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Analytical Performance Specifications for Lipoprotein(a), Apolipoprotein B-100, and Apolipoprotein A-I Using the Biological Variation Model in the EuBIVAS Population

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BACKGROUND: With increased interest in lipoprotein(a) (Lp[a]) concentration as a target for risk reduction and growing clinical evidence of its impact on cardiovascular disease (CVD) risk, rigorous analytical performance specifications (APS) and accuracy targets for Lp(a) are required. We investigated the biological variation (BV) of Lp(a), and 2 other major biomarkers of CVD, apolipoprotein A-I (apoA-I) and apolipoprotein B-100 (apoB), in the European Biological Variation Study population.

METHOD: Serum samples were drawn from 91 healthy individuals for 10 consecutive weeks at 6 European laboratories and analyzed in duplicate on a Roche Cobas 8000 c702. Outlier, homogeneity, and trend analysis were performed, followed by CV-ANOVA to determine BV estimates and their 95% CIs. These estimates were used to calculate APS and reference change values. For Lp(a), BV estimates were determined on normalized concentration quintiles.

RESULTS: Within-subject BV estimates were significantly different between sexes for Lp(a) and between women aged <50 and >50 years for apoA-I and apoB. Lp(a) APS was constant across concentration quintiles and, overall, lower than APS based on currently published data, whereas results were similar for apoA-I and apoB.

CONCLUSION: Using a fully Biological Variation Data Critical Appraisal Checklist (BIVAC)–compliant protocol, our study data confirm BV estimates of Lp(a) listed in the European Federation of Clinical Chemistry and Laboratory Medicine database and reinforce concerns expressed in recent articles regarding the suitability of older APS recommendations for Lp(a) measurements. Given the heterogeneity of Lp(a), more BIVAC-compliant studies on large numbers of individuals of different ethnic groups would be desirable.

Introduction

Although cardiovascular disease (CVD) still represents 31% of deaths worldwide *(1)*, advances in research over the past decade have led to great progress in its prevention and treatment. Novel therapies, such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, have resulted in significant reduction of patients' risk of developing premature CVD *(2)*. However, even after achieving optimal cholesterol concentrations, a substantial number of patients still experience acute cardiovascular events *(3)*. These observations have stimulated further research on residual CVD risk, and different biomarkers have been suggested, based on cholesterol, apolipoprotein concentrations, or lipoprotein particle number. A strong correlation between apolipoprotein B-100 (apoB) concentration and residual risk was consistently identified *(4, 5)*, and study of apoB physicochemical properties has further confirmed the important role of apoB in the formation of atherosclerotic plaques—the first stage of developing CVD *(6)*.

ApoB is a constituent of many lipoproteins: low-density lipoprotein (LDL), intermediate-density lipoprotein, very low-density lipoprotein, and lipoprotein(a) (Lp[a]). These lipoproteins differ by their lipid

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compositions, sizes, and physicochemical properties that result in different atherogenicity *(7)*. In particular, small dense LDL and Lp(a), due to their high sensitivity to oxidation, are strongly associated with increased risk of developing CVD *(8–10)*. Lp(a) is a highly complex lipoprotein formed by an LDL-like particle but containing an additional protein, apolipoprotein(a) (apo[a]), covalently bound to apoB *(11)*. This glycoprotein, similar in structure to plasminogen, imparts particular properties to Lp(a), differentiating it from LDL and increasing its atherogenicity *(12–14)*. Circulating Lp(a) levels in serum are largely genetically determined by the *LPA* gene and do not substantially vary over time *(15)* although physiologic, dietary, hormonal, and environmental factors play modest roles in its variation *(16, 17)*. Several clinical trials recently showed the strong potential of Lp(a) concentration as a high prediction target for CVD risk intervention and PCSK9 therapies *(18, 19)*, resulting in renewed and growing interest in this biomarker. However, concerns have been raised regarding the suitability of current analytical performance specifications (APS) and accuracy recommendations for Lp(a) *(13)*.

The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) has defined 3 privileged models to set APS: the model based on clinical outcomes, the biological variation (BV) model, and the "state-of the-art" model *(20)*. Compared with the other 2 models, the BV model is advantageous in that it is applicable to a wide range of biomarkers. Moreover, BV data can be used to define significance of change in a measurement series within an individual *(21)* or to assess the utility of conventional population-based reference intervals *(22)*. BV determination of a biomarker consists in the evaluation of within-subject BV (CV_I)—that is, the natural fluctuation of the concentration within an individual—and between-subject BV (CV_G)—the variation in concentration among healthy individuals *(23)*. Using these 2 components, desirable precision, or desirable allowable imprecision (CV_{APS}), and desirable allowable bias (B_{APS}) can be calculated *(24)*. Evaluating BV components and associated SDs also provides information to calculate the reference change value (RCV), defined as the minimum significant difference in results that can be detected given the analytical and preanalytical variation and the BV for the considered analyte *(21, 25)*.

A review of the online BV databases revealed that for Lp(a), and for apolipoprotein A-I (apoA-I) and apoB, the APS listed relies on results of studies performed >25 years ago and involving mostly outdated analytical methods to assess BV *(26)*. The application of the Biological Variation Data Critical Appraisal Checklist (BIVAC) *(27)* to these studies further resulted in all of them being judged as quality C, with the exception of one judged as quality B *(28)*. It thus appeared timely to address this issue with up-to-date BV estimates to deliver revised APS and RCV recommendations for these biomarkers.

This article aimed at providing a BIVAC-compliant, rigorous evaluation of BV components and associated APS and RCV specifications for Lp(a), apoB, and apoA-I in the European Biological Variation Study (EuBIVAS) population *(29)*.

Materials and Methods

SAMPLE COLLECTION AND HANDLING

BV estimates for Lp(a), apoB, and apoA-I were evaluated in the EuBIVAS population. The health status and inclusion and exclusion criteria of the individuals enrolled in the EuBIVAS study and the protocol used to collect, process, and store the samples were previously reported in detail by Carobene et al. *(29)*.

Briefly, EuBIVAS involved 6 European laboratories from 5 different countries (Italy, Norway, Spain, the Netherlands, and Turkey) that enrolled 91 healthy volunteers (38 male and 53 female, aged 21–69 years). All involved laboratories followed the same protocol for the preanalytical phase *(29)*. Included participants completed an enrollment questionnaire to verify their health status and to provide information on lifestyle. Eligibility of study participants was further verified by a selection of laboratory tests performed during the first collection *(29)*. For each eligible individual, fasting blood samples were drawn for 10 consecutive weeks (April-June 2015). Eighty-three participants completed all 10 collections, 11 completed 9 collections, 3 completed 8 collections, and 2 completed 7 collections. Serum samples collected from all individuals by each laboratory were aliquoted and sent frozen on dry ice to the coordinating center at the San Raffaele Hospital in Milan, Italy, and stored in a freezer at −80 °C until analysis in 2016 (*29*). The EuBIVAS protocol was approved by the institutional ethics review board of San Raffaele Hospital in agreement with the World Medical Association Declaration of Helsinki and by the ethics board or regional ethics committee for each center collecting samples.

ANALYTICAL METHODS

All analyses were performed in duplicate within a single run on a Roche Cobas 8000 c702 in the laboratory of San Raffaele Hospital. Lp(a) measurements were performed using the Roche reagent LPA Gen 2 (code 5852633190), an immunoturbidimetric assay involving latex particles coated with anti-Lp(a) antibodies. The calibration was performed using the Preciset Lp(a) Gen 2 Roche calibrator set consisting of 5 different points ranging from 12.2 to 185 nmol/L. PreciControl Lp(a) Gen 2, Level I Low and Level II High, were analyzed in duplicate in each single run as internal QC materials.

ApoA-I and apoB concentrations were measured using Roche reagents apoA-I Tina Quant version 2 (code 5950686190) and Roche reagents apoB Tina Quant version 2 (code 5950694190). These assays similarly involve latex particles coated with either anti–apoA-I or anti–apoB antibodies, respectively. Calibration was performed using Roche Calibrators f.a.s Lipids (code 12172623122). The QC materials PreciControl ClinChem Multi 1 and 2 were analyzed in duplicate in each run for apoA-I and apoB.

DATA ANALYSIS

Calculation of CV_I was performed using CV-ANOVA, a method in which data are first transformed using CV transformation *(30)*. CV-ANOVA is a nonparametric, robust, and largely distribution-independent procedure used to estimate analytical and within-subject BV coefficients (respectively, CV_A and CV_I) in 3-level nested random models *(30)*. Before CV-ANOVA, assessment of outliers, variance homogeneity, normality, and steady-state were performed as detailed below, with outlier identification and removal performed for replicates and samples on the CV-transformed data *(31)*.

Homogeneities of the analytical CVs (CV_A, between replicates) and of CV_I were examined using the Bartlett test and the Cochran test, respectively *(25)*. The Shapiro–Wilk test was used to verify the normality of the residuals. To examine whether individuals were at steady-state, linear regressions on the 10 pooled mean group sample concentrations were performed for each measure. Participants were considered to be in steady-state if the 95% CIs of the slope of the regression line included zero. If the slope of the regression did not include the zero, the data were adjusted by adding the slope value multiplied by S 1, where S is the sample number. Larger individual systematic changes were identified by the homogeneity test of the CV_I (Cochran test) *(25)*. CV_G was estimated by ANOVA on natural log-transformed data after applying the Dixon q test to detect outliers between individuals and the Shapiro–Wilk test to verify the normality assumption *(25)*.

For Lp(a), components of BV were estimated for men and women separately. To evaluate differences in mean concentrations between participants from the different countries or of different sexes and ages, data were visually inspected. If different between subgroups, BV was estimated for the different subgroups. Given the wide distribution of Lp(a) levels in individuals, data were also partitioned in concentration quintiles, as suggested by Marcovina and Albers *(32)* (<50 nmol/L, between 50 and 100, between 100 and 150, between 150 and 200, >200 nmol/L). For apoA-I and apoB, components of BV were also estimated based on sex and age, with female participants partitioned into 2 categories: <50 and >50 years old.

To calculate APS and RCVs, the overall BV estimates were used unless estimates were significantly different between the subgroups. In those cases, the lowest BV estimates of all subgroups were used, provided that the subgroup was reasonably large for the estimate to be reliable *(33)*. Desirable APS for CV_{APS} and B_{APS} were calculated according to the following equations:

$$CV_{APS} = 0.5 \times CV_I \quad [1]$$

$$B_{APS} = 0.25 \times \sqrt{CV_I^2 + CV_G^2} \quad [2]$$

RCVs were identified for an increase and a decrease in the measurand using the log normal approach delivering asymmetric values for rise and fall *(34)*, using the formulas below and CV_A were estimated based on duplicate measurements of study samples from all participants:

$$SD^2_{A,log} = \log_e\left(CV_A^2 + 1\right) \quad [3]$$

$$SD^2_{I,log} = \log_e\left(CV_I^2 + 1\right) \quad [4]$$

$$SD_{combined,log} = \sqrt{SD^2_{A,log} + SD^2_{I,log}} \quad [5]$$

$$RCV\ (\%) = 100\% \times \left(e^{\left(\pm Z_\alpha \times \sqrt{2} \times SD_{combined,log}\right)} - 1\right), \quad [6]$$

where $Z_\alpha = 1.65$ for the probability level of significant unidirectional change set at 95%. Data analyses were performed using Excel 2010 and IBM SPSS statistics version 23.

Results

For Lp(a), 24 individuals had a mean concentration below the limit of detection of the Roche assay (7 nmol/L); therefore, their results were excluded from the data set. One individual was also excluded because of a significant negative trend, with decreasing concentrations over the study period. After exclusion of these 25 individuals, the population to estimate Lp(a) BV components consisted of 66 participants: 41 female and 25 male (see online Supplemental Tables 1 and 2). The median number of participants per center was 10 (range, 9–15). Participants were physically active in general and did not take any drugs, and 18.2% were regular smokers. Median body mass index was 22.5, ranging from 17.6 to 32.5. For apoA-I and apoB, 1 individual was excluded from the 91 study participants because of missing data. Data excluded for the calculation of BV estimates for Lp(a), apoA-I, and apoB are detailed in online Supplemental Table 2. Overall, 10.5% of the results were excluded for Lp(a) ($n = 66$) following homogeneity analyses, and 1.3% and 1.1% of the results were excluded for apoA-I and apoB ($n = 90$), respectively.

No overall significant trends in the concentrations of Lp(a) and apoA-I were observed during the study

period. For apoB, however, the overall means of the results showed a significant negative drift during the 10-week sampling period. This trend, probably originating from seasonal changes, was corrected accordingly before calculation of BV estimates. Data were adjusted by adding the slope value multiplied by *S* 1, where *S* is the sample number. A similar trend was previously observed for total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides in the EuBIVAS study *(35)*. The Shapiro–Wilk test used to analyze data distribution yielded a normal distribution for the residuals of the individuals' variation around their homeostatic set points for all measures.

ESTIMATES OF LP(A) BIOLOGICAL VARIATION

The distribution of Lp(a) concentrations is shown in Figure 1, ordered by age and sex, after exclusion of the outliers. Data are presented as the median Lp(a) concentration (nmol/L) over the 10 samplings, with the range of values measured to illustrate the BV for each individual. Results of the components of BV estimates and their 95% CIs are detailed in Table 1.

Overall, a CV_I estimate of 8.9% (95% CI, 8.3%–9.5%) was obtained for all individuals, whereas CV_I estimates of 6.7% (95% CI, 5.9%–7.5%) and 10.6% (95% CI, 9.8%–11.6%) were obtained for men and women, respectively (Table 1). CV_I estimates were homogeneous across countries (data not shown). Given the very wide distribution of Lp(a) values (see online Supplemental Figure 1), data were further partitioned into concentration quintiles, and estimated CV_I for each quintile were constant across the different concentrations, excluding the last one at higher Lp(a) concentration (see online Supplemental Table 3). Given the extremely wide range of Lp(a) concentrations that is genetically determined, CV_G for Lp(a) was not calculated.

ESTIMATES OF APOA-I AND APOB BIOLOGICAL VARIATION

The distributions of apoA-I and apoB concentrations in the 10 samplings after exclusion of outliers are shown in Figures 2 and 3, and detailed results are presented in Table 1. Considering all individuals, CV_I estimates of 4.8% (95% CI, 4.5%–5.1%) and 6.7% (95% CI, 6.4%–7.0%) were obtained for apoA-I and apoB, respectively, with no significant differences between countries (data not shown) or between men and women (Table 1). However, a significant difference was observed between women aged <50 and >50 years, for both

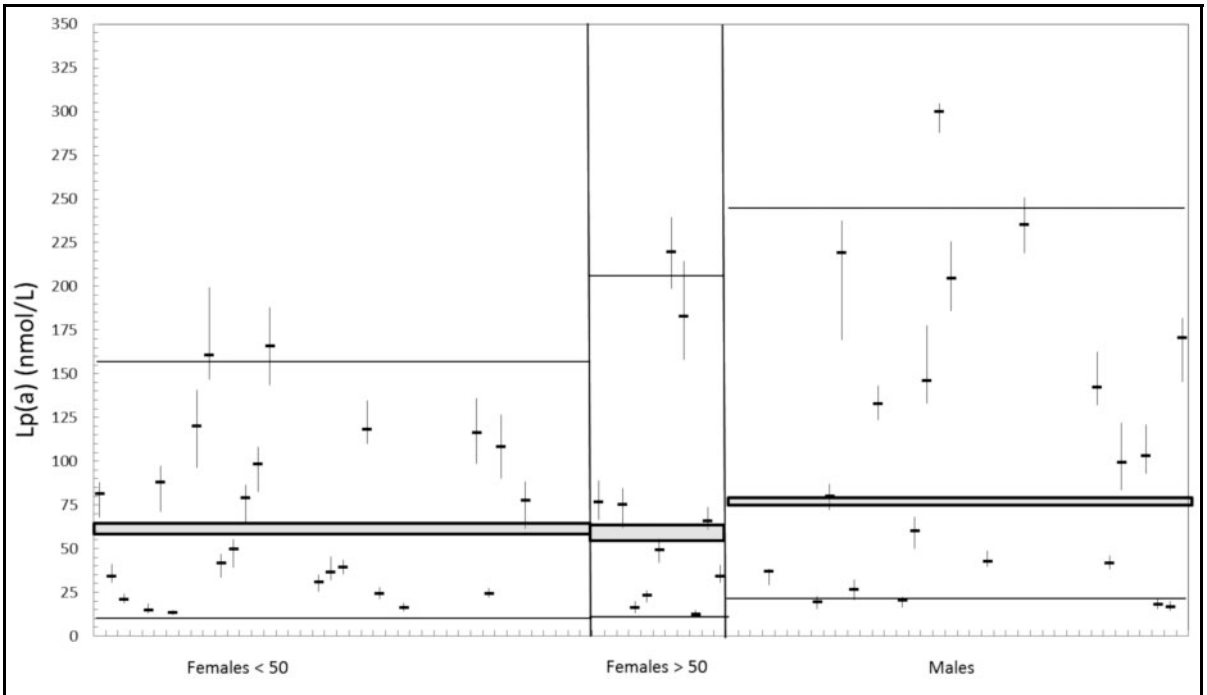

Fig. 1. Median values (dots) and range of Lp(a) concentrations (vertical error bars) for each individual included in the study, after exclusion of outliers, ordered by sex and age. Horizontal gray rectangles indicate the 95% CI of the mean, and the continuous horizontal lines indicate 5th and 95th percentiles for women <50 years old, women >50 years old, and men.

Table 1. BV estimates for Lp(a), apoA-1, and apoB partitioned by sex and age of the individuals involved in the study.[a]

		No. of individuals	Total No. of results	Mean No. of samples/ individuals	Mean No. of replicates/ samples	Mean value (95% CI)[b]	CV_A % (95% CI)[b]	CV_I % (95% CI)[b]	CV_G % (95% CI)[b]	EFLM BV database	
										CV_I %	CV_G %
Lp(a) (nmol/L)	All	66	1093	8.6	1.9	73.1 (68.4–77.7)	3.1 (3.0–3.4)	8.9 (8.3–9.5)	Not calculated	7.6[c] (5.1–10.6)	135.8[c] (94.8–238.4)
	M	25	376	7.8	1.9	92.7 (82.8–102.6)		**6.7** (5.9–7.5)	Not calculated		
	F	41	720	8.9	2.0	61.6 (57.0–66.2)		**10.6** (9.8–11.6)	Not calculated		
ApoA-1 (mg/dL)	All	90	1665	9.4	2.0	152.0 (150.5–153.4)	1.7 (1.6–1.8)	**4.8** (4.5–5.1)	17.3 (14.9–20.2)	5.8[d] (3.6–10.4)	11.2[d] (8.0–15.9)
	M	38	394	9.3	1.9	135.2 (133.4–136.9)		4.7 (4.4–5.2)	14.6 (11.8–18.9)		
	F	52	971	9.4	2.0	164.0 (162.3–165.7)		4.9 (4.5–5.2)	13.5 (11.3–16.8)		
	F < 50	42	776	9.3	2.0	159.4 (157.5–161.2)		5.1 (4.7–5.5)	**14.1** (11.4–17.9)		
	F > 50	10	195	9.8	2.0	182.6 (180.2–185.0)		3.6 (3.1–4.3)	7.3 (5.0–13.7)		
ApoB (mg/dL)	All	90	1669	9.4	2.0	90.0 (88.7–91.3)	1.5 (1.4–1.6)	**6.7** (6.4–7.0)	25.9 (22.7–30.9)	7.4[d] (4.2–13.5)	20.2[d] (10.7–27.2)
	M	38	699	9.3	1.9	94.9 (92.7–97.1)		6.8 (6.2–7.3)	28.5 (23.3–37.8)		
	F	52	970	9.4	2.0	86.4 (84.9–87.9)		6.7 (6.2–7.1)	23.6 (19.7–29.6)		
	F < 50	42	775	9.3	2.0	81.3 (79.8–82.7)		6.9 (6.4–7.5)	**20.8** (17.0–26.8)		
	F > 50	10	195	9.8	2.0	107.0 (103.7–110.2)		5.6 (4.9–6.6)	20.6 (14.1–38.9)		

CV_A, CV_I, and CV_G are shown with 95% CIs and BV estimates available in the EFLM BV database (*26*). The data used for the calculation of APS reported in Table 2 are shown in boldface.
[a]CV_A estimates are based on CV-ANOVA of duplicate analysis of all study samples.
[b]Results from a single paper (*28*).
[c]Results derived from meta-analysis.

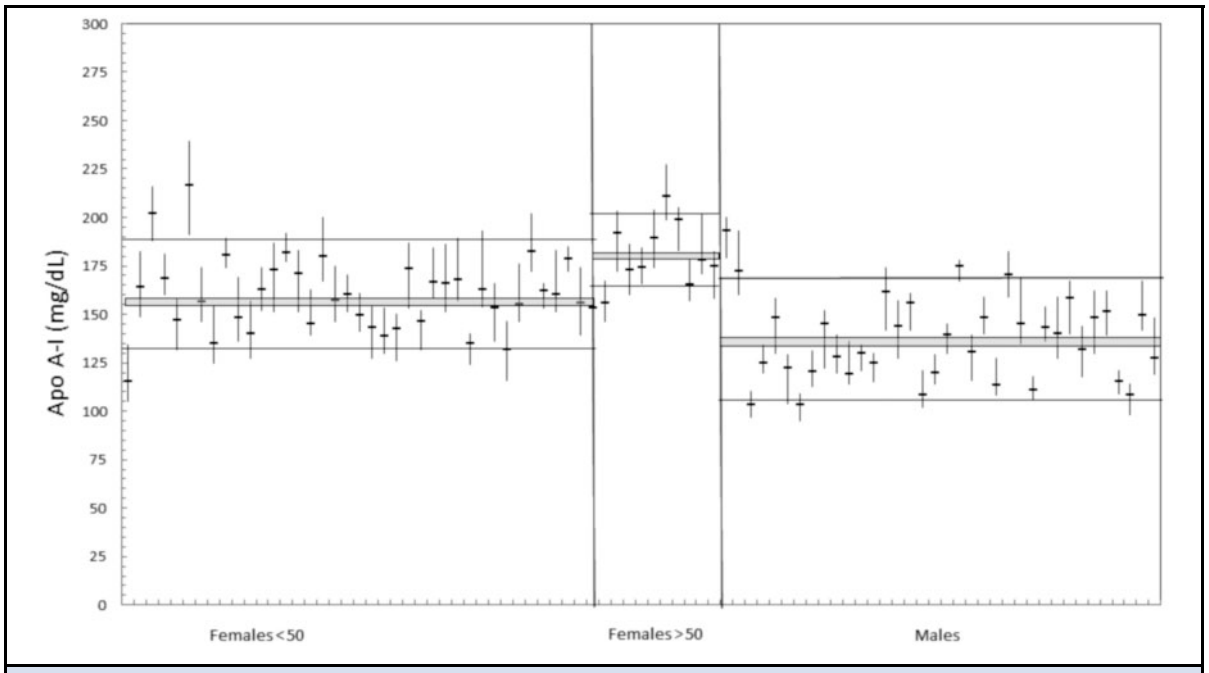

Fig. 2. Median values (dots) and range of apoA-I concentrations (error bars) for each individual included in the study, after exclusion of outliers and ordered by sex and age. Horizontal gray rectangles indicate the 95% CI of the mean, and the continuous horizontal lines indicate 5th and 95th percentiles for women <50 years old, women >50 years old, and men.

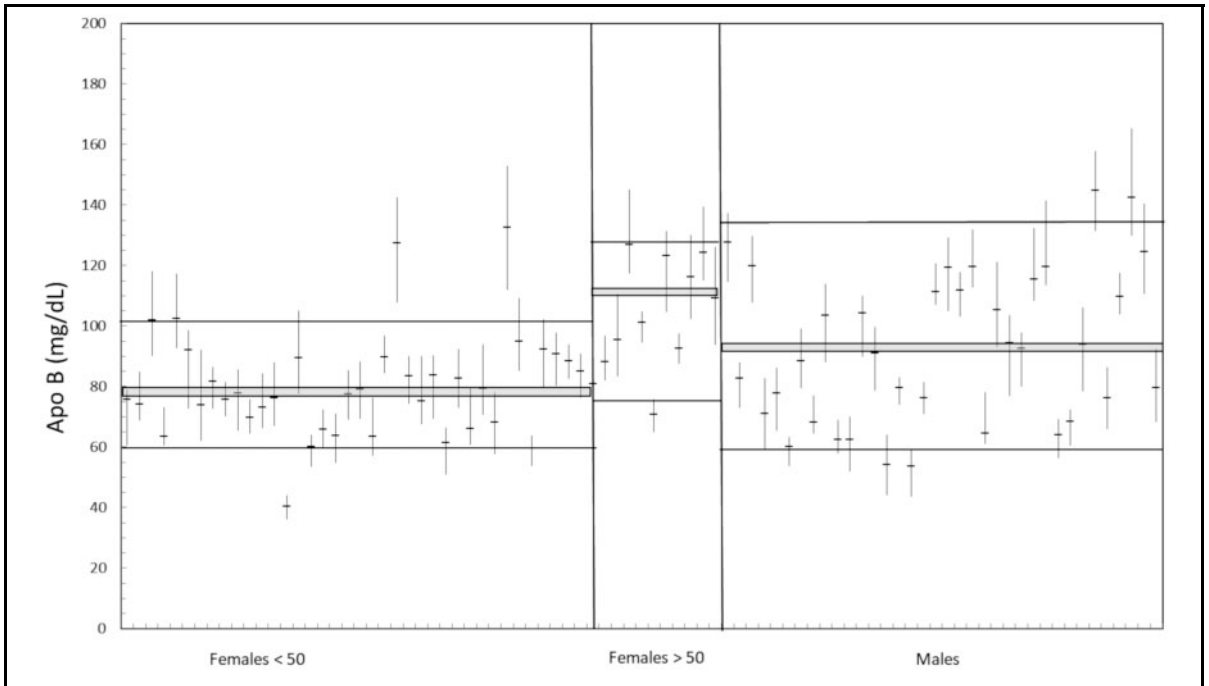

Fig. 3. Median values (dots) and range of apoB concentrations (error bars) for each individual included in the study, after exclusion of outliers and ordered by sex and age. Horizontal gray rectangles indicate the 95% CI of the mean, and the continuous horizontal lines indicate 5th and 95th percentiles for women <50 years old, women >50 years old, and men.

Table 2. APS for imprecision (CV_{APS}) and bias (B_{APS}) and RCVs based on the BV estimates as reported in Table 1, compared with the desirable APS reported in the EFLM BV database *(26)*.

					EFLM BV database	
		CV_{APS} (%)	B_{APS} (%)	RCV (%) decrease/increase	CV_{APS} %	B_{APS} %
Lp (a)	Male	3.3	NA[a]	−15.8/18.7	3.8[b]	34[b]
	Female	5.3	NA[a]	−22.6/29.2		
ApoA-I		2.4	3.7	−11.1/12.5	2.9[c]	3.2[c]
ApoB		3.4	5.5	−14.7/17.3	3.7[c]	5.4[c]

[a]Not applicable (NA) given that CV_G for Lp(a) was not calculated.
[b]Based on results from a single paper *(28)*.
[c]Based on results derived from meta-analysis.

apoA-I and apoB. However, this observation needs to be interpreted cautiously because the subgroup of women aged >50 years comprises only 10 individuals.

ANALYTICAL PERFORMANCE SPECIFICATIONS

BV estimates were used to calculate APS for imprecision of Lp(a), apoA-I, and apoB, and APS was used for bias of apoA-I and apoB concentration measurements and RCVs (Table 2). For apoA-I and apoB, the recommendations that can be calculated using data available in the EFLM BV database are shown in Table 2. CV_{APS} results had good agreement for apoA-I and apoB.

Discussion

With the growing clinical evidence of the impact of increased circulating Lp(a) on CVD risk and heightened interest in Lp(a) concentration as a target for risk reduction, the currently available APS and accuracy targets for the measurement of Lp(a) concentrations have been questioned *(13)*. To address these issues, we investigated the BV of Lp(a) together with those of apoA-I and apoB—well-established biomarkers of CVD.

For apoA-I and apoB, results of this study were in very good agreement with the meta-analysis reported in the EFLM BV database *(26)*. However, significant differences for these 2 biomarkers were identified in women above and below the age of 50 years, a finding consistent with previously published results *(36)*. It has been demonstrated that the reliability of BV estimates largely depends on the number of individuals involved and that smaller populations have more uncertain estimates *(33)*. For that reason, B_{APS} for apoB and apoA-I was calculated using only the CV_G of the subgroup of women aged <50 years. Nevertheless, considering the importance of age as a risk factor for developing CVD, performing an evaluation of its impact on BV estimates for apoB and apoA-I in an appropriately powered study could be of interest. Interestingly, BV estimates obtained for apoA-I and apoB compare well with those obtained in the same population of the EuBIVAS study for high-density lipoprotein (HDL) and LDL cholesterol. However, apoA-I CV_G is significantly lower than that reported for HDL cholesterol, and overall, CV_I for both apoA-I and apoB tended to be lower than that for HDL and LDL cholesterol *(35)*.

For Lp(a), the EFLM BV database currently cites only 1 article, by Pagani and Panteghini *(28)*, that reports a CV_I estimate (7.6%) similar to the one we obtained, even though the estimate was obtained in a much smaller population (10 healthy participants), with a shorter observation time (5 weeks). In our present study, the large number of participants involved and the large range of Lp(a) concentrations allowed us to further partition data into concentration quintiles. Interestingly, CV_{APS} was consistent across these quintiles, although a surprising very low CV_I was observed for the high concentrations. However, the large proportion of excluded results for this high-Lp(a)-concentration subgroup, combined with the small number of individuals, does not allow us to draw reliable conclusions about this particular result (see online Supplemental Table 3).

In addition to the study by Pagani and Panteghini *(28)*, 3 articles that formally assessed BV of Lp(a) were published between 1994 and 1998 *(17, 37, 38)*. These articles present substantially higher estimates of BV components. The most probable explanation for these differences in calculated APS would be that previous estimates rely on results obtained using outdated assays *(17, 28, 37)*. However, the study by Marcovina et al. *(38)* was performed with a well-characterized, in-house-developed, monoclonal antibody–based ELISA that accurately measures Lp(a) independently of apo(a) size variability and is considered the gold standard method for the quantitation of Lp(a). In this particular case, the difference of approach to the evaluation of BV estimates

and the more inclusive statistical criteria for calculation could explain the larger APS obtained.

To our knowledge, few recent data are available on BV for Lp(a). However, in a recently published article on the temporal variability of Lp(a) in serum, Marcovina et al. *(39)* reported a mean percentage change from baseline ranging from −13.1% up to +21.6% on 3 different placebo groups involved in 3 independent clinical trials over a period of 30 up to 190 days. Although the detailed components of BV are not available, results we report in terms of RCV closely agree (see Table 2). With the recent clinical trial outcomes recommending the use of Lp(a) concentration as a new target for CVD risk reduction *(13, 18)*, it can be expected that follow-up of on-treatment patients for Lp(a) concentration will become more frequent in clinical practice. However, as highlighted by Fraser *(21)*, a result within a patient-measurement series can lie within a reference interval but be highly unusual for this specific individual. Therefore, the development of reliable RCVs, estimated in a well-characterized and controlled population with a fully compliant statistical analysis, represents valuable information.

This study was performed following the recommendations of the Biological Variation Working Group (BV-WG) of the EFLM for the generation of comprehensible and reliable BV data *(31)*. Results were obtained on the large-scale EuBIVAS population, using a validated and standardized protocol with well-defined inclusion and exclusion criteria and strictly controlled factors influencing preanalytical variability *(29)*. Statistical analyses were also done following the BIVAC checklist issued by the BV-WG and included exclusion of outliers, evaluation of data homogeneity, and ANOVA *(31)*. Thanks to its strict approach to design and delivery, the results of BV estimates obtained on the EuBIVAS population are in full compliance with the current quality standards established for reporting BV data and should be considered more reliable than those published previously.

Nevertheless, some limitations to this study may be identified, one being the large proportion of results excluded for the evaluation of Lp(a) BV estimates. Indeed, some individuals demonstrated very large variability over the 10-week sampling period, resulting in nonhomogeneous data identified as outliers by the CV-ANOVA. For 3 individuals in particular, 5 samples were detected as outliers across the 10 samplings, without any specific trend identified. In addition, many individuals showed Lp(a) concentrations well below the limit of detection of the Roche assay.

The use of methods from a single manufacturer and a single lot of reagents for all analyses could also be considered a limitation of this study. Even though it is well known that methods and reagents from different manufacturers may perform differently, it is unlikely that this factor may significantly affect the BV estimates. Finally, samples from different participants were measured on different days, but between-run variation was much smaller than the CV_G and could not have influenced it. Moreover, any contribution from within-run analytical variability was eliminated by the study design and statistical approach because all analyses of each participant were performed in a single run.

Conclusion

Our results show that CV_I for apoA-I and apoB is lower than results obtained in previous studies, thus driving more stringent criteria for desirable precision. For Lp(a), previous literature reports discordant data; our results are in line with those reported by Pagani and Panteghini *(28)* and included in the EFLM database but are much lower than those reported in other publications *(17, 37, 38)*. In terms of CV_G estimates for apoA-I and apoB, our results are in line with or slightly higher than previous findings. For Lp(a), CV_G was not estimated because of the extreme heterogeneity of the molecule and the lack of genetic information for our population. This study further confirms issues already noted in recent articles *(13, 19, 32)* and supports the conclusions of these authors that APS targets need to be redefined for accurate and precise quantitation of Lp(a) to be used effectively as individual risk classification for CVD. Data provided in this article also deliver revised and more precise APS and RCVs for Lp(a); this information is highly important for clinical use. However, given the heterogeneity of Lp(a) and the extremely large range of values, more BIVAC-compliant studies performed with larger numbers of individuals and different racial/ethnic groups would be desirable.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: CVD, cardiovascular disease; PCSK9, proprotein convertase subtilisin/kexin type 9; apoB, apolipoprotein B; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); APS, analytical performance specifications; EFLM, European Federation of Clinical Chemistry and Laboratory Medicine; BV, biological variation; CV_I, within-subject variation; CV_G, between-subject biological variation; CV_{APS}, analytical performance specification for imprecision; B_{APS}, analytical performance specification for bias; RCV, reference change value; ApoA-I, apolipoprotein A-I; BIVAC, Biological Variation Data Critical Appraisal Checklist; EuBIVAS, European Biological Variation Study; CV_A, analytical variation coefficient; BV-WG, Biological Variation Working Group.

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requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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