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ORIGINAL INVESTIGATIONS

Time Course of LDL Cholesterol Exposure and Cardiovascular Disease Event Risk

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ABSTRACT

BACKGROUND Incident cardiovascular disease (CVD) increases with increasing low-density lipoprotein cholesterol (LDL-C) concentration and exposure duration. Area under the LDL-C versus age curve is a possible risk parameter. Data-based demonstration of this metric is unavailable and whether the time course of area accumulation modulates risk is unknown.

OBJECTIVES Using CARDIA (Coronary Artery Risk Development in Young Adults) study data, we assessed the relationship of area under LDL-C versus age curve to incident CVD event risk and modulation of risk by time course of area accumulation—whether risk increase for the same area increment is different at different ages.

METHODS This prospective study included 4,958 asymptomatic adults age 18 to 30 years enrolled from 1985 to 1986. The outcome was a composite of nonfatal coronary heart disease, stroke, transient ischemic attack, heart failure hospitalization, cardiac revascularization, peripheral arterial disease intervention, or cardiovascular death.

RESULTS During a median 16-year follow-up after age 40 years, 275 participants had an incident CVD event. After adjustment for sex, race, and traditional risk factors, both area under LDL-C versus age curve and time course of area accumulation (slope of LDL-C curve) were significantly associated with CVD event risk (hazard ratio: 1.053; $p < 0.0001$ per 100 mg/dl $\times$ years; hazard ratio: 0.797 per mg/dl/year; $p = 0.045$, respectively).

CONCLUSIONS Incident CVD event risk depends on cumulative prior exposure to LDL-C and, independently, time course of area accumulation. The same area accumulated at a younger age, compared with older age, resulted in a greater risk increase, emphasizing the importance of optimal LDL-C control starting early in life.
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ABBREVIATIONS AND ACRONYMS

AUC = area under the curve

CHD = coronary heart disease

CVD = cardiovascular disease

LDL-C = low-density lipoprotein cholesterol

Elevated low-density lipoprotein cholesterol (LDL-C) confers risk for cardiovascular disease (CVD) that increases with increasing LDL-C concentration and longer duration of exposure (1,2). One approach suggested for quantification of the risk burden imposed by LDL-C exposure over time is to compute the area under the curve relating LDL-C concentration to age as a metric of cumulative risk consequent upon exposure to LDL-C (mg/dl × years) (3). Incorporating both the LDL-C concentration and exposure duration into a single risk parameter for future CVD events is intuitively appealing, although a data-based demonstration of the utility of this metric is not available. Also unclear is whether the time course of area accumulation is important in modulating the risk conferred by a given area. For instance, does the risk of an adverse cardiovascular event in an individual subsequent to a particular landmark age (e.g., age 40 years [4]) differ based only on the total area, or is there a different risk (despite the same total area) depending on whether more of the area is accumulated at earlier rather than later ages prior to the landmark age?

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The possible influence of the time course of area accumulation, in addition to the total area, with incident CVD event risk may be inferred from data derived from primary prevention trials of LDL-C lowering (5–9). For example, in the long-term follow-up of the West of Scotland Coronary Prevention Study, participants randomized to statins during the original 5-year trial had a significant and persistent reduction in all-cause mortality at 20-year follow-up due primarily to a 21% reduction in cardiovascular mortality (6). The long-term beneficial effect of lowering exposure to LDL-C using statins for the active treatment group versus placebo group has also been shown in the ASCOT-LLA (Anglo-Scandinavian Cardiac Outcomes Trial-the Lipid-Lowering Arm) (7), LIPID (Long-term Intervention with Pravastatin in Ischaemic Disease) (8), and PROSPER (PROspective Study of Pravastatin in the Elderly at Risk) (9) trials. More notably, recent data examining long-term exposure to lower levels of LDL-C (due to the presence of genetic polymorphisms or healthier lifestyle), suggest that the concentration of LDL-C over time is an important determinant of risk for CVD (10–13), but these data do not fully quantify and explore the potential age-dependent nature of the risk associated with LDL-C exposure. With the recent advent of electronic health records, longitudinal data are increasingly available for risk prediction in clinical practice and provide new opportunities to examine longitudinal data rather than using single baseline measures (14).

In this study, we examine: 1) the association of the area under the LDL-C versus age curve with incident CVD events; and 2) the modulation of this risk by the time course of LDL-C using data from the CARDIA (Coronary Artery Risk Development on Young Adults) study (15,16). We hypothesized that both cumulative exposure to LDL-C and the time course of LDL-C concentration would be independently associated with subsequent risk of CVD events; that is, area accumulated earlier in life would confer greater risk than the same amount of area accumulated later.

METHODS

PARTICIPANTS. The CARDIA study is a multicenter longitudinal cohort study that enrolled 5,115 healthy Black and White men and women from 1985 to 1986 (year 0: Y0) from 4 U.S. cities (Birmingham, Alabama; Oakland, California; Chicago, Illinois; and Minneapolis, Minnesota). The study design has been previously described (15,16). The study protocols were approved by the institutional review boards at each study site, and written informed consent was obtained from all participants. Participant samples were approximately balanced by race, sex, education (less or more than high school) status, and age (≤24 or ≥25 years) at Y0 for each site. CARDIA participants have undergone 9 in-person examinations at Y0, Y2, Y5, Y7, Y10, Y15, Y20, Y25, and Y30. Retention rate among surviving participants at each in-person examination was 91%, 86%, 81%, 79%, 74%, 72%, 72%, and 71%, respectively. Contact is maintained with participants via telephone, mail, or e-mail every 6 months, with annual interim medical history ascertainment.

STUDY OUTCOME AND ASCERTAINMENT. The primary outcome of this study was a composite of incident CVD events that included nonfatal coronary

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC* author instructions page.

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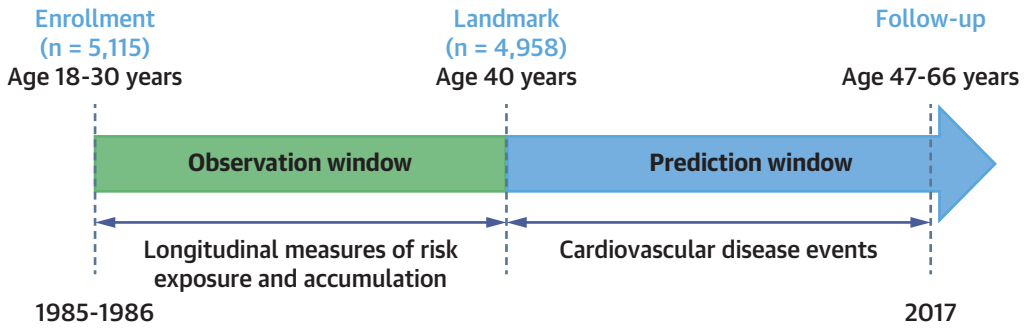
FIGURE 1 Study Design



The CARDIA study enrolled 5,115 healthy Black and White men and women from 1985 to 1986 and the participants underwent 9 in-person examinations between years 0 and 30. We set age 18 to 40 years as the "observation window" for the cumulative cardiovascular risk factor burden prior to the start of event follow-up, and age 40 to 66 years as the "prediction window" for event follow-up and prediction.

heart disease (CHD) (myocardial infarction and acute coronary syndrome), stroke, transient ischemic attack, hospitalization for heart failure, cardiac revascularization, intervention for peripheral arterial disease, or death from cardiovascular causes.

New cardiovascular and cerebrovascular events were recorded from the first examination through August 2017. During their scheduled study examinations and yearly telephone interviews, each participant or designated proxy was asked about interim hospital admissions, outpatient procedures, and deaths. Medical records were requested for participants who had been hospitalized or received an outpatient revascularization procedure. Vital status was assessed every 6 months; medical and other death records were requested after consent had been obtained from the next of kin. Over the last 5 years, >90% of the surviving cohort members have been directly contacted, and follow-up for vital status is virtually complete through related contacts and intermittent National Death Index searches. Two physician members of the endpoints committee independently reviewed medical records and recorded information to adjudicate possible cardiovascular or cerebrovascular events or underlying causes of death using specific definitions and manual of operations (17). If disagreement occurred, the case was reviewed by the full committee.

COVARIATES AND RISK EXPOSURE. At the first and subsequent follow-up examinations, physical measurements, lifestyle factors, medical and family history, and laboratory measures were measured according to standard protocols (16). Participants self-reported medication use. Diabetes mellitus was determined by use of diabetic medication, fasting glucose ≥126 mg/dl (each of the 9 examinations), 2-h glucose ≥200 mg/dl (years 10, 20, and 25), or HbA1c ≥6.5% (years 20 and 25) when available. Body mass index (BMI) was calculated as weight (kg) divided by height in meters squared (m^2). The estimated glomerular filtration rate (eGFR) was calculated from serum creatinine using the CKD-EPI equation (18). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken as the average of the second and third of 3 blood pressure readings after the participant had been sitting quietly for 5 min in a still room. Pulse pressure was calculated by subtracting DBP from SBP; mean arterial pressure (MAP) was calculated as the weighted average of SBP and DBP with one-third and two-thirds weights, respectively. Hypertension was defined by SBP ≥140 mm Hg or DBP ≥90 mm Hg or use of antihypertensive medication. Lipid measurements on fasting blood samples were carried out at each study examination. Total cholesterol and triglyceride levels were measured enzymatically, high-density lipoprotein cholesterol (HDL-C) was determined after precipitation with dextran sulfate/magnesium chloride, and LDL-C was calculated by the Friedewald equation (16). For glucose, creatinine, and blood pressure, calibration studies were conducted to ensure comparability of the same factor across the follow-up examinations (19,20). The recalibrated measures were used in our analysis (Supplemental Appendix).

STATISTICAL ANALYSIS. We sought to study the association of cumulative exposure to LDL-C with subsequent CVD events in a landmark analysis (21) for 2 specific aims: first, we determined whether the area under LDL-C versus age curve accumulated prior to a specific landmark age was related to subsequent risk of

TABLE 1 Characteristics of the Participants at Age 40 Years

	All (N = 4,958)	Women (n = 2,740)	Men (n = 2,218)
Age at enrollment, yrs	24.9 ± 3.7	24.9 ± 3.7	24.8 ± 3.6
Race			
White	2,430 (49.0)	1,297 (47.3)	1,133 (51.1)
Black	2,528 (51.0)	1,443 (52.7)	1,085 (48.9)
Education: never went to college (grade 12 or less)	1,115 (22.5)	549 (20.0)	566 (25.5)
Never met physical activity recommendations	970 (19.6)	749 (27.3)	221 (10.0)
Family history of coronary heart disease	1,244 (25.1)	701 (25.6)	543 (24.5)
Ever-smoker	2,492 (50.3)	1,365 (49.8)	1,127 (50.8)
Diabetes mellitus	220 (4.4)	132 (4.8)	88 (4.0)
Hypertension	1,355 (27.3)	727 (26.5)	628 (28.3)
Body mass index, kg/m^2	28.0 ± 6.7	28.4 ± 7.7	27.6 ± 5.4
Fasting glucose, mg/dl	91.9 ± 21.1	90.2 ± 21.1	94.1 ± 20.8
Creatinine, mg/dl	0.85 ± 0.27	0.75 ± 0.20	0.97± 0.31
eGFR, ml/min/1.73 m^2	109.1 ± 18.3	109.9 ± 18.3	108.2 ± 18.3
eGFR <60 ml/min/1.73 m^2	12 (0.2)	6 (0.2)	6 (0.2)
Systolic blood pressure, mm Hg	111.9 ± 13.7	109.2 ± 14.0	115.3 ± 12.5
Diastolic blood pressure, mm Hg	72.6 ± 11.0	70.9 ± 11.0	74.7 ± 10.7
Pulse pressure, mm Hg	39.3 ± 9.0	38.3 ± 8.7	40.6 ± 9.2
Mean arterial pressure, mm Hg	85.7 ± 11.2	83.7 ± 11.4	88.2 ± 10.5
Total cholesterol, mg/dl	181.6 ± 35.4	179.1 ± 32.7	184.8 ± 38.2
HDL cholesterol, mg/dl	51.5 ± 14.8	55.4 ±14.5	46.7 ± 13.7
Triglyceride, mg/dl	97.9 ± 78.7	83.1 ± 56.0	116.1 ± 96.8
LDL cholesterol, mg/dl	110.7 ± 32.5	106.9 ± 30.6	115.3 ± 34.0
Area under the LDL-C curve over age 18-40 yrs, mg/dl × yrs*	2,520.6 ± 577.7	2,463.7 ± 547.3	2,591.0 ± 606.0

Values are mean ± SD or n (%). *The area under the LDL-C versus age curve was calculated in the unit of mg/dl × years (integrating LDL-C measured in mg/dl over age in years) to describe the cumulative LDL-C exposure.

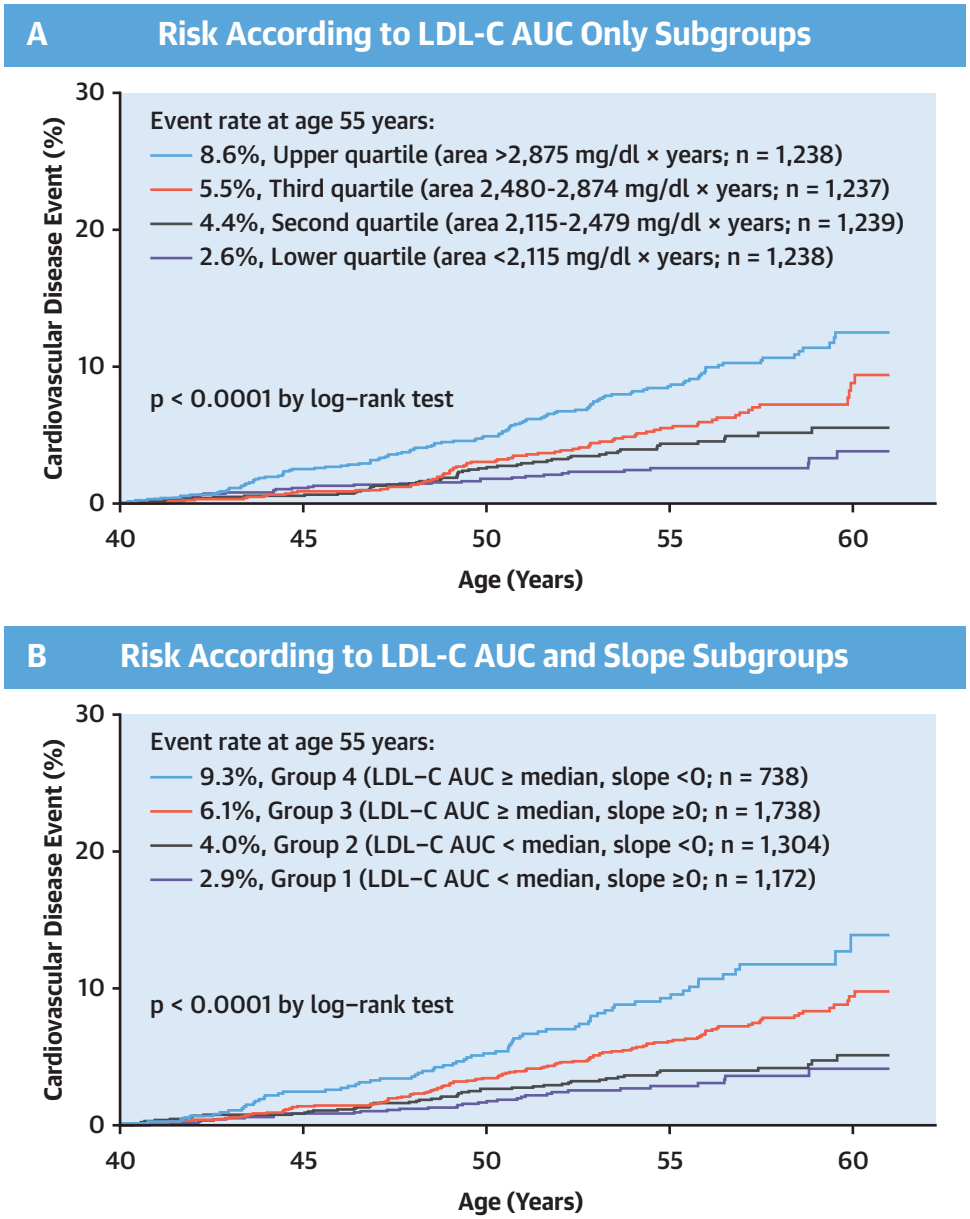
eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein, LDL-C = low-density lipoprotein cholesterol.

CVD events; and second, we assessed whether the time course of area accumulation affected the subsequent CVD risk. We set age 40 years as the landmark age (4,22), that is, the beginning of event follow-up, so that: 1) we had 18 to 40 years (max 22 years) as the "observation window" for the cumulative cardiovascular risk factor burden prior to the start of event follow-up; and 2) we could associate the risk exposure with the development of the CVD events later in life during a long-term follow-up time up to 26 years ("prediction window": ages 40 to 66 years) after age 40 years (**Figure 1**). With this design, the study included 4,958 participants with follow-up data after age 40 years, after excluding 157 (3.1%) participants who withdrew consent (n = 1), died (n = 132), or had a CVD event (n = 24) before age 40 years. Other landmark times were explored in a sensitivity analysis.

To quantify the risk associated with cumulative LDL-C exposure over time, we used an age-based analysis instead of an exam-time-based analysis. We first represented the examination visit times by participants' age, then a nonparametric cubic spline-based mixed-effects model was used to estimate the subject-specific LDL-C from age 18 to 40 years for all participants (23). The details of the statistical approach are provided in the Supplemental Appendix and Supplemental Figure 1. The area under LDL-C versus age curve from 18 to 40 years ($AUC_{[18\text{-}40y]}$) was calculated as a cumulative exposure to LDL-C, and was expressed in mg/dl × years. The time course of LDL-C exposure was characterized in 2 ways: a slope of LDL-C versus age relationship from age 18 to 40 years was estimated, with a positive (or negative) slope indicating an increase (or decrease) in LDL-C over time; or alternatively, the AUC of LDL-C over age 18 to 30 years ($AUC_{[18\text{-}30y]}$) and 30 to 40 years ($AUC_{[30\text{-}40y]}$) were calculated as the early and the late LDL-C exposure measures, respectively.

The CVD event probabilities were estimated by the Kaplan-Meier method and compared by log-rank test between subgroups. We assessed the relationship of these LDL-C risk exposure measures to CVD events after age 40 years in multivariable Cox proportional hazards regression models, adjusted for participants' sex, race, and other traditional cardiovascular risk factors measured as close as possible to age 40 years, including education, smoking history, physical activity, family history of CHD, BMI, glucose, blood pressure, eGFR, HDL-C, triglyceride, and the presence of diabetes mellitus and hypertension (24). For smoking

CENTRAL ILLUSTRATION **Kaplan-Meier Curves of Incident Cardiovascular Disease Event Rates**



Domanski, M.J. et al. J Am Coll Cardiol. 2020;76(13):1507–16.

(A) Incident event rates following the landmark age stratified by area under the curve (AUC) of low-density lipoprotein cholesterol (LDL-C) versus age from 18 to 40 years. Increased area was associated with increased risk of an incident event following the landmark age of 40 years. **(B)** Incident event rates following the landmark age stratified by both AUC and time course (slope) of LDL-C versus age from 18 to 40 years. The risk of an incident event depended on both the cumulative exposure to LDL-C, measured by area under LDL-C versus age curve, and the time course of area accumulation.

and medical history, data collected from the first to the last clinical visits prior to age 40 years were examined. To develop a prognostic CVD risk prediction algorithm based on a participant's risk profile at age 40 years, we used the backward variable selection to determine the important factors in the final model and evaluated the model performance with C-statistics and modified Hosmer-Lemeshow test (25,26). The interaction

TABLE 2 Cox Regression of Cumulative LDL-C Exposure and LDL-C at Age 40 Years Adjusted for Sex and Race

	Model 2A		Model 2B	
	Hazard Ratio (95% CI)	p Value	Hazard Ratio (95% CI)	p Value
LDL-C at age 40 yrs, mg/dl	1.011 (1.007–1.014)	<0.0001	1.003 (0.996–1.009)	0.42
LDL-C $AUC_{[18\text{-}40y]}$ (per 100 mg/dl × yrs)	–	–	1.054 (1.017–1.094)	0.005
Male vs. Female	1.780 (1.394–2.272)	<0.0001	1.764 (1.382–2.253)	<0.0001
Black vs. White	1.713 (1.342–2.185)	<0.0001	1.698 (1.331–2.167)	<0.0001

The C-statistics for models 2A and 2B were 0.657 and 0.669, respectively.
AUC = area under the curve; CI = confidence interval; LDL-C= low-density lipoprotein cholesterol.

between sex and race and their interactions with other risk factors were tested. In sensitivity analyses, we fit the multivariable models after excluding participants taking cholesterol lowering medications before age 40 years, and also explored Fine and Gray competing risk models by considering non-CVD deaths as competing risk events (27). Statistical analyses were performed using the SAS version 9.4 (SAS Institute, Cary, North Carolina) and R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

PARTICIPANT CHARACTERISTICS. The characteristics of the study participants at age 40 years are presented in Table 1. The mean age at study enrollment was 24.9 ± 3.7 years; 2,740 participants (55.3%) were women, 2,218 (44.7%) were men, 2,528 (51.0%) were Black, and 2,430 (49.0%) were White. At age 40 years, approximately one-half of the participants had a smoking history, 25.1% had a family history of CHD, 4.4% had diabetes mellitus, and 27.3% had hypertension. Risk factors at age 40 years, including fasting glucose, creatinine or eGFR, blood pressure, and lipid levels, were different between men and women. The characteristics at age 40 years stratified by enrollment age are presented in Supplemental Table 1.

FOLLOW-UP AND CVD EVENTS. During a median follow-up of 16 years (range 0.2 to 26.3 years) after age 40 years, 275 individuals (of 4,958 participants who were free of CVD events before age 40 years) experienced their first CVD event (including 134 coronary heart disease events, 78 strokes, 27 congestive heart failure events, and 38 deaths due to CVD). The mean age for the surviving participants was 55.8 ± 3.6 years (range 46.5 to 66.3 years). For our study cohort, the first CVD events occurred at a median age of 49.4 years (range 40 to 60 years), and the CVD event rate was 3.1% at age 50 years and 7.8% at age 60 years. The CVD event rate estimates differed by sex and race, and the estimates for the 4 sex-race groups are displayed in Supplemental Figure 2 ($p < 0.0001$).

ANALYSIS OF LDL-C EXPOSURE. For each participant, the cumulative LDL-C exposure before age 40 years was calculated by the area under LDL-C curve over age 18 to 40 years, with a mean of 2,520.6 ± 577.7 mg/dl × years (integrating LDL-C measured in mg/dl over 22 years of exposure), that is, an average of yearly exposure of 114.6 ± 26.3 mg/dl (Table 1). We note that the mean ± SD of the slope of LDL-C time course was 0.10 ± 0.53 mg/dl/year, with a range of −2.75 to 3.36 mg/dl/year. Thus, although mean LDL-C levels were relatively stable during this time, individual patterns of the LDL-C levels varied considerably from a decreasing trend of LDL-C (negative slope) to an increasing trend (positive slope).

The CVD event rates stratified by 4 quartiles of $AUC_{[18\text{-}40y]}$ are shown in Central Illustration A. These data suggest that greater cumulative LDL-C exposure

TABLE 3 Cox Regression of LDL-C Overall Exposure and Accumulation Adjusted for Sex and Race

Model 3A			Model 3B			Model 3C		
Variable	Hazard Ratio (95% CI)	p Value	Variable	Hazard Ratio (95% CI)	p Value	Variable	Hazard Ratio (95% CI)	p Value
LDL-C $AUC_{[18\text{-}40y]}$ (per 100 mg/dl × yrs)	1.068 (1.049– 1.088)	<0.0001	LDL-C $AUC_{[18\text{-}40y]}$ (per 100 mg/dl × yrs)	1.077 (1.057–1.098)	<0.0001	LDL-C $AUC_{[18\text{-}30y]}$ (per 100 mg/dl × yrs)	1.279 (1.104–1.483)	0.001
–	–	–	Slope of LDL-C vs. age (per mg/dl/yr)	0.709 (0.567–0.885)	0.0024	LDL-C $AUC_{[30\text{-}40y]}$ (per 100 mg/dl × yrs)	0.858 (0.716–1.027)	0.096
Male vs. Female	1.757 (1.377–2.241)	<0.0001	Male vs. Female	1.822 (1.427–2.327)	<0.0001	Male vs. Female	1.815 (1.421–2.318)	<0.0001
Black vs. White	1.682 (1.320–2.145)	<0.0001	Black vs. White	1.667 (1.308–2.126)	<0.0001	Black vs. White	1.672 (1.312–2.132)	<0.0001

The C-statistics for models 3A to 3C were 0.668, 0.676, and 0.673, respectively.
Abbreviations as in Table 2.

TABLE 4 Multivariable Cox Regression of LDL-C Overall Exposure and Accumulation Adjusted for Traditional CVD Risk Factors

	Model 4A			Model 4B		
	β*	Hazard Ratio (95% CI)	p Value	β*	Hazard Ratio (95% CI)	p Value
LDL-C $AUC_{[18\text{-}40y]}$ (per 100 mg/dl × yrs)	0.04475	1.046 (1.026–1.066)	<0.0001	0.05149	1.053 (1.032–1.074)	<0.0001
Slope of LDL-C vs. age (per mg/dl/yr)		–	–	−0.22697	0.797 (0.638–0.995)	0.045
Male vs. Female	0.33034	1.391 (1.080–1.793)	0.011	0.34879	1.417 (1.099–1.828)	0.007
Black vs. White	0.31070	1.364 (1.050–1.773)	0.020	0.29899	1.348 (1.038–1.752)	0.025
Family history of coronary heart disease	0.50725	1.661 (1.299–2.123)	<0.0001	0.51430	1.672 (1.308–2.138)	<0.0001
Ever smoked	0.51286	1.670 (1.292–2.158)	<0.0001	0.51371	1.671 (1.293–2.161)	<0.0001
Never attending college (grade 12 or less)	0.34439	1.411 (1.083–1.838)	0.011	0.35182	1.422 (1.091–1.852)	0.009
Fasting glucose, mg/dl, log-transformed	0.94019	2.560 (1.313–4.994)	0.006	0.93854	2.556 (1.312–4.982)	0.006
Diabetes mellitus	0.60899	1.839 (1.111–3.042)	0.018	0.58041	1.787 (1.078–2.961)	0.024
Pulse pressure, mm Hg	0.01595	1.016 (1.004–1.028)	0.008	0.01616	1.016 (1.004–1.028)	0.007
Mean arterial pressure, mm Hg	0.03318	1.034 (1.024–1.044)	<0.0001	0.03246	1.033 (1.023–1.043)	<0.0001
Triglyceride, mg/dl, log-transformed	0.29930	1.349 (1.084–1.679)	0.007	0.29234	1.340 (1.078–1.665)	0.008

The C-statistic was 0.770 for model 4A and 0.772 for model 4B. The estimated baseline survival at age 50 years, S_0(age 50 years), was 0.97984 for model 4A and 0.97993 for model 4B, respectively. For each continuous variable, the hazard ratio (HR) corresponds to HR per 1-unit increment. Both fasting glucose and triglyceride were skewed and were log-transformed to improve normality. Their corresponding HRs were HRs per 1-U increment in the log-transformed values. *β is the estimated coefficient in Cox regression model.

CVD = cardiovascular disease; other abbreviations as in Table 2.

(area under the LDL-C curve) from age 18 to 40 years was associated with more CVD events after age 40 years ($p < 0.0001$). The CVD event rates also differed in the groups of participants categorized by a $AUC_{[18\text{-}40y]}$ < or ≥ its median (2,479 mg/dl × years) and a positive (or negative) slope of the LDL-C versus age relationship indicating either an increasing (or decreasing) trend over time of the LDL-C (**Central Illustration B**) ($p < 0.0001$). The characteristics of participants stratified by the 4 LDL-C AUC and slope groups are presented in Supplemental Table 2. The results suggest participants with a negative slope and similar LDL-C AUC tended to have lower education, increased diabetes and hypertension, and higher BMI, glucose, blood pressure, and lipid levels.

MULTIVARIABLE-ADJUSTED ANALYSIS. **Tables 2 and 3** show that both LDL-C at age 40 years and cumulative LDL-C exposure from age 18 to 40 years (LDL-C $AUC_{[18\text{-}40y]}$) were significantly associated with risk of incident CVD event, adjusted for sex and race (models 2A and 3A). However, LDL-C $AUC_{[18\text{-}40y]}$ was a better metric to measure the cumulative exposure to LDL-C compared with the LDL-C level at age 40 years alone. The LDL-C level at age 40 years was not significant in the multivariable model adjusted for LDL-C $AUC_{[18\text{-}40y]}$ ($p = 0.42$, model 2B) (**Table 2**).

The association of the time course of LDL-C exposure was evaluated by adding the slope of LDL-C time course over age 18 to 40 years to the analysis, or by splitting the overall area between ages 18 years and 40 years into an early area over age 18 to 30 years ($AUC_{[18\text{-}30y]}$) and later area over age 30 to 40 years ($AUC_{[30\text{-}40y]}$) in the models (**Table 3**). After adjustment for cumulative LDL-C exposure using $AUC_{[18\text{-}40y]}$, participants with negative slope of LDL-C time course (decreasing LDL-C trend) had elevated CVD risk, compared with participants with positive slope of LDL-C time course (increasing LDL-C trend) (model 3B in **Table 3**, hazard ratio: 0.709 per mg/dl/year; 95% confidence interval: 0.567 to 0.885; $p = 0.002$). Consistent with this finding, model 3C in **Table 3** indicates that the later area $AUC_{[30\text{-}40y]}$ was not associated with CVD outcome after adjusted for early LDL-C areas ($AUC_{[18\text{-}30y]}$) in the multivariable model ($AUC_{[18\text{-}30y]}$, $p = 0.001$; $AUC_{[30\text{-}40y]}$, $p = 0.096$). This means that the early LDL-C areas contributed more to later CVD risk, compared with the later area. Individuals with higher LDL-C early (18 to 30 years of age) followed by lower LDL-C between ages 30 and 40 years had a greater risk of an incident CVD event after age 40 years than individuals with lower LDL-C between ages 18 and 30 years but then higher LDL-C when both groups had achieved the same cumulative area at age 40 years. Taken altogether, these data show that the risk of CVD depended on both the cumulative exposure to LDL-C and also, importantly, the time course of area accumulation. Individuals with the same cumulative LDL-C exposure at age 40 years (area under the LDL-C vs. age curve) but with a greater fraction of that exposure occurring earlier in life had a greater risk of incident CVD event risk subsequent to age 40 years. That is, area under LDL-C versus age curve accumulated early was associated with greater risk compared with when the same area was accumulated later in life.

The univariate analysis results of traditional risk factors (**Table 1**) on the risk of CVD are shown in Supplemental Table 3. Then, the

multivariable-adjusted associations of cumulative LDL-C exposure and pattern of accumulation were examined after adjusting for the additional cardiovascular risk factors at age 40 years. The levels of physical activity, BMI, HDL-C, hypertension, and eGFR were not statistically significant and were thereby removed by backward selection. The 2-way interactions between sex, race, and other risk factors were not significant. The multivariable-adjusted regression analysis for our final CVD risk prediction models based on the cumulative LDL-C exposure and other risk factors at age 40 years is presented in **Table 4**. Both the overall exposure to LDL-C and the slope of LDL-C versus age remained significantly associated with the CVD outcome, supporting the independent effects of cumulative exposure and time course of LDL-C on the CVD risk in addition to traditional risk factors.

Compared with models 3A and 3B in **Table 3**, the models in **Table 4** had better discrimination with C-statistics improved from 0.668 to 0.770 for model 4A, and from 0.676 to 0.772 for model 4B after adjusting for multiple risk factors. We then evaluated the calibration of our CVD risk prediction models by comparing the observed and model-based predicted probabilities of CVD event by age 50 years in deciles of model-based probabilities (Supplemental Figure 3). Both model 4A (using overall LDL-C exposure $AUC_{[18\text{-}40y]}$) and model 4B (using $AUC_{[18\text{-}40y]}$ and slope of LDL-C time course) had excellent goodness of fit (calibration chi-square statistic = 4.76; p = 0.78 for the lack of fit of model 4A, and chi-square statistic was 9.65; p = 0.29 for the lack of fit of model 4B). Therefore, the results in **Table 4** provide a clinical tool to predict the individuals' risk of developing incident CVD after age 40 years in a population similar to the CARDIA participants based on their cumulative LDL-C exposure as well as time course of LDL-C and other risk factors. These risk prediction algorithms to quantify the risk of CVD at a given age are presented in the Supplemental Appendix.

Finally, in a sensitivity analysis, we excluded 85 participants (10 CVD events) who reported ever taking cholesterol-lowering medications before age 40 years; the multivariable-adjusted analysis of this subset of participants produced virtually no change in the results (Supplemental Table 4). We performed the multivariable-adjusted association analysis of LDL-C exposure on CVD risk based on a landmark age 45 years, which yielded very similar results for LDL-C exposure compared with **Table 4** (Supplemental Table 5). We also explored competing risk models of LDL-C exposures, which showed similar findings compared to **Tables 3 and 4** (Supplemental Tables 6 and 7).

DISCUSSION

This study shows that the risk of a future incident CVD event at a given age increases with the total accumulated area under the LDL-C versus age curve. Importantly, this risk is modulated by the time course of area accumulation. Specifically, the data suggest that area accumulated early confers greater risk than when the same area is accumulated later. Hence, for instance, 2 individuals age 40 years with the same LDL-C and/or the same area under the LDL-C versus age curve could have different risk for a future incident CVD if the time course of area accumulation was different. This underscores the importance of optimal LDL-C early in life, because lower LDL-C later, even when low enough to result in the same area at a landmark age, does not fully reverse risk acquired earlier. It is important to recognize that these data do not in any way suggest that there is no primary prevention benefit to lowering LDL-C no matter when elevated LDL-C lowering is started; the data only suggest an apparent persistent increase in later CVD risk conferred by high LDL-C levels experienced early in life. Interestingly, after adjustment for conventional risk factors, men with the same area under the LDL-C versus age curve had a greater risk of subsequent incident CVD than women, and Blacks had a greater risk than Whites.

A stronger association of lower LDL-C early in life with lower CVD risk has also been suggested by Mendelian randomization studies (28–30). These studies suggest lower risk with lower LDL-C that is far greater than the risk reduction observed in primary prevention studies of older patients with drug therapy and is attributable to lower LDL-C dating from birth rather than beginning later in life (3,31).

There are some interesting implications of this study, particularly when viewed in the context of other studies. The first is that assessment of risk of future CVD events is informed by considering not just the area under the LDL-C versus age curve, but also the time course of area accumulation. Using these data from CARDIA, we developed a risk model that takes into account both of these descriptors of longitudinal LDL-C exposure through young adulthood. In current practice, the LDL-C at the time is used without trying to incorporate the modulation of that risk by the time course of the patient's LDL-C level. The results presented here both underscore the dependence of risk, not just on the present LDL-C level, but also the LDL-C versus time history, and offer a model to quantify the modulation of risk by the time course of LDL-C. Finally, these data suggest that clinical trials of lowering LDL-C in young, even

teenage, populations might show a major reduction in CVD incidence compared to risk reduction started later.

STUDY LIMITATIONS. First, the CARDIA study enrolled a young and healthy population at the inception of the study. Our study cohort had a mean age of 56 years at the current follow-up, with 275 fatal and nonfatal CVD events (5.5% of 4,958 participants). Thus, this population is too young to be studied for each component of the composite endpoint separately due to limited statistical power. Second, we selected age 40 years in our landmark analysis (4) so that we have approximately equal intervals for measuring LDL-C exposure and accumulation during young adulthood and follow-up for subsequent events in middle-age and older life. However, our results appear to be robust with respect to the choice of specific landmark time. Specifically, the analysis of LDL-C based on a landmark age 45 years suggested very similar results (Supplemental Table 5). Furthermore, the CVD event rate is expected to increase as CARDIA continues follow-up of the participants beyond 60 years of age. Finally, we focused on assessing the relationship of the time course of LDL-C versus age curve to incident CVD event risk in this study. The exploration of the time courses of multiple risk factors may lead to other important interpretations and will be an interesting extension of the current research.

An important strength of this study is the very long-term follow-up of healthy individuals who had repeated measurements of LDL-C and other CVD risk factors over 30 years. Also, the results of this study are consistent with other studies, as discussed previously, showing that LDL-C lowering results in risk reduction that lasts beyond the time when treatments between a statin-treated versus placebo group become similar following the end of a randomized trial ("legacy" effect).

CONCLUSIONS

Incident CVD event risk depends on cumulative prior exposure to LDL-C, measured by the area under the LDL-C versus age curve and, independently, on the time course of area accumulation. The same area accumulated at a younger age, compared with an older age, resulted in a greater risk increase, emphasizing the importance of optimal LDL-C control starting early in life.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The risk of cardiovascular disease depends on both the cumulative exposure to LDL-C, measured as the area under the LDL-C versus age curve, and on the time course of area accumulation. These data and Mendelian randomization studies suggest that early optimization of LDL-C level may be more beneficial than later intervention. Late LDL-C intervention does not overcome risk accumulated during early LDL-C exposure.

TRANSLATIONAL OUTLOOK: Future trials of maintaining very low LDL-C at a young age may support this strategy to reduce the prevalence of coronary heart disease.

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APPENDIX For an expanded Methods section as well as supplemental figures and tables, please see the online version of this paper.