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Inaccurately Reported Statin Use Affects the Assessing of Lipid Profile Measures and Their Association with Coronary Artery Disease Risk

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Abstract

BACKGROUND: Lipid profiling is central for coronary artery disease (CAD) risk assessment. Nonadherence or unreported use of lipid-lowering drugs, particularly statins, can significantly complicate the association between lipid profile measures and CAD clinical outcomes. By

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combining medication history evaluation with statin analysis in plasma, we determined the effects of inaccurately reported statin use on lipid profile measures and their association with CAD risk.

METHODS: We compared medication history of statin use with statin concentration measurements, by liquid chromatography–tandem mass spectrometry, in 690 participants undergoing coronary angiography (63 ± 11 years of age). Nominal logistic regression was employed to model CAD diagnosis with statin measurements, phenotypic, and lipid profile characteristics.

RESULTS: Medication history of statin use was confirmed by statin assay for 81% of the patients. Surprisingly, statins were detected in 46% of patients without statin use records. Nonreported statin use was disproportionately higher among older participants. Stratifying samples by statin history resulted in underestimated LDL-lipid measures. Apolipoprotein B concentrations had a significant inverse CAD association, which became nonsignificant upon re-stratification using the statin assay data.

CONCLUSIONS: Our study uncovered prominent discrepancies between medication records and actual statin use measured by mass spectrometry. We showed that inaccurate statin use assessments may lead to overestimation and underestimation of LDL levels in statin user and nonuser categories, exaggerating the reverse epidemiology association between LDL levels and CAD diagnosis. Combining medication history and quantitative statin assay data can significantly improve the design, analysis, and interpretation of clinical and epidemiological studies.

Introduction

Since the 1960s, coronary artery disease (CAD)-related deaths in the United States have decreased about 4-fold (1). This reduction is in large part due to the benefits of cholesterol-lowering agents (2). The decline in CAD-associated mortality was further improved following the approval in the late 1980s of the first statins. Statins are highly effective for reducing the levels of atherogenic low-density lipoprotein (LDL)-related biomarkers, such as low-density lipoprotein cholesterol (LDL-C), LDL particles, and apolipoprotein B (APOB) (1, 2).

The beneficial effect of LDL-reducing therapy through statin use is reinforced by a reduction of myocardial infarction in randomized clinical trials that compare statin-treated vs control groups (3, 4). The newest clinical guidelines from the American Heart Association and the American College of Cardiology were developed based on data from numerous randomized clinical trials. The guidelines advocate the calculated percent risk of atherosclerotic cardiovascular disease (ASCVD) over a subsequent 10 years (pooled cohort equation score) as the main criterion for statin prescription, further expanding the number of persons eligible for statin therapy (5). According to the most recent US government survey covering the years 2003 to 2012, 23% of adults used some brand of statin medications (6), and the prevalence of statin use is increasing worldwide (7).

With increasing statin prescription rates, poor statin adherence has emerged as a major barrier to achieving LDL-C target levels, increasing the risk of CAD-associated death (8–10). Consequently, an urgent need for significant improvement in statin adherence became commonly recognized (9, 11). To achieve this goal, it is vital to accurately stratify statin

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users and nonusers during CAD risk assessment. However, the collection of information about medication compliance or adherence (i.e., whether a patient or study participant actually takes the drug as prescribed) is challenging (6, 12–15). Efforts to develop general standard operating procedures for collecting medication adherence information have started only in recent years (16). Patient nonadherence to the prescribed statin-dose regimen is a widespread problem recognized by clinicians (1, 16, 17). Medication history reports by patients might not accurately reflect statin use. That is partly related to the clinical settings where the information is collected (i.e., in primary care or during surgical treatments), which can vary across age groups and risk categories. In addition, reliance on assays that allow confirmation of statin use by plasma or urine concentration measurements is challenging because each statin has different pharmacologic characteristics (e.g., half-life, excretion pathway, etc.) (17).

In this study, we address these challenges by evaluating medication information based on a laboratory-developed mass spectrometry assay to screen plasma samples for detectable statin concentrations (18). We studied the plasma specimens and the medical histories of 690 participants of the CATHeterization GENetics (CATHGEN) coronary catheterization cohort (19). Combining the demographic/health information and medication history with measured statin and lipid concentration data, we examined the confounding effects of statin history vs assay-based data on the associations between traditional lipid profile measures and CAD diagnosis. Our findings demonstrate the application of a high-throughput statin screening assay for large sample cohorts and may inform the design, data analysis, and interpretation of CAD-related clinical and observational studies.

Materials and Methods

STUDY POPULATION

We used 690 plasma samples from the CATHGEN sample archive collected from participants during their coronary artery catheterization procedure (19). Age, sex, race, body mass index (BMI), history of hypertension (BP), diabetes (DM), smoking (SMK), and name of reported statin medication were acquired from the CATHGEN database. Specimens were classified based on a CAD index of <32 and ≥32 as primary association variables CAD[−] and CAD[+], respectively (20). Medication history, including statin type and dose, was collected by the treating physicians at the time of catheterization. Sources were history recorded during the patient interview and the medical record, where medication history was obtained at the time of hospital admission for cardiac catheterization. Samples with recorded statin use were categorized as h[+], and those without recorded statin medication were categorized as h[−] (h: history). The study population included 442 males (64%) and 248 females (36%), with a mean age of 63 ± 11 years. All samples were de-identified before shipment. Table 1 shows participant characteristics, including demographic data. The use of CATHGEN study data was approved by the Duke Health Institutional Review Board.

CHEMICALS AND REAGENTS

Solvents and chemicals used in this study are listed in the online Supplemental Material.

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PROTEIN AND LIPID PROFILE MEASUREMENTS

Total cholesterol (TC), total triglycerides (TG), total APOB, and total apolipoprotein A1 (APOA1) were measured with published liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods (21, 22). The lipoprotein class fractions, high-density lipoprotein cholesterol (HDL-C), LDL-C, and very-low-density lipoprotein cholesterol (VLDL-C) were acquired by size fractionation using an asymmetric flow field-flow fractionation method (23), collecting 40 fractions from 50 μL plasma injections. Each fraction was analyzed for TC with the same LC-MS/MS methods used for the whole plasma analysis (21). Summing concentrations after recovery correction in corresponding size ranges gave HDL-C, LDL-C, VLDL-C, and non-HDL-C (non-high-density lipoprotein cholesterol: TC minus HDL-C) concentrations in plasma (23).

STATIN PLASMA CONCENTRATION ASSAY

The carboxylic acid form of the statin parent compounds or metabolites was measured by an LC-MS/MS method published elsewhere (18). Briefly, an aliquot of 10 μL plasma sample was mixed with 10 μL of water, and then 80 μL of acetone spiked with deuterated internal standards, vortexed, and centrifuged. The supernatant was transferred onto a 96-well polypropylene plate, then evaporated and reconstituted with 25 μL of 30:70 acetonitrile:water. The reconstituted extracts were analyzed via LC-MS/MS in scheduled multiple reaction monitoring mode with ≤1 ng/mL limits of detection (LODs) in plasma for the following 7 generic statin compounds: atorvastatin, simvastatin, pravastatin, lovastatin, rosuvastatin, fluvastatin, and pitavastatin. We calculated the percentage of confirmed statin users out of all reported users of a particular compound. The analysis confirmed statin use for atorvastatin (90%), pravastatin (73%), lovastatin (83%), rosuvastatin (64%), fluvastatin (67%), simvastatin (69%), but not pitavastatin (0%) (18). All compounds with concentrations above the LOD were counted as detected (labeled a[+]), and below the LOD were counted as non-detects (labeled a[−]).

STATISTICAL ANALYSIS

The LC-MS/MS data acquired on the different analytical platforms were exported and merged using JMP software (SAS Institute). Logistic regression algorithms implemented in JMP were used to model CAD[+] vs CAD[−] diagnoses. All models incorporated standardized continuous variables for age and BMI, and categorical variables for sex, race, SMK, BP, and DM. When the models included an additional binary nominal variable for statin use history or assay, labels h[+]/[−] or a[+]/[−] were used, respectively. The model sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) were calculated as follows: Sensitivity = TP/(TP + FN); Specificity = TN/(TN + FP); Accuracy = (TP + TN)/(TP + TN + FP + FN); PPV = TP/(TP + FP); NPV = TN/(TN + FN) where TP is the number of true-positive values, TN is the number of true-negative values, FP is the number of false-positive values, and FN is the number of false-negative values.

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Results

DISCREPANCIES IN THE DETERMINATION OF STATIN USE BASED ON MEDICATION RECORDS AND DETECTABLE STATIN CONCENTRATIONS

Based on medication histories, 261 out of 690 participants were statin users (labeled h[+], Fig. 1). In the 261 h[+] specimens, the LC-MS/MS assay confirmed some type of statin use for 212 participants (81%, labeled a[+]). In 7 out of 690 samples, a second statin was detected near the LOD of that drug. The most frequently detected statins by LC-MS/MS assay were atorvastatin (133 samples) and simvastatin (82 samples). Among the 429 specimens without a recorded history of statin use (labeled h[−]), the LC-MS/MS assay agreed with non-detectable levels of statins for 231 specimens (53.8%, labeled a[−]). For 198 (46.2%) h[−] specimens, statins were detectable, labeled a[+], and the mean and range of statin concentrations were similar to the [h+] group (18). The samples also can be stratified into concordant and discordant categories: 33.5% concordant statin nonusers h[−]/a[−], 7.1% discordant h[+]/a[−], 28.7% discordant h[−]/a[+], and 30.7% concordant statin users h[+]/a[+].

LIPID MEASURES IN CONCORDANT AND DISCORDANT HISTORY/ASSAY CATEGORIES

The patients' statin use was reflected by relative LDL measures. The stratification into concordant and discordant categories revealed significant differences in APOB and LDL-C concentrations between the groups (Fig. 2, Table 2). Corresponding group characteristics are shown in online Supplemental Table 1. The concentration difference between the h[−]/a[−] and h[+]/a[−] subsets was less prominent than between the h[−]/a[+] and h[+]/a[+] subsets. These observations confirmed the notion that the h[−] group included a substantial number of actual a[+], while the h[+] group included relatively few a[−] participants. Overall, there were decreasing dose–response-like trends in LDL-related lipid measures across the groups in the following order: h[−]/a[−] ≍ h[+]/a[−] > h[−]/a[+] > h[+]/a[+].

EFFECT OF STATIN USE ON THE ASSOCIATION OF LDL MEASURES WITH CAD BY AGE GROUPS

The consequence of statin history-based vs assay-based determination of statin use was revealed by comparison of APOB concentrations in h[−], a[−], h[+], and a[+] samples (online Supplemental Table 2), especially after further stratification into CAD[+]/[−] and 4 age subgroups (Fig. 3, online Supplemental Fig. 1, and online Supplemental Table 4). The most significant differences in CAD[+] vs CAD[−] were found for participants in the 40 to 54 years and 75 to 86 years age groups. In the 40 to 54 years age group, APOB levels were positively associated with CAD[+], most significantly in the h[−] group ($P = 0.005$). In the 75 to 86 years age group, however, APOB levels showed negative associations with CAD[+], most significant in the h[+] ($P = 0.008$) and a[+] ($P = 0.005$) groups. Similar trends were found for the LDL-related measures and others, LDL-C, VLDL-C, and non-HDL-C (Supplemental Fig. 1).

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ASSOCIATION OF RISK COVARIATES WITH STATIN USE

The percentage differences in the prevalence of risk covariates were small within the h[+], h[−], a[+], and a[−] groups (Supplemental Table 1, online Supplemental Fig. 2). However, logistic regression analysis showed a significant association between statin use (as dependent variable (h[+]/[−] or a[+]/[−]) and certain covariates (as independent variable). Sex, BP, and DM were significantly associated with h[+]/[−], while age with a[+]/[−] (*P*-values range 0.010 to 0.034). These differences in associations between statin use and risk covariates were probably the result of changes in the prevalence of risk factors after re-stratification into a[+]/[−] groups, and highlight the importance of balancing the prevalence of risk covariates after accurate statin use and adherence assessment.

EFFECT OF STATIN USE ON CAD MODEL CHARACTERISTICS

The percentage of CAD[+] prevalence was similar in h[+] and a[+] groups, 64% and 63%, respectively, $P = 0.831$, but the CAD[+] prevalence was higher in the h[−] group (48%) than in the a[−] group (40%), $P = 0.048$. To assess the whole model consequence of using h[+]/[−] vs a[+]/[−] as a covariate, 3 nominal logistic models were constructed (Table 3 and online Supplemental Table 3). All models were built with the whole sample set (n = 690). Model 1 was built without statistical correction for statin use. Model 2 was calculated with h[+]/[−] correction. Model 3 was built using statin detection by LC-MS/MS with a[+]/[−] correction. The models were evaluated based on the partial odds ratio of h[+]/[−] and a[+]/[−] covariates, and whole model sensitivity, specificity, accuracy, PPV, NPV, and area under the receiver operating characteristic (ROC) curve (AUC) (Table 3). The partial odds ratio for h[+]/[−] in Model 2 was 1.74 (95% confidence interval [CI], 1.23–2.44) and for a[+]/[−] in Model 3 it was 2.42 (95% CI, 1.73–3.37) (Table 3, Supplemental Table 3). Although Models 1 to 3 had similar whole model sensitivity and specificity, the partial odds ratio was higher for the a[+]/[−] covariate than for the h[+]/[−] covariate.

Discussion

Statins are the first-line lipid-lowering pharmaceutical therapy against CAD. However, inaccurately reported statin adherence may complicate the CAD risk assessment based on the standard lipid panel measures, posing a barrier to clinical and epidemiological studies. Here, using a quantitative LC-MS/MS-based approach we evaluated the accuracy of statin medication history records and determined the effects of misreported statin use on assessing lipid profile measures and their association with CAD risk.

One of the main strengths of our study was measuring the concentration of 7 commonly used statin drugs and their metabolites. Although statin detectability can be limited by pharmacologic characteristics (e.g., half-life, target organ, excretion pathway, etc.), it was compensated for by the relatively low LOD of the LC-MS/MS assay (LOD of 0.1 ng/mL for atorvastatin, 1 ng/mL for other statins). We assumed that other sources of variability such as statin dose, time since initiation of statin use, and initial LDL-related measures were randomly distributed within the h[+]/[−] and a[+]/[−] categories. Another important strength of our study was that 690 samples were analyzed both for statins and lipid profile measures, which allowed for stratification into treated/nontreated groups and subgroups by

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age. Furthermore, the CAD status of the study participants was defined based on their CAD index, determined by cardiac catheterization, which was able to confirm CAD status with high PPV. Meanwhile, the samples were collected at the time of cardiac catheterization, which potentially can limit a broad extrapolation of the specific findings from the study to other populations in different settings.

Previously, as a validation of our LC-MS/MS method on the same study population, we presented evidence that our assay can determine statin use with high accuracy in cases of reported statin use (h[+]/a[+]), where the detected statin compounds matched the expected statin medication (18). Unexpectedly, in this study, we also found a substantial number of cases where the study participant did not report statin use but we found detectable statin concentrations (h[−]/a[+]). The concordant h[+]/a[+] and discordant h[−]/a[+] samples had a statistically similar normal distribution of statin concentrations (mean, median, and range). Furthermore, the number of cases where we did not detect statin concentrations despite a reported history of statin use was relatively small (Fig. 1). Therefore, in our study, the population's lack of reported statin use seemed to be a more serious problem than the nonadherence to statins.

Importantly, we showed that unreported statin use can significantly affect LDL measures with consequences on the assessment of CAD risk.

Statin use is expected to decrease LDL particle number-related measures, such as APOB, LDL-C, non-HDL-C, and TC. Inaccurate assessment of statin use can result in underestimation and overestimation of the LDL-C levels, and biased associations between LDL particle-related measures and CAD diagnosis. We found that LDL measures were significantly higher in the concordant h[−]/a[−] group than in the discordant h[−]/a[+] group (Fig. 2) with the following order of significantly different mean concentrations: h[−]/a[−] > h[+]/a[−] > h[−]/a[+] > h[+]/a[+]. This order of LDL levels was most likely a result of differences in response to statin use in general: nontreatment > nonadherent-treatment > treatment, where nonadherent-treatment corresponds with h[−]/a[+] and h[+]/a[−] groups. These data suggest that a lack of agreement between the medication history of statin use vs the statin assay results may be used as an indicator of nonadherent statin use. These results also highlight a less recognized nonadherence problem, when patients use a statin, perhaps at irregular intervals, but report no statin use at all (h[−]/a[+]). Our data illustrate the consequence of inaccurate statin-use assessment through the comparison of APOB concentrations after stratification into h[−], a[−], h[+], and a[+] groups and further subgroups by CAD[+]/[−] diagnosis and age (Fig. 3). In the h[−] group particularly, we observed declining relative APOB levels with age in the h[−]/CAD[+] group as opposed to increasing APOB levels with age in the h[−]/CAD[−] group. In the 75 to 86 years age group, there was a significant inverse association between CAD[+] diagnosis and APOB concentrations. A similar inverse association between LDL measures and CAD diagnosis has been reported for older populations, leading to the so-called "lipid paradox" or "inverse epidemiology" hypothesis (24–28). However, our results demonstrate that the false inclusion of statin users into the h[−] group can cause a significant underestimation of APOB levels, mainly among older CAD[+] participants. On the other hand, the inclusion of nonusers into the h[+] group resulted in an overestimation of APOB levels, mainly among older CAD[−]

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participants. In both scenarios, underestimation among CAD[+] and overestimation among CAD[−] participants contributed to the inverse association between APOB and CAD[+] diagnosis. Thus, our findings demonstrate that the inaccurate assessment of statin-use medication history can lead to an exaggerated inverse epidemiology or lipid paradox trend, especially in the >75 years of age group.

To some extent, the lipid paradox was observable in the 75 to 86 years age group regardless of stratification by statin use (Fig. 3). However, we noticed that CAD[−] participants in the 75 to 86 years age group had a lower prevalence of DM (22%) and SMK (28%) as compared to the younger 40 to 54 and 55 to 64 years age groups (DM: 25% to 35%, SMK: 41% to 47%). There was also a higher representation of female (61% vs 36% to 54%) and White (72% vs 51% to 62%) participants in the 75 to 86 years age group (online Supplemental Fig. 3). The lack of diabetes and smoking history combined with the prevalence of White female patients contribute to decreased rates of CAD-associated death. Meanwhile, the average LDL-related measures were relatively higher in older people. Thus, these phenotypic characteristics together may have favored the probability of CAD[−] in the 75 to 86 years age group even with high LDL-related measures.

In most epidemiological studies, statin use is determined from self-reports (29). To avoid statistical biases, and to define associations between CAD presence and lipid profile measures, most studies include participants who either do or do not use lipid-lowering statin medications (6, 29–31). Another approach is to include user and nonuser participants and apply a 2-level categorical [+]/[−] covariate in the prediction model (32). Using our data, by correcting with either h[+]/[−] or a[+]/[−] binary covariates, in addition to other demographic and risk covariates, we expected to see substantial differences in the partial odds ratios of both h[+]/[−] and a[+]/[−] covariates. Indeed, the partial odds ratio for the a[+]/[−] was 0.68 higher than for the h[+]/[−] covariate (Table 3). This false-positive association between statin use and CAD[+] was likely due to referral bias. In this study, we evaluated a cohort of patients who were referred for cardiac catheterization because of their expected elevated CAD risk, which also resulted in receiving a statin prescription before the examination occurred.

Interestingly, the h[+]/[−]-corrected Model 2 and the a[+]/[−]-corrected Model 3 both resulted in whole model characteristics (sensitivity and specificity) that were very similar to the uncorrected Model 1 (Table 3). The lack of differences in the whole model output was in contrast to the differences in the h[+]/[−] and a[+]/[−] odds ratios. This observation can be understood in view of the numerically different cross-effects or confounding effects between h[+]/[−] or a[+]/[−] and the other model covariates. During the optimization of the nominal logistic prediction equations, the inaccuracy of the [+]/[−] statin-use variable-containing term is numerically compensated by adjustments in the other covariate-containing terms. Therefore, the consequence on the whole model output from the lack of inclusion or accuracy of a [+]/[−] statin-use variable is minimal if demographic and risk covariates are also included in the model.

The results of this study demonstrate the feasibility of our statin assay for application in large-scale studies. The assay requires only 10 μL plasma, performs reproducibly on a high-

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throughput 96-well sample preparation platform (18), and allows quantitative screening for 7 statin drugs or metabolites, which can be further expanded to the carboxylic acid forms of any other drug. Including a statin panel in future public health surveys, such as the National Health and Nutrition Examination Survey (NHANES), may be highly desirable. Meanwhile, the assessment of medication history remains an unsolved problem. The development and implementation of new practices to improve the quality of the medication history collection is urgently needed. The development of standardized methods, highly trained personnel, and constant reevaluation of records may lead to significant improvement in this area. Electronic monitoring, electronic medication packaging (EMP) devices, and pill counts were suggested as promising approaches (33–35). For example, the US government surveys suggest that the patients are asked whether they had taken a prescription medication in the past 30 days. Those who answered “yes” are further asked to show the interviewer the medication containers of all their prescription medications (6). The use of electronic health records (EHR) can also improve the medication history accuracy (36). However, the multi-measure approach, including the quantitative assessment, would likely provide the most accurate measure of therapeutic adherence (37).

In summary, our findings suggest that collecting both medication history and statin assay data can enhance the assessment of statin use and adherence. Medication history should be assessed following standard operating procedures. Screening plasma samples for detectable statin levels can be performed with high efficacy by using sensitive and high-throughput mass spectrometry detection-based methods. The discrepancy between medication reports and statin assay data can be a flag for nonadherent statin use and inform the design and evaluation of clinical studies. Specifically, for the CATHGEN study, given the mechanism whereby medication history was obtained, some of the discordances may be due to inaccurate patient reporting or inaccuracies in the medical record. Inaccurate statin-use assessments may lead to overestimation and underestimation of LDL-related measures in statin user and nonuser categories, exaggerating the reverse epidemiology association between LDL-related measures and CAD diagnosis. In the case of evaluation of lipid metabolism-related biomarkers, independent assessment with stratification by age is also highly important due to the confounding cross-effects among age, risk factors, and statin use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement:

The datasets used and/or analyzed during the current study are contained within the manuscript and available from the corresponding author upon reasonable request.

Nonstandard Abbreviations:

CAD	coronary artery disease
LDL-C	low-density lipoprotein cholesterol
APOB	apolipoprotein B
CATHGEN	CATHeterization GENetics
DM	diabetes
SMK	smoking
HDL-C	high-density lipoprotein cholesterol
non-HDL-C	total cholesterol minus HDL-C
LOD	limits of detection

References

1. Mensah GA, Wei GS, Sorlie PD, Fine LJ, Rosenberg Y, Kaufmann PG, et al. Decline in cardiovascular mortality: possible causes and implications. Circ Res 2017;120:366–80. [PubMed: 28104770]
2. Endo A A historical perspective on the discovery of statins. Proc Jpn Acad Ser B Phys Biol Sci 2010;86:484–93.
3. Leening MJG. Who benefits from taking a statin, and when?: on fundamentally restructuring our thinking regarding primary prevention of cardiovascular disease. Circulation 2020;142:838–40. [PubMed: 32866062]
4. Silverman MG, Ference BA, Im K, Wiviott SD, Giugliano RP, Grundy SM, et al. Association between lowering LDL-C and cardiovascular risk reduction among different therapeutic interventions: a systematic review and meta-analysis. JAMA 2016;316:1289–97. [PubMed: 27673306]
5. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/A DA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on clinical practice guidelines. J Am Coll Cardiol 2019; 73:3168–209.
6. Gu Q, Paulose-Ram R, Burt VL, Kit BK. Prescription cholesterol-lowering medication use in adults aged 40 and over: United States, 2003–2012. NCHS Data Brief 2014;177:1–8.
7. Liu Y, Lv X, Xie N, Fang Z, Ren W, Gong Y, et al. Time trends analysis of statin prescription prevalence, therapy initiation, dose intensity, and utilization from the hospital information system

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of Jinshan Hospital, Shanghai (2012–2018). BMC Cardiovasc Disord 2020;20:201. [PubMed: 32334525]

8. Drexel H, Coats AJS, Spoletini I, Bilato C, Mollace V, Perrone Filardi P, Rosano GMC. An expert opinion paper on statin adherence and implementation of new lipid-lowering medications by the ESC working group on cardiovascular pharmacotherapy: barriers to be overcome. Eur Heart J Cardiovasc Pharmacother 2020;6: 115–21. [PubMed: 31873726]
9. Vonbank A, Agewall S, Kjeldsen KP, Lewis BS, Torp-Pedersen C, Ceconi C, et al. Comprehensive efforts to increase adherence to statin therapy. Eur Heart J 2017;38:2473–9. [PubMed: 28077470]
10. Colantonio LD, Rosenson RS, Deng L, Monda KL, Dai Y, Farkouh ME, et al. Adherence to statin therapy among US adults between 2007 and 2014. J Am Heart Assoc 2019;8:e010376. [PubMed: 30616455]
11. Lansberg P, Lee A, Lee ZV, Subramaniam K, Setia S. Nonadherence to statins: individualized intervention strategies outside the pill box. Vasc Health Risk Manag 2018;14:91–102. [PubMed: 29872306]
12. Blackburn DF, Dobson RT, Blackburn JL, Wilson TW. Cardiovascular morbidity associated with nonadherence to statin therapy. Pharmacotherapy 2005;25:1035–43. [PubMed: 16207093]
13. Gu Y, Zalkikar A, Liu M, Kelly L, Hall A, Daly K, Ward T. Predicting medication adherence using ensemble learning and deep learning models with large scale health-care data. Sci Rep 2021;11:18961. [PubMed: 34556746]
14. Jackevicius CA, Mamdani M, Tu JV. Adherence with statin therapy in elderly patients with and without acute coronary syndromes. JAMA 2002;288:462–7. [PubMed: 12132976]
15. Lee JK, Grace KA, Taylor AJ. Effect of a pharmacy care program on medication adherence and persistence, blood pressure, and low-density lipoprotein cholesterol: a randomized controlled trial. JAMA 2006;296:2563–71. [PubMed: 17101639]
16. McDermott J, Granger B, Thompson S, Baker D, D'Aoust R. Improving advanced practice provider knowledge and assessment of medication adherence in patients with cardiovascular disease. J Nurse Pract 2022;18:86–91.
17. Duke Margolis Center for Health Policy. Medication adherence: landscape, strategies, and evaluation methods. Washington (DC): Duke University; 2019.
18. Kusovschi JD, Ivanova AA, Gardner MS, McGarrah RW 3rd, Kraus WE, Kuklenyik Z, et al. Confirmation of statin and fibrate use from small-volume archived plasma samples by high-throughput LC-MS/MS method. Int J Mol Sci 2023;24:7931. [PubMed: 37175638]
19. Kraus WE, Granger CB, Sketch MH Jr, Donahue MP, Ginsburg GS, Hauser ER, et al. A guide for a cardiovascular genomics biorepository: the CATHGEN experience. J Cardiovasc Transl Res 2015;8:449–57. [PubMed: 26271459]
20. Felker GM, Shaw LK, O'Connor CM. A standardized definition of ischemic cardiomyopathy for use in clinical research. J Am Coll Cardiol 2002;39:210–8. [PubMed: 11788209]
21. Gardner MS, McWilliams LG, Jones JI, Kuklenyik Z, Pirkle JL, Barr JR. Simultaneous quantification of free cholesterol, cholesteryl esters, and triglycerides without ester hydrolysis by UHPLC separation and in-source collision induced dissociation coupled MS/MS. J Am Soc Mass Spectrom 2017;28:2319–29. [PubMed: 28801822]
22. Toth CA, Kuklenyik Z, Jones JI, Parks BA, Gardner MS, Schieltz DM, et al. On-column trypsin digestion coupled with LC-MS/MS for quantification of apolipoproteins. J Proteomics 2017;150:258–67. [PubMed: 27667389]
23. Kuklenyik Z, Jones JI, Gardner MS, Schieltz DM, Parks BA, Toth CA, et al. Core lipid, surface lipid and apolipoprotein composition analysis of lipoprotein particles as a function of particle size in one workflow integrating asymmetric flow field-flow fractionation and liquid chromatography-tandem mass spectrometry. PLoS One 2018;13:e0194797. [PubMed: 29634782]
24. Lindbohm JV, Kaprio J, Korja M. Survival bias explains improved survival in smokers and hypertensive individuals after aSAH. Neurology 2019;93:e2105–e9. [PubMed: 31719131]
25. Lu YW, Lu SF, Chou RH, Wu PS, Ku YC, Kuo CS, et al. Lipid paradox in patients with acute myocardial infarction: potential impact of malnutrition. Clin Nutr 2019;38: 2311–8. [PubMed: 30482563]

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26. Orkaby AR. The highs and lows of cholesterol: a paradox of healthy aging? J Am Geriatr Soc 2020;68:236–7. [PubMed: 31930729]
27. Reddy VS, Bui QT, Jacobs JR, Begelman SM, Miller DP, French WJ. Relationship between serum low-density lipoprotein cholesterol and in-hospital mortality following acute myocardial infarction (the lipid paradox). Am J Cardiol 2015;115: 557–62. [PubMed: 25727079]
28. Sittiwet C, Simonen P, Gylling H, Strandberg TE. Mortality and cholesterol metabolism in subjects aged 75 years and older: the Helsinki businessmen study. J Am Geriatr Soc 2020;68:281–7. [PubMed: 31930737]
29. Bergmann MM, Jacobs EJ, Hoffmann K, Boeing H. Agreement of self-reported medical history: comparison of an in-person interview with a self-administered questionnaire. Eur J Epidemiol 2004;19: 411–6. [PubMed: 15233312]
30. Rochat M, Delabays B, Marques-Vidal PM, Vollenweider P, Mach F, Vaucher J. Ten-year evolution of statin eligibility and use in a population-based cohort. Am J Cardiol 2023;187:138–47. [PubMed: 36459737]
31. Yi SW, An SJ, Park HB, Yi JJ, Ohrr H. Association between low-density lipoprotein cholesterol and cardiovascular mortality in statin non-users: a prospective cohort study in 14.9 million Korean adults. Int J Epidemiol 2022;51:1178–89. [PubMed: 35218344]
32. Chang CH, Yeh ST, Ooi SW, Li CY, Chen HF. The relationship of low-density lipoprotein cholesterol and all-cause or cardiovascular mortality in patients with type 2 diabetes: a retrospective study. PeerJ 2023;11:e14609. [PubMed: 36643628]
33. Genelin MP, Helmkamp LJ, Steiner JF, Maertens JA, Hanratty R, Vupputuri S, et al. Patient pill organization strategies and adherence measured in a cross-sectional study of hypertension. Patient Prefer Adherence 2023;17:817–26. [PubMed: 36992865]
34. Kurup R, Martinez JPD, Doucet M, Tyrrell PN. Effectiveness of electronic medication packaging devices on medication adherence: a scoping review. J Gerontol Nurs 2020;46:27–36.
35. Lee JR, Leo S, Liao S, Ng WR, Tay TYN, Wang Y, et al. Electronic adherence monitoring devices for children with asthma: a systematic review and meta-analysis of randomised controlled trials. Int J Nurs Stud 2021;122:104037. [PubMed: 34391027]
36. Emani S, Ting DY, Healey M, Lipsitz SR, Karson AS, Einbinder JS, et al. Physician beliefs about the impact of meaningful use of the EHR: a cross-sectional study. Appl Clin Inform 2014;5: 789–801. [PubMed: 25298817]
37. Lam WY, Fresco P. Medication adherence measures: an overview. Biomed Res Int 2015;2015:217047. [PubMed: 26539470]

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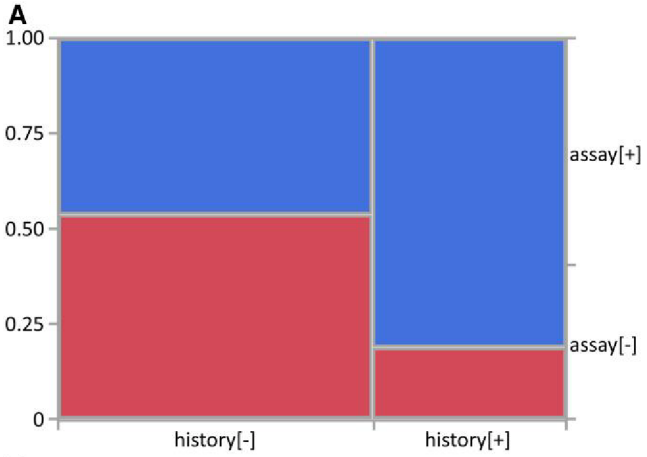
A



B

Count	assay [-]	assay [+]	Total
history [-]			
Count	231	198	429
% of Total	33.5	28.7	62.17
% of Total assay[-]([+])	82.5	48.3	
% of history[-]	53.8	46.2	
history [+]			
Count	49	212	261
% of Total	7.1	30.7	37.83
% of Total assay [-]([+])	17.5	51.7	
% of history[+]	18.8	81.2	
Total			
Count	280	410	690
%	40.6	59.4	

Fig. 1.

Discrepancies in statin use based on medication history vs liquid chromatography–tandem mass spectrometry analysis. (A), Mosaic plot; (B), contingency table.

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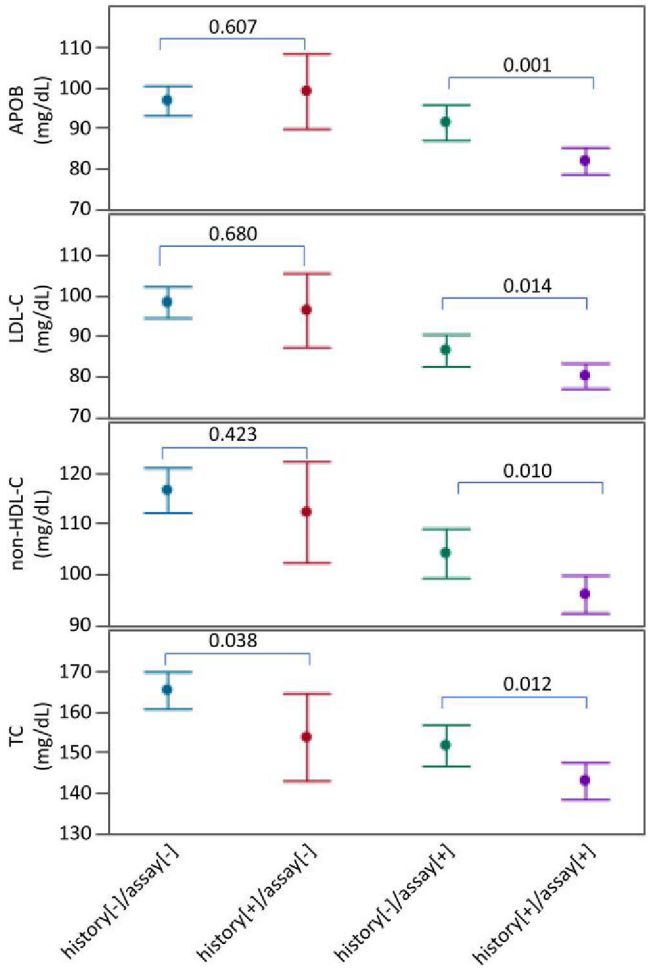

Fig. 2.

Lipid profile measures of apolipoprotein B (APOB), low-density lipoprotein cholesterol (LDL-C), non–high-density lipoprotein cholesterol (non-HDL-C), and total cholesterol (TC) in the h[−]/a[−], h[+]/a[−], h[−]/[a+], and h[+]/a[+] groups; *P*-values for mean differences between groups of samples are shown in the graph. The measures are shown in mg/dL. To convert to SI units from mg/dL to mmol/L, divide the results for TC, LDL-C, HDL-C, very-low-density lipoprotein cholesterol (VLDL-C), and non-HDL-C by 38.6; for triglycerides (TG), divide by 88.6.

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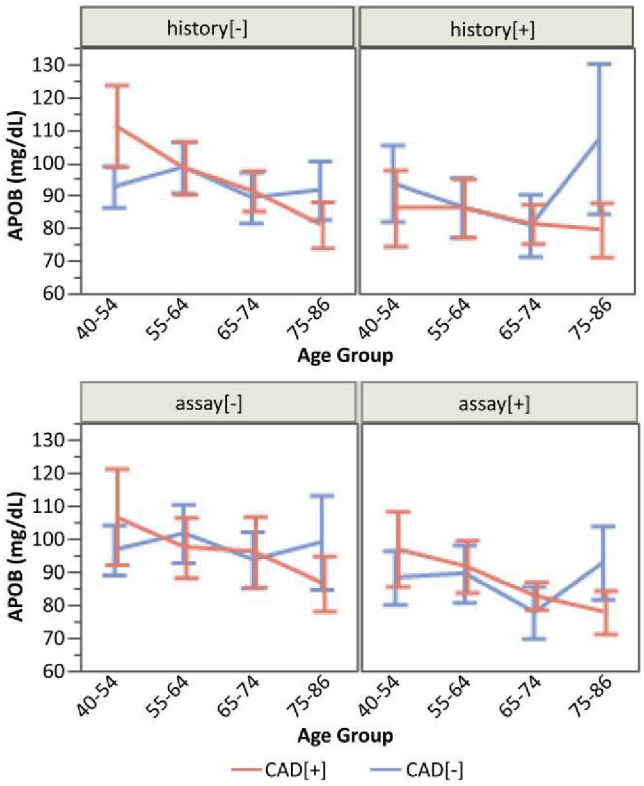

Fig. 3.

Apolipoprotein B (APOB) measures stratified by age groups and CAD[−]/[+] diagnosis in h[−], h[+], a[−], and a[+] groups. The *P*-values are shown in Supplemental Table 4.

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Table 1.

General characteristics of the cohort.

Variable	Values
Demographics	
Number of samples	690
Age, years	63 ± 11[a]
40 to 54	155
55 to 64	212
65 to 74	218
75 to 86	105
Body mass index (BMI), kg/m^2	30 ± 7[a]
Sex: male/female	442/248 (64%/36%)
Race: White/Black/Other	494/146/50 (72%/21%/7%)
Conditions	
Cardiovascular disease (CAD): no/yes	319/371 (46%/54%)
Diabetes (DM): no/yes	482/208 (70%/30%)
Hypertension (BP): no/yes	211/479 (31%/69%)
Current smokers (SMK): no/yes	337/353 (49%/51%)

[a]Mean ± standard deviation (SD).

The percent prevalence of CAD by age category is shown in Supplemental Table 1.

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Table 2.

Mean lipid measures (with CI 5th–95th) in sample categories based on the concordance of medication records with liquid chromatography-tandem mass spectrometry analysis confirmed statin-use.[a,b]

	history[−]/assay[−]	history[+]/assay[−]	history[−]/assay[+]	history[+]/assay[+]	*P*-value
n (% of total 690)	**231 (33%)**	**49 (7%)**	**198 (29%)**	**212 (31%)**	
Mean lipid profile measures (mg/dL) within record/statin categories without correction for phenotypic covariates (CI 5th–95th)					
APOB	96.8 (93.1–100.4)	99.1 (89.9–108.3)	91.5 (87.2–95.8)[c]	82.0 (78.7–85.3)[c]	<0.0001
LDL-C	98.4 (94.6–102.2)	96.4 (87.3–105.6)	86.6 (82.7–90.5)[c]	80.4 (77.2–83.5)[c]	<0.0001
Non-HDL-C	116.6 (112.3–121)	112.4 (102.5–122.2)	104.3 (99.4–109.2)[c]	96.3 (92.5–100)[c]	0.0003
VLDL-C	18.2 (16.9–19.6)	15.9 (13.7–18.1)	17.6 (16.2–19.1)	15.9 (14.7–17.1)[c]	0.0344
TC	165.3 (160.8–169.8)	153.8 (143–164.5)	151.7 (146.7–156.8)[c]	143.1 (138.6–147.6)[c]	0.0002
TG	109.5 (100.4–118.7)	127.3 (103.9–150.8)	126.2 (114.8–137.5)	112.6 (103.7–121.5)	0.0167
APOA1	117.0 (113.6–120.4)	113.0 (105.6–120.4)	116.2 (112.6–119.8)	114.7 (111.2–118.2)	0.5115
HDL-C	48.7 (46.7–50.6)	41.4 (38.3–44.5)[c]	47.5 (45.6–49.3)	46.9 (44.9–48.8)	0.0033

[a]Mean lipid profile measures reflected in mg/dL within record/statin categories without correction for phenotypic covariates (CI 5th–95th). To convert to SI units from mg/dL to mmol/L, divide the results for TC, LDL-C, HDL-C, VLDL-C, and non-HDL-C by 38.6; for triglycerides (TG), divide by 88.6.

[b]Abbreviations: APOB, apolipoprotein B; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol; TC, total cholesterol; TG, total triglycerides; APOA1, apolipoprotein A1; HDL-C, high-density lipoprotein cholesterol.

[c]Relative to the global mean.

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Table 3.

Summary of nominal logistic models for CAD[+].[a]

	Partial OR for statin-use[b]		Whole CAD model characteristics[c]					
Models	**history[+]/[−] (CI)**	**assay[+]/[−] (CI)**	**Sensitivity, %**	**Specificity, %**	**AUC**	**Accuracy, %**	**PPV, %**	**NPV, %**
Model 1 (no correction for statin)			72.4	56.5	0.717	66.0	66.5	65.3
Model 2 (correction by h[+]/[−])	1.74 (1.23–2.44)		72.3	57.0	0.724	65.2	66.1	63.9
Model 3 (correction by a[+]/[−])		2.42 (1.73–3.37)	72.5	59.8	0.739	66.6	67.7	65.2

[a] Abbreviations: CAD, coronary artery disease; AUC, area under the receiver operating characteristic curve; PPV, positive predictive value, NPV, negative predictive value.

[b] h[+]/[−] and a[+]/[−] are binary covariates.

[c] For each model, n = 690.

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