

# Associations between very low concentrations of low density lipoprotein cholesterol, high sensitivity C-reactive protein, and health outcomes in the Reasons for Geographical and Racial Differences in Stroke (REGARDS) study

Peter E. Penson<sup>1</sup>, D. Leann Long<sup>2</sup>, George Howard<sup>2</sup>, Peter P. Toth<sup>3,4</sup>, Paul Muntner<sup>5</sup>, Virginia J. Howard<sup>5</sup>, Monica M. Safford<sup>6</sup>, Steven R. Jones<sup>3</sup>, Seth S. Martin<sup>3</sup>, Mohsen Mazidi<sup>7</sup>, Alberico L. Catapano<sup>8</sup>, and Maciej Banach<sup>9,10,11\*</sup>

<sup>1</sup>School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK; <sup>2</sup>Department of Biostatistics, University of Alabama at Birmingham School of Public Health, 1665 University Boulevard, Birmingham, AL 35294-0022, USA; <sup>3</sup>The Johns Hopkins Ciccarone Center for the Prevention of Heart Disease, 600 N. Wolfe St, Carnegie 565-G, Baltimore, MD 21287, USA; <sup>4</sup>Preventive Cardiology, CGH Medical Center, 01 East Miller Road, Sterling, IL 61081, USA; <sup>5</sup>Department of Epidemiology, University of Alabama at Birmingham, 700 University Boulevard, Suite LHL 450, Birmingham, AL 35294, USA; <sup>6</sup>Department of Medicine, Weill Cornell Medicine, 1320 York Avenue, HT-621 New York, NY 10021, USA; <sup>7</sup>Department of Biology and Biological Engineering, Food and Nutrition Science, Chalmers University of Technology, Kemigården 4, SE-412 96 Gothenburg, Sweden; <sup>8</sup>Department of Pharmacological and Biomolecular Sciences, University of Milan and IRCCS Multimedica, Via Balzaretti 9, 20133 Milan, Italy; <sup>9</sup>Department of Hypertension, Chair of Nephrology and Hypertension, Medical University of Lodz, Zeromskiego 113, 90-549 Lodz, Poland; <sup>10</sup>Polish Mother's Memorial Hospital Research Institute, Rzgowska 281/288; 93-338 Lodz, Poland; and <sup>11</sup>Cardiovascular Research Centre, University of Zielona Gora, Zyty 28; 65-046 Zielona Gora, Poland

Received 12 July 2018; revised 28 July 2018; editorial decision 8 August 2018; accepted 9 August 2018; online publish-ahead-of-print 28 August 2018

## Aims

Recent findings have demonstrated the important contribution of inflammation to the risk of cardiovascular disease (CVD) in individuals with optimally managed low density lipoprotein cholesterol (LDL-C). We explored relationships between LDL-C, high sensitivity C-reactive protein (hs-CRP), and clinical outcomes in a free-living US population.

## Methods and results

We used data from the REasons for Geographical And Racial Differences in Stroke (REGARDS), and selected individuals at 'high risk' for coronary events with a Framingham Coronary Risk Score of  $\geq 10\%$  or atherosclerotic cardiovascular disease (ASCVD) risk  $\geq 7.5\%$  in order to explore relationships between low LDL-C [ $< 70$  mg/dL (1.8 mmol/L) in comparison to  $\geq 70$  mg/dL (1.8 mmol/L)]; hs-CRP  $< 2$  compared with  $\geq 2$  mg/L and clinical outcomes [all-cause mortality, incident coronary heart disease (CHD), and incident stroke]. To assess the association between the LDL-C and hs-CRP categories and each outcome, a series of incremental Cox proportional hazards models were employed on complete cases. To account for missing observations, the most adjusted model was used to interrogate the data using multiple imputation with chained equations (MICE). In this analysis, 6136 REGARDS high-risk participants were included. In the MICE analysis, participants with high LDL-C ( $\geq 70$  mg/dL) and low hs-CRP ( $< 2$  mg/L) had a lower risk of incident stroke [hazard ratio (HR) 0.69, 0.47–0.997], incident CHD (HR 0.71, 0.53–0.95), and CHD death (HR 0.70, 0.50–0.99) than those in the same LDL-C category high hs-CRP ( $\geq 2$  mg/L). In participants with high hs-CRP ( $\geq 2$  mg/dL), low LDL-C [ $< 70$  mg/dL (1.8 mmol/L)] was not associated with additional risk reduction of any investigated outcome, but with the significant increase of all-cause mortality (HR 1.37, 1.07–1.74).

## Conclusions

In this high-risk population, we found that low hs-CRP ( $< 2$  mg/L) appeared to be associated with reduced risk of incident stroke, incident CHD, and CHD death, whereas low LDL-C ( $< 70$  mg/dL) was not associated with protective

\* Corresponding author. Tel: +48 42 639 3771, Fax: +48 42 639 3771, Email: maciejbanach77@gmail.com

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author(s) 2018. For permissions, please email: journals.permissions@oup.com.

effects. Thus, our results support other data with respect to the importance of inflammatory processes in the pathogenesis of CVD.

## Keywords

Coronary heart disease • LDL-cholesterol • mortality • stroke • hs-CRP

## Introduction

Low density lipoprotein cholesterol (LDL-C) is a causative factor in the development of atherosclerotic cardiovascular disease (ASCVD).<sup>1</sup> In randomized controlled trials, reduction in plasma LDL-C concentration by statins has repeatedly been shown to reduce the mortality and morbidity associated with cardiovascular disease (CVD) in a variety of primary<sup>2–4</sup> and secondary<sup>4,5</sup> prevention settings. However, risk is not eliminated when therapeutic LDL-C targets are met.<sup>6,7</sup> Residual risk may result from different variables, including elements of risk owing to LDL-C and risk due to inflammation.<sup>8</sup>

Recent therapeutic advances have enabled unprecedented reductions in plasma LDL-C (and other apolipoprotein B containing lipoproteins), thus decreasing the lipid component of residual risk.<sup>8</sup> Such approaches have included combination therapy of statins with ezetimibe<sup>9</sup> or other lipid-lowering drugs.<sup>10</sup> Notable success in lipid-lowering has been achieved with the pharmacological attenuation of the action of proprotein convertase subtilisin/kexin type 9 (PCSK9) by monoclonal antibodies such as alirocumab<sup>11,12</sup> and evolucumab,<sup>13,14</sup> and by the small interfering RNA, inclisiran.<sup>15</sup> In the Further Cardiovascular Outcomes research with PCSK9 Inhibition in subjects with elevated risk (FOURIER) study, evolucumab decreased LDL-C to a median of 30 mg/dL (0.78 mmol/L) and reduced CVD events by 15% [hazard ratio (HR) 0.85, 0.79–0.92]<sup>16</sup> without evidence of serious adverse effects.<sup>17</sup> Importantly, treatment was not associated with cognitive decline<sup>18</sup> or new-onset diabetes.<sup>19</sup>

The concept that residual inflammatory risk exists and can be treated has recently been demonstrated in the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS), where investigators showed that treatment with canakinumab (a monoclonal antibody targeting interleukin-1 $\beta$ ) reduced the circulating levels of C-reactive protein (CRP), and significantly reduced recurrent cardiovascular (CV) events.<sup>8</sup>

In contrast to the extensive evidence from clinical trials, the correlation between plasma concentrations of LDL-C and mortality rates in free living populations is less well documented. This is particularly the case in individuals with unusually high or low concentrations of LDL-C, and especially in primary prevention populations of various ethnic origins who have been underrepresented in clinical trials and observational studies. Therefore, we used data from the REasons for Geographical And Racial Differences in Stroke (REGARDS) study to explore relationships between low LDL-C, high sensitivity C-reactive protein (hs-CRP), and clinical outcomes [all-cause mortality, incident coronary heart disease (CHD), and incident stroke] with a particular focus on participants with baseline LDL-C <70 mg/dL (1.8 mmol/L) in order to improve our understanding of the associations between lipids, inflammatory markers, and the risk of CVD and death in this population. We limited our analysis to participants with high baseline

10 year risk (Framingham-CHD  $\geq 10\%$  or ASCVD  $\geq 7.5\%$ ) to make our study results comparable to populations typically included in lipid-lowering intervention studies.

## Methods

### REGARDS study population

The REGARDS longitudinal cohort study recruited 30 239 community-dwelling participants between January 2003 and October 2007. Participants were selected from commercial lists and recruited through a combination of mail and telephone contact. Because of a focus on geographic and racial disparities in stroke mortality, blacks were oversampled (44%), as were residents of the southeastern US Stroke Belt states (56%). The Stroke Belt states were defined as NC, SC, GA, TN, AL, MS, AR, and LA, with the remaining 44% of the participants being selected from the remaining 40 contiguous US states. Eligibility criteria included non-Hispanic black or white race, aged 45 years and older, absence of conditions associated with a life expectancy of less than 5 years, and not being on a waiting list for a nursing home. Potential participants with diagnosed malignancy at baseline were not eligible to take part in the study. Participation rate was estimated as 33%, a similar value to that seen in other studies. For those agreeing to participate, the telephone interviewers conducted an interview to assess CVD risk factors and medical history. An in-person assessment for direct measurement of risk factors (blood pressure, anthropomorphic characteristics, and electrocardiogram) and collection of blood and urine samples was conducted after the interview. Participants were followed by telephone at 6 months intervals to detect suspected cardiovascular events. Details of the study design are provided elsewhere.<sup>20</sup>

In this analysis, we included participants with a 2002 Framingham CHD 10 year risk score<sup>21</sup> of  $\geq 10\%$  and, in a separate analysis those with a ASCVD 10 year risk score  $\geq 7.5\%$  who fasted overnight prior to their study visit, had valid measurements of total cholesterol, high density lipoprotein cholesterol (HDL-C), and triglycerides, and with follow-up for incident CHD (as well as other health outcomes).

### Laboratory methods

Laboratory assays were conducted as previously described.<sup>22</sup> Samples were centrifuged an average of 97 min after collection and serum or plasma separated and shipped overnight on ice packs to the University of Vermont as previously described.<sup>22</sup> On arrival, samples were centrifuged at 30 000 g at 4 C and either analysed (general chemistries) or stored at below -80 C. High sensitivity C-reactive protein was analysed in batches by particle-enhanced immunonephelometry using the BNII nephelometer (N hs-CRP; Dade Behring, Deerfield, IL, USA) with interassay coefficients of variation of 2.1–5.7%. Cholesterol, HDL-C, triglycerides, and glucose were measured by colorimetric reflectance spectrophotometry using the Ortho Vitros Clinical Chemistry System 950IRC instrument (Johnson & Johnson Clinical Diagnostics, New Brunswick, NJ, USA).<sup>23</sup> Low density

lipoprotein cholesterol was calculated using the Friedewald formula from total cholesterol, HDL-C, and triglycerides.<sup>24</sup>

## Statistical methods

The primary exposure of interest was LDL-C, with particular interest in those individuals with very low LDL-C measurements [ $<70$  mg/dL (1.8 mmol/L)]. In addition, we explored hs-CRP concentration dividing participants into those with concentrations of  $\geq 2$  mg/L and  $< 2$  mg/L. The outcomes of interest were all-cause mortality, incident CHD, CHD mortality, and incident stroke each at or before 31 December 2013. Incident CHD was defined as either a definite or probable myocardial infarction (MI) or a definite or probable acute CHD death. The participants were contacted every 6 months for CV event information, and medical records were sought for suspected events and adjudicated by physicians. Expanded details of the study follow-up and stroke adjudication are found elsewhere.<sup>25</sup> For all analysis of incident CHD, those participants with a history of heart disease [self-reported MI, coronary artery bypass grafting, angioplasty, or stenting OR evidence of MI via ECG (from interview and ECG)] were excluded. Similarly, the analysis of incident stroke excludes those participants with reported stroke at baseline.

Participant age, race (black/white), region of residence, and sex were included as demographic variables. Self-reported income level ( $< \$20k$ ,  $\$20k$ – $\$35k$ ,  $\$35k$ – $\$75k$ , and  $> \$75k$ ) and education level (less than high school, high school graduate, some college, and college graduate) were used as measures of socioeconomic status. Alcohol consumption (some/none), physical activity (none/1–3 times per week/4 or more times per week), current smoking were measured through self-reported questionnaires. Diabetes was defined as self-reported glucose-control medication use or fasting glucose  $\geq 126$  mg/dL. Body mass index (BMI) ( $\text{kg}/\text{m}^2$ ) and systolic blood pressure (SBP) (mmHg) were measured at the in-home visit. Albumin-to-creatinine ratio (ACR) ( $\geq 30$  mg/g vs.  $< 30$  mg/g), estimated glomerular filtration rate (eGFR) through the chronic kidney disease (CKD)-Epi equation,<sup>26</sup> hs-CRP ( $< 2$  mg/L and  $\geq 2$  mg/L), HDL-C, and triglycerides were measured through specimens. Information regarding the use of statins and other lipid-lowering medications (fibrates or niacin) by participants was obtained from their medication inventory at baseline.

To assess the association between LDL-C, hs-CRP, and each outcome, Cox proportional hazards models with penalized splines were employed, both unadjusted and adjusted for: demographic factors (age, race, sex), income level, education level, alcohol consumption, physical activity, smoking, BMI, diabetes, eGFR, ACR, hs-CRP, statin use, other lipid-lowering medication use, HDL-C, and triglycerides. The penalized spline allows the relationship between LDL-C and the log-hazard of each outcome to vary in a non-linear fashion, offering more modelling flexibility. Likelihood ratio tests were used to assess the statistical significance between LDL-C and each outcome.

In an additional analysis, LDL-C categories were defined by fasting LDL-C measurement into the following categories  $< 50$  mg/dL (1.3 mmol/L),  $50$ – $< 70$  mg/dL (1.3–1.8 mmol/L), and  $\geq 70$  mg/dL (1.8 mmol/L). To assess the association between the LDL-C categories and each outcome, a series of incremental Cox proportional hazards models were employed on complete cases: Model 1—adjustment for age, sex, race, and region of residence; Model 2—additional adjustment for education, income, alcohol use, physical activity, smoking, and BMI; Model 3—additional adjustment for diabetes, ACR, eGFR, SBP, use of antihypertensive medications, use of lipid-lowering medications, use of beta blockers, and hs-CRP; and Model 4—additional adjustment for HDL-C and triglycerides. Model 4 was also used to interrogate the data using multiple imputation with chained equations (MICE).<sup>27–29</sup>

A further analysis was performed as above with the following categories of LDL-C and hs-CRP: LDL-C  $< 70$ , hs-CRP  $< 2$ ; LDL-C  $< 70$ , hs-CRP  $\geq 2$ ; LDL-C  $\geq 70$ , hs-CRP  $< 2$ ; LDL-C  $\geq 70$ , and hs-CRP  $\geq 2$ . The series of incremental Cox proportional hazards models were employed on complete cases and using MICE as above, but the correction for hs-CRP was excluded from Model 3. Sensitivity analysis focused on the stratification by statin users. SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) and R<sup>30</sup> were used for all statistical analyses.

## Results

### Baseline characteristics of participants

Overall, 6136 REGARDS participants with Framingham-CHD 10 year risk scores  $> 10\%$  were eligible for inclusion into this study. Of these, 95% were found to have an LDL-C  $\geq 70$  mg/dL, and 5% had an LDL-C  $< 70$  mg/dL. Demographic characteristics were broadly similar between the two groups. Compared to the higher LDL-C group, participants in the low LDL-C group were more likely to have a diagnosis of diabetes (67.2% vs. 32.6%) and more likely to be on statins (57.1% vs. 24.9%) or other lipid-lowering therapy (6.8% vs. 3.9%). Additionally, participants in the low LDL-C group were more likely to be black (46.8% vs. 41.2%), less likely to consume alcohol (30.8% vs. 37.3%), and more likely to smoke tobacco (31.2 vs. 25.0%) (Table 1).

### Association between low density lipoprotein cholesterol, high sensitivity C-reactive protein, and all-cause mortality

Over the 7.14 year average follow-up, 1376 (22.4%) participants suffered a fatal event. We found a significant non-linear relationship between LDL and all-cause mortality, which remained after adjustment for all covariates in all participants, but not in subgroups of statin users and non-users (Table 2). The overall tests of association (likelihood ratio tests) indicated a significant association between LDL-C and all-cause mortality in both unadjusted and fully adjusted models of all participants and subgroups of statin users and non-users (Table 3). Inspection of spline plots revealed that LDL measurements between approximately 70 mg/dL (1.8 mmol/L) and 200 mg/dL (5.2 mmol/L) were protective against all-cause mortality (Figure 1) compared with LDL measurements equal to 70 mg/dL (1.8 mmol/L), with levels below 70 mg/dL (1.8 mmol/L) not being associated with decreased risk for mortal events.

When participants with Framingham 10 year risk  $> 10\%$  were categorized according to three LDL-C categories ( $< 50$  mg/dL,  $50$ – $< 70$  mg/dL, and  $\geq 70$  mg/dL), LDL-C  $50$ – $< 70$  mg was associated with increased risk of all-cause mortality: HR 1.40 (1.10–1.78) compared with the referant group of  $\geq 70$  mg/dL in a minimally adjusted model. The effect size was attenuated in adjusted models, but a statistically significant effect was observed when a fully adjusted MICE was used (Supplementary material online, Table S1). Similar patterns were seen in subgroups of participants not taking (Supplementary material online, Table S1a) and those taking (Supplementary material online, Table S1b) statins, although the HRs for the LDL-C categories did not differ significantly in these subgroups.

In the participants with ASCVD 10-year risk  $> 7.5\%$ , both low LDL-C categories were associated with greater risk for mortality

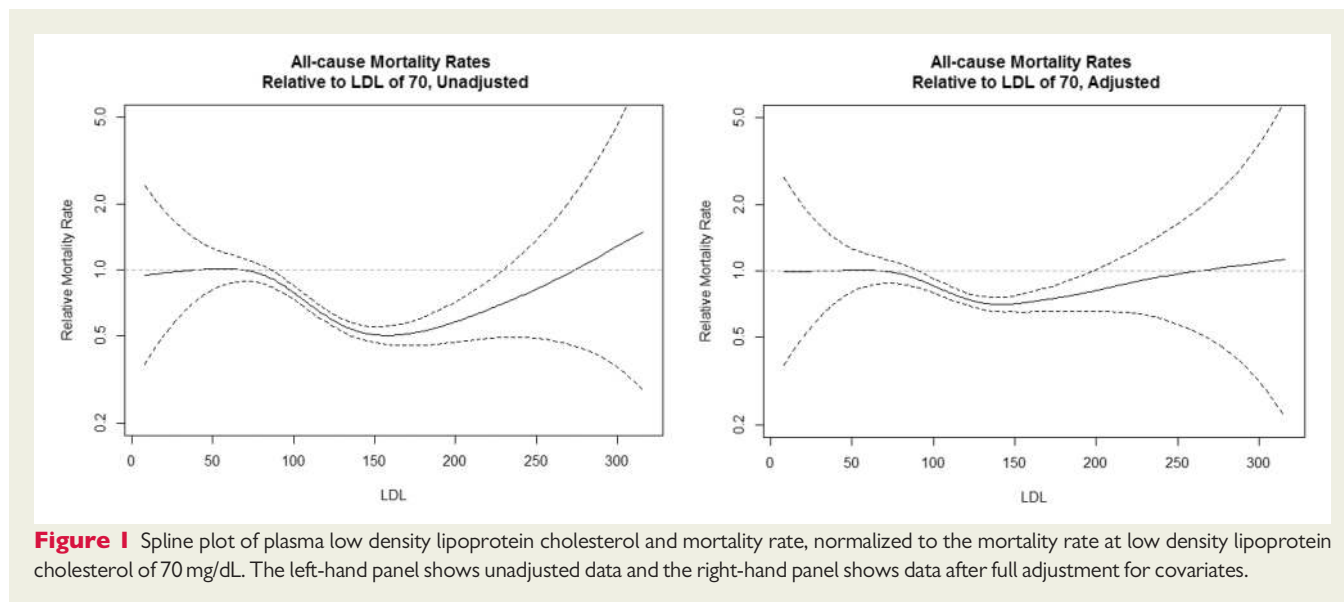
**Table 1** Baseline characteristics of participants (N = 6136)

	LDL-C <70 mg/dL/1.8 mmol/L	LDL-C ≥70 mg/dL/ 1.8 mmol/L	Total
Overall N (%)	308 (5.0)	5828 (95.0)	6136
Continuous variables, mean (SD)			
Age (years)	69.2 (8.15)	67.6 (8.65)	67.6 (8.64)
Body mass index (kg/m <sup>2</sup> )	30.1 (6.07)	29.6 (5.85)	29.6 (5.86)
Estimated GFR (CKD-Epi equation)	78.5 (23.56)	82.9 (19.47)	82.6 (19.72)
HDL cholesterol (mg/L)	42.2 (14.96)	44.6 (12.01)	44.5 (12.18)
Triglycerides (mg/L)	149.0 (93.87)	145.4 (68.11)	145.6 (69.62)
Categorical variables, N (%)			
Men	204 (66.2)	3876 (66.5)	4080 (66.5)
Black	144 (46.8)	2401 (41.2)	2545 (41.5)
Education			
Less than high school	56 (18.2)	865 (14.8)	921 (15.0)
High school graduate	79 (25.7)	1519 (26.1)	1598 (26.0)
Some college	87 (28.3)	1498 (25.7)	1585 (25.8)
College graduate and more	86 (27.9)	1946 (33.4)	2032 (33.1)
Income			
<\$20k	59 (19.2)	1081 (18.6)	1140 (18.6)
\$20k–\$34k	96 (31.2)	1536 (26.4)	1632 (26.6)
\$35k–\$74k	86 (27.9)	1805 (31.0)	1891 (30.8)
\$75k and above	34 (11.0)	765 (13.1)	799 (13.0)
Refused	33 (10.7)	641 (11.0)	674 (11.0)
Alcohol consumption	95 (30.8)	2174 (37.3)	2269 (37.0)
Physical activity			
None	135 (43.8)	1991 (34.2)	2126 (34.7)
1–3 times per week	86 (27.9)	2046 (35.1)	2132 (34.8)
4 or more times per week	87 (28.3)	1791 (30.7)	1878 (30.6)
Current smoking	96 (31.2)	1460 (25.0)	1556 (25.4)
Diabetes	207 (67.2)	1899 (32.6)	2106 (34.3)
Statin use	176 (57.1)	1451 (24.9)	1627 (26.5)
Other lipid-lowering medication use	21 (6.8)	227 (3.9)	248 (4.0)
Urinary albumin/creatinine ratio >30 mg/g	73 (23.7)	1142 (19.6)	1215 (19.8)
hs-CRP			
<1 mg/L	81 (26.3)	1302 (22.3)	1383 (22.5)
1–<3 mg/L	95 (30.8)	2006 (34.4)	2101 (34.2)
3+ mg/L	132 (42.9)	2520 (43.2)	2652 (43.2)

than the referent group. This observation persisted across all models including the fully adjusted MICE model: HR (LDL-C 50–<70 mg/dL) 1.17 (1.04–1.33); HR (LDL-C <50 mg/dL) 1.32 (1.06–1.65) (Supplementary material online, Table S2). In the subgroup of participants who did not take statins, a similar result was seen across all models: fully adjusted MICE model: HR (LDL-C 50–<70 mg/dL) 1.32 (1.12–1.56); HR (LDL-C <50 mg/dL) 1.53 (1.13–2.06) (Supplementary material online, Table S2a, Figure 4). In statin-users, low LDL-C was associated with higher mortality in a minimally adjusted model HR (LDL-C 50–<70 mg/dL) 1.32 (1.12–1.56); HR (LDL-C <50 mg/dL) 1.53 (1.13–2.06) this effect was attenuated with progression of adjustment models (Supplementary material online, Table S2b, Figure 4).

Categorization of participants with Framingham 10 year risk >10% into four LDL-C/hs-CRP groups (LDL-C <70, hs-CRP <2; LDL-C

<70, hs-CRP ≥2; LDL-C ≥70, hs-CRP <2; LDL-C ≥70, hs-CRP ≥2) revealed that the combination of LDL-C <70 mg/dL and hs-CRP ≥2 was associated with greater risk of mortality than the referent group (LDL-C ≥70, hs-CRP ≥2) across all complete-case models and MICE: HR 1.37 (1.07–1.74). Participants with the combination of LDL-C ≥70 and hs-CRP <2 were at lowest risk of death in a fully adjusted complete-case model: HR 0.75 (0.67–0.85), but the difference was not statistically significant in the MICE model (Table 4). Similar trends were observed in the subgroup of participants who were not taking statins. In this subgroup, LDL-C ≥70 and hs-CRP <2 was associated with lower risk across all adjusted models and MICE: HR 0.79 (0.63–0.98) but the increased risk seen in participants with LDL-C <70 mg/dL and hs-CRP ≥2 in a minimally adjusted model was attenuated with progression of models (Supplementary material online, Table S3a). In



**Table 2** Tests of non-linearity of low density lipoprotein cholesterol penalized spline

Outcomes	Unadjusted model			Adjusted model <sup>a</sup>		
	All participants	Statin use	No statin use	All participants	Statin use	No statin use
All-cause mortality	<0.001	0.003	<0.001	0.002	0.17	0.07
Incident CHD	0.18	0.20	0.97	0.47	0.14	0.01
Incident stroke	0.81	0.17	0.18	0.94	0.14	0.89

<sup>a</sup>Includes all adjustment variables in Model 4: age, sex, race, region of residence, education, income, alcohol use, physical activity, smoking, BMI, diabetes, ACR, eGFR, SBP, use of antihypertensive medications, use of lipid-lowering medications, use of beta blockers, hs-CRP, HDL, and triglycerides.

**Table 3** Overall tests of association (Likelihood ratio test) between low density lipoprotein cholesterol and outcomes

Outcome	Unadjusted model			Adjusted model <sup>a</sup>		
	All participants	Statin use	No statin use	All participants	Statin use	No statin use
All-cause mortality	<0.001	<0.001	<0.001	<0.001	0.005	<0.001
Incident CHD	0.06	0.07	0.35	0.07	0.048	0.42
Incident stroke	0.83	0.20	0.48	0.66	0.15	0.26

<sup>a</sup>Includes all adjustment variables in Model 4: age, sex, race, region of residence, education, income, alcohol use, physical activity, smoking, BMI, diabetes, ACR, eGFR, SBP, use of antihypertensive medications, use of lipid-lowering medications, use of beta blockers, hs-CRP, HDL, and triglycerides.

participants who were taking statins LDL-C <70 mg/dL and hs-CRP ≥2 was associated with increased risk in a MICE model, but the combination of LDL-C ≥70 and hs-CRP <2 was not associated with reduced risk (Supplementary material online, Table S3b). Similar results were observed with the group of participants with ASCVD 10 year risk >7.5% in the whole population (Table 5), in those not taking statins (Supplementary material online, Table S4a) and participants who were taking statins (Supplementary material online, Table S4b).

### Association between low density lipoprotein cholesterol, high sensitivity C-reactive protein, and coronary heart disease death

In participants with Framingham 10 year risk >10% the risk of CHD death did not differ significantly between the three LDL-C categories in the whole population (Supplementary material online, Table S1, Figure 2) or in

**Table 4** Hazard ratios (and 95% confidence interval) for each outcome by low density lipoprotein cholesterol/high sensitivity C-reactive protein category among REGARDS participants with Framingham coronary heart disease score  $\geq 10\%$ 

	Number of events/population	Model 1	Model 2	Model 3	Model 4	Model 4 (MI)
All-cause mortality	1322/5842					
LDL-C/hs-CRP Category						
LDL-C <70, hs-CRP <2	31/137	0.81 (0.56–1.16)	0.77 (0.54–1.10)	0.70 (0.49–1.01)	0.72 (0.50–1.03)	0.85 (0.59–1.23)
LDL-C <70, hs-CRP $\geq 2$	59/159	1.57 (1.20–2.04)	1.47 (1.12–1.91)	1.31 (1.00–1.71)	1.32 (1.01–1.73)	1.37 (1.07–1.74)
LDL-C $\geq 70$ , hs-CRP <2	464/2377	0.68 (0.60–0.76)	0.73 (0.65–0.82)	0.76 (0.67–0.86)	0.75 (0.67–0.85)	0.86 (0.71–1.03)
LDL-C $\geq 70$ , hs-CRP $\geq 2$	768/3169	Ref	Ref	Ref	Ref	Ref
Incident stroke	285/5458					
LDL-C/hs-CRP category						
LDL-C <70, hs-CRP <2	7/125	0.91 (0.43–1.94)	0.87 (0.41–1.86)	0.90 (0.42–1.95)	0.90 (0.42–1.94)	0.85 (0.39–1.84)
LDL-C <70, hs-CRP $\geq 2$	6/136	0.72 (0.32–1.62)	0.72 (0.32–1.62)	0.65 (0.29–1.49)	0.65 (0.28–1.48)	0.74 (0.36–1.54)
LDL-C $\geq 70$ , hs-CRP <2	102/2239	0.72 (0.56–0.92)	0.71 (0.55–0.92)	0.75 (0.58–0.97)	0.75 (0.58–0.97)	0.69 (0.47–0.997)
LDL-C $\geq 70$ , hs-CRP $\geq 2$	170/2958	Ref	Ref	Ref	Ref	Ref
Incident CHD	475/5717					
LDL-C/hs-CRP category						
LDL-C <70, hs-CRP <2	9/134	0.65 (0.34–1.27)	0.67 (0.34–1.30)	0.61 (0.31–1.20)	0.62 (0.32–1.22)	0.55 (0.27–1.10)
LDL-C <70, hs-CRP $\geq 2$	16/154	1.16 (0.70–1.92)	1.11 (0.67–1.84)	0.95 (0.57–1.59)	0.96 (0.58–1.61)	1.05 (0.67–1.64)
LDL-C $\geq 70$ , hs-CRP <2	167/2330	0.66 (0.54–0.80)	0.70 (0.57–0.86)	0.71 (0.58–0.87)	0.71 (0.58–0.87)	0.71 (0.53–0.95)
LDL-C $\geq 70$ , hs-CRP $\geq 2$	283/3099	Ref	Ref	Ref	Ref	Ref
CHD death	372/5842					
LDL-C/hs-CRP category						
LDL-C <70, hs-CRP <2	7/137	0.62 (0.29–1.32)	0.58 (0.27–1.24)	0.52 (0.24–1.11)	0.51 (0.24–1.10)	0.58 (0.28–1.20)
LDL-C <70, hs-CRP $\geq 2$	17/159	1.48 (0.91–2.43)	1.42 (0.86–2.33)	1.18 (0.72–1.96)	1.19 (0.72–1.97)	1.26 (0.81–1.97)
LDL-C $\geq 70$ , hs-CRP <2	122/2377	0.62 (0.50–0.78)	0.65 (0.52–0.82)	0.68 (0.54–0.86)	0.67 (0.54–0.85)	0.70 (0.50–0.99)
LDL-C $\geq 70$ , hs-CRP $\geq 2$	226/3169	Ref	Ref	Ref	Ref	Ref

Model 1: Adjusted for age, sex, race, and region of residence. Model 2: Model 1 + education, income, alcohol use, physical activity, smoking, and BMI. Model 3: Model 2 + diabetes, ACR, eGFR, SBP, use of antihypertensive medications, use of lipid-lowering medications, and use of beta blockers. Model 4: Model 3 + HDL and triglycerides.

subgroups of participants who did not (Supplementary material online, Table S1a) or did take statins (Supplementary material online, Table S1b).

In the participants with ASCVD 10 year risk  $>7.5\%$ , LDL-C 50– $<70$  mg/dL was associated with greater risk for mortality, HR 1.43 (1.16–1.75) than the referant group in a minimally adjusted model in the whole population (Supplementary material online, Table S2) and in the subgroup of statin users (Supplementary material online, Table S2b). However, this relationship was not observed after adjustment for covariables or in the subgroups of statin non-users (Supplementary material online, Table S2a).

Categorization of participants with Framingham 10 year risk  $>10\%$  into four LDL-C/hs-CRP groups (LDL-C  $<70$ , hs-CRP  $<2$ ; LDL-C  $<70$ , hs-CRP  $\geq 2$ ; LDL-C  $\geq 70$ , hs-CRP  $<2$ ; LDL-C  $\geq 70$ , hs-CRP  $\geq 2$ ) revealed that the combination of LDL-C  $<70$  mg/dL and hs-CRP  $\geq 2$  was associated with greater risk of CHD mortality than the referent group (LDL-C  $\geq 70$ , hs-CRP  $\geq 2$ ) across all complete-case models and MICE: HR 1.35 (0.97–1.88). Participants with the combination of LDL-C  $\geq 70$  and hs-CRP  $<2$  were at lower risk of death in a fully

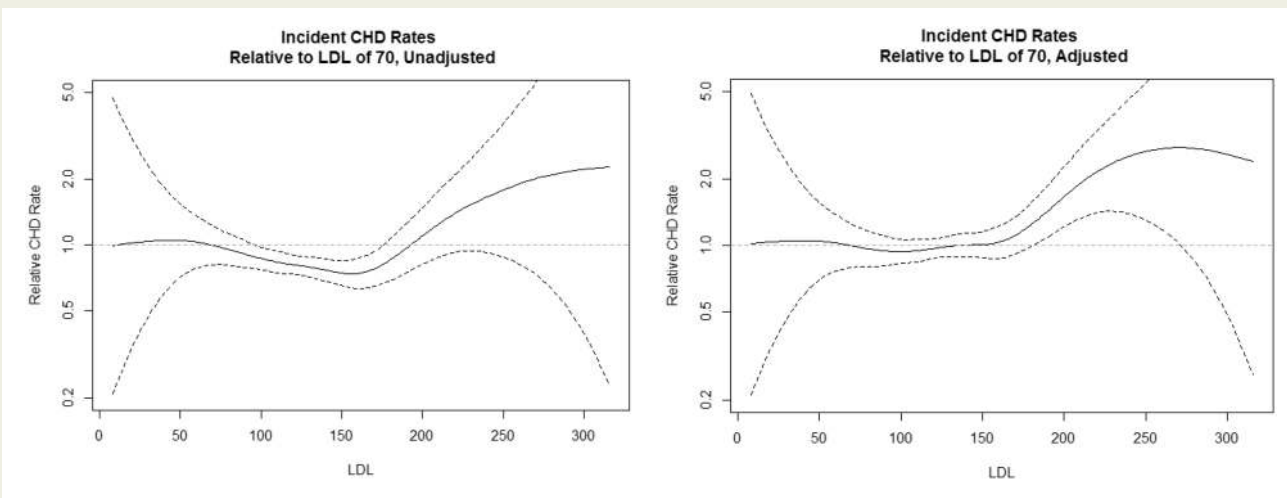
adjusted complete-case model: 0.67 (0.54–0.85); this association remained significant in the MICE model: 0.70 (0.50–0.99) (Table 4). Similar trends were observed in the subgroup of participants who were not taking statins. In this subgroup, LDL-C  $\geq 70$  and hs-CRP  $<2$  was associated with lower risk across all adjusted models and MICE: HR 0.62 (0.41–0.95) but the increased risk seen in participants with LDL-C  $<70$  mg/dL and hs-CRP  $\geq 2$  in a minimally adjusted model was attenuated with progression of models (Supplementary material online, Table S3a). In participants who were taking statins LDL-C  $<70$  mg/dL and hs-CRP  $\geq 2$  was associated with increased risk in a MICE model: HR 2.09 (1.09–3.71), but the combination of LDL-C  $\geq 70$  and hs-CRP  $<2$  was not associated with significant risk reduction (Supplementary material online, Table S3b).

Similar results were observed with the group of participants with ASCVD 10-year risk  $>7.5\%$  in the whole population (Table 5), in those not taking statins (Supplementary material online, Table S4a, Figure 4) and participants who were taking statins (Supplementary material online, Table S4b, Figure 4).

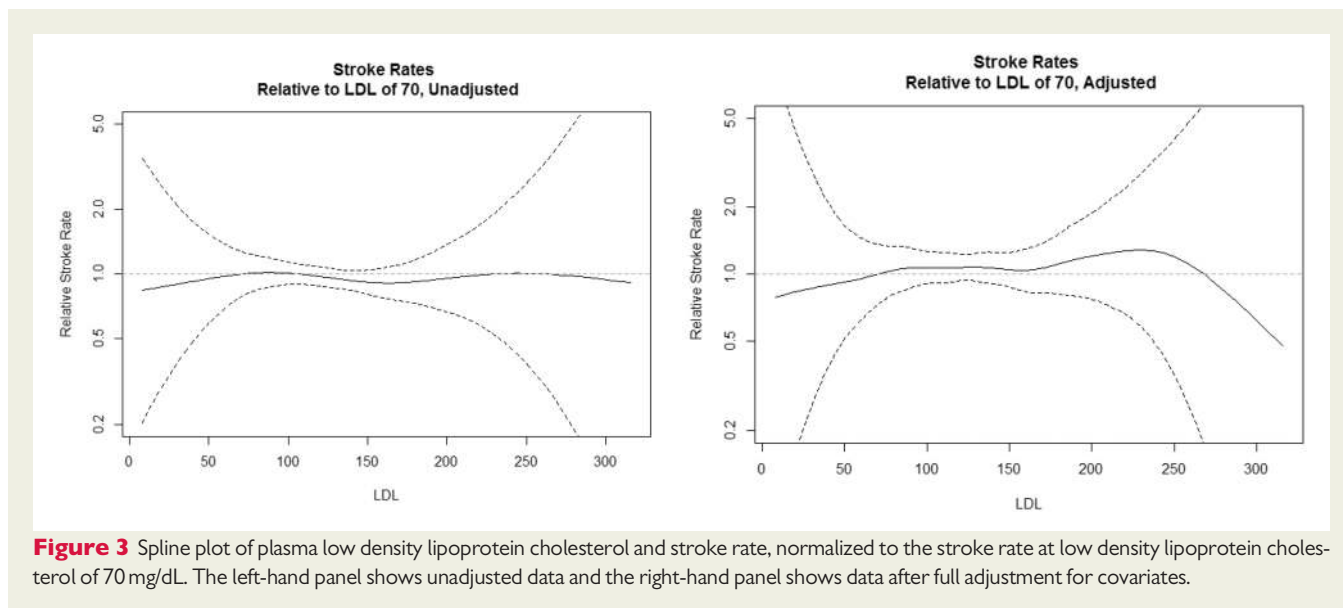
**Table 5** Hazard ratios (and 95% confidence interval) for each outcome by low density lipoprotein cholesterol/high sensitivity C-reactive protein category among REGARDS participants with atherosclerotic cardiovascular disease risk score  $\geq 7.5\%$

	Number of events/population	Model 1	Model 2	Model 3	Model 4	Model 4 (MI)
All-cause mortality	3156/14 469					
LDL-C/hs-CRP category						
LDL-C <70, hs-CRP <2	162/625	0.91 (0.77–1.07)	0.95 (0.80–1.11)	0.91 (0.77–1.07)	0.90 (0.77–1.07)	1.08 (0.90–1.30)
LDL-C <70, hs-CRP $\geq 2$	203/608	1.40 (1.21–1.62)	1.36 (1.18–1.58)	1.25 (1.08–1.45)	1.25 (1.08–1.45)	1.27 (1.11–1.46)
LDL-C $\geq 70$ , hs-CRP <2	1101/5996	0.65 (0.60–0.70)	0.72 (0.67–0.78)	0.76 (0.70–0.83)	0.76 (0.70–0.82)	0.91 (0.81–1.03)
LDL-C $\geq 70$ , hs-CRP $\geq 2$	1685/7240	Ref	Ref	Ref	Ref	Ref
Incident stroke	690/13 418					
LDL-C/hs-CRP category						
LDL-C <70, hs-CRP <2	33/564	0.96 (0.67–1.38)	0.97 (0.68–1.39)	0.97 (0.67–1.39)	0.97 (0.67–1.40)	1.12 (0.76–1.65)
LDL-C <70, hs-CRP $\geq 2$	29/534	0.92 (0.63–1.34)	0.90 (0.62–1.32)	0.86 (0.59–1.26)	0.86 (0.59–1.26)	0.83 (0.58–1.21)
LDL-C $\geq 70$ , hs-CRP <2	258/5628	0.77 (0.66–0.91)	0.79 (0.67–0.93)	0.83 (0.70–0.98)	0.83 (0.70–0.98)	0.92 (0.71–1.18)
LDL-C $\geq 70$ , hs-CRP $\geq 2$	370/6692	Ref	Ref	Ref	Ref	Ref
Incident CHD	710/11 117					
LDL-C/hs-CRP category						
LDL-C <70, hs-CRP <2	20/353	0.68 (0.44–1.07)	0.71 (0.45–1.12)	0.69 (0.44–1.09)	0.69 (0.44–1.10)	0.67 (0.41–1.09)
LDL-C <70, hs-CRP $\geq 2$	33/372	1.27 (0.89–1.81)	1.21 (0.85–1.73)	1.09 (0.76–1.56)	1.08 (0.76–1.55)	1.11 (0.80–1.54)
LDL-C $\geq 70$ , hs-CRP <2	254/4743	0.63 (0.54–0.74)	0.71 (0.60–0.84)	0.73 (0.62–0.86)	0.73 (0.62–0.87)	0.75 (0.59–0.96)
LDL-C $\geq 70$ , hs-CRP $\geq 2$	403/5649	Ref	Ref	Ref	Ref	Ref
CHD death	1035/14 469					
LDL-C/hs-CRP category						
LDL-C <70, hs-CRP <2	49/625	0.82 (0.61–1.10)	0.85 (0.63–1.14)	0.77 (0.57–1.04)	0.77 (0.57–1.04)	0.75 (0.45–1.25)
LDL-C <70, hs-CRP $\geq 2$	75/608	1.52 (1.19–1.93)	1.46 (1.15–1.86)	1.36 (0.98–1.60)	1.26 (0.98–1.61)	1.35 (0.97–1.88)
LDL-C $\geq 70$ , hs-CRP <2	341/5996	0.60 (0.53–0.69)	0.67 (0.58–0.77)	0.72 (0.62–0.82)	0.71 (0.62–0.82)	0.72 (0.54–0.96)
LDL-C $\geq 70$ , hs-CRP $\geq 2$	570/7240	Ref	Ref	Ref	Ref	Ref

Model 1: Adjusted for age, sex, race and region of residence. Model 2: Model 1 + education, income, alcohol use, physical activity, smoking, and BMI. Model 3: Model 2 + diabetes, ACR, eGFR, SBP, use of antihypertensive medications, use of lipid-lowering medications, and use of beta blockers. Model 4: Model 3 + HDL and triglycerides.



**Figure 2** Spline plot of plasma low density lipoprotein cholesterol and coronary heart disease rate, normalized to the coronary heart disease rate at low density lipoprotein cholesterol of 70 mg/dL. The left-hand panel shows unadjusted data and the right-hand panel shows data after full adjustment for covariates.



**Figure 3** Spline plot of plasma low density lipoprotein cholesterol and stroke rate, normalized to the stroke rate at low density lipoprotein cholesterol of 70 mg/dL. The left-hand panel shows unadjusted data and the right-hand panel shows data after full adjustment for covariates.

### Association between low density lipoprotein cholesterol, high sensitivity C-reactive protein, and incident coronary heart disease

Over the 6.91 year average follow-up, 508 (8.3%) participants suffered a coronary event. Inspection of fully adjusted spline plots indicates an approximate doubling of incident CHD risk between baseline LDL-C concentrations of 150 mg/dL and 250 mg/dL (Figure 2). Non-linearity was not observed in either unadjusted or adjusted data (Table 2) and the overall tests of association (likelihood ratio tests) did not indicate a significant association between LDL-C and incident CHD (Table 3).

In Cox proportional hazards models, the two lower categories of LDL were not associated with reduced risk of CHD in participants with high CVD risk as calculated by Framingham (Supplementary material online, Tables S1, S1a, and S1b) or ACSVD (Supplementary material online, Tables S2, S2a, and S2b) scores. However, when participants were categorized according to LDL-C and CRP, it was found that risk was the lowest in the group of participants with LDL-C  $\geq 70$  and CRP  $< 2$  in Framingham high-risk participants. This effect was statistically significant in the whole study population (Table 4) as well as subgroups of those not taking statins (Supplementary material online, Table S3a, Figure 4) and statin users (Supplementary material online, Table S3b, Figure 4). Similar results were observed in ASCVD high-risk participants (Table 5, Supplementary material online, Tables S4a and S4b).

### Association between low density lipoprotein cholesterol, high sensitivity C-reactive protein, and incident stroke

Over the 8.63 year average follow-up, 352 (5.7%) participants suffered a stroke event. Non-linearity was not observed in either unadjusted or adjusted data (Table 2) and the overall tests of association (likelihood ratio tests) did not indicate a significant association between LDL-C

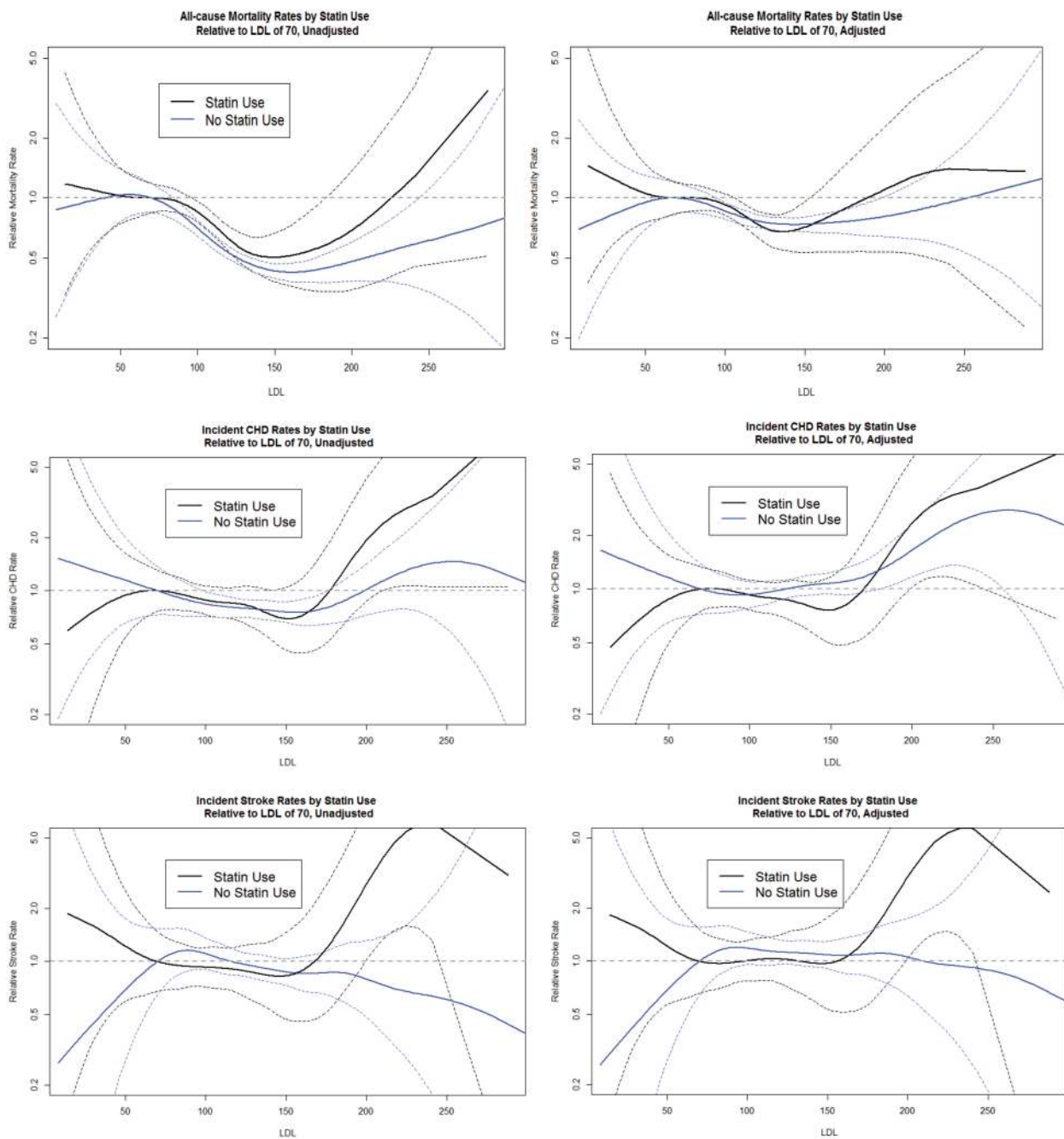
and incident stroke (Table 3, Figure 3). No significant differences with respect to stroke were observed across any of the LDL-C categories in Framingham (Supplementary material online, Tables S1, S1a, and S1b) or ASCVD (Supplementary material online, Tables S2, S2a, and S2b) high-risk participants. When participants were stratified by LDL-C and hs-CRP, stroke risk was lowest in the group with LDL-C  $\geq 70$  and hs-CRP  $< 2$  in both Framingham (Table 4) and ASCVD (Table 5) populations. Subgroup analysis indicated that this effect was significant in participants who did not use statins (Supplementary material online, Tables 3a and 4a, Figure 4), but not in those who used statins (Supplementary material online, Tables 3b and 4b, Figure 4).

## Discussion

This study has demonstrated a non-linear association between LDL-C and all-cause mortality in high-risk primary prevention individuals with an inverse relationship evident between approximately 70 mg/dL (1.8 mmol/L) and 200 mg/dL (5.2 mmol/L), with higher risk of fatal events below 70 mg/dL (1.8 mmol/L) and for LDL-C levels in this range. Recently, a systematic review was conducted of 19 cohort studies totalling 68 094 participants aged 60 years or older. An inverse association between all-cause mortality and LDL-C was seen in 16 studies representing 92% of the number of participants.<sup>31,32</sup>

We identified 70 mg/dL (1.8 mmol/L) as a convenient reference point for our analyses because American College of Cardiology (ACC)/American Heart Association (AHA) guidelines on lipid reduction suggests 70 mg/dL (1.8 mmol/L) as the lowest value at which lipid-lowering therapy is recommended in individuals without diabetes (although statin therapy should be considered for diabetic individuals with LDL-C below this value, taking into account patient preferences and comorbidities).<sup>33</sup> The 2016 European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) guidelines for the management of dyslipidaemia recommend a target of  $< 70$  mg/dL (or  $> 50\%$  reduction in LDL-C) in patients with very high risk of



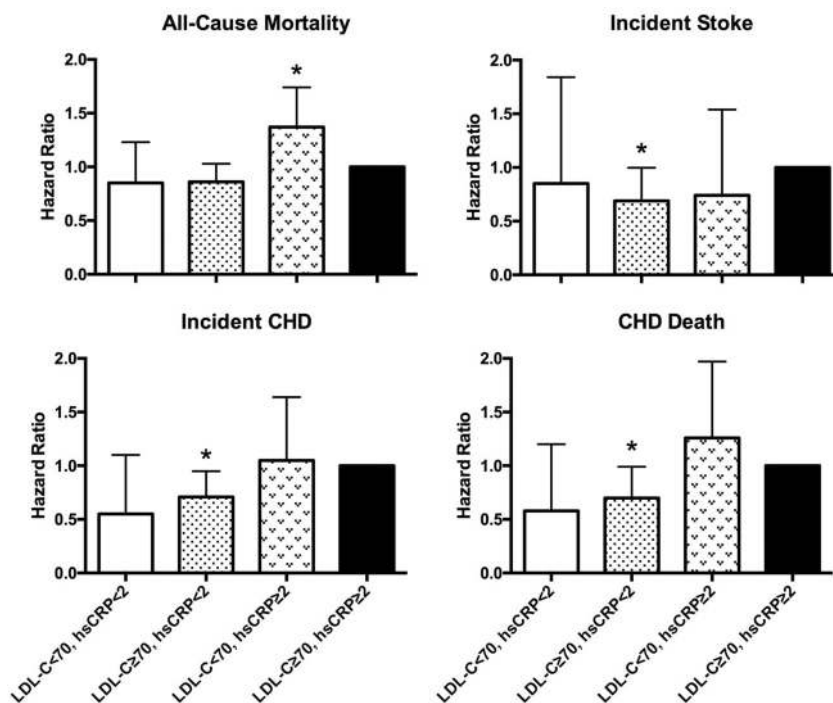


**Figure 4** Sensitivity analysis (by statin use).

CVD.<sup>34</sup> Thus these very low values of LDL-C are of increasing importance in a primary prevention population. Furthermore, relationships between LDL-C and CV events have been extensively studied for values of LDL-C above 70 mg/dL (1.8 mmol/L). Finally, a large meta-analysis found that 40% of patients treated with high-intensity statin therapy failed to reach a target of 70 mg/dL (1.8 mmol/L).<sup>35</sup>

The absence of a statistically significant relationship between LDL-C and CHD was indeed surprising. Applying the fully adjusted MICE models to study the relationships between LDL-C categories and all-cause

mortality, incident stroke, incident CHD, and CHD death, we found that the lower LDL-C categories were not associated with reduced risk of any of the outcomes compared to the reference [ $\geq 70$  mg/dL (1.8 mmol/L)] group. Using the same MICE models to study participants classified by both hs-CRP and LDL-C, we found that in participants with LDL-C  $\geq 70$  mg/dL (1.8 mmol/L) and with low hs-CRP ( $< 2$  mg/L) the risk of incident stroke, incident CHD and CHD death was significantly lower than those with higher LDL-C and hs-CRP in the Framingham high-risk population. A similar pattern of results was



**Take home figure** Hazard ratios for all-cause mortality and cardiovascular outcomes amongst REGARDS participants categorized according to baseline levels of low density lipoprotein cholesterol and high sensitivity C-reactive protein. These analyses were performed on participants with Framingham-coronary heart disease 10 year risk score of >10% and represent fully adjusted models with missing data accounted for using multiple imputation with chained equations. \*Statistically significant ( $P < 0.05$ ) differences from the referant group (LDL-C  $\geq 70$  mg/dL, hs-CRP  $\geq 2$  mg/L).

seen in the ASCVD high-risk group. The combination of high hs-CRP and low LDL-C was not associated with reduced risk of any outcome in either high-risk group, indeed significantly higher all-cause mortality was observed. Participants with low values of both hs-CRP and LDL-C were not at lower risk for any of the outcomes compared with participants with high hs-CRP and high LDL-C (*Take home figure*).

These data might appear to be at odds with the preponderance of evidence from interventional studies, which strongly suggest that 'lower is better' with respect to LDL-C. In the FOURIER study, evolocumab reduced LDL-C to a median of 30 mg/dL and significantly reduced CVD events [HR 0.85, 95% confidence interval (CI): 0.79–0.92].<sup>16</sup> The secondary analysis of the same trial revealed that the benefits were observed also at LDL-C levels <20 mg/dL (0.5 mmol/L).<sup>18</sup> The same results were indeed observed in the recent ODYSSEY OUTCOMES trial with alirocumbab, suggesting however that the higher baseline LDL-C [ $\geq 100$  mg/dL (2.5 mmol/L)] showed greater risk reduction.<sup>36</sup> A pooled analysis of ODYSSEY studies with alirocumbab has demonstrated the feasibility of LDL-C reduction to below 50 mg/dL, which was achieved in one third of the cohort.<sup>37</sup> The investigators found an inverse relationship between LDL-C achieved during treatment and major CV events. The composite endpoint included CHD death, non-fatal MI, ischaemic stroke, or unstable angina requiring hospitalization.<sup>37</sup> Furthermore, a large meta-analysis of studies employing statins found LDL-C reduction to be associated with reduced CV risk, even as low as 50 mg/dL in the patients for whom this was achievable.<sup>35</sup>

However, despite the fact that we limited our analysis to participants with high risk of CVD (calculated using two different methods), our results cannot be directly compared with those of interventional studies for a number of reasons. Firstly, by excluding participants with CHD and stroke at baseline, we studied only a primary prevention population (as opposed to secondary prevention participants in many interventional trials). Based on the data from many available studies, we are aware these are different populations taking into account the risk stratification as well as cardiovascular outcomes observed in hitherto studies.<sup>38</sup> Secondly, the free living population of the REGARDS study is likely to be more heterogeneous than that of interventional studies with numerous inclusion criteria. Thirdly, our follow-up of participants after a single LDL-C-measurement at baseline is not equivalent to controlled studies, whereby a LDL-C lowering intervention is employed. Low density lipoprotein cholesterol which is inherently low and LDL-C, which has been lowered by pharmacological agents will not necessarily lead to similar effects on outcomes. Much work has been carried out investigating the pleiotropic effects of statins,<sup>4</sup> although less is known about non-LDL-mediated effects of newer lipid-lowering drugs. Finally, because of many biochemical roles of cholesterol and concern that low levels of plasma lipids may therefore, cause deleterious side-effects beyond the CV system, we chose all-cause mortality as our primary outcome in contrast to the CHD endpoints used in many trials.<sup>37</sup>

While the association of lipid levels with stroke risk remains somewhat controversial,<sup>39</sup> our results contrast with those obtained from a

larger subset of participants in the REGARDS study, which found that baseline concentrations of LDL-C and non-HDL-C baseline levels were associated with the risk of ischaemic stroke.<sup>32</sup> A very large meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths has also demonstrated only a very weak association between LDL-C and stroke mortality.<sup>38</sup> Perhaps the weaker association of lipid levels with stroke than for coronary disease is a product of the multiple non-atherogenic pathways for stroke, especially as stratifying participants by hs-CRP in addition to LDL-C gave better prediction of stroke risk.

This study focused only on LDL-C, as this measurement was taken at baseline in the REGARDS study. Since then, lipidology has become more sophisticated and a greater appreciation is given to the importance of LDL-C quality, encompassing particle size and number<sup>40</sup>; other atherogenic lipoproteins such as Lp(a)<sup>41</sup>; and combined dyslipidaemia.<sup>42</sup> It is possible that participants with high cholesterol at baseline were later started on statin therapy thus confounding the analysis.<sup>31</sup>

The large sample size, long period of follow-up, and rigorous approach to data-collection in the REGARDS study make this cohort an extremely useful tool to explore relationships between biomarkers and risks of disease. Nevertheless, such an approach to research has several limitations. Observational studies such as this are vulnerable to bias by unknown or unmeasured factors and cannot demonstrate causality. By definition, extreme values of any statistic are rare. We found that only 5% of our study population had LDL-C <70 mg/dL (1.8 mmol/L) at baseline; this resulted in small numbers of events within participants with LDL-C <50 mg/dL, yielding relatively wide CIs. This limits the statistical power of our analysis. Due to the limited number of participants that met the inclusion criteria, we could not analyse participants with Framingham risk score >20%.

We cannot entirely exclude the possibility of reverse causality, whereby low cholesterol secondary to other disease (e.g. malignancy) is associated with poor prognosis.<sup>43</sup> On this basis, Collins *et al.*<sup>4</sup> have suggested censoring the early period of follow-up in this type of analysis. However, the potential for reverse causality in our study is reduced by the exclusion of participants with diagnosed malignancy from the REGARDS cohort. In common with all epidemiological studies, which collect participant data at baseline, we cannot be also certain about the interventions and treatments the participants received thereafter. Our results may be confounded by patients with high LDL-C concentrations at baseline initiating lipid-lowering therapy. Similarly, a proportion of those patients taking statins at baseline will have stopped during the follow-up period. Finally, the great improvements in the diagnosis and management of dyslipidaemias over recent years means that the REGARDS population is likely to have received better care with respect to LDL-C (but not necessarily inflammation and hs-CRP) towards the end of the follow-up period, than they did at the start of the study.

## Conclusions

In primary prevention participants from REGARDS study, we found a significant non-linear relationship between LDL-C and all-cause mortality, which remained after adjustment for all measured covariates. Low density lipoprotein cholesterol between approximately 70 mg/dL (1.8 mmol/L) and 200 mg/dL (5.2 mmol/L) was protective against

all-cause mortality, with levels lower than 70 mg/dL (1.8 mmol/L) not showing any further benefit. We did not find significant associations between LDL-C and incident CHD or incident stroke. Classifying participants by both hs-CRP and LDL-C, we found that that low hs-CRP (<2 mg/L) appeared to be associated with reduced risk of incident stroke, incident CHD and CHD death, whereas low LDL-C (<70 mg/dL) was not associated with protective effects. Whilst, this is maybe to be expected in a population selected for high cardiovascular risk and low LDL-C, our results support those of the recent CANTOS trial with respect to the importance of inflammatory processes in the pathogenesis of CVD.<sup>8</sup>

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

## Acknowledgements

The authors thank the other investigators, the staff, and the participants of the REGARDS study for their valuable contributions. A full list of participating REGARDS investigators and institutions can be found at <http://www.regardsstudy.org>.

## Funding

This work is supported by a cooperative agreement (U01 NS041588) from the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Department of Health and Human Service. Additional support was provided by grant R01 HL080477 from the National Heart, Lung, and Blood Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke or the National Institutes of Health. Representatives of the funding agency have been involved in the review of the manuscript but not directly involved in the collection, management, analysis, or interpretation of the data.

**Conflict of interest:** P.P. owns four shares in Astra Zeneca PLC and has received travel/speaker's fees from Amgen; M.B.: speakers bureau: Abbott/Mylan, Abbott Vascular, Actavis, Akcea, Amgen, Biofarm, KRKA, MSD, Sanofi-Aventis, Servier, and Valeant; consultant to Abbott Vascular, Akcea, Amgen, Daichii Sankyo, Esperion, Lilly, MSD, Resverlogix, Sanofi-Aventis; Grants from Sanofi and Valeant; A.L.C. has received honoraria, lecture fees, or research grants from: Abbot, Aegerion, Amgen, AstraZeneca, Bayer, Eli Lilly, Genzyme, Ionis, Kowa, Mediolanum, Meda, Menarini, Merck, Pfizer, Recordati, Regeneron, Sanofi, and SigmaTau. All other authors do not declare any conflict of interest related to the results of this study.

## References

1. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, Hegele RA, Krauss RM, Raal FJ, Schunkert H, Watts GF, Boren J, Fazio S, Horton JD, Masana L, Nicholls SJ, Nordestgaard BG, van de Sluis B, Taskinen MR, Tokgozogl L, Landmesser U, Laufs U, Wiklund O, Stock JK, Chapman MJ, Catapano AL. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J* 2017;**38**:2459–2472.
2. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med* 1995;**333**:1301–1307.
3. Vallejo-Vaz AJ, Robertson M, Catapano AL, Watts GF, Kastelein JJ, Packard CJ, Ford I, Ray KK. Low-density lipoprotein cholesterol lowering for the primary

- prevention of cardiovascular disease among men with primary elevations of low-density lipoprotein cholesterol levels of 190 mg/dL or above: analyses from the WOSCOPS (West of Scotland Coronary Prevention Study) 5-year randomized trial and 20-year observational follow-up. *Circulation* 2017;**136**:1878–1891.
4. Collins R, Reith C, Emberson J, Armitage J, Baigent C, Blackwell L, Blumenthal R, Danesh J, Smith GD, DeMets D, Evans S, Law M, MacMahon S, Martin S, Neal B, Poulter N, Preiss D, Ridker P, Roberts I, Rodgers A, Sandercock P, Schulz K, Sever P, Simes J, Smeeth L, Wald N, Yusuf S, Peto R. Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet* 2016;**388**:2532–2561.
  5. The Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994;**344**:1383–1389.
  6. Serban MC, Banach M, Mikhailidis DP. Clinical implications of the IMPROVE-IT trial in the light of current and future lipid-lowering treatment options. *Expert Opin Pharmacother* 2016;**17**:369–380.
  7. Sampson UK, Fazio S, Linton MF. Residual cardiovascular risk despite optimal LDL cholesterol reduction with statins: the evidence, etiology, and therapeutic challenges. *Curr Atheroscler Rep* 2012;**14**:1–10.
  8. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P, Glynn RJ; CANTOS Trail Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;**377**:1119–1131.
  9. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, Darius H, Lewis BS, Ophuis TO, Jukema JW, De Ferrari GM, Ruzyllo W, De Lucca P, Im K, Bohula EA, Reist C, Wiviott SD, Tershakovec AM, Musliner TA, Braunwald E, Califf RM; IMPROVE-IT Investigators. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med* 2015;**372**:2387–2397.
  10. Penson P, McGowan M, Banach M. Evaluating bempedoic acid for the treatment of hyperlipidaemia. *Expert Opin Investig Drugs* 2017;**26**:251–259.
  11. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, Stroes ES, Langset G, Raal FJ, El Shahawy M, Koren MJ, Lepor NE, Lorenzato C, Pordy R, Chaudhari U, Kastelein JJ; ODYSSEY Investigators. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015;**372**:1489–1499.
  12. Robinson JG, Rosenson RS, Farnier M, Chaudhari U, Sasiela WJ, Merlet L, Miller K, Kastelein JJ. Safety of very low low-density lipoprotein cholesterol levels with alirocumab: pooled data from randomized trials. *J Am Coll Cardiol* 2017;**69**:471–482.
  13. Nicholls SJ, Puri R, Anderson T, Ballantyne CM, Cho L, Kastelein JJ, Koenig W, Somaratne R, Kassahun H, Yang J, Wasserman SM, Scott R, Ungi I, Podolec J, Ophuis AO, Cornel JH, Borgman M, Brennan DM, Nissen SE. Effect of evolocumab on progression of coronary disease in statin-treated patients: the GLAGOV randomized clinical trial. *JAMA* 2016;**316**:2373–2384.
  14. Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, Ballantyne CM, Somaratne R, Legg J, Wasserman SM, Scott R, Koren MJ, Stein EA; Open-Label Study of Long-Term Evaluation against LDL-Cholesterol (OSLER) Investigator. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015;**372**:1500–1509.
  15. Ray KK, Landmesser U, Leiter LA, Kallend D, Dufour R, Karakas M, Hall T, Troquay RP, Turner T, Visseren FL, Wijngaard P, Wright RS, Kastelein JJ. Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. *N Engl J Med* 2017;**376**:1430–1440.
  16. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, Sever PS, Pedersen TR; Fourier Steering Committee Investigators. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med* 2017;**376**:1713–1722.
  17. Giugliano RP, Mach F, Zavitz K, Kurtz C, Im K, Kanevsky E, Schneider J, Wang H, Keech A, Pedersen TR, Sabatine MS, Sever PS, Robinson JG, Honarpour N, Wasserman SM, Ott BR; Ebbinghaus Investigators. Cognitive function in a randomized trial of evolocumab. *N Engl J Med* 2017;**377**:633–643.
  18. Giugliano RP, Pedersen TR, Park J-G, De Ferrari GM, Gaciong ZA, Ceska R, Toth K, Gouni-Berthold I, Lopez-Miranda J, Schiele F, Mach F, Ott BR, Kanevsky E, Pineda AL, Somaratne R, Wasserman SM, Keech AC, Sever PS, Sabatine MS. Clinical efficacy and safety of achieving very low LDL-cholesterol concentrations with the PCSK9 inhibitor evolocumab: a prespecified secondary analysis of the FOURIER trial. *Lancet* 2017;**390**:1962–1971.
  19. Sabatine MS, Leiter LA, Wiviott SD, Giugliano RP, Deedwania P, De Ferrari GM, Murphy SA, Kuder JF, Gouni-Berthold I, Lewis BS, Handelsman Y, Pineda AL, Honarpour N, Keech AC, Sever PS, Pedersen TR. Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial. *Lancet Diabetes Endocrinol* 2017;**5**:941–950.
  20. Howard VJ, Cushman M, Pulley L, Gomez CR, Go RC, Prineas RJ, Graham A, Moy CS, Howard G. The reasons for geographic and racial differences in stroke study: objectives and design. *Neuroepidemiology* 2005;**25**:135–143.
  21. National Cholesterol Education Program (NCEP) Expert Panel. Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 2002;**106**:3143–3421.
  22. Lakoski SG, Le AH, Muntner P, Judd SE, Safford MM, Levine DA, Howard G, Cushman M. Adiposity, inflammation, and risk for death in black and white men and women in the United States: the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study. *J Clin Endocrinol Metab* 2011;**96**:1805–1814.
  23. Cushman M, McClure LA, Howard VJ, Jenny NS, Lakoski SG, Howard G. Implications of increased C-reactive protein for cardiovascular risk stratification in black and white men and women in the US. *Clin Chem* 2009;**55**:1627–1636.
  24. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;**18**:499–502.
  25. Howard VJ, Kleindorfer DO, Judd SE, McClure LA, Safford MM, Rhodes JD, Cushman M, Moy CS, Soliman EZ, Kissela BM, Howard G. Disparities in stroke incidence contributing to disparities in stroke mortality. *Ann Neurol* 2011;**69**:619–627.
  26. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;**150**:604–612.
  27. Rubin DB. Multiple imputation after 18+ years. *J Am Stat Assoc* 1996;**91**:473.
  28. Rubin DB, Schenker N. Multiple imputation in health-care databases: an overview and some applications. *Stat Med* 1991;**10**:585–598.
  29. White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. *Stat Med* 2011;**30**:377–399.
  30. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2016.
  31. Ravnskov U, Diamond DM, Hama R, Hamazaki T, Hammarskjold B, Hynes N, Kendrick M, Langsjoen PH, Malhotra A, Mascitelli L, McCully KS, Ogushi Y, Okuyama H, Rosch PJ, Schersten T, Sultan S, Sulndberg R. Lack of an association or an inverse association between low-density-lipoprotein cholesterol and mortality in the elderly: a systematic review. *BMJ Open* 2016;**6**:e010401.
  32. Glasser SP, Mosher A, Howard G, Banach M. What is the association of lipid levels and incident stroke? *Int J Cardiol* 2016;**220**:890–894.
  33. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, Goldberg AC, Gordon D, Levy D, Lloyd-Jones DM, McBride P, Schwartz JS, Shero ST, Smith SC Jr, Watson K, Wilson PW, Eddleman KM, Jarrett NM, LaBresh K, Nevo L, Wnek J, Anderson JL, Halperin JL, Albert NM, Bozkurt B, Brindis RG, Curtis LH, DeMets D, Hochman JS, Kovacs RJ, Ohman EM, Pressler SJ, Sellke FW, Shen WK, Smith SC Jr, Tomaselli GF; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2014;**129**:S1–S45.
  34. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, Hoes AW, Jennings CS, Landmesser U, Pedersen TR, Reiner Z, Riccardi G, Taskinen MR, Tokgozoglul L, Verschuren WMM, Vlachopoulos C, Wood DA, Zamorano JL, Cooney MT. 2016 ESC/EAS guidelines for the management of dyslipidaemias. *Eur Heart J* 2016;**37**:2999–3058.
  35. Boekholdt SM, Hovingh GK, Mora S, Arsenault BJ, Amarencu P, Pedersen TR, LaRosa JC, Waters DD, DeMicco DA, Simes RJ, Keech AC, Colquhoun D, Hitman GA, Betteridge DJ, Clearfield MB, Downs JR, Colhoun HM, Gotto AM Jr, Ridker PM, Grundy SM, Kastelein JJ. Very low levels of atherogenic lipoproteins and the risk for cardiovascular events: a meta-analysis of statin trials. *J Am Coll Cardiol* 2014;**64**:485–494.
  36. Schwartz G, Szarek M, Bhatt D, Bittner V, Diaz R, Edelberg J, Goodman S, Hanotin C, Harrington R, Jukema J, Lecorps G, Moryusef A, Pordy R, Roe M, White H, Zeiher A, Steg GP. The ODYSSEY OUTCOMES Trial: topline results alirocumab in patients after acute coronary syndrome. In: *ACC.18 67th Annual Scientific Session & Expo. ACC.18 Joint ACC/JACC Late-Breaking Clinical Trials*, Orlando, 2018. <https://accscien.tifcsession.acc.org/features/2018/03/video-sanofi-regeneron> (11 April 2018).
  37. Ray KK, Ginsberg HN, Davidson MH, Pordy R, Bessac L, Minini P, Eckel RH, Cannon CP. Reductions in atherogenic lipids and major cardiovascular events: a pooled analysis of 10 ODYSSEY trials comparing alirocumab with control. *Circulation* 2016;**134**:1931–1943.
  38. Prospective Studies Collaboration, Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R, Collins R. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* 2007;**370**:1829–1839.

39. Howard G, Goff DC Jr. Lipids and stroke: looking for risk in all the wrong places? *Ann Neurol* 2011;**69**:597–599.
40. Mikhailidis DP, Elisaf M, Rizzo M, Berneis K, Griffin B, Zambon A, Athyros V, de Graaf J, Marz W, Parhofer KG, Rini GB, Spinass GA, Tomkin GH, Tselepis AD, Wierzbicki AS, Winkler K, Florentin M, Liberopoulos E. "European panel on low density lipoprotein (LDL) subclasses": a statement on the pathophysiology, atherogenicity and clinical significance of LDL subclasses: executive summary. *Curr Vasc Pharmacol* 2011;**9**:531–532.
41. Kotani K, Serban MC, Penson P, Lippi G, Banach M. Evidence-based assessment of lipoprotein(a) as a risk biomarker for cardiovascular diseases - Some answers and still many questions. *Crit Rev Clin Lab Sci* 2016;**53**:370–378.
42. Rizzo M, Baryliski M, Rizvi AA, Montalto G, Mikhailidis DP, Banach M. Combined dyslipidemia: should the focus be LDL cholesterol or atherogenic dyslipidemia? *Curr Pharm Des* 2013;**19**:3858–3868.
43. Law MR, Thompson SG, Wald NJ. Assessing possible hazards of reducing serum cholesterol. *BMJ* 1994;**308**:373–379.

## CARDIOVASCULAR FLASHLIGHT

doi:10.1093/eurheartj/ehy414

Online publish-ahead-of-print 17 July 2018

### Frank's sign: diagonal earlobe crease

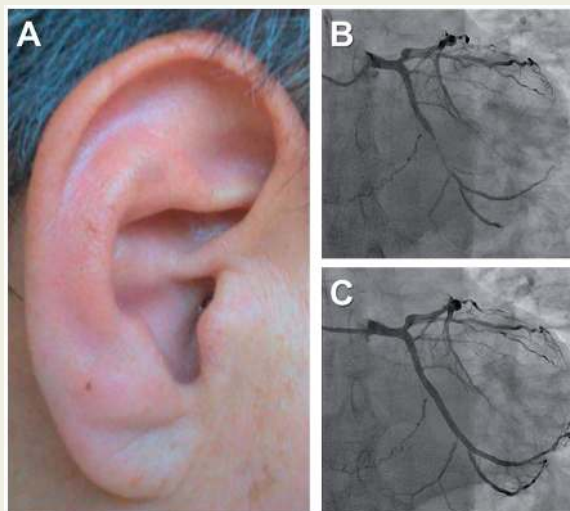
Takanori Aizawa, Hiroki Shiomi\*, Kazuki Kitano, and Takeshi Kimura

Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

\* Corresponding author. Tel: +81-75-751-4254, Fax: +81-75-751-3289, Email: hishiomi@kuhp.kyoto-u.ac.jp

A 59-year-old man presented to the outpatient clinic with frequent chest pain at rest and mild exertion for a few days. An electrocardiogram showed only equivocal minimal ST-segment depression without abnormal Q wave, and cardiac enzyme test was normal. He had several coronary risk factors such as hypertension, dyslipidaemia, and diabetes. In addition to xanthelasma palpebrarum, his bilateral ears showed a diagonal earlobe crease (ELC) running down from the tragus to the edge of the auricle at a 45° angle, which is so-called Frank's sign (Panel A). He underwent coronary angiography for suspected unstable angina. Angiography revealed critical stenosis in the mid-circumflex coronary artery, which was treated by percutaneous coronary intervention (PCI) with stent implantation (Panels B and C). After the procedure, he was relieved from chest pain and discharged 3 days after PCI.

Frank's sign was first described by Sanders T. Frank in 1973, as a predictive dermatological finding of coronary artery disease. The detailed mechanisms of developing ELC is uncertain, although it is speculated to be derived from loss or degeneration of elastic fibres by diminishing blood supply to the earlobes in a pathological study. Earlobe crease is reported to have significant association with coronary artery disease and other atherosclerotic disease in several studies and thought to be one of the physiological sign predicting atherosclerotic disease.



Published on behalf of the European Society of Cardiology. All rights reserved. © The Author(s) 2018. For permissions, please email: journals.permissions@oup.com.