10–3.51 mmol/L), HDL phospholipid (0.89–2.89 mmol/L), plasma triglyceride (0.82–2.60 mmol/L) (**Table 2**); percent of HDL as large (10–13 nm) particles (26–78%), and relatively modest variation in HDL peak size (Stokes diameter 9.01–10.31 nm) (**Table 3**). Approximately 54–88% of the total cholesterol was found in the HDL-cholesterol fraction, with the remainder residing in the VLDL and LDL fractions.

To fully explore the significance of these observed differences we need to consider that the various mouse strains differ in their relatedness to one another. Among these 15 mouse strains there are six, largely unrelated groups: Castaneus, which is recently inbred from wild mice; AKR/J as a representative from Furth's stocks; DBA and related strains including DBA/2J, A/HeJ, A/J, BALB/cJ, and C3H/HeSnJ; Swiss mice, which include SWR/J and SJL/J; C57 from Miss Lathrop's stocks which include C57BL/6J, C57BLKS/J, and C57L/J; and NZB/BINJ because it is unrelated to the other groups. SM/J and 129/SvJ were excluded from the groups, because they were derived from a cross utilizing a mixture of strains. The Castaneus mice had the lowest PLTA and HDL phospholipid, nearly the lowest HDL cholesterol levels and very small

TABLE 1. Selected plasma and lipoprotein lipids and PLTP activity among mouse strains on chow and high fat diet[a]

Strain[a]	Sex	PLTP Activity		Cholesterol Total		Cholesterol HDL		Cholesterol non-HDL	
		Chow	Fat	Chow	Fat	Chow	Fat	Chow	Fat
Cast/Ei[b]	M	15.2	41.1	1.45	8.22	0.98	2.40	0.47	5.82
Cast/EI[b]	F	14.0	35.7	1.49	20.21	0.80	0.93	0.69	19.28
AKR/J[b]	M	20.2	37.2	1.49	4.16	1.14	1.81	0.34	2.35
AKR/J[b]	F	21.5	38.2	1.32	4.80	0.98	1.53	0.34	3.26
A/J	M	20.5	50.2	1.76	6.65	1.32	1.95	0.44	4.70
A/J	F	18.2	57.7	1.49	6.85	1.00	1.86	0.48	4.99
DBA/2J[b]	M	23.2	41.1	2.00	4.87	1.45	2.88	0.56	1.99
DBA/2J[b]	F	20.9	36.0	1.50	4.03	0.93	1.09	0.57	2.94
A/HeJ	M	23.6	52.3	1.77	7.42	1.45	2.20	0.32	5.22
A/HeJ	F	18.0	54.0	1.42	5.22	1.15	2.10	0.27	3.12
BALB/CJ	M	23.6	39.4	1.95	4.14	1.72	2.07	0.23	2.07
BALB/CJ	F	19.2	34.1	1.77	5.12	1.39	1.55	0.38	3.57
C3H/HeSnJ	M	31.3	44.7	2.93	7.90	2.27	2.55	0.67	5.35
C3H/HeSnJ	F	36.9	44.5	2.24	9.82	1.78	1.66	0.45	8.16
SJL/J	M	26.3	n.d.	1.90	n.d.	1.31	n.d.	0.59	n.d.
SJL/J	F	22.4	43.6	1.44	3.84	0.99	1.19	0.45	2.65
SWR/J[b]	M	28.7	39.8	2.13	7.58	1.52	1.40	0.61	6.17
SWR/J[b]	F	21.3	41.8	1.49	5.95	0.94	0.93	0.55	5.02
C57BLKS/J	M	25.9	40.9	1.92	9.79	1.46	0.93	0.46	8.86
C57BLKS/J	F	24.4	44.1	1.12	7.56	0.73	1.06	0.38	6.50
C57BL/6J[b]	M	31.8	45.7	1.85	8.97	1.60	1.46	0.26	7.51
C57BL/6J[b]	F	29.4	39.0	1.46	7.31	1.12	0.86	0.34	6.45
C57L/J	M	33.9	36.2	1.91	4.29	1.37	1.30	0.54	3.00
C57L/J	F	26.2	32.8	1.55	4.85	0.99	0.54	0.56	4.31
NZB/BLNJ[b]	M	28.3	60.9	3.26	12.65	2.51	4.29	0.75	8.35
NZB/BLNJ[b]	F	25.8	59.3	3.04	10.76	2.31	3.52	0.73	7.24
SM/J	M	21.9	39.8	1.97	8.15	1.58	1.71	0.39	6.44
SM/J	F	16.5	29.0	1.44	8.37	1.09	2.40	0.35	5.97
129/SVJ	M	18.0	45.0	2.60	6.40	2.15	2.46	0.45	3.94
129/SVJ	F	17.8	47.9	2.17	6.39	1.73	2.11	0.44	4.28

Lipid values are expressed in mmol/L and phospholipid transfer protein activity (PLTA) is expressed in μmol/ml/h. Lipid analyses were performed in duplicate and PLTP activity in triplicate on pooled plasma obtained on eight male or eight female mice that were fasted 4 h prior to blood draw.

[a] Strains are arranged by six unrelated groups. SM/J and 129/SVJ were excluded from the groups because these two strains were derived from a cross utilizing multiple strains.

[b] Representative of unrelated groups.

TABLE 2. Selected plasma and lipoprotein lipids among mouse strains on chow and high fat diet

Strain[a]	Sex	Phospholipid Total		Phospholipid HDL		Phospholipid non-HDL		Triglyceride Total	
		Chow	Fat	Chow	Fat	Chow	Fat	Chow	Fat
Cast/Ei[b]	M	1.27	3.32	1.02	2.10	0.25	1.22	1.49	1.17
Cast/EI[b]	F	1.10	3.23	0.89	0.62	0.22	2.61	1.19	1.62
AKR/J[b]	M	1.94	2.63	1.67	1.87	0.27	0.77	1.61	1.26
AKR/J[b]	F	1.66	2.47	1.43	1.74	0.24	0.73	1.55	0.96
A/J	M	2.34	3.69	1.94	2.10	0.40	1.59	1.77	1.15
A/J	F	1.78	3.39	1.49	2.12	0.29	1.27	1.27	1.02
DBA/2J[b]	M	2.32	3.60	1.75	2.86	0.56	0.75	1.91	1.30
DBA/2J[b]	F	1.72	1.97	1.28	1.30	0.44	0.68	2.00	0.85
A/HeJ	M	2.30	3.75	2.03	2.42	0.26	1.34	1.25	0.89
A/HeJ	F	1.80	3.14	1.63	2.25	0.17	0.89	1.18	0.81
BALB/CJ	M	2.34	2.49	2.18	1.86	0.16	0.63	1.48	0.79
BALB/CJ	F	1.94	2.24	1.82	1.58	0.11	0.66	1.39	0.82
C3H/HeSnJ	M	3.51	3.81	2.89	2.63	0.62	1.18	2.59	1.08
C3H/HeSnJ	F	2.64	3.15	2.30	1.72	0.34	1.42	1.83	1.04
SJL/J	M	2.53	n.d.	1.99	n.d.	0.54	n.d.	2.04	n.d.
SJL/J	F	1.73	2.04	1.43	1.37	0.30	0.67	1.32	1.10
SWR/J[b]	M	2.53	2.74	2.02	1.32	0.52	1.42	1.89	0.91
SWR/J[b]	F	1.77	2.11	1.39	1.05	0.37	1.06	1.79	0.91
C57BLKS/J	M	2.14	2.93	1.82	1.06	0.32	1.87	1.20	0.98
C57BLKS/J	F	1.27	2.50	1.06	1.11	0.21	1.39	0.82	0.83
C57BL/6J[b]	M	2.38	2.98	2.11	1.40	0.27	1.58	0.94	0.76
C57BL/6J[b]	F	1.82	1.94	1.56	0.82	0.26	1.12	0.88	0.84
C57L/J	M	2.28	2.32	1.84	1.47	0.44	0.85	1.24	0.81
C57L/J	F	1.74	1.43	1.40	0.58	0.34	0.85	1.09	0.95
NZB/BLNJ[b]	M	3.27	5.32	2.77	3.31	0.50	2.02	1.61	0.94
NZB/BLNJ[b]	F	2.90	4.60	2.49	2.89	0.42	1.71	1.40	0.76
SM/J	M	2.41	3.10	2.08	1.55	0.34	1.54	1.49	1.20
SM/J	F	1.54	3.31	1.34	2.09	0.20	1.22	1.29	1.25
129/SVJ	M	2.41	3.18	2.04	2.22	0.37	0.97	1.54	0.87
129/SVJ	F	1.94	2.83	1.69	1.95	0.25	0.88	0.98	0.78

Lipid values are expressed in mmol/L. Lipid analyses were performed in duplicate on pooled plasma obtained on eight male or eight female mice that were fasted 4 h prior to blood draw; TG, total glycerides.

[a] Strains are arranged by six unrelated groups. SM/J and 129/SVJ were excluded from the groups because these two strains were derived from a cross utilizing multiple strains.

[b] Representative of unrelated groups.

HDL size, whereas NZB had the highest HDL cholesterol, nearly the highest HDL phospholipid, and quite high PLTA and large HDL size. The AKR strain had low PLTA, HDL cholesterol and phospholipid levels, and small HDL size. In contrast, the C57 strains had relatively high PLTA and HDL cholesterol and phospholipid levels. In general, the DBA and Swiss strains tended to have somewhat intermediate PLTA and HDL cholesterol and phospholipid levels although, within each of these groups, there was considerable variation in these lipoprotein parameters. When the strains were fed a high fat diet, they continued to exhibit a considerable variation in these lipoprotein parameters, but the levels of PLTA and HDL were generally higher.

Among representative unrelated strains fed the chow diet, we found a highly significant relationship between PLTA and plasma phospholipid ($r_s = 0.727$, $P < 0.01$), HDL phospholipid ($r_s = 0.762$, $P < 0.01$) and HDL cholesterol ($r_s = 0.699$, $P < 0.02$) (**Table 4**). PLTA was also positively correlated with the percent of large (from 10 to 13 nm) HDL particles ($r_s = 0.699$, $P < 0.02$), and the HDL peak size ($r_s = 0.776$, $P < 0.01$). Among all strains the correlation between PLTA and total phospholipid, HDL phospholipid, or HDL cholesterol was still significant, although the strength of the correlations was weaker. Among the representative unrelated strains fed the high fat diet, PLTA again was found to be significantly correlated to the levels of plasma phospholipid ($r_s = 0.573$, $P = 0.05$), HDL phospholipid ($r_s = 0.622$, $P < 0.05$), HDL cholesterol ($r_s = 0.567$, $P = 0.05$), percent of large HDL particles ($r_s = 0.685$, $P < 0.02$) and HDL peak size ($r_s = 0.720$, $P < 0.01$). Furthermore, among all strains the correlation of PLTA with each of these lipoprotein parameters was highly significant. Interestingly, among all strains and among the representative unrelated strains fed a chow diet, HDL peak size was positively correlated with HDL cholesterol ($r_s = 0.598$, $P = 0.001$; $r_s = 0.685$, $P = 0.014$) and HDL phospholipid ($r_s = 0.529$, $P = 0.003$; $r_s = 0.776$, $P = 0.003$). Similarly, the percent of large HDL particles (10–13 nm) was significantly correlated with HDL cholesterol

TABLE 3. Percent of large HDL particles and HDL peak size among mouse strains on chow and high fat diet

Strain[a]	Sex	% Large[b] HDL		HDL Size[c]	
		Chow	Fat	Chow	Fat
Cast/Ei[d]	M	26.3	27.6	9.01	9.12
Cast/EI[d]	F	27.1	34.0	9.18	9.23
AKR/J[d]	M	37.6	27.6	9.32	9.07
AKR/J[d]	F	38.6	25.0	9.35	9.16
A/J	M	36.6	36.3	9.36	9.32
A/J	F	40.5	39.9	9.26	9.25
DBA/2J[d]	M	31.1	35.2	9.37	9.70
DBA/2J[d]	F	28.1	26.1	9.41	9.19
A/HeJ	M	40.5	46.7	9.24	9.44
A/HeJ	F	43.6	45.6	9.60	9.71
BALB/CJ	M	41.5	44.3	9.70	9.52
BALB/CJ	F	62.8	51.9	9.93	9.89
C3H/HeSnJ	M	55.1	30.6	9.93	9.39
C3H/HeSnJ	F	78.2	28.9	10.31	9.26
SJL/J	M	nd	nd	nd	nd
SJL/J	F	35.9	28.3	9.15	9.16
SWR/J[d]	M	31.6	32.8	9.37	9.20
SWR/J[d]	F	39.7	32.0	9.30	9.23
C57BLKS/J	M	36.6	29.7	9.15	9.25
C57BLKS/J	F	32.2	29.4	9.27	9.28
C57BL/6J[d]	M	44.6	35.9	9.56	9.30
C57BL/6J[d]	F	44.6	34.3	9.67	9.21
C57L/J	M	32.2	27.3	9.29	9.28
C57L/J	F	40.4	30.5	9.34	9.35
NZB/BLNJ[d]	M	56.5	67.8	9.83	10.15
NZB/BLNJ[d]	F	66.2	73.7	9.86	10.46
SM/J	M	33.2	29.9	9.06	9.08
SM/J	F	29.6	27.2	9.32	9.29
129/SVJ	M	40.4	54.2	9.71	9.85
129/SVJ	F	56.2	65.5	10.10	10.07

[a] Strains are arranged by six unrelated groups. SM/J and 129/SVJ were excluded from the groups because these two strains were derived from a cross utilizing multiple strains.
[b] % of HDL 10 to 13 nm.
[c] HDL peak size in Stokes diameter (nm).
[d] Representative of unrelated groups.

(r_s = 0.624, $P < 0.001$; r_s = 0.636, P = 0.026) and HDL phospholipid (r_s = 0.620, $P < 0.001$; r_s = 0.741, P = 0.006).

To further explore our observations in these inbred mouse strains, strain SM females with relatively low PLTA and HDL levels were crossed with strain NZB males with high PLTA and HDL levels. Interestingly, strains SM and NZB differ not only in PLTA and HDL levels, but also in HDL composition (**Table 5**). The F1 females were mated to SM males to produce 41 male backcross progeny. The PLTA, HDL lipids, and HDL CH/HDL PL ratio in the F1 and backcross progeny were intermediate between those observed in NZB and SM (Table 5). PLTA exhibited a normal distribution in the backcross animals, suggesting that PLTA is determined by multiple genes (**Fig. 1**). Among the backcross animals (n = 41), PLTA was significantly correlated to plasma phospholipid (r_s = 0.508, P = 0.001), HDL phospholipid (r_s = 0.566, $P < 0.001$), HDL cholesterol (r_s = 0.532, $P < 0.001$), and percent of large HDL particles (r_s = 0.446, P = 0.02). Additionally, the percent of large HDL particles was highly correlated to HDL cholesterol (r_s = 0.874, $P < 0.001$) and HDL phospholipid (r_s = 0.816, $P < 0.001$).

Gender differences

The females in all strains but AKR/J and C3H/HeSnJ, fed a chow diet, had slightly lower PLTA than males ($\bar{x}$ = 22.1 μmol/ml per h vs. 24.7 μmol/ml per h, $P < 0.01$). Female mice consistently had lower plasma cholesterol ($\bar{x}$ = 1.66 mmol/L vs. 2.07 mmol/L, $P < 0.001$) and HDL cholesterol ($\bar{x}$ = 1.19 mmol/L vs. 1.58 mmol/L, $P < 0.001$), and HDL phospholipid ($\bar{x}$ = 1.55 mmol/L vs. 2.02 mmol/L, $P < 0.001$), tended to have a greater proportion of large HDL (44.2% vs. 38.9%, $P < 0.04$) and bigger HDL peak size (9.54 nm vs. 9.42 nm, $P < 0.01$) than male mice. Plasma phospholipids were consistently lower in females than in males (1.82 mmol/L vs. 2.40 mmol/L, $P < 0.001$) and correlated well with plasma total cholesterol (r = 0.88, $P < 0.001$). The non-HDL phospholipid was lower in females than in males ($\bar{x}$ = 0.27 mmol/L vs. 0.39 mmol/L, $P < 0.001$), and the plasma triglyceride concentrations were lower in females than in males (Table 2). No consistent differences between males and females, fed the chow diet, were observed for non-HDL cholesterol. Between males and females fed a high fat diet, there were no consistent differences in PLTA, total cholesterol, non-HDL cholesterol, non-HDL phospholipid, percent of large HDL, or HDL peak size. In contrast, in most strains, female mice on the high fat diet had lower HDL cholesterol ($P < 0.02$), HDL phospholipid ($P < 0.02$), and plasma phospholipid ($P < 0.02$) than their male counterparts.

Changes in PLTA, lipids, lipoproteins, and HDL size distribution in response to high fat, high cholesterol diet

In response to the high fat, high cholesterol diet, plasma cholesterol levels increased for males and females of all strains (Table 1), and plasma triglyceride concentrations decreased for all strains except Cast/Ei and C57BLKS/J females (Table 2). The changes in plasma phospholipid varied by strain and gender (−18% to +192%). Changes in HDL cholesterol concentrations were quite variable among strains and sometimes between genders (−45% to +145%), and only 5–59% of the total cholesterol was found in the HDL fractions, when mice were fed the high fat, high cholesterol diet. The changes in HDL phospholipid were also quite variable (−59% to +105%). Although in most strains the HDL cholesterol and phospholipid levels increased in response to fat feeding, the HDL lipid levels decreased with fat feeding in all C57 strains except C57BLKS/J females. It also should be noted that in some strains the changes in HDL were quite different between males and females of the same strain. For example, the HDL lipid levels increased considerably in Castaneus and DBA males but not in the females from these strains. The non-HDL cholesterol (Table 1) and non-HDL phospholipid (Table 2) levels increased dramatically in all strains. HDL peak sizes changed relatively little except in two strains (C3H and NZB), when mice were fed the high fat, high cholesterol diet (Table 3).

PLTA increased in all strains fed the high fat, high cholesterol diet ($\bar{x}$ = 94%), but the amount of increase varied over a wide range, from 6% for C57L/J males to 221% for A/J females. Interestingly, the percent change

TABLE 4. Correlations of PLTP activity with lipoprotein parameters among mouse strains

		Lipoprotein Parameter									
		Total PL		HDL PL		HDL CH		HDL % Large[a]		HDL Size[b]	
Strains	n	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P
PLTP activity vs. lipoprotein parameter among strains fed chow diet											
All	30	0.583	0.001	0.566	0.001	0.405	<0.05	0.339	0.07	0.234	0.22
Selected[c]	12	0.727	<0.01	0.762	<0.01	0.699	<0.02	0.699	<0.02	0.776	<0.01
Group average[d]	12	0.713	<0.01	0.706	<0.02	0.636	<0.05	0.531	0.08	0.629	<0.05
PLTP activity vs. lipoprotein parameter among strains fed high fat diet											
All	29	0.617	<0.001	0.614	<0.001	0.526	<0.01	0.609	<0.001	0.441	<0.02
Selected	12	0.573	0.05	0.622	<0.05	0.567	0.05	0.685	<0.02	0.720	<0.01
Group average	12	0.629	<0.05	0.699	<0.02	0.657	<0.05	0.629	<0.05	0.699	<0.02
% Change in PLTP activity vs. % change of lipoprotein parameter from chow to fat diet											
All	29	0.730	<0.001	0.614	<0.001	0.647	<0.001	0.601	0.001	0.516	<0.01
Selected	12	0.874	<0.001	0.587	<0.05	0.608	<0.05	0.476	0.12	0.671	<0.02
Group average	12	0.797	<0.01	0.545	0.07	0.580	<0.05	0.594	<0.05	0.573	0.05

[a] Percent of HDL 10 to 13 nm.
[b] HDL distribution peak size.
[c] Selected representative strains include: Cast/Ei, AKR/J, DBA/2J, SWR/J, C57BL/6J, and NZB/BLNJ.
[d] Strains have been assigned to the following six groups: Cast—Cast/Ei; AKR—AKR/J; DBA—A/J, DBA/2J, A/HeJ, BALB/CJ, C3H/HeSnJ; C57 -C57BLKS/J, C57BL/6J, C57L/J; SWR—SJL/J, SWR/J; NZB—NZB/BLNJ. SM/J and 129/SVJ were excluded from the group average analysis because these two strains are derived from a cross utilizing multiple strains.

in PLTA was highly negatively correlated to the PLTA on chow diet ($r_s = -0.752$, $P < 0.001$, all strains; $r_s = -0.671$, $P < 0.02$, unrelated strains). In other words, the higher the basal PLTA, the smaller was the observed change in PLTA in response to the high fat, high cholesterol diet. In addition, the percent change in PLTA was significantly positively correlated to the percent change in plasma phospholipid, HDL phospholipid, HDL cholesterol, and HDL peak size among all strains and among the unrelated selected strains (Table 4). Thus, the changes in PLTP activity in response to the high fat diet were significantly related to the changes in HDL lipids and HDL size.

DISCUSSION

The inbred mouse strains are particularly useful models for studies of the role of apolipoproteins, lipolytic enzymes, and lipid transfer proteins in lipoprotein metabolism and atherosclerosis. The strain specific genetic and phenotypic differences can be used to advantage to delineate the genes controlling specific traits related to atherosclerosis susceptibility and genes controlling dietary responsiveness. Additionally, through the use of knockout, transgenic, and knockin (7) mice, we have a unique opportunity to explore the role of the proteins of lipid transport in biological processes and atherosclerosis. However, in these latter studies we must be cognizant of the possibility that our findings may be strain and/or model specific and may not apply to all mice in general and, more importantly, the findings may not be relevant to human lipoprotein metabolism or human atherosclerosis. In order to select the appropriate strain, develop the optimal experimental protocol, and correctly interpret the findings, it is essential to know the genetic and phenotypic differences among mouse strains, between males and females of a given strain, and between mice and humans. In spite of the proliferation of mouse models for the understanding of human lipoprotein metabolism, there is a relative paucity of comprehensive surveys of lipoprotein metabolism among inbred mouse strains. Furthermore, relatively few studies have critically considered how the fundamental differences

TABLE 5. PLTP activity, HDL lipids, and plasma phospholipids

	NZB (n = 10)	SM (n = 10)	F1 (n = 7)	(SM × NZB) F1 × SM (n = 41)
PLTP activity	27.7 ± 2.7	18.4 ± 2.5[a]	24.8 ± 2.1[b,c]	25.4 ± 3.6[b,c]
HDL CH	2.57 ± 0.15	1.37 ± 0.11[a]	2.30 ± 0.13[a,c]	2.23 ± 0.44[b,c]
HDL PL	2.44 ± 0.16	1.70 ± 0.11[a]	2.32 ± 0.12[c]	2.32 ± 0.36[c]
HDL CH/HDL PL	0.53 ± 0.03	0.40 ± 0.01[a]	0.49 ± 0.02[a,c]	0.48 ± 0.03[a,c]
Total PL	3.01 ± 0.20	2.16 ± 0.14[a]	2.80 ± 0.14[b,c]	2.79 ± 0.42[c]

Male mice were fed a chow diet for 14±2 weeks. PLTP activity (mean ± SD) expressed in μmol/ml per h and plasma and HDL lipids (mean ± SD) expressed in mmol/L. Significant differences are indicated as follows.
[a] Significantly different from NZB, $P < 0.01$.
[b] Significantly different from NZB, $P < 0.05$.
[c] Significantly different from SM, $P < 0.01$.

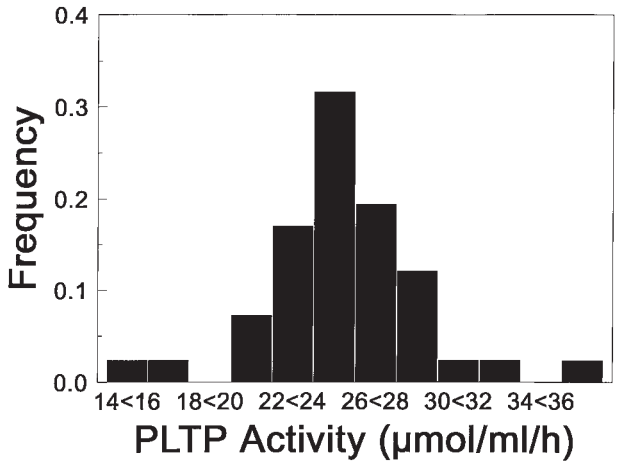

Fig. 1. Frequency distribution of PLTP activity in the (SM × NZB) F1 × SM backcross.

between a given mouse strain and a human population may limit the extrapolation of the findings to humans.

Because of our long-standing interest in HDL metabolism and the plasma lipid transfer proteins and, more specifically, the plasma phospholipid transfer protein (PLTP) and our recent interest in pursuing the use of mouse models to explore the role of PLTP in lipid metabolism, we embarked upon this survey of inbred mouse strains. Given the near complete lack of data on lipoprotein phospholipid levels among mouse strains and the putative role of PLTP in phospholipid transport, it was important to obtain data on plasma and lipoprotein phospholipids in addition to cholesterol. It is of considerable interest that among the measured lipid and lipoprotein parameters, PLTP activity (PLTA) was most strongly associated with the plasma phospholipid levels. Thus, among unrelated mouse strains fed a chow diet, PLTA explained approximately 50% (0.727 × 0.727 × 100) of the variance in plasma phospholipid levels. Additionally, the change in PLTA that occurred with fat feeding explained over 50% of the variance in the change in plasma phospholipid in response to fat feeding. As most of the plasma phospholipid is associated with the HDL fraction in mice fed a chow diet, it is not surprising that PLTA levels were significantly associated with HDL phospholipid levels. PLTA was also significantly correlated with HDL cholesterol levels, HDL size distribution, and HDL peak size. Although it is granted that these significant associations do not prove causality, considering the in vitro data demonstrating that PLTP facilitates the transformation of HDL into larger and smaller particles (3) and facilitates the net mass transfer of phospholipid from VLDL and LDL to HDL (1, 24), it is reasonable to postulate that PLTA has a direct effect on both HDL levels and size. Additionally, the significant positive correlation between PLTA and HDL level and size among the (SM × NZB) F1 × SM backcross mice provides strong support to the premise that PLTP is a determinant of HDL level and size in mice.

Our survey clearly documents that nearly all strains female mice, fed chow, have lower plasma cholesterol, triglyceride, and phospholipid levels and consistently have lower HDL cholesterol and phospholipid levels. It is important that these inherent sex differences be considered when evaluating the effects of transgenes on lipid metabolism. In contrast to these findings in mice, women have higher HDL levels than men, primarily due to differences in the sex hormones. The basis for the lower HDL levels in female mice has yet to be elucidated.

Although the mechanism of PLTP-mediated conversion is not well understood, it has been reported to involve displacement and release of apoA-I from the HDL surface and formation of larger particles by inter-particle fusion (29). HDL to HDL transfers constitute the bulk of PLTP-mediated transfer in the plasma compartment (30). These PLTP-mediated transfers facilitate the conversion of discoidal HDL particles into vesicular structures (31). Furthermore, we have shown that incubation of mouse HDL with mouse recombinant PLTP in vitro produces larger HDL particles (32). Also, purified PLTP from pig plasma converts pig HDL into HDL populations of larger and smaller particles (33). Recently, we have found that plasma PLTP activity was highly correlated with the percentage of plasma apoA-I in the HDL subclass Lp(A-I) ($r = 0.514$, $P < 0.001$, $n = 52$) among patients with low HDL and cardiovascular disease (34). The present study has shown that HDL phospholipid and HDL cholesterol levels are positively correlated with the percentage of large HDL particles among unrelated inbred mouse strains fed a chow diet and among the (SM × NZB) F1 × SM backcross mice. We speculate that PLTP-mediated HDL conversion in vivo leads to larger HDL with a slower catabolic rate which, in turn, gives rise to an increase in HDL levels.

Consistent with our hypothesis that PLTP plays a role in determining HDL levels and HDL size distribution, we observed that the change in PLTP activity, after the mice were fed a high fat diet for 6 weeks, was significantly correlated with the change in HDL cholesterol, HDL phospholipid, and HDL size. A recent report indicates that adenovirus-mediated human PLTP overexpression in mice leads to decreased plasma HDL levels (35). We have developed a mouse model in which the introduction of the human transgene resulted in a reduction of PLTP mRNA and PLTP activity on a high fat, high cholesterol diet (36). In this model, the reduced PLTA is associated with reduced HDL level and size. Thus, the available in vivo and in vitro data suggest that the modulation of HDL particle size and level could be a key physiological function of PLTP.

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