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Plasma Post-Heparin Lipase Activities in the HERITAGE Family Study: The Reproducibility, Gender Differences, and Associations with Lipoprotein Levels

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Objectives: Examine the reproducibility of plasma lipid and lipoprotein measurements in the HERITAGE Family Study.
Design and Methods: In a sample of 379 subjects (191 men and 188 women), reproducibility was determined for lipids, lipoproteins (done on two occasions) and post-heparin lipase assays using an Intracenter Quality Control study by generating split samples from an additional 60 subjects (35 men and 25 women), which were assayed in a blind fashion by the lipid core laboratory. Reproducibility was estimated using intraclass correlation coefficients (ICC) for the selected variables. Analytical error (ANER) and coefficient of variation (CV) were also calculated. Day-to-day variation for 10 variables including plasma cholesterol and triglycerides (TG), HDL-cholesterol and its subfractions HDL_2-cholesterol and HDL_3-cholesterol, LDL-cholesterol and VLDL-cholesterol, as well as apoprotein (apo) A-I, apo B, and LDL-apo B were assessed.
Results: In the HERITAGE study, all lipid and lipoprotein variables had ICC above 0.79. Plasma VLDL-cholesterol (31%) and TG (23%) levels, which are well known to be highly variable from one day to another, had CVs greater than 20%. Other variables had CVs lower than 10% except for HDL_2-cholesterol which reached 16%. In the intracenter reliability sub-study, the measurement errors were found to be low except for HDL_2-cholesterol. For the lipases, the reproducibility of repeated samples was very high, with ICC over 0.95. The within-assay CV corresponded to 2.1 and 5.3% for hepatic lipase (HL) and lipoprotein lipase (LPL), respectively, whereas the between-assay CV reached 8–12% for HL and about 15% for LPL. Due to the complexity of these two assays, the results are considered to be quite satisfactory.
Conclusions: The reproducibility of plasma lipid and lipoprotein measurements, as well as of post-heparin lipase activities, is good in the multicenter HERITAGE Family Study. In addition, the well-documented gender difference in the plasma lipoprotein profile was confirmed in the present study, women having lower fasting triglyceride and LDL-cholesterol levels than men as well as reduced cholesterol/HDL-cholesterol and increased HDL_2-cholesterol/HDL_3-cholesterol ratios compared to men. Results of the present study support the notion that the higher LPL and low HL activities found in women compared to men are important factors contributing to explain gender difference in the lipoprotein profile. However, additional factors not examined in the present study are involved beyond the contribution of post-heparin lipase to the sex dimorphism in plasma lipoprotein levels. Copyright © 1999 The Canadian Society of Clinical Chemists

KEY WORDS: cholesterol; LDL-cholesterol; HDL-cholesterol; apolipoprotein; lipoprotein lipase; hepatic triglyceride lipase.

Introduction

It is now well accepted that the measurement of plasma lipid and lipoprotein levels is a relevant procedure that provides further information on the patient's risk of coronary heart disease (CHD). There is now convincing evidence available indicating that elevated cholesterol and low-density lipoprotein (LDL) cholesterol levels are associated with an increased CHD risk, whereas elevated high-density lipoprotein (HDL) cholesterol concentrations appear to provide protection against premature CHD (1–3). Accordingly, clinical trials, in which LDL-cholesterol concentrations were reduced and HDL-cholesterol levels increased, have been associated with a reduced incidence of CHD providing additional support for the lipoprotein-lipid vs. CHD hypothesis (4,5). Therefore, the study of the effects of lifestyle changes (diet, exercise) on plasma li-

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poprotein-lipid levels is a topic of considerable importance from a public health standpoint.

Several studies have shown that activities of two lipolytic enzymes that can be measured in post-heparin plasma, namely lipoprotein lipase (LPL) and hepatic-triglyceride lipase (HL), are important correlates of plasma lipoprotein-lipid levels (6,7). Indeed, low plasma triglycerides (TG) levels and increased HDL-cholesterol concentrations have generally been reported among subjects with high post-heparin LPL activities (6,7). However, low plasma HDL-cholesterol levels, especially the HDL_2 subfraction, have been noted among subjects with elevated HL activity in post-heparin plasma (8). Therefore, the balance between the activity of these two enzymes has been suggested to be an important correlate of plasma HDL-cholesterol levels, ultimately modulating the risk of CHD (6). Large multicenter studies dealing with the plasma lipoprotein-lipid profile have all measured and reported the reproducibility of lipids and lipoproteins (9–13).

However, the ability to accurately track changes in the plasma lipoprotein-lipid profile and in relevant enzymes involved in the modulation of plasma lipoprotein-lipid levels such as post-heparin plasma LPL and HL following an exercise training program or dietary intervention is critically dependent on the reproducibility of these measurements, which reflects both measurement error and day-to-day biological variability. When the error associated with the assay itself and with the biological variation is close to the expected change in the variable of interest following a given intervention, there is a high likelihood that any true change may not be detected, *i.e.,* it may not achieve statistical significance.

The HEalth, RIsk factors, exercise Training And GEnetics (HERITAGE) Family Study is a study of the genetic and non-genetic determinants of the response to endurance training for several cardiovascular and diabetes risk factors in sedentary families. The participants are measured before and after a 20-week standardized exercise training program. Details of the HERITAGE Family Study aims, design, and measurement protocols have been presented in a previous publication (14). The protocol requires a multicenter approach so as to achieve the desired sample size within a reasonable period of time. The multicenter approach, coupled with the need to be able to detect fairly small changes in response to training, required that extensive quality assurance and quality control measures be implemented (15). To detect relatively small changes in important variables such as the plasma lipid and lipoprotein profile and post-heparin lipases activities (PHLA) following the training program and to measure the genetic basis for these changes, it was critical to document the reproducibility of the measurements performed. Therefore, the purpose of this study was to determine the reproducibility of the plasma lipid and lipoprotein profile as well as PHLA in the HERITAGE Family Study. In addition, we examined to what extent the well-documented gender dimorphism in the plasma lipoprotein-lipid profile could be explained by gender differences in plasma post-heparin LPL and HL activities.

Methods

Subjects

Reproducibility study

HERITAGE pre-training data. The HERITAGE Family Study cohort has been previously described (14). The HERITAGE subjects came from families that included the natural mother and father (aged 65 or less) and at least three adult offspring 17 years of age or older. This article describes the results of the pre-training data from the first 379 subjects (191 men and 188 women; 82 blacks and 297 whites) studied at the four Clinical Centers (Arizona State University [Indiana University since January 1996], Laval University, University of Minnesota and The University of Texas at Austin). Subjects were healthy and sedentary and met a number of inclusion and exclusion criteria (14). The study protocol had been previously approved by the Institutional Review Board at each of the four Clinical Centers. Informed consent was obtained from each subject.

Intracenter Quality Control substudy data. For this substudy, subjects were not participants of the HERITAGE Family Study due to the nature of the study design. Each of the four Clinical Centers recruited five additional subjects every 6 months over three 6-month periods to participate in this Intracenter Quality Control substudy. Subjects were required to meet all criteria for admission in the HERITAGE Family Study, with the exception of family membership. Data were available for 60 subjects from all four centers (15 volunteers/center). The study protocol had been previously approved by each Clinical Center's Institutional Review Board, and informed consent was obtained from each subject.

Gender differences in PHLA and associations with lipoprotein levels

Two hundred forty-seven men and 240 women took part in the PHLA study. For this subanalysis, all subjects were of Caucasian origin and were all part of the HERITAGE Family Study (14).

Assays

For both HERITAGE subjects and the Intracenter Quality Control substudy, the lipid assays were performed twice with blood samples drawn at least 24 hours apart. Fasting blood samples were drawn at each Clinical Center and prepared according to a standard protocol before being sent to the Project Director in Québec who brought them to the central lipid core laboratory (Lipid Research Center, Laval University Medical Center) for analysis.

Specimen procurement and processing. Blood samples were obtained twice on subjects after a 12-hour overnight fast. Blood samples on EDTA tubes were drawn at each Clinical Center in the morning on two different days at least 24 hours apart and not more than 7 days between them. Plasma was prepared according to a standard protocol. For women, samples were obtained in the early follicular phase. Fresh samples kept on ice were then sent refrigerated using ice pack to the lipid core laboratory at the Lipid Research Center of the Laval University Medical Center for determination of plasma lipids and lipoproteins. For PHLA determination, blood samples were obtained on one occasion in subjects following blood draw for lipids. Plasma LPL and HL activities were measured on one occasion in subjects, early in the morning, 10 minutes after an intravenous injection of heparin (60 IU/kg body weight), on blood samples drawn on heparinized tube kept on ice until centrifugation (1000g, 10 min at 4 °C). Plasma samples were then stored at −80 °C and sent frozen to the Project Director in Québec, once a month.

Plasma lipids, lipoproteins and post-heparin lipolytic activities. Cholesterol and TG levels were determined, on fresh samples within 10 days, in plasma and in lipoprotein fractions by enzymatic methods using the Technicon RA-500 (Bayer Corporation, Tarrytown, NY, USA) analyzer as previously described (16). Plasma very low-density lipoprotein (VLDL) ($d < 1.006$ g/mL) were isolated by ultracentrifugation and the HDL fraction obtained after precipitation of LDL in the infranatant ($d > 1.006$ g/mL) with heparin and $MnCl_2$ (17). The cholesterol and TG contents of the infranatant fraction were measured before and after the precipitation step. Apolipoprotein (apo) B concentration was measured in plasma (total apo B) and in the infranatant (LDL-apo B) by the rocket immunoelectrophoretic method of Laurell (18), as previously described (19). Apo A-I concentration was also measured in the infranatant fraction. The lyophilized serum standards for apolipoprotein measurements were prepared in our laboratory and calibrated with reference standards obtained from the Centers for Disease Control (Atlanta, GA, USA). Plasma LDL-cholesterol, LDL-TG, and VLDL-apo B concentrations were obtained by difference. The cholesterol content of HDL_2 and HDL_3 subfractions was also determined after additional precipitation of HDL_2 with dextran sulfate (20). Ten of these variables were selected for analysis: plasma cholesterol, plasma TG, LDL-cholesterol, HDL-cholesterol, HDL_2-cholesterol, HDL_3-cholesterol, apo A-I, apo B, and LDL-apo B. To eliminate subjects who did not follow the required fasting protocol, samples that contained chylomicrons were not used in this analysis. The PHLA were measured using a modification of the method of Nilsson-Ehle and Ekman (21), as previously described (22). The two lipolytic enzyme activities were expressed as nmoles of oleic acid released per mL of plasma per min. In the present reproducibility study, only baseline measures for plasma lipoprotein and PHLA are reported and analyzed.

Internal quality control. The following procedures were applied to ensure quality of plasma lipids, lipoproteins and PHLA assays at the lipid core laboratory. For cholesterol and TG measurements, repeated samples were assayed in 5% of all samples. Pooled plasma was prepared and stabilized by the addition of protease inhibitor and antibacterial agent. This pooled plasma was used as a control for every 20 samples placed in the ultracentrifuge. The recovery of cholesterol and TG in the lipoprotein fractions was monitored by this procedure. In addition, the ultracentrifugation and assays were repeated in 5% of all cases using HERITAGE samples. For apo A-I and B measurements, one out of every 20 samples was completely reassayed. For measurement of PHLA, post-heparin plasma was collected from one normal subject and frozen in aliquots at −80 °C. One aliquot was used for determination of both LPL and HL activities for every 5 samples. To cover the 4-year period, four different lots were used (1 lot/year on average). This represents a total of 176 measurements. Once again, using HERITAGE subjects, assays were repeated in 5% of all cases. Repeated assays represent a total of 82 samples for the lipid data and 73 samples for the PHLA assays.

External quality control. In addition to the preceeding quality control measures instituted by the lipid core laboratory, the Data Coordinating Center implemented the following external quality control. Split samples were prepared at each clinical center from the participants of the Internal Quality Control study. The Data Coordinating Center assigned phantom IDs to be used by each Clinical Center for shipping blinded duplicate blood samples from 60 volunteer subjects (15 subjects/Clinical Center) to the lipid core laboratory. The data entry system provided for the identification of the pair-wise correspondence, so that only the Data Coordinating Center and not the core laboratory could break the code and assess the reliability of the lipid core laboratory for the lipid, lipoproteins, and PHLA assays. There was a total of 120 samples for the lipid data (measured of two occasions) and 60 samples for the PHLA assays (only done once).

Statistical methodology

Reproducibility study

All data were analyzed using the SAS statistical package. Intraclass correlations (ICC), analytical errors (ANER), and coefficients of variation (CV) were computed to evaluate the reproducibility of the sample. In the pre-training data, subjects seen at each of the four centers had duplicate measurements for selected variables such as plasma lipids and lipoproteins that were obtained on two separate days; this allowed us to estimate the day-to-day biological variability in addition to the laboratory measurement error. Additionally, the use of dupli-

TABLE 1
Characteristics of the Subject Population

Variable	Unit	HERITAGE Study[a] (n = 379)		Intracenter Quality Control Substudy[b] (n = 60)	
		Mean	SD	Mean	SD
Age	year	35.1	14.4	28.0	9.0
Weight	kg	75.8	16.8	71.4	12.8
Cholesterol	mmol/L	4.46	0.95	3.97	0.89
Triglycerides	mmol/L	1.30	0.81	1.04	0.47
HDL-cholesterol	mmol/L	1.04	0.26	1.08	0.25
HDL_2-cholesterol	mmol/L	0.35	0.19	0.36	0.20
HDL_3-cholesterol	mmol/L	0.69	0.13	0.71	0.11
LDL-cholesterol	mmol/L	2.99	0.81	2.56	0.76
VLDL-cholesterol	mmol/L	0.42	0.17	1.18	0.16
Apolipoprotein A-I	g/L	1.17	0.17	1.18	0.16
Apolipoprotein B	g/L	0.86	0.24	0.72	0.22
LDL-apolipoprotein B	g/L	0.78	0.21	0.66	0.20
Hepatic Lipase	nmol/min/mL	201.2	71.8	178.5	75.8
Lipoprotein Lipase	nmol/min/mL	61.5	35.2	52.2	33.1

[a]The pre-training plasma lipoprotein-lipid profile was obtained in a total of 379 subjects (82 blacks and 297 whites). The sample included 191 men (mean age ± SD: 35.5 ± 14.8 years) and 188 women (34.8 ± 14.0 years).
[b]The Intracenter Quality Control lipid data included a total of 60 Caucasian subjects (35 men and 25 women). The average plasma lipoprotein-lipid profile of this sample was not different from that of the overall sample.

cate samples, both internal (5% of all samples were repeated) and external (blinded split-duplicate samples) allowed us to estimate the measurement error by itself. There was one important difference in the internal and external samples in that the internal sample was always repeated in the same assay. ICC is defined as the correlation between two or more measurements in a given subject (23). Using this model, the ith measurement on the jth subject is given by:

$$X_{ij} = \mu + \beta_j + w_{ij}$$

where μ is the population mean of the ratings, β_j is the difference from μ of the mean of the measurements on the jth subject, and w_{ij} is the difference from $\mu+\beta_j$ of the ith measurement on the jth subject. Both β_j and w_{ij} are assumed to be random and normally distributed and independent, with standard deviations of σ_τ and σ_ω, respectively. σ_ω is the within-subjects standard deviation, also called ANER. The CV within subjects was computed as:

$$CV = (100 \bullet \sigma_\omega)/\mu$$

To compute the ICC, PROC GLM in SAS was used to run an ANOVA, providing a between-subjects mean square (BMS) and a within-subjects mean square (WMS). These values were used to estimate the ICC according to (23):

$$ICC = (BMS - WMS)/[BMS + (k - 1) \bullet WMS]$$

where k is the number of replicate measurements on a single subject.

Gender differences in PHLA and associations with lipoprotein levels

Pearson product-moment correlation coefficients were used to quantify the relationships among variables. Student t-tests were used for differences between genders. General linear models were used to examine the regression of PHLA to lipoprotein levels in men and women. Finally, gender differences in plasma lipoprotein levels were also examined after adjustment by analysis of covariance for the variation in lipase activities.

RESULTS

Reproducibility study

Table 1 shows the characteristics of the subjects and the variables that were analyzed in both HERITAGE and in the Intracenter Quality Control studies. The reproducibility of measurements for day-to-day biological variability is presented in Table 2 and the laboratory measurement error is presented in Table 3 for lipid and lipoprotein variables. Reproducibility of the post-heparin lipolytic activities of HL and LPL is shown in Table 4. All measurements appeared to be characterized by high ICCs as they ranged from 0.71 to 0.95 for day-to-day variation and were all over 0.93 for the analytical errors using split samples.

The coefficient of variation, however, gives a relative measure of dispersion which is 100 times the ANER scaled by the mean of the variable examined. The CVs ranged from 4.2–32.5% for the biological variability (Table 2) and ranged from 0.7–10% for the measurement error of the lipid data (Table 3).

TABLE 2
Reproducibility of the Lipids and Lipoproteins for Day-to-Day Variation in the Pre-training Data of the HERITAGE Family Study and the Intracenter Quality Control Substudy

	Day-to-Day Variation Plus Measurement Error					
	HERITAGE Study (n = 379)			Intracenter Quality Control Substudy (n = 60)		
Variable	ICC	ANER	CV	ICC	ANER	CV
Cholesterol	0.95	0.21	4.7	0.93	0.24	6.0
Triglycerides	0.87	0.21	22.9	0.79	0.23	21.8
HDL-cholesterol	0.94	0.06	6.2	0.94	0.06	6.0
HDL_2-cholesterol	0.92	0.06	15.6	0.92	0.06	16.3
HDL_3-cholesterol	0.79	0.06	9.1	0.71	0.06	8.8
LDL-cholesterol	0.94	0.19	6.5	0.92	0.22	8.6
VLDL-cholesterol	0.87	0.13	31.1	0.78	0.11	32.5
Apolipoprotein A-I	0.89	0.06	4.9	0.91	0.05	4.2
Apolipoprotein B	0.95	0.05	6.2	0.93	0.06	8.2
LDL-apolipoprotein B	0.94	0.05	6.6	0.92	0.06	8.6

ICC, intraclass correlation; ANER, analytical error; CV, coefficient of variation.

Results of similar analyses performed on the PHLA of the intracenter quality control are shown in Table 4. Both variables were characterized by high reliability coefficients (>0.95). Within-assay CVs ranged from 2–5%, with between-assay coefficients varying between 8.4–15.4%. These results confirm that our measurements of post-heparin plasma LPL and HL activities were characterized by an adequate level of reliability.

Gender differences in PHLA and associations with lipoprotein levels

Results of comparison studies between men and women are illustrated in Figure 1. Women of the study were characterized by significantly higher LPL activity and lower hepatic lipase activity compared to men. These reciprocal differences in lipase activities led to a substantially higher HL/LPL ratio in men than in women.

Table 5 shows the correlations between post-heparin lipase activities and plasma lipoprotein levels. Overall, high HL and low LPL activities were associated with reduced HDL-cholesterol levels, especially for the HDL_2 subfraction. The regression slopes of LPL and HL activities to HDL_2-cholesterol levels were then compared in both men and women and significant gender differences were noted (Figure 2).

Finally, expected gender differences in the lipoprotein profile are shown in Figure 3, before and after adjustment for LPL and HL activities. Statistical adjustment of lipoprotein-lipid levels for post-heparin lipase activities did not eliminate the gender difference in the lipoprotein-lipid profile.

TABLE 3
Reproducibility of the Lipids and Lipoproteins in the Intracenter Quality Control Substudy and for Repeated Samples (5%)

	Measurement Error					
	Internal Repeated Samples (5%) (n = 82)			External Split Samples (n = 120)		
Variable	ICC	ANER	CV	ICC	ANER	CV
Cholesterol	0.99	0.03	0.7	0.99	0.05	1.3
Triglycerides	0.99	0.02	1.6	0.99	0.04	3.5
HDL-cholesterol	0.99	0.02	2.0	0.99	0.03	2.4
HDL_2-cholesterol	0.99	0.02	5.7	0.97	0.04	10.0
HDL_3-cholesterol	0.98	0.02	3.2	0.93	0.03	4.4
LDL-cholesterol	0.99	0.02	1.0	0.99	0.04	1.5
VLDL-cholesterol	0.99	0.01	4.3	0.99	0.02	5.3
Apolipoprotein A-I	0.99	0.02	2.0	0.95	0.03	3.0
Apolipoprotein B	0.99	0.01	1.9	0.99	0.02	3.3
LDL-apolipoprotein B	0.99	0.01	1.9	0.99	0.02	3.2

ICC, intraclass correlation; ANER, analytical error; CV, coefficient of variation.

TABLE 4
Reproducibility of the Plasma Post-Heparin Lipase Assays in the Intracenter Quality Control Substudy and for Repeated Samples (5%)

	Internal Quality Control						External		
	Intra-Assay[a] (n = 73)			Inter-Assay[b] (n = 176)			Split Samples[c] (n = 60)		
Variable	ICC	ANER	CV	ICC	ANER	CV	ICC	ANER	CV
Hepatic lipase	0.99	3.9	2.1	0.98	16.0	12.0	0.96	15.0	8.4
Lipoprotein lipase	0.99	3.2	5.3	0.95	8.1	15.4	0.95	7.6	14.7

ICC, intraclass correlation; ANER, analytical error; CV, coefficient of variation.
[a]Repeated samples in 5% of all cases in the HERITAGE Study.
[b]PHLA is collected from one normal subject and frozen in aliquots at −80° C. One aliquot is used in every assay. Four different lots were used (1 lot/year for 4 years).
[c]Blinded duplicate blood samples from 60 volunteers subjects (15 volunteers/Clinical Center) were shipped to the lipid core laboratory to be assayed.

DISCUSSION

Reproducibility study

The biological variability found in the present HERITAGE Study is well within the variation previously reported (9–13). Indeed, Bookstein *et al.* (24) found that day-to-day variability reached 5% for plasma cholesterol, 10% for HDL-cholesterol and was as high as 20% for TG concentrations. Results of the present study indicate that the biological variation of apo A-I and B as well as of LDL-apo B concentrations is well within what has been reported for conventional lipid and lipoprotein measurements. Additionally, the day-to-day variation of plasma HDL_2-cholesterol is greater than for HDL- or HDL_3-cholesterol levels. Obviously, the analytical error itself may contribute to the day-to-day variation. This issue is particularly relevant for HDL_2-cholesterol concentrations which was calculated as the difference between HDL-cholesterol and measured HDL_3-cholesterol after the precipitation step. However, the measurement error was quite low for TG concentration (Table 3), which suggests that this variable was mostly affected by biological factors rather than by technical issues.

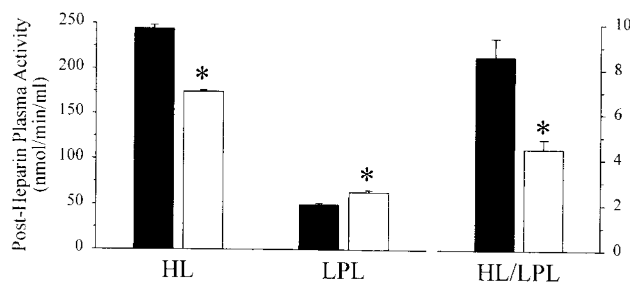

Figure 1 — Post-heparin hepatic lipase (HL) and lipoprotein lipase (LPL) activities of the 247 men (black bars) and 240 women (white bars) of the study. Values are means ± SEM. *Significantly different from men at $p < 0.0001$.

It is also quite striking that the measurement error for apo A-I and B levels as well as for LDL-apo B concentrations was well within the range (about 2%) of measurement error values obtained for cholesterol and TG levels measurements performed in the plasma and in lipoprotein fractions (from 0.7–5.7%). Therefore, results of the present study suggest that we will be in an advantageous position for the interpretation of our apolipoprotein data as the related analytical error was as low as for lipoprotein-lipids.

TABLE 5
Associations Between Post-Heparin Hepatic and Lipoprotein Lipase Activities and Fasting Lipoprotein-Lipid Concentrations

	Men (n = 247)			Women (n = 240)		
Variable	HL	LPL	HL/LPL	HL	LPL	HL/LPL
Triglycerides	−0.10	−0.20***	0.06	−0.11	−0.14*	−0.03
HDL-cholesterol	−0.07	0.32§§§	−0.16*	−0.37§§§	0.46§§§	−0.22§
HDL_2-cholesterol	−0.20***	0.27§§§	−0.19***	−0.34§§§	0.44§§§	−0.20***
HDL_3-cholesterol	0.07	0.26§§§	−0.08	−0.21§	0.25§§§	−0.13*
HDL_2-c/HDL_3-c	−0.25§§§	0.18***	−0.17**	−0.23§§	0.31§§§	−0.13*
Cholesterol/HDL-c	−0.03	−0.18***	0.08	0.19*	−0.18**	0.04

HL, hepatic lipase; LPL, lipoprotein lipase.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.005$; §$p < 0.001$; §§$p < 0.0005$; §§§$p < 0.0001$.

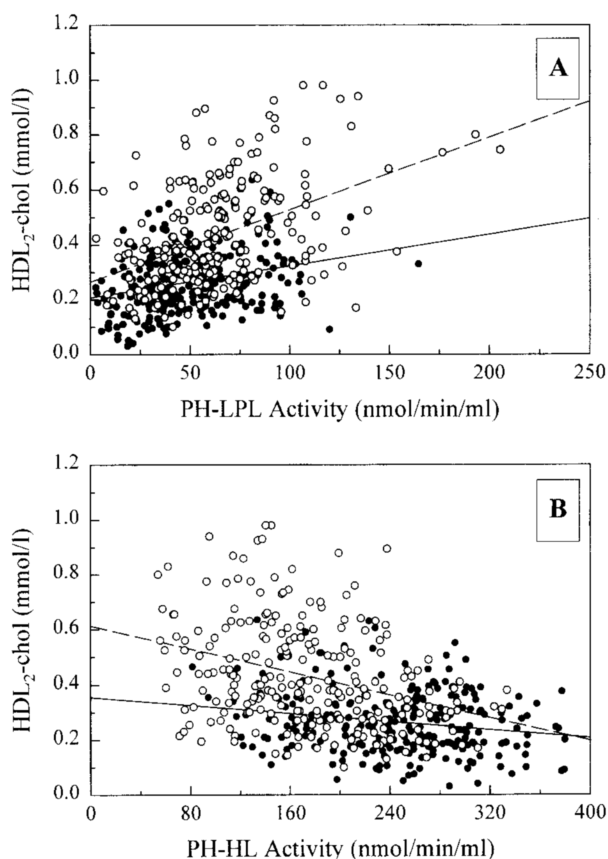

Figure 2 — Associations of fasting HDL_2-cholesterol concentrations with post-heparin (**A**) lipoprotein lipase and (**B**) hepatic lipase activities in the 247 men (black circles) and 240 women (white bars) of the study.

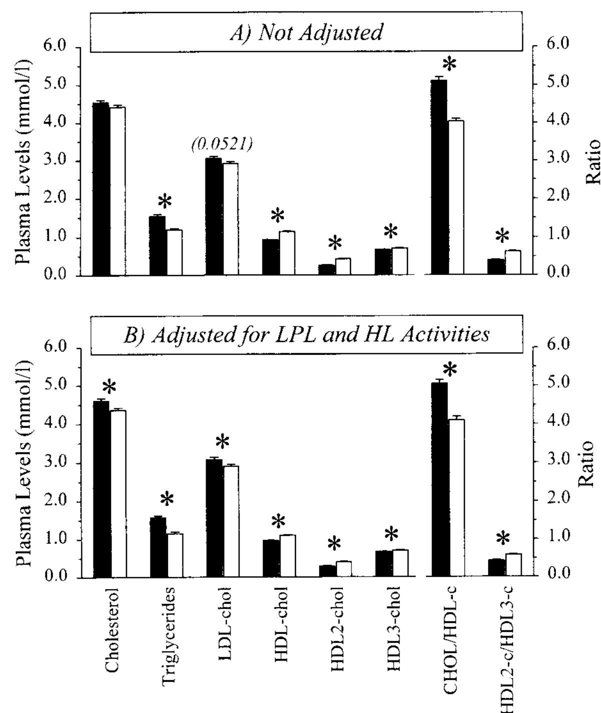

Figure 3 — Metabolic profile of the 247 men (black bars) and 240 women (white circles) of the study (**A**) before and (**B**) after statistical adjustment for post-heparin plasma activities. Values are means ± SEM. *Significantly different at the $p < 0.0001$ level.

A rather unique feature of the HERITAGE Study was our repeated analyses of post-heparin LPL and HL activities from the same sample in 5% of all subjects ($n = 73$ individuals). This analysis revealed rather low CVs (<5.5%) for the two measurements. In addition, the internal interassay and the external split samples yielded comparable results. Indeed, when the CVs of the lipase assays performed on a pooled reference sample ($n = 176$) over 4 years were examined (12.0% and 15.4% for HL and LPL, respectively), values obtained were essentially similar to the CVs of the Intracenter Quality Control study where duplicate samples were shipped and analyzed in a blinded fashion (8.4% and 14.7% for HL and LPL, respectively). In addition, ICCs were all above 0.95, indicating that our ability to classify (*i.e.*, rate) our subjects for lipase activities was excellent. We could not, however, perform the heparin infusion test twice within a week for ethical reasons. Indeed, participants of the HERITAGE Family Study were subjected to numerous tests performed on several days and it was not possible to obtain information on the day-to-day variability for the plasma PHLA. However, as these two enzymes (LPL and HL) are good correlates of plasma HDL-cholesterol levels, it is proposed that their day-to-day variation mainly results from ANER rather than biological variation, because the day-to-day variation including measurement error was about 6.0% for HDL-cholesterol levels.

The National Cholesterol Education Program Laboratory Standardization Panel has recommended that the coefficient of variation for plasma cholesterol concentration should be within 5% in order to allow a 95% probability that the value is within ±10% of the true value (25). With the exception of fasting TG and HDL_2-cholesterol levels, results of the present study indicate that CVs for lipoprotein, lipid and apolipoproteins measurements varied between 4.7% and 9.1%. To improve the precision of a given estimated lipoprotein variable, it is necessary to reduce the analytical variation (0.7–5.7% in the present study) and to increase the number of patient specimens (26). We believe that the rather small analytical error reported for our variables reflecting the lipoprotein-lipid profile, combined with a procedure in which the baseline plasma lipoprotein-lipid profile was assessed on two separate occasion provide adequate conditions to examine the effects of genetics and exercise training on cardiovascular disease risk factors including plasma lipoprotein levels in the HERITAGE Family Study.

Gender differences in PHLA and associations with lipoprotein levels

Our results clearly demonstrate the contribution of PHLA to the variation in fasting plasma lipoprotein-lipid concentrations. Indeed, in both men and women, an increased post-heparin LPL activity was

associated with a favorable plasma lipoprotein-lipid profile as decreased TG levels and higher HDL-, HDL_2-, and HDL_3-cholesterol concentrations were noted among subjects with a high LPL activity. In contrast, increased post-heparin HL activity was detrimental to the plasma lipoprotein-lipid profile as a high post-heparin HL activity was related to decreased HDL-, HDL_2-, and HDL_3-cholesterol levels in women and low HDL_2-cholesterol concentrations in men. These observations are concordant with previous studies from our group (8,27) and from others (28).

Men were characterized by a higher post-heparin HL activity as well as by a lower LPL activity compared to women, which were both associated with a less favorable plasma lipoprotein-lipid profile compared to women (8,27). Therefore, the possibility was tested that differences in post-heparin HL and LPL activities between men and women could explain, at least in part, the sex dimorphism in plasma lipoprotein-lipid concentrations. However, statistical adjustment for both post-heparin HL and LPL activities failed to eliminate the differences in plasma lipoprotein levels between men and women. Therefore, other factors could contribute to the gender differences in plasma lipoprotein-lipid concentrations. Well-known gender differences in regional adipose tissue accumulation, insulin sensitivity as well as in the sex steroid profile are likely candidates to explain this sex dimorphism in plasma lipoprotein-lipid levels (29).

In summary, the HERITAGE Family Study provides us with a unique and reliable dataset on fasting lipoprotein-lipid concentrations as well as post-heparin lipase activities. These features have rarely been assembled in the past in such a large sample of men and women. Results of the present study provide support to the notion that gender differences in plasma lipoprotein levels are explained to a certain extent by differences in post-heparin LPL and HL activities. However, PHLA could not explain entirely the gender differences in plasma lipoprotein-lipid levels reported in the present study.

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