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# Lipoprotein lipase and hepatic lipase:

## their relationship with HDL subspecies Lp(A-I) and Lp(A-I,A-II)

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## Abstract

HDL subspecies Lp(A-I) and Lp(A-I,A-II) have different anti-atherogenic potentials. To determine the role of lipoprotein lipase (LPL) and hepatic lipase (HL) in regulating these particles, we measured these enzyme activities in 28 healthy subjects with well-controlled Type 1 diabetes, and studied their relationship with Lp(A-I) and Lp(A-I,A-II). LPL was positively correlated with the apolipoprotein A-I (apoA-I), cholesterol, and phospholipid mass in total Lp(A-I), and with the apoA-I in large Lp (A-I) ($r \geq 0.58$, $P \geq 0.001$). HL was negatively correlated with all the above Lp(A-I) parameters plus Lp(A-I) triglyceride ($r \geq -0.53$, $P \leq 0.003$). No correlation was detected between LPL and Lp(A-I,A-II). However, HL was inversely correlated with total Lp(A-I,A-II) phospholipid, and with large Lp (A-I,A-II) ($r \geq 0.50$, $P \leq 0.006$). Similar studies were performed with phospholipid transfer protein (PLTP). Only total Lp(A-I) triglyceride in women (not men) ($r = 0.71$, $P = 0.009$) was significantly correlated with PLTP activity. These observations indicate that LPL and HL play major roles in determining the level and composition of plasma Lp(A-I), particularly large Lp(A-I), but not with Lp(A-I,A-II) level. Furthermore, select correlations of LPL and/or HL with the apoA-I, cholesterol, and triglyceride of Lp(A-I) but not Lp(A-I,A-II) imply that the apoA-I and lipid of Lp(A-I) and Lp (A-I,A-II) are not fully equilibrated.

### Keywords

phospholipid transfer protein; high density lipoprotein size profile; Type 1 diabetes

Epidemiological studies performed in the past few decades have firmly established a negative association between human plasma HDL level and the incidence of coronary artery disease (CAD). Recent case-control and angiographic studies have found that this inverse relationship involves primarily species of certain sizes within $HDL_2$ and $HDL_3$ (1,2). Apolipoprotein A-I (apoA-I) and apoA-II are the major protein constituents of HDL. Two major populations of particles distinguishable by the presence or absence of apoA-II [Lp(A-I,A-II) and Lp(A-I)] exist in human HDL (3). Both can be found in the $HDL_2$ and $HDL_3$ density subfractions (4, 5). They are catabolized at different rates (6,7), and have been shown to respond differently to pharmacological (8,9), dietary (10,11), and hormonal (12,13) perturbations, suggesting that they are distinct metabolic entities. Insofar as there is evidence that Lp(A-I) and Lp(A-I,A-II) and their components differ in their antiatherogenic potential and functional properties (14–

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16), and that CAD is more strongly associated with abnormalities in HDL particle size than with low HDL cholesterol level (1,2), it is important to know how their level, composition, and particle size profile are regulated.

There are a number of proteins currently believed to play important roles in HDL metabolism. These include lipoprotein lipase (LPL), hepatic lipase (HL), plasma phospholipid transfer protein (PLTP), cholesteryl ester transfer protein (CETP), and lecithin cholesterol acyltransferase (LCAT) [reviewed in refs. (17,18)]. In this study, we focus on three of them: LPL, HL, and PLTP, and their relationship with HDL particles with and without apoA-II. LPL has been consistently shown to be positively, and HL inversely, associated with $HDL_2$ (19–24). We hypothesized that both are major but opposing determinants of large Lp(A-I) particles. In vitro HL has been shown to convert the Lp(A-I) and Lp(A-I,A-II) in $HDL_2$ to smaller particles (16), and PLTP has been shown to promote the conversion of both Lp(A-I) and Lp (A-I,A-II) into larger and smaller particles (25). Thus, we also hypothesized that both HL and PLTP participate in regulating the particle size of these two populations of HDL. To test these hypotheses, we measured the postheparin plasma LPL and HL activities and plasma PLTP activity in 28 healthy subjects with well-controlled Type 1 diabetes, and determined the association of their activities with Lp(A-I) and Lp(A-I,A-II) level and composition. This report presents our findings, and discusses their metabolic implications on these apo-specific HDL particles.

## METHODS

### Subjects and blood samples

The subjects with well-controlled Type 1 diabetes (16 men and 12 women) were 28 of the 61 participants of the Diabetes Control and Complications Trials (DCCT) recruited between 1983 and 1989 who were enrolled in the Seattle cohort of the Epidemiology of Diabetes Intervention and Complication (EDIC) trial at the time of this study. EDIC is an ongoing observational follow-up study of the DCCT, a clinical trial conducted between 1982 and 1993 that involved 1,441 subjects with Type 1 diabetes for 1 to 15 years at study entry. The DCCT cohort was randomly assigned to intensive or conventional diabetes treatment and followed for 6.5 years on average (26). Subjects were excluded from the DCCT at baseline if they had total cholesterol level greater than three standard deviations above the mean for sex and age as defined by the Lipid Research Clinics Population Studies (27), a calculated LDL above 190 mg/dl, a cardiovascular abnormality (such as major electrocardiogram abnormalities, peripheral vascular disease symptoms, a clinical history of cardiovascular disease, or hypertension), or a body weight >30% above ideal body weight as defined by the 1983 Metropolitan Life Insurance norms (28). At the end of the DCCT in 1993, all surviving participants were eligible for EDIC. The study was approved by the Human Subject Review Committee of the University of Washington, and informed consent was obtained from all subjects before entering the study. Two blood samples were collected from each subject after a 12–14 h fast, one before and one 10 min after a heparin bolus of 60 U/kg was given. Preheparin and postheparin blood samples were collected into EDTA-containing and lithium heparin-containing tubes, respectively. The blood samples were promptly centrifuged at 4°C at 3,000 rpm for 15 min to obtain plasma. Fresh preheparin plasma was used for lipid and apolipoprotein determination, and for HDL particle isolation, or immediately frozen at -70°C for the measurement of PLTP activity. Postheparin plasma samples that were promptly frozen at -70°C were used for measuring lipase activities.

### HDL particles

The HDL particles containing both apoA-I and apoA-II [Lp(A-I, A-II)] and those containing apoA-I but not A-II [Lp(A-I)] were isolated from fresh plasma samples by established

sequential dextran sulfate, anti-A-II, and anti-A-I chromatography (3,29). The distribution of plasma apoA-I between Lp(A-I) and Lp(A-I,A-II) was determined by quantifying the apoA-I in these lipoproteins with proper adjustment for recovery (2,3). The HDL size species were separated by nondenaturing gradient polyacrylamide gel electrophoresis using precast 4–30% gels (Alamo Gel, Inc., San Antonio, TX), and thyroglobulin, apoferritin, lactate dehydrogenase, and bovine albumin as calibration proteins. Gels were visualized for proteins with Coomassie Blue G-250, and scanned with a laser densitometer. The LKB 2400 GelScan XL® software was used to integrate and calculate the percent distribution of Lp(A-I) and Lp(A-I,A-II) in four size intervals: small, 7.0–8.2 nm; medium, 8.2–9.2 nm; large, 9.2–11.2 nm; and very large, 11.2–17.0 nm Stokes diameter as described (2). These size intervals were established based on the clustering of particles seen in healthy, normolipidemic subjects (30). The concentration of apo(A-I+A-II) and apoA-I in HDL particles with and without apoA-II, respectively, in each size interval was calculated by multiplying the plasma concentration of apoA-I and apoA-II associated with these particles by the relative percent of particles in each size interval.

### Postheparin lipase activity

Postheparin plasma samples that had been immediately frozen and stored at -70°C were used for the determination of total lipolytic activity as previously described (31). Glycerol tri [1-$^{14}$C]oleate (Amersham Pharmacia Biotech, Arlington Heights, IL) -labeled triglyceride, lecithin, and albumin were incubated with postheparin plasma for 60 min at 37°C, and the liberated $^{14}$C-labeled free fatty acids were then extracted and counted. LPL activity was calculated as the lipolytic activity removed from plasma by incubation with a specific monoclonal antibody against LPL (5D2), and HL activity was determined as the activity remaining after incubation with the LPL antibody. Enzyme activity is expressed as nanomoles of free fatty acid released per min/ml plasma at 37°C. For each assay, a bovine milk LPL standard was used to correct for interassay variation, and a human post-heparin plasma standard was included to monitor interassay variation.

### PLTP activity

The plasma phospholipid transfer activity mediated by PLTP was determined by measuring the transfer of [$^{14}$C]phosphatidylcholine from phospholipid liposomes to HDL using an established radioassay (32). Three plasma samples, stored at -70°C until use, were included in each assay to control for interassay variation.

### Other analytical methods

Standard enzymatic methods performed on a Hitachi 917 automatic analyzer (Roche Diagnostics, Indianapolis, IN) were used to determine fasting blood glucose and cholesterol, triglyceride, and phospholipid in plasma or fractionated lipoproteins (33). Plasma HDL cholesterol concentration was measured from the supernatant fraction, following precipitation by dextran sulfate and magnesium chloride (34). Cholesterol in VLDLs and LDLs was calculated using the Friedewald equation (35). ApoA-I, apoA-II, and apoB were measured with a Behring nephelometer using Behring reagents (Behring Diagnostic, Inc., Somerville, NJ) and calibrated with the Northwest Lipid Research Laboratories calibrator. The concentration of plasma Lp(A-I) and Lp(A-I,A-II) was determined by measuring the apoA-I, apoA-II, and lipid in these particles isolated as described above. Glycosylated hemoglobin was measured as hemoglobin A1c (HbA1c) using ion-exchange HPLC (BioRad).

### Statistical analysis

The Mann-Whitney U test was used to compare the various measured parameters between men and women. Spearman rank order correlation analyses were used to determine the relationship between LPL, HL, or PLTP activity and lipoproteins. All analyses were carried out using SPSS

statistical software. In view of multiple comparison, only *P* values of ≤0.01 (two-tailed) are reported as significant unless otherwise indicated.

# RESULTS

## Subject characteristics

The characteristics of the 28 subjects are summarized in Table 1. They were between 33 and 54 years old, with a mean age of 44.6 years, mean HbA1c of 7.6%, fasting glucose of 154 mg/dl, and body mass index (BMI) of 26.4 kg/m$^2$. No gender differences were observed in these parameters.

## Plasma lipid and HDL particles

All subjects had normal plasma cholesterol, with the highest being 218 mg/dl. All but two men had plasma triglyceride <180 mg/dl. Men and women had comparable total cholesterol, triglyceride, LDL, and apoB. However, men had significantly lower $HDL_2$ and $HDL_3$ cholesterol (Table 1). They also had lower plasma apoA-I (Table 1) and fewer Lp(A-I) particles (Table 2), particularly those in the medium-(8.2–9.2 nm) and large-(9.2–11.2 nm) sized range.

Although plasma Lp(A-I,A-II) levels were similar in men and women, differences in the size profile of these particles were observed. Men had significantly fewer large (9.2–11.2 nm) particles ($P = 0.007$), and tended to have more small particles with Stokes diameter <8.2 nm ($P = 0.03$) (Table 2).

## Postheparin lipolytic activities and lipoproteins

Postheparin plasma LPL and HL activities of the subjects are shown in Table 1. Men and women had comparable LPL activity, but men had significantly higher HL activity. Neither activity had any significant correlation with total or non-HDL cholesterol and triglyceride, and neither had any significant association with HbA1c, a measure of glycemic control (data not shown). As expected, LPL activity was positively associated ($r = 0.59$, $P = 0.001$) and HL activity was negatively associated ($r = -0.50$, $P = 0.007$) with HDL cholesterol. The correlations between these lipolytic activities and HDL particles are shown in Table 3. Both activities were strongly associated with the amounts of apoA-I, cholesterol, and phospholipid in Lp(A-I), and with the level of these particles with Stokes diameter >9.2 nm, but only HL activity demonstrated a significant association with Lp(A-I) triglyceride. All correlations were positive with LPL ($r$ between 0.51 and 0.62, $P \leq 0.006$) and negative with HL ($r$ between -0.48 and -0.56, $P \leq 0.01$). A significant inverse relationship was also detected between HL activity and Lp(A-I,A-II) phospholipid, as well as the amounts of these particles in the 9.2–11.2 nm size interval. In contrast, no correlation was found between LPL activity and the lipid and size profile of Lp(AI,A-II). Furthermore, no relationship was detected between the two major protein components of Lp(A-I,A-II) and the lipases.

## PLTP activity and lipoproteins

Recently, we found a clear gender difference in the association of PLTP activity with lipoproteins and metabolic parameters in a population of 134 subjects (36). In view of this, correlation analysis between PLTP and lipoproteins was performed with all 28 subjects as well as with the gender subgroups. In the group of all subjects, PLTP was significantly and positively correlated with total and LDL cholesterol ($r = 0.52$, $P = 0.004$ and $r = 0.48$, $P = 0.01$, respectively) (Table 4). Gender subgroup analysis revealed that these correlations were statistically significant only in women. Furthermore, in the women, PLTP activity was also positively associated with BMI ($r = 0.76$, $P = 0.005$), apoB ($r = 0.72$, $P = 0.008$), Lp(A-I) triglyceride ($r = 0.71$, $P = 0.009$), and the amounts of small (>8.2 nm) Lp(A-I) ($r = 0.68$, $P =$

0.015) and small Lp(A-I,A-II) particles ($r = 0.66$, $P = 0.02$). No correlation was observed between PLTP and age, glycosylated hemoglobin, total A-I, A-II, HDL, $HDL_2$, and $HDL_3$ cholesterol in either men or women or the entire group.

## DISCUSSION

LPL is the key enzyme that hydrolyzes core triglyceride in circulating chylomicrons and VLDLs [reviewed in refs. (37,38)]. Although the transfer of surface protein and lipid from chylomicron and VLDL remnant particles to HDL induced by LPL hydrolysis of triglyceride is the accepted mechanism behind the positive association between LPL and HDL, the extent to which these lipoprotein surface remnants affect the level and composition of Lp(A-I) and Lp(A-I,A-II) in vivo is not known. Our observation that LPL activity is significantly and positively associated with the apoA-I, cholesterol, and phospholipid of Lp(A-I) but not of Lp (A-I,A-II) suggests that HDL particles without apoA-II may be the preferential acceptors of surface remnants of triglyceride-rich lipoproteins. Consistent with this, LPL activity is positively and significantly associated with the amounts of large (>9.2 nm) Lp(A-I) particles but not with large Lp(A-I,A-II) particles. Thus these data provide evidence for the first time that the efficiency of triglyceride hydrolysis by LPL results in greater impact (direct or indirect) on the level, composition, and particle size of Lp(A-I) than on those of Lp(A-I,A-II).

HL can hydrolyze both triglyceride and phospholipids. Its physiological substrates were originally believed to be the core remnants of triglyceride-rich lipoproteins and LDL [reviewed in refs. (37,39)]. However, the increase in HDL level and size in HL deficiency (37,39,40) and the inverse association between HL and $HDL_2$ consistently observed in normolipidemic and dyslipidemic individuals (19,21,22,24,40) indicate that HL is a key enzyme in processing the triglyceride and phospholipid in these lipoproteins, especially in large HDLs. What is not known, however, is whether HL hydrolyzes the phospholpid and triglyceride in Lp(A-I) and Lp(A-I,A-II) to the same extent, and what the ultimate impact of HL on these apospecific HDL particles is in vivo.

Mowri et al. reported that the lipolysis of triglycerides and phospholipid by HL was higher in $HDL_2$ particles with apoA-II than in those without apoA-II in vitro. Also, the different lipolytic rates in $HDL_2$ particles with and without apoA-II correlated with the size reduction of these HDL particles (16). On the basis of these observations, we had expected that HL would show a stronger correlation with the phospholipid, triglyceride, and size profile of Lp(A-I,A-II) than with those of Lp(A-I). Our data that HL activity was significantly correlated with the triglyceride of Lp(A-I) but not with that of Lp(A-I,A-II) are inconsistent with that report. This discrepancy could perhaps be explained by the studies of Hime, Barter, and Rye (41) and their prediction that at low substrate concentrations (such as in the in vitro experiments of Mowri et al.), the amount of HL interacting with HDL and hydrolyzing HDL lipids might be much greater in particles with apoA-II than in those without apoA-II. At high substrate concentrations (such as in the vascular compartment), HL would hydro-lyze more lipids in HDL particles without apoA-II than in those with apoA-II.

In contrast, HL activity was significantly correlated with the phospholipid in both Lp(A-I) and Lp(A-I,A-II). These data imply that the rate of HL-mediated phospholipid hydrolysis is comparable between Lp(A-I) and Lp(A-I,A-II). Alternatively, this may mean that phospholipids are transferred more readily than triglycerides between these two populations of HDL particles. As HL depletes the lipid components of HDL, it also reduces HDL size. We had expected that HL would therefore be negatively correlated with large HDL particles and positively correlated with small HDLs. Here we find that it correlated more with large Lp(A-I) and Lp(A-I,A-II) than with small particles. This could be explained by our observation that PLTP also plays some roles in regulating the level of small HDL particles (Table 4), and the

current concept that the conversion of HDL from large to small HDL upon HL-mediated hydrolysis requires further processing by other factors, such as CETP (17,42). Enhanced uptake and degradation of small HDL particles mediated by CETP and the lipases could also contribute to the weaker correlation between HL and small HDL (43).

Plasma PLTP has been shown to be instrumental in the net mass transfer of phospholipid and free cholesterol from the lower density lipoproteins to HDL in vitro (32,44–46) and in PLTP-deficient mice (47). It has also been shown to induce the fusion of HDL to form particles larger and smaller than the parent molecules in vitro (25,48,49). These observations suggest that PLTP may be involved in the metabolism of HDL as well as VLDL and LDL. However, its physiological significance in humans has been difficult to confirm in the absence of any known case of PLTP deficiency, and results of studies performed to determine its relationship to the different classes of lipoproteins have been inconsistent (21,36,50,51). Because we have shown that PLTP can cause size changes in both Lp(A-I) and Lp(A-I,A-II) (25), we had expected to see a significant association between plasma PLTP activity and the size profile of these particles. However, among the four size species in Lp(A-I) and Lp(A-I,A-II), PLTP only revealed a trend of correlation with Lp(A-I) particles smaller than 8.2 nm in the 28 subjects. Because we have recently found that gender-related factors can modify the relationship between PLTP and lipoproteins in 134 nondiabetic subjects (36), correlation between PLTP activity and the various metabolic and lipid parameters was analyzed further with the two gender subgroups. This additional analysis revealed that in women but not in men, ~45% of the variation in Lp(A-I) and Lp(A-I,A-II) particles smaller than 8.2 nm could be attributed to plasma PLTP activity. Association between PLTP activity and the three size species of Lp(A-I) and Lp(A-I,A-II) with Stokes diameter >8.2 nm was less evident. Taken together, our data suggest that among HL, LPL, and PLTP, the lipases appeared to be stronger determinants of the amounts of large HDL particles while PLTP appeared to have a greater impact on small HDL particles. Furthermore, as previously reported for nondiabetic subjects, the impact of PLTP on HDL in subjects with well-controlled Type 1 diabetes is also gender dependent.

In addition to particle size, there was also a significant positive correlation between PLTP activity and Lp(A-I) triglyceride. We do not have any explanation for this relationship at present, but it may be related to an earlier observation that PLTP can promote CETP-mediated cholesteryl ester and triglyceride exchange between VLDL and HDL in vitro (44). If this is the case in vivo, then the significant correlation between PLTP and Lp(A-I) triglyceride would further suggest that Lp(A-I) is the preferential HDL acceptor of triglyceride transferred from the lower density lipoproteins. In women but not in men, PLTP activity was also significantly and positively associated with BMI, apoB, and LDL cholesterol. These associations are entirely consistent with those observed in 134 nondiabetic subjects (36). The strong association between PLTP and apoB or LDL cholesterol supports the recent suggestion that PLTP may play a role in regulating the secretion of apoB-containing lipoproteins (52).

The relationships among HDL particles are highly complex and have yet to be fully elucidated. The original concept of reversible interconversion of $HDL_2$- and $HDL_3$- density subclasses (17) was recently challenged when kinetic studies showed that small HDL particles were converted in a unidirectional manner to medium or large HDLs (53,54). This may be relevant to the differential correlation between LPL, HL, or PLTP and small or large particles observed in this study. Although apoA-I transfers among HDL particles, there has been evidence of the existence of exchangeable and nonexchangeable pools of apoA-I in HDL (8,55). Little is known regarding the movements of lipid among HDL particles. The present study provides some insight into this area. Specifically, significant correlations of LPL and HL with the cholesterol and/or triglyceride of Lp(A-I) but not of Lp(A-I,A-II) suggest that the lipids in these two populations of particles are not fully equilibrated.

Although we have based the focus of the discussion on the lipolytic activity of LPL and HL and the lipid transfer ability of PLTP, both LPL and HL can also enhance the binding of lipoproteins to cells and promote cellular lipoprotein uptake and degradation (56–58), and there is evidence that PLTP plays a role in cell cholesterol efflux (59). Thus, it is possible that the observed correlation between LPL, HL, or PLTP and HDL particles also reflects, in part, the roles of these proteins in lipoprotein-cell interaction, uptake, degradation, and efflux.

While all the subjects in this study have Type 1 diabetes, their HbA1c level was not significantly associated with HL, LPL, or Lp(A-I) level (data not shown). Therefore, the correlations between the lipases and Lp(A-I) observed in this study are independent of glycemic control. Insofar as neither LPL, HL, nor PLTP appeared to be major determinants of plasma Lp(A-I,A-II) level, the regulation of this population of HDL particles in the vascular compartment remains to be investigated. We observed over a decade ago that both LCAT and the apoB-containing lipoproteins are required to induce significant changes in Lp(A-I,A-II) in vitro (29). Recently, Clay et al. found that LCAT can promote the formation of Lp(A-I,A-II) in the presence of LDL through a fusion process (60). On the basis of these studies, we speculate that apoB-containing lipoproteins and LCAT may be important regulators of plasma Lp(A-I, A-II).

In summary, we have studied the postheparin plasma LPL and HL activities, plasma PLTP activity, and HDL particles in 28 subjects with well-controlled Type 1 diabetes, and determined the relationship of these lipolytic and lipid transfer activities with the characteristics of Lp(A-I) and Lp(A-I,A-II) to gain further insights into the role of these proteins in the metabolism of apo-specific HDL particles. Our observations suggest that Lp(A-I)s may be the preferred acceptors of excess surface remnants of triglyceride-rich lipoproteins, and that both LPL and HL play important but opposing regulatory roles in plasma Lp(A-I) concentration and lipid composition, and the amounts of large Lp(A-I). In contrast, PLTP appears to exert a greater impact on the amounts of small HDL rather than of large HDL. The lack of correlation between LPL, HL, or PLTP and the apoA-I and A-II in Lp(A-I,A-II) suggests that none of these three proteins are major determinants of total amounts of plasma Lp(A-I,A-II), even though HL is inversely correlated with Lp(A-I,A-II) phospholipid and large Lp(A-I,A-II). Select correlation of LPL and HL with the apoA-I, cholesterol and/or triglyceride of Lp(A-I) but not of Lp(A-I,A-II) implies that the apoA-I and lipid of Lp(A-I), and Lp(A-I,A-II) are not fully equilibrated. The present data also confirm our observation in an earlier study (38) that PLTP may play a role in the metabolism of apoB-containing lipoproteins, and that gender-related factors such as differences in HL activity seen in this study can modulate the impact of PLTP on lipoprotein.

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## TABLE 1

Subject characteristics

| | All (n = 28) | Women (n = 12) | Men (n = 16) |
|---|---|---|---|
| Age, years | 44.6 ± 5.6 | 44.3 ± 5.4 | 44.9 ± 5.9 |
| Body mass index, kg/m$^2$ | 26.4 ± 3.4 | 25.9 ± 3.2 | 26.7 ± 3.6 |
| Hemoglobin A1c, % | 7.6. ±1.0 | 7.7 ± 1.0 | 7.6 ± 1.1 |
| Fasting glucose, mg/dl | 154 ± 78 | 136 ± 48 | 166 ± 94 |
| Cholesterol, mg/dl | 172 ± 32 | 177 ± 26 | 169 ± 37 |
| Triglyceride, mg/dl | 82.4 ± 67.1 | 68.8 ± 38.2 | 92.6 ± 82.3 |
| ApoB, mg/dl | 80.3 ± 20.4 | 77.4 ± 18.7 | 82.5 ± 22.0 |
| LDL cholesterol, mg/dl | 98.5 ± 25.4 | 96.3 ± 20.6 | 100.1 ± 29.0 |
| ApoA-I, mg/dl | 143 ± 28 | 161 ± 25 | 129 ± 21[a] |
| $HDL_2$ cholesterol, mg/dl | 12.9 ± 6.8 | 16.7 ± 5.9 | 10.1 ± 6.2[b] |
| $HDL_3$ cholesterol, mg/dl | 44.0 ± 10.2 | 50.2 ± 8.3 | 39.4 ± 9.1[c] |
| HDL triglyceride, mg/dl | 13.3 ± 10.8 | 16.0 ± 9.8 | 11.2 ± 11.3 |
| LPL activity, nmol/ml/min | 380 ± 120 | 417 ± 114 | 353 ± 121 |
| HL activity, nmol/ml/min | 324 ± 158 | 245 ± 54 | 384 ± 184[b] |
| PLTP activity, μmol/ml/h | 14.6 ± 1.7 | 14.6 ± 2.0 | 14.6 ± 1.5 |

HL, hepatic lipase; LPL, lipoprotein lipase; PLTP, phospholipid transfer protein. Numbers are mean ± SD. Values denote significant difference between men and women by Mann-Whitney U test.

[a] $P \leq 0.001$.

[b] $P \leq 0.01$.

[c] $P \leq 0.005$.

## TABLE 2

Particle size profiles of Lp(A-I) and Lp(A-I,A-II)

| | All (n = 28) | Women (n = 12) | Men (n = 16) |
|---|---|---|---|
| | | *mean* ± *SD* | |
| Lp(A-I) particles | | | |
| ApoA-I (mg/dl) | 54.3 ± 21.2 | 69.6 ± 15.2 | 42.9 ± 17.6[a] |
| Size species (mg/dl)[d] | | | |
| Small (7.0–8.2 nm) | 10.6 ± 3.5 | 11.6 ± 4.8 | 9.8 ± 1.9 |
| Medium (8.2–9.2 nm) | 14.4 ± 6.6 | 20.0 ± 5.4 | 10.2 ± 3.7[a] |
| Large (9.2–11.2 nm) | 23.2 ± 13.8 | 30.9 ± 10.2 | 17.4 ± 13.7[b] |
| Very large (11.2–17.0 nm) | 6.2 ± 2.1 | 7.1 ± 1.6 | 5.5 ± 2.2 |
| Lp(A-I,A-II) particles | | | |
| ApoA-I (mg/dl) | 87.2 ± 13.6 | 90.0 ± 16.0 | 85.1 ± 11.5 |
| ApoA-II (mg/dl) | 32.5 ± 5.0 | 33.7 ± 6.3 | 31.5 ± 3.6 |
| Size species (mg/dl)[d] | | | |
| Small (7.0–8.2 nm) | 37.1 ± 14.4 | 30.9 ± 11.5 | 41.7 ± 15.0 |
| Medium (8.2–9.2 nm) | 53.4 ± 12.3 | 59.2 ± 14.4 | 49.0 ± 8.5 |
| Large (9.2–11.2 nm) | 22.1 ± 8.4 | 26.1 ± 6.0 | 19.1 ± 8.9[c] |
| Very large (11.2–17.0 nm) | 7.1 ± 2.4 | 7.4 ± 2.5 | 6.9 ± 2.4 |

Values denote significant difference between men and women by Mann-Whitney U test.

[a] $P \leq 0.001$.

[b] $P \geq 0.005$.

[c] $P \leq 0.01$.

[d] Calculated as apoA-I and apo(A-I+A-II) in Lp(A-I) and Lp(A-I,A-II), respectively, in each size interval.

**TABLE 3**

Correlations between post heparin plasma lipolytic activities and HDL particle composition and size

| | LPL Activity | | | | HL Activity | | | |
|---|---|---|---|---|---|---|---|---|
| | Lp(A-I) | | Lp(A-I,A-II) | | Lp(A-I) | | Lp(A-I,A-II) | |
| **(n = 28)** | ***r*** | ***P*** | ***r*** | ***P*** | ***r*** | ***P*** | ***r*** | ***P*** |
| Particle protein and lipid (mg/dl) | | | | | | | | |
| ApoA-I | 0.60 | **0.001** | 0.18 | 0.368 | -0.56 | **0.002** | -0.16 | 0.424 |
| ApoA-II | - | - | 0.03 | 0.880 | - | - | -0.19 | 0.339 |
| Cholesterol | 0.62 | <**0.001** | 0.41 | 0.030 | -0.55 | **0.003** | -0.38 | 0.048 |
| Phospholipid | 0.58 | **0.001** | 0.22 | 0.265 | -0.55 | **0.002** | -0.50 | **0.006** |
| Triglyceride | 0.20 | 0.299 | -0.37 | 0.053 | -0.53 | **0.003** | -0.16 | 0.405 |
| Size species (mg/dl)[a] | | | | | | | | |
| Small (7.0–8.2 nm) | 0.07 | 0.707 | -0.08 | 0.692 | -0.21 | 0.293 | 0.30 | 0.119 |
| Medium (8.2–9.2 nm) | 0.41 | 0.031 | 0.17 | 0.388 | -0.44 | 0.019 | -0.22 | 0.267 |
| Large (9.2–11.2 nm) | 0.60 | **0.001** | 0.36 | 0.062 | -0.56 | **0.002** | -0.69 | <**0.001** |
| Very large (11.2–17.0 nm) | 0.51 | **0.006** | 0.22 | 0.252 | -0.48 | **0.010** | -0.28 | 0.146 |

Numbers in bold type denote significant correlation at $P \leq 0.01$.

[a] Correlation was performed with the mass of apoA-I and apo(A-I+A-II) in Lp(A-I) and Lp(A-I,A-II), respectively.

**TABLE 4**

Gender-specific correlations between plasma PLTP activity and metabolic or lipoprotein parameters

| | All (n = 28) | | Women (n = 12) | | Men (n = 16) | |
|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* |
| Body mass index | 0.34 | 0.079 | 0.76 | **0.005** | 0.00 | 0.996 |
| Fasting glucose | 0.22 | 0.278 | 0.61 | 0.047 | 0.01 | 0.965 |
| Cholesterol (CH) | 0.52 | **0.004** | 0.68 | **0.016** | 0.27 | 0.305 |
| Triglyceride (TG) | 0.21 | 0.289 | 0.47 | 0.124 | 0.00 | 0.996 |
| ApoB | 0.35 | 0.064 | 0.72 | **0.008** | -0.029 | 0.914 |
| LDL CH | 0.48 | **0.010** | 0.72 | **0.008** | 0.29 | 0.282 |
| TG in Lp(A-I) | 0.33 | 0.088 | 0.71 | **0.009** | 0.00 | 0.991 |
| TG in Lp(A-I,A-II) | 0.21 | 0.280 | 0.44 | 0.152 | 0.01 | 0.978 |
| Small Lp(A-I) (7.0–8.2 nm) | 0.40 | 0.035 | 0.68 | **0.015** | 0.12 | 0.668 |
| Small Lp(A-I,A-II) (7.0–8.2 nm) | 0.04 | 0.858 | 0.66 | **0.020** | -0.39 | 0.140 |

Units of parameters are listed in Table 1. $P \leq 0.02$ are in bold font.