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Peter Penson, PhD[1], Leann Long, PhD[2], George Howard, DrPh[2], Virginia J. Howard, PhD[3], Steven R. Jones, MD[4], Seth S. Martin, MD[4], Dimitri P. Mikhailidis, MD[5], Paul Muntner, PhD[3], Manfredi Rizzo, PhD[6,7], Daniel J. Rader, MD[8], Monika M. Safford, MD[9], Amirhossein Sahebkar, PharmD[10,11], Peter P. Toth, PhD[4,12], Maciej Banach, PhD[13-15*]

[1]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK; [2]Department of Biostatistics, UAB School of Public Health, Birmingham, AL, USA; [3]Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL, USA; [4]The Johns Hopkins Ciccarone Center for the Prevention of Heart Disease, Baltimore, MD, USA; [5]Department of Clinical Biochemistry, Royal Free Campus, University College London Medical School, University College London (UCL), London, UK; [6]Biomedical Department of Internal Medicine and Medical Specialties, University of Palermo, Palermo, Italy; [7]Euro-Mediterranean Institute of Science and Technology, Palermo, Italy; [8]Department of Medicine, Perelman School of Medicine of the University of Pennsylvania, Philadelphia, PA, USA; [9]Department of Medicine, Weill Cornell Medicine, New York, NY, USA; [10]Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; [11]Metabolic Research Centre, Royal Perth Hospital, School of Medicine and Pharmacology, University of Western Australia, Perth, Australia; [12]Preventive Cardiology, CGH Medical Center, Sterling, Illinois, USA; [13]Department of Hypertension, Chair of Nephrology and Hypertension, Medical University of Lodz, Poland; [14]Polish Mother's Memorial Hospital Research Institute, Lodz, Poland; [15]Cardiovascular Research Centre, University of Zielona Gora, Zielona Gora, Poland.

Running Head: *Very low high-density lipoprotein (HDL) and health outcomes*

***Correspondence to:** Prof. Maciej Banach, MD, PhD, FNLA, FAHA, FESC, FASA, Head, Department of Hypertension, WAM University Hospital in Lodz, Medical University of Lodz, Zeromskiego 113; 90-549 Lodz, Poland. **Phone:** +48 42 639 37 71; **Fax:** +48 42 639 37 71; **E-mail:** maciejbanach77@gmail.com

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ABSTRACT:

AIMS: Relatively little is known about the health outcomes associated with very low plasma concentrations of high density lipoprotein cholesterol (HDL-C) mainly because of the small numbers of individuals with such extreme values included in clinical trials. We therefore investigated the association between low and very low HDL-C concentration at baseline and incident all-cause-mortality, death from malignant disease (i.e. cancer), and with fatal or non-fatal incident coronary heart disease (CHD) in individuals from the Reasons for Geographical And Racial Differences in Stroke (REGARDS) study.

METHODS AND RESULTS: Analysis was based on 21,751 participants from the REGARDS study who were free of CHD, other cardiovascular disease and cancer at baseline and were categorized by baseline HDL-C into <30 mg/dL (very low), 30 -<40 mg/dL (low), and ≥40 mg/dL (reference). A series of incremental Cox proportional hazards models were employed to assess the association between the HDL-C categories and outcomes. Statistical analysis was performed using both complete case methods and multiple imputations with chained equations. After adjustment for age, race and sex, the hazard ratios (HRs) comparing the lowest and highest HDL-C categories were 1.48 (95% confidence interval [CI]: 1.28, 1.73) for all-cause mortality, 1.35 (95%Cl: 1.03, 1.77) for cancer-specific mortality and 1.39 (95%Cl: 0.99, 1.96) for incident CHD. These associations became non-significant in models adjusting for demographics, cardiovascular risk factors and treatment for dyslipidemia. We found evidence for an '*HDL paradox*' whereby low HDL (30-<40 mg/dL) was associated with reduced risk of incident CHD in black participants in a fully-adjusted complete case model (HR 0.63; 95%CI: 0.46, 0.88) and after multiple imputation analyses (HR 0.76; 95%CI 0.58, 0.98). HDL-C (<30 mg/dL) was significantly associated with poorer outcomes in women for all outcomes, especially with respect to cancer mortality (HR 2.31; 95%Cl: 1.28, 4.16) in a fully-adjusted complete case model, replicated using multiple imputation (HR 1.81; 95%CI 1.03, 3.20).

CONCLUSIONS: Low HDL-C was associated with reduced risk of incident CHD in black participants suggesting a potential *HDL paradox* for incident CHD. Very low HDL-C in women was significantly associated with cancer mortality in a fully-adjusted complete case model.

Key words: Cholesterol, Coronary Heart Disease, HDL, Malignant Disease, Mortality.

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INTRODUCTION

In the 1970s, the Framingham Heart Study (FHS) demonstrated an inverse correlation between plasma high density lipoprotein cholesterol (HDL-C) concentrations and coronary heart disease (CHD) risk[1], an observation that was consistent with previous descriptions of the role of HDL in reverse cholesterol transport [2] and which prompted investigations into the therapeutic potential of HDL-elevating interventions. Despite early promise [3], recent trials with niacin or cholesteryl ester transfer protein (CETP) inhibitors failed to demonstrate that treatment to raise HDL-C resulted in improved CV health outcomes[4, 5]. A study employing Mendelian randomization demonstrated that several polymorphisms, which raised HDL-C did not reduce the risk of myocardial infarction (MI) [6]. Furthermore, it has recently been shown that a rare variant of the scavenger receptor B1 is associated with increased HDL-C and an increased risk of CHD [7]. These data suggest that HDL-C is not implicated in the causal pathway of atherosclerosis.

The accumulation of evidence therefore casts doubt on HDL-elevation as a therapeutic strategy. However, the risk conferred by low HDL-C can be ameliorated – a *post-hoc* analysis of the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial has shown benefit of statins in this respect [8, 9]. Thus, the inverse relationship between HDL-C and clinical outcomes necessitates careful study to enable identification of patients at risk and to offer risk reduction therapies where they are available. While individuals with HDL-C <40 mg/dl are recognized at being increased risk of cardiovascular (CV) events, currently relatively little is known about the health outcomes associated with very low (<30 mg/dl) HDL-C. Most studies conducted to date have been underpowered to detect such differences. One observational study including 43,368 subjects, 429 of whom had HDL-C <15 mg/dl, showed that most cases of very low HDL-C were

associated with secondary causes and that mortality was significantly elevated when HDL-C concentrations were <15 mg/dl [10].

We analyzed data from the REasons for Geographical And Racial Differences in Stroke (REGARDS) study to investigate the relationships between low (30 to 39.9 mg/dL) and very low (<30 mg/dl) concentrations of HDL-C and CHD incidence, death and all-cause mortality. In light of previous observations of inverse relationship between HDL-C and cancer[11] we also included malignant disease as an endpoint in our analysis. The large size of the cohort and the recruitment strategy of the REGARDS study allowed these relationships to be investigated in racial subgroups and for comparisons to be made between the sexes.

METHODS

REGARDS study population

The REGARDS longitudinal cohort study recruited 30,239 community-dwelling subjects between January 2003 and October 2007 [12]. Participants were selected from commercially available 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 U.S. Stroke Belt states (56%) [12, 13]. The Stroke Belt states were defined as North Carolina, South Carolina, Georgia, Tennessee, Alabama, Mississippi, Arkansas, and Louisiana, with the remaining 44% of the participants selected from the remaining 40 contiguous U.S. states [12, 13]

Eligibility criteria included having a name and telephone number in the Gensys database, black or white race, English-speaking, aged 45 and older, absence of conditions associated with a life expectancy of less than 5 years, living in the community, and not being in or on a waiting list for a nursing home. Potential participants with diagnosed malignancy at baseline were excluded, those with medical conditions that would preclude long-term participation, and being

cognitive impairment as judged by the telephone interviewer [12]. The participation rate was estimated as 33%, similar to other studies [13] .

The REGARDS study protocol was approved by the Institutional Review Boards governing research in human subjects at the participating centers. All participants provided informed consent.

Data collection

For those agreeing to participate, the telephone interviewers conducted an interview to assess cardiovascular disease (CVD) risk factors and medical history. An in-person assessment for direct measurement of risk factors (blood pressure, anthropomorphic characteristics, electrocardiogram) and collection of blood and urine samples was conducted approximately 2 to 3 weeks after the telephone interview. Participants (or their surrogates) were contacted by telephone at 6-month intervals to detect suspected CVD events and death, with medical records associated with suspected events retrieved and adjudicated by a physician panel. Additionally, surveillance for death was performed by use of online sources such as the Social Security Death Index and the National Death Index. Cause of death was established by physician review of medical history, medical records (when available), interviews with next-of-kin or proxies, autopsy reports, death certificates, and the National Death Index. Details of the study design are provided elsewhere [12].

In this analysis, we included REGARDS study participants who fasted overnight prior to their study visit, were not missing any explanatory variables of interest, had valid measurements of total cholesterol, HDL-C and triglycerides. Because the complete case method of analysis has been shown to underestimate risks, especially in black women, we then reanalyzed the data, imputing missing values using multiple imputation with chained equations

(MICE) [14, 15]. REGARDS participants with a history of CHD at baseline were excluded from the incident CHD analysis.

Laboratory assays were conducted as previously described [16]. 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[17]. On arrival, samples were centrifuged at 30,000 g at 4^0C and either analyzed (general chemistries) or stored at below -80^0C. C-reactive protein (CRP) was analyzed in batches by particle enhanced immunonephelometry using the BNII nephelometer (N High Sensitivity CRP; Dade Behring, Deerfield, IL) 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) [18]. LDL-C was calculated using the Friedewald formula from total cholesterol, HDL-C and triglycerides [19]

Demographic factors included participant age, race (black/white) and sex. Measures of socio-economic status (SES) included self-reported income level (<$20k, $20k-$34k, $35k-$74k, ≥$75k) and education level (less than high school, high school graduate, some college, college graduate). Alcohol consumption (some, none), physical activity (none, 1-3 times/week, 4 or more times/week), and current cigarette smoking were assessed during the baseline telephone interview. Diabetes was defined as self-reported diabetes medication use or fasting glucose ≥126 mg/dL. Body mass index (BMI) (kg/m^2) and systolic blood pressure (mmHg) were measured during the in-home visit. Albumin-to-creatinine ratio (ACR ≥30 *vs* <30 mg/g), estimated glomerular filtration rate (eGFR) through the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [20], CRP <1 mg/L, 1-3 mg/L, ≥3 mg/L), low density lipoprotein (LDL) and triglycerides were measured through specimens. Use of statins, other lipid-lowering medications (fibrates or niacin) and steroids was based on medication inventory.

Endpoints

The three endpoints of interest were: 1) death from any cause, 2) death from malignant disease, and, 3) incident fatal or non-fatal CHD, each at or before December 31, 2013 (the last date where adjudication of the cause of death was available). The definition of incident CHD has been previously described as an incident definite or probable non-fatal MI or CHD death [17]. MI was classified based on published guidelines and consideration of clinical signs and symptoms consistent with ischemia; a rising and/or falling pattern of biomarkers over at least 6h with a peak at least twice the upper limit of normal; and electrocardiogram (ECG) or other imaging findings consistent with ischemia. REGARDS study participants or proxy respondents were contacted every 6 months *via* telephone to assess incident CHD events. Medical records were retrieved for adjudication for suspected events. When fatal CHD events were reported, interviews with next-of-kin or proxies, medical records in the last year of life, death certificates and autopsy reports were examined to determine if a CHD event was the main underlying cause of death. Non-fatal MIs and fatal CHD events were adjudicated by trained clinicians following published guidelines [21-23]. For all analysis of incident CHD, those participants with baseline CHD (self-reported MI, coronary artery bypass grafting, angioplasty or stenting, or evidence of MI *via* ECG) were excluded.

Cancer mortality was recorded regardless of cancer type, as previously described [24]. Cancer mortality was assessed through semi-annual telephone follow up, death information from participant proxies, linkages with the Social Security Death Index (SSDI) as well as the National Death Index (NDI). Date of death was confirmed using death certificates, SSDI and/or NDI, and cause of death was adjudicated by a committee of experts using all available information as recommended by national guidelines [17].

Statistical analysis

HDL-C categories were defined by fasting HDL-C measurement into the following categories: 'very low' HDL-C (<30 mg/dL), 'low' HDL-C (30-<40 mg/dL) and 'normal' HDL-C (≥40 mg/dL) (reference value). To assess the association between the HDL-C categories and each outcome, a series of incremental Cox proportional hazards models were employed on complete cases: Model 1) adjustment for demographic factors (age, race, sex); Model 2) additional adjustment for SES (income level and education level), alcohol consumption, physical activity, smoking and BMI; Model 3) additional adjustment for diabetes, estimated eGFR, ACR, CRP, statin use, other lipid-lowering medication use, steroid use, and, Model 4) additional adjustment for LDL-C and triglycerides. Statistical interactions in the minimally-adjusted model (Model 1) and final model (Model 4) were used to examine whether the associations between HDL-C category and the outcomes varied by sex and race, separately. In a separate analysis, Model 4 was used to interrogate the data using multiple imputation with chained equations (MICE) [14, 15]. For the incremental proportional hazards models, the level of significance was set at 0.05, and 0.10 for the interaction analyses [25]. A sensitivity analysis further explored whether the association between HDL-C category and each outcome changes over time, using a joint Wald test of time-varying HDL-C effects in Model 4. Additional sensitivity analyses examined continuous HDL-C using restricted cubic splines in Model 4 as well as HDL-C quintiles in Models 1 and 4. SAS 9.4 (SAS Institute, Inc.) and R [26] were used for all complete case statistical analyses and Stata 14.2 for multiple imputation analyses.

RESULTS

Baseline characteristics

Of the 21,751 participants that met the complete case inclusion criteria (**Supplemental Figure 1)**, 45% of them were male and 39% were black. The mean age was 64.6 (±9.4) years.

With respect to HDL-C, 748 (3.4%) participants were in the very low (<30 mg/dL) HDL-C category; 4038 (18.6%) in the low (30 - <40 mg/dL), and 16965 (78.0%) were in the normal (≥40 mg/dl) category.

Age, BMI, eGFR, educational status, income, physical activity, smoking, statin use and CRP were broadly similar between the categories. Participants in the low and very low HDL categories were more likely to be male, white, and to have diabetes than participants with normal HDL. HDL category was directly correlated with LDL-C and inversely correlated with triglycerides. Baseline characteristics for the population (stratified by HDL-C category) are shown in **Supplemental Table 1.** Detailed number of events and populations for each HDL*race and HDL*sex group is presented in **Supplemental Table 2.** Hazard ratios (HRs) describing the association between HDL-C category and risk of all-cause mortality, mortality from malignant disease and incident CHD are presented in **Supplemental Table 3**, and HRs describing the association between HDL-C quintile and each outcome of interest by race and sex are presented in **Supplemental Tables 4 and 5**, respectively. Relative hazard of each outcome using continuous HDL-C through restricted cubic splines by race and sex are presented in **Supplementary Figures 2-7.**

Relationship between HDL-C category and all-cause-mortality

The mean person-years follow-up (SD) for all-cause mortality was 7.2 (2.5) years. Unadjusted Kaplan-Meier curves showed that, compared with participants in the normal HDL-C category, all-cause mortality was higher in patients with low, or very low HDL-C (**Figure 1**). In Cox proportional hazard models adjusted for age, race, and sex (Model 1), participants in the low category of HDL-C had greater risk of death with the HR 1.15 (95% confidence interval [CI]: 1.06, 1.25) and the mortality in the very low HDL-C group was greater still 1.48 (95%CI: 1.28, 1.73), thus demonstrating a monotonic relationship between HDL-C and

mortality. Similar results were seen after further adjustment for education level, income level, alcohol consumption, physical activity, smoking and BMI (Model 2). Further adjustment (Models 3 and 4) attenuated this relationship, as did analysis by MICE (**Supplemental Table 3**). There is no evidence that the association between HDL-C category and all-cause mortality changes over time (p=0.12).

In the fully-adjusted model (Model 4), statistically significant differences were observed between males and females in the relationship between HDL-C and all-cause mortality (p for interaction = 0.08) with numerically larger HR in females (HR 1.31, 95%CI: 0.88, 1.95 for HDL-C <30 mg/dL). However, no statistically significant differences between sexes were observed when MICE was employed (**Table 1**). Neither complete case analysis (Model 4) nor MICE demonstrated a statistically significant interaction of race with respect to all-cause mortality **(Table 2)**. Treating HDL-C continuously, no differences in association with all-cause mortality were observed by race (p=0.65; **Supplemental Figure 2**), but the interaction between HDL-C and sex was statistically significant (p<0.01; **Supplemental Figure 3**).

Relationship between HDL-C and cancer-specific mortality

The mean person-years follow-up (SD) for cancer-specific mortality was 7.8 (2.8) years. Unadjusted Kaplan-Meier curves showed that, compared with participants in the normal HDL-C category, the rate of cancer mortality was increased in patients with low or very low HDL-C (**Figure 2**), which were apparent before 2 years and extend through 10 years of follow up. In Cox proportional hazard models adjusted for age, race, and sex (Model 1), participants in the low and very low categories of HDL-C had increased risk of cancer-specific mortality - HR 1.14 (95%CI: 0.99, 1.32) and 1.35 (95%CI: 1.03, 1.77), respectively, and this trend continued through models 2, 3 and 4 with gradual effect attenuation, and the effect was not observed with MICE (**Supplemental Table 3**). There is no evidence that the association between HDL-C

category and cancer-specific mortality changes over time (p=0.08). In the fully-adjusted model (Model 4), using complete case analysis, statistically significant differences were observed between males and females in the relationship between HDL-C and cancer-specific mortality (p for interaction = 0.014). In females, the very low category of HDL-C was strongly associated with the higher risk of cancer (HR 2.31, 95%CI: 1.28, 4.16) compared with men (HR 0.88, 95%CI: 0.64, 1.21) (**Table 1**). The difference between cancer mortality between males and females was also seen when MICE was employed (p=0.033). Neither complete case analysis (Model 4) nor MICE demonstrated a statistically significant interaction of race with respect to cancer-specific mortality **(Table 2)**. Examining HDL-C continuously, no differences in association with cancer-specific mortality were observed by race (p=0.62; **Supplemental Figure 4**), but the interaction between HDL-C and sex was statistically significant (p<0.01; **Supplemental Figure 5**).

Relationship between HDL-C and incident CHD

The mean person-years follow-up (SD) for all-cause mortality was 7.0 (2.6) years. Unadjusted Kaplan-Meier curves showed that, compared with participants in the normal HDL-C category, rates of incident CHD were increased in patients with low, or very low HDL-C (**Figure 3**), which were apparent before 2 years and extend through 10 years of follow up. In adjusted Cox proportional hazard models (Models 1-4) the observed effect was gradually attenuated together with subsequent adjustments (**Supplemental Table 3**). There is no evidence to suggest that the association between HDL-C category and incident CHD varies over time (p=0.08).

Subgroup analysis of the fully-adjusted model (Model 4) using the complete case method demonstrated statistically significant effects of sex (**Table 1**) (p for interaction = 0.008) and race **(Table 2)** (p for interaction = 0.018). The analysis of the relationship between HDL-C and

incident CHD in females showed that low HDL-C (30-39.9 mg/dL) was significantly associated with a reduced risk of incident CHD (HR 0.57, 95%CI: 0.38, 0.86); conversely, very low HDL-C was non-significantly associated with greater risk (HR: 1.57, 95%CI: 0.69, 3.58). In males, a different relationship was seen with no effect of low HDL-C (HR 1.04, 95%CI: 0.85, 1.28), or very low HDL-C (HR: 0.82, 95%CI: 0.55, 1.22) on incident CHD. However, these sex differences were not replicated when analysis was performed using MICE (**Table 1**). In whites, participants in the low HDL-C category had a similar incidence of CHD as those in the normal (reference) category (HR 1.09, 95%CI: 0.88, 1.35), similar results were seen with very low HDL-C (HR 0.92, 95%CI: 0.61, 1.39). Black participants in the low HDL-C category were at significant lower risk of incident CHD than those with normal HDL-C (HR 0.63: 95%CI: 0.46, 0.88), and a similar trend (however not significant) was seen with the very low HDL-C group (HR: 0.82, 95%CI: 0.40, 1.68). The significant interaction of race in complete case analysis was preserved when MICE analysis was employed (p for interaction = 0.054) (**Table 2**). Significant differences in the association between continuous HDL-C and incidence of CHD were observed by both race (p=0.02; **Supplemental Figure 6**) and sex (p=0.04; **Supplemental Figure 7**).

DISCUSSION

Our study has demonstrated that HDL-C tertiles were inversely monotonically associated with all-cause mortality and cancer-specific mortality in a minimally-adjusted model. A similar trend was seen with the relationship between HDL-C and the risk of incident CHD. Analysis of racial subgroups revealed that blacks in the 'low' and 'very low' HDL-C categories experienced fewer incident CHD events than those with 'normal' HDL-C using both complete case and MICE analysis methods. Thus, in this population, our data suggest an '*HDL paradox'*, raising the possibility of the existence of an inverted *U*- or *J-curve* phenomenon. Subgroup

analysis of a fully-adjusted complete case model revealed striking differences between males and females. The interaction with sex was significant for all three outcomes, with low HDL-C being prognostic of poorer outcomes in females than in males in each case. In particular, in females, 'very low' HDL was strongly associated with cancer-specific mortality, whereas such an effect was not seen in males. This interaction of sex was also observed in MICE analysis, however the effect was slightly attenuated (HR goes from 2.31 in complete case to 1.81 in MICE). It has long been recognized that population-level HDL-C concentrations are higher in adult females than males [27]. Thresholds for diagnosing low HDL-C differ between the genders (<40 mg/dL in men and <50 mg/dL in women) [28]. A similar inverse relationship between HDL-C and incident cancer has been previously observed in a Chinese cohort study including 17,779 participants[29]. Furthermore, a study-level meta-analysis of 625,000 participants demonstrated a 36% lower incidence of cancer for every 10 mg/dl increase in HDL-C[11]. The fact that our study complements findings conducted in very different demographic groups and using different methodologies is reassuring.

The results of this study suggest the possibility that HDL-C may be prognostically useful in clinical practice beyond the calculation of CVD risk, particularly for women. The poor predictive value of HDL-C against CHD among the whole population in this study is consistent with results obtained by Mendelian randomization [6] that demonstrate that a causal relationship between low HDL-C and MI is unlikely. Our results are consistent with those of Tada *et al.* who conducted an observational study of subjects attending hospital in Japan and whose HDL-C was measured for any reason [10]. Out of a cohort of 43,368 patients, 429 were found to have 'extremely low' HDL-C (<15 mg/L), and mortality was greatest in this group. During the median 175 days follow up period, 106 patients in this group died. It is possible that infectious diseases are partially responsible for excess mortality in participants with very low HDL-C. Our study did not investigate this hypothesis, however recent findings from the Copenhagen

City Heart Study indicate that HDL-C <31 mg/dl (almost identical to our 'very low' HDL-C group) was associated with a HR of 1.75 for infectious disease compared with normal HDL-C[30]. This likely reflects the important roles of HDL in immunity and the modulations of HDL function during infectious and inflammatory states. HDL particles influence the activity of monocytes, macrophages, dendritic cells and T- and B- lymphocytes by altering the cholesterol content of lipid rafts[31]. HDL limits the potential for bacterial lipopolysaccharide to induce inflammatory reactions[31]. However, rapid reductions of HDL-C have been observed during acute infections[31], and inflammatory states can result in 'dysfunctional' HDL which can exert pro-inflammatory effects[32]. Similarly, cytokine release associated with the inflammatory response to tumours has been associated with reduced plasma concentrations of HDL-C[33].

Probably the remaining question is whether the relationship between low HDL-C and poor outcomes is causal, or whether low HDL-C occurs secondary to another condition, which results in morbidity and mortality. Rader and de Goma reviewed the causes of low HDL-C, which they divided into artifactual causes (e.g. assay interference by paraproteinemia), primary (monogenic) causes (e.g. ApoA-I deficiency or mutation, Tangier disease, heterozygous deficiency of ATP-binding cassette transporter A1 [ABCA1], lecithin cholesterol acyltransferase [LCAT] deficiency), all of which are uncommon and secondary causes (e.g. anabolic androgenic steroids, malignancy and idiosyncratic response to fibrates) [34]. In the recent study by Tada *et al.*, most cases of low HDL-C were attributable to secondary causes. As many as 80 (75%) of the causes of death were either from malignancies, inflammatory diseases, or major bleeding, in contrast to a relatively low mortality from CVD (10%) [10]. That observation is consistent with the finding of this study that low HDL-C was a better predictor of all-cause mortality and cancer mortality than it was for incident CHD in complete case analysis. However, the results of these studies may not be directly comparable in this respect because Tada *et al.* included in their analysis all patients found to have low HDL-C, whereas

in this study, participants with malignancy at baseline were not included in REGARDS and patients with CHD at baseline were excluded from the analysis. Thus, in contrast to the study by Tada *et al*, in which most of the very low cases of HDL-C were attributable to secondary causes, our study might be considered to focus on the lowest identifiable levels of primary low HDL-C. This is reflected in different cut-off points in the categorization of 'extremely low' HDL: <20 mg/dL in Tada *et al*, compared with 'very low' <30 mg/dl in this study, which resulted in 3.5% of our population being included in this group.

Recent findings suggest that the inverse relationship between HDL-C and triglycerides low HDL-C could be attributed to hypertriglyceridemia *via* augmentation of CETP activity [35]. Furthermore, the incidence of diabetes was much greater in participants with low and very low HDL-C, than those in the 'normal' category. The existence of dysfunctional, pro-inflammatory HDL-C in diabetes has been described elsewhere[36]. In this study, the prognostic effects of HDL-C in the whole study population were attenuated when the Cox-regression model 2 correcting for BMI was applied, and further attenuation was seen with Model 3, which corrects for diabetes (among other factors).

Our results suggest a protective effect of HDL-C against cancer. Several lines of investigation indicate that such an effect is biologically plausible. Apolipoprotein A-1, the constituent lipoprotein of HDL-C has been demonstrated to inhibit tumour development in mouse models of ovarian cancer[37] and melanoma[38] and L-5F, an apolipoprotein mimetic has been shown to inhibit tumor angiogenesis[39]. Other components of the cholesterol efflux / reverse cholesterol transport pathway may be involved in the regulation of malignancy. It has recently been demonstrated in mouse models that knockout of ATP-binding cassette transporters ABCA1 and ABCG1 is protective against melanoma growth and metastasis [40].

Few populations have a sufficient number of black participants to powerfully assess racial differences in the role of HDL and disease risk. A potentially new finding of this study is the

association of lower CHD risk in blacks with HDL-C levels <40 mg/dl, thus apparently exhibiting an *'HDL paradox'*. These results, however, should be interpreted with caution, especially as the relatively small numbers of participants in the racial subgroups of patients with low HDL-C weakened the statistical power of these analyses. Nevertheless, it should be noted that the phenomenon was observed after both complete case analysis and MICE, reducing the likelihood of an artifactual finding. This is an interesting finding that requires further investigation, perhaps in other large cohorts and registries. Racial differences in the prognostic utility of HDL-C in CVD risk prediction have been observed before, and a similar paradoxical effect of very low HDL-C has been observed in Asians [41]. A previous analysis of data from black participants in the REGARDS study failed to find an association between low HDL-C (defined as <40 mg/dl in men or <50 mg/dl in women) and incident CHD [42]. Trends indicating similar paradoxically protective effects of 'low' or 'extremely low' HDL-C were seen in other subgroups (all-cause mortality in males and whites; cancer mortality in males; and incident CHD in males). None of these latter trends were statistically significant, however it is possible that the study was underpowered to detect such effects because of the relatively small number of participants in the 'low' and 'extremely low' HDL-C categories. Differences in outcomes in different racial groups may reflect varying prevalence of genetic traits relating to HDL-C. Associations of HDL-C, CVD, and genetic variants have been discussed elsewhere [43, 44] and recently a rare variant of the scavenger receptor B1 has been found to be associated with increased HDL-C and an increased risk of CHD [7]. Further investigations of this type may open the possibility for personalized risk prediction after genetic testing.

Study strengths and limitations

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, cannot demonstrate causality and are vulnerable to bias by unknown or unmeasured factors. In this analysis, we have performed both complete case and MICE analysis in order to address these difficulties. Complete case analysis has the limitation that it can underestimate risks in some subgroups. MICE analysis assumes that the probability a variable is missing depends only on observed covariates is based on the missing-at-random assumption and is sensitive to departures from this assumption, increasingly so with larger amounts of missing data [45]. Thus, we have provided both analyses for comparison and for completeness.

In common with many other studies, our analyses are based upon measurement of plasma lipids at a single point in time, and could be confounded by undiagnosed disease (particularly malignancies) at baseline. LDL-C in this study was calculated by the Friedewald equation, and therefore likely represents the sum of LDL-C, lipoprotein (a) and intermediate-density lipoprotein cholesterol [19]. Greater precision could have been obtained by direct measurement of LDL-C, however as the focus of this study was HDL-C, this would be unlikely to affect our findings. Other investigators have described a phenomenon of falsely low measurements of HDL[46], although such artifactual errors are likely to be rare. However, even with this potential measurement error the associations discussed were sufficiently strong for detection; and importantly we do not know why measurement error would differ between racial groups, and as such would not affect the interesting observations with respect to race. In this investigation, similar to the results of other investigators [10], there was a very uneven distribution of patients across the categories of HDL-C, limiting statistical power, in particular in the very low category of HDL, and precluding the study of even lower categories of HDL-C. Since our analysis includes all available participants for effect estimation, we are unable to investigate the potential confounding of female-specific measures such as menopause or hormone replacement

therapy on the relationship between HDL-C and our endpoints. Our study focuses only on health outcomes associated with low HDL-C. Health outcomes associated with exceptionally high HDL-C will be investigated in future studies.

Conclusions

Low HDL-C was associated with increased risk for all-cause mortality, cancer mortality, and incident CHD in a minimally-adjusted model, however the effect was attenuated in fully-adjusted model. When complete case analysis was used, for all three outcomes considered, the sex-HDL-C interaction was significant with poorer outcomes associated with low HDL-C in women than men. Further, the relationship with cancer mortality appears to be specific to women. Using both complete case analysis and MICE, we observed the existence of an '*HDL paradox'*, whereby low HDL-C associated with lower risk of incident CHD was observed in black participants of the REGARDS study.

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Table 1: Hazard ratios (HRs) describing the association between HDL-C category and risk of all-cause mortality, mortality from malignant disease and incident CHD by sex.

			Complete Case Model 1*		**Complete Case Model 4***		**MICE Model 4***	
			Female	*Male*	*Female*	*Male*	*Female*	*Male*
All-cause mortality	HDL Strata	<30 mg/dL	2.42 (1.64, 3.56)	1.35 (1.15, 1.59)	1.31 (0.88, 1.95)	0.90 (0.81, 1.07)	1.14 (0.83, 1.57)	0.79 (0.56, 1.11)
		30 - < 40 mg/dL	1.48 (1.27, 1.72)	1.04 (0.94, 1.15)	1.04 (0.89, 1.22)	0.90 (0.81, 1.00)	1.05 (0.91, 1.21)	0.88 (0.75, 1.04)
		>40 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*<0.0001		*p*=0.08		*p*=0.16	
Cancer-specific mortality	HDL Strata	<30 mg/dL	3.31 (1.87, 5.88)	1.13 (0.84, 1.54)	2.31 (1.28, 4.16)	0.88 (0.64, 1.21)	1.81 (1.03, 3.20)	0.44 (0.24, 0.83)
		30 - < 40 mg/dL	1.33 (1.01, 1.76)	1.07 (0.90, 1.27)	1.07 (0.80, 1.42)	0.97 (0.82, 1.16)	1.09 (0.83, 1.45)	0.86 (0.63, 1.19)
		>40 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*=0.003		*p*=0.014		*p*=0.033	
Incident CHD	HDL Strata	<30 mg/dL	2.75 (1.23, 6.18)	1.29 (0.89, 1.88)	1.57 (0.69, 3.58)	0.82 (0.55, 1.22)	1.07 (0.60, 1.90)	0.92 (0.50, 1.70)
		30 - < 40 mg/dL	0.86 (0.58, 1.28)	1.29 (1.06, 1.57)	0.57 (0.38, 0.86)	1.04 (0.85, 1.28)	0.82 (0.63, 1.07)	1.23 (0.92, 1.63)
		>40 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*=0.041		*p*=0.008		*p*=0.35	

Abbreviations: HDL-C – high density lipoprotein cholesterol; CHD – coronary heart disease; MICE – Multiple Imputation by Chained Equations

*Model 1 estimates are adjusted for age, race and sex. Model 4 estimates are additionally adjusted for education level, income level, alcohol consumption, physical activity, current smoking, body mass index (BMI), diabetes, albumin-to-creatine ratio (ACR), estimated glomerular filtration rate (eGFR), use of statins, use of other lipid-lowering medications (fibrates/niacin), use of steroids, high sensitivity C-reactive protein (hsCRP), low density lipoprotein cholesterol (LDL-C), and triglycerides.

Table 2. Hazard ratios (HRs) describing the association between HDL-C category and risk of all-cause mortality, mortality from malignant disease and incident CHD by race.

			Complete Case Model 1*		**Complete Case Model 4***		**MICE Model 4***	
			Black	*White*	*Black*	*White*	*Black*	*White*
All-cause mortality	HDL Strata	<30 mg/dL	1.67 (1.26, 2.22)	1.43 (1.20, 1.70)	1.19 (0.89, 1.59)	0.88 (0.74, 1.06)	0.96 (0.72, 1.28)	0.96 (0.82, 1.12)
		30 - < 40 mg/dL	1.15 (1.00, 1.32)	1.15 (1.04, 1.27)	0.93 (0.81, 1.07)	0.94 (0.84, 1.05)	0.93 (0.81, 1.07)	0.99 (0.90, 1.10)
		>40 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*=0.64		*p*=0.21		*p*=0.61	
Cancer-specific mortality	HDL Strata	<30 mg/dL	1.43 (0.82, 2.50)	1.32 (0.97, 1.80)	1.11 (0.63, 1.95)	0.99 (0.72, 1.37)	1.03 (0.58, 1.81)	0.93 (0.66, 1.31)
		30 - < 40 mg/dL	1.17 (0.91, 1.49)	1.13 (0.95, 1.35)	1.05 (0.82, 1.34)	0.98 (0.81, 1.18)	0.97 (0.72, 1.32)	1.00 (0.84, 1.19)
		>40 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*=0.95		*p*=0.87		*p*=0.98	
Incident CHD	HDL Strata	<30 mg/dL	1.18 (0.58, 2.39)	1.51 (1.02, 2.22)	0.82 (0.40, 1.68)	0.92 (0.61, 1.39)	0.76 (0.50, 1.17)	1.06 (0.83, 1.35)
		30 - < 40 mg/dL	0.81 (0.59, 1.12)	1.42 (1.16, 1.74)	0.63 (0.46, 0.88)	1.09 (0.88, 1.35)	0.76 (0.58, 0.98)	1.06 (0.91, 1.24)
		>40 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*=0.014		*p*=0.018		*p*=0.054	

Abbreviations: HDL-C – high-density lipoprotein cholesterol; CHD – coronary heart disease; MICE – Multiple Imputation by Chained Equations. * Model 1 estimates are adjusted for age, race and sex. Model 4 estimates are additionally adjusted for education level, income level, alcohol consumption, physical activity, current smoking, body mass index (BMI), diabetes, albumin-to-creatine ratio (ACR), estimated glomerular filtration rate (eGFR), use of statins, use of other lipid-lowering medications (fibrates/niacin), use of steroids,

FIGURES' LEGENDS:

Figure 1: Unadjusted Kaplan-Meier survival curves with estimates for survival probability for all-cause mortality for each of the three HDL-C categories.

Figure 2: Unadjusted Kaplan-Meier survival curves with estimates for survival probability for cancer mortality for each of the three HDL-C categories.

Figure 3: Unadjusted Kaplan-Meier survival curves with estimates for survival probability for incident coronary heart disease for each of the three HDL categories.

Supplementary Table 1. Baseline characteristics of participants by HDL category (n = 21751).

	HDL-C < 30 mg/dL	**HDL-C 30 - <40 mg/dL**	**HDL-C 40 + mg/dL**
Overall n (%)	748 (3.4)	4038 (18.6)	16965 (78.0)
Continuous Variables, Mean (±SD)			
Age (years)	65.2 (9.0)	64.5 (9.2)	64.6 (9.4)
Body Mass Index (kg/m^2)	30.6 (5.5)	30.5 (5.7)	28.9 (6.2)
Estimated GFR (CKD-EPI equation)	79.1 (21.8)	83.4 (19.9)	86.4 (19.3)
LDL-C (mg/dL)	99.4 (33.9)	111.4 (33.1)	116.5 (34.8)
Triglycerides (mg/dL)	197.0 (82.5)	157.5 (71.6)	114.2 (54.7)
Categorical Variables, N (Column %)			
Men	633 (84.6)	2854 (70.7)	6282 (37.0)
Black	179 (23.9)	1283 (31.8)	6997 (41.2)
Education			
Less than High School	94 (12.6)	477 (11.8)	1882 (11.1)
High School Graduate	172 (23.0)	1041 (25.8)	4374 (25.8)
Some College	209 (27.9)	1091 (27.0)	4540 (26.8)
College Graduate and More	273 (36.5)	1429 (35.4)	6169 (36.4)
Income			
Less than $20k	111 (14.8)	619 (15.3)	2894 (17.1)
$20k-$34k	168 (22.5)	945 (23.4)	4092 (24.1)
$35k-$74k	273 (36.5)	1330 (32.9)	5095 (30.0)
$75k and above	121 (16.2)	698 (17.3)	2858 (16.8)
Refused	75 (10.0)	446 (11.1)	2026 (11.9)
Alcohol Consumption	234 (31.3)	1411 (34.9)	6685 (39.4)
Physical Activity			
None	251 (33.6)	1378 (34.1)	5584 (32.9)
1-3 times per week	274 (36.6)	1455 (36.0)	6228 (36.7)
4 or more times per week	223 (29.8)	1205 (29.8)	5153 (30.4)
Current Smoking	128 (17.1)	680 (16.8)	2265 (13.4)
Diabetes	256 (34.2)	1071 (26.5)	2861 (16.9)
Fibrate Use	49 (6.6)	149 (3.7)	257 (1.5)
Statin Use	255 (34.1)	1449 (35.9)	5135 (30.3)
Other Lipid-lowering Medication Use	69 (9.2)	268 (6.6)	511 (3.0)
Steroid Use	18 (2.4)	112 (2.8)	577 (3.4)
Urinary Albumin/Creatinine Ratio>30 mg/g	154 (20.6)	680 (16.8)	2221 (13.1)
C-reactive protein			
< 1 mg/L	160 (21.4)	898 (22.2)	4714 (27.8)
1 - < 3 mg/L	236 (31.6)	1387 (34.4)	5695 (33.6)
3+ mg/L	352 (47.1)	1753 (43.4)	6556 (38.6)
LDL and TG Combination Category			
LDL <100, TG <150	129 (17.3)	933 (23.1)	4627 (27.3)
LDL ≥100, TG <150	120 (16.0)	1307 (32.4)	8934 (52.7)
LDL <100, TG ≥150	266 (35.6)	650 (16.1)	1002 (5.9)
LDL ≥100, TG ≥150	233 (31.2)	1148 (28.4)	2402 (14.1)

***Abbreviations:** HDL-C – high density lipoprotein cholesterol; GFR – glomerular filtration rate; CKD-EPI equation – chronic kidney disease – epidemiology collaboration equation; LDL-C – low density lipoprotein cholesterol; TG – triglycerides.

Supplementary Table 2. Number of events and populations for each HDL*race and HDL*sex group.

			Overall		***Black***		***White***		***Female***		***Male***	
			Number of Events / Population	*Crude Incidence Rates per 10,000 person-years follow-up*	*Number of Events / Population*	*Crude Incidence Rates per 10,000 person-years follow-up*	*Number of Events / Population*	*Crude Incidence Rates per 10,000 person-years follow-up*	*Number of Events/Population*	*Crude Incidence Rates per 10,000 person-years follow-up*	*Number of Events / Population*	*Crude Incidence Rates per 10,000 person-years follow-up*
All-cause mortality	**HDL Strata**	<30 mg/dL	192/748	357.2 (311.7, 409.4)	50/179	421.6 (321.7, 553.5)	142/569	339.0 (289.5, 397.0)	26/115	353.8 (242.7, 515.9)	166/633	357.8 (309.1, 414.1)
		30 - < 40 mg/dL	761/4038	260.7 (243.4, 279.4)	252/1283	282.7 (250.8, 318.6)	509/2755	251.1 (230.8, 273.3)	187/1184	229.6 (200.0, 263.8)	574/2854	272.8 (251.9, 295.4)
		>40 mg/dL	2557/16965	209.5 (201.8, 217.6)	1114/6997	229.5 (216.7, 243.0)	1443/9968	196.4 (186.7, 206.5)	1293/10683	169.7 (160.9, 178.9)	1264/6282	275.9 (261.5, 291.1)
	Overall Event		3510/21751	224.2 (217.1, 231.5)	1416/8459	241.4 (229.5, 254.0)	2094/13292	213.8 (205.1, 222.9)	1506/11982	177.0 (168.5, 185.9)	2004/9769	280.3 (268.6, 292.5)
Cancer-specific mortality	**HDL Strata**	<30 mg/dL	58/748	100.5 (77.8, 129.8)	13/179	103.9 (60.6, 178.2)	45/569	99.6 (74.5, 133.2)	12/115	145.1 (82.3, 255.9)	46/633	92.4 (69.3, 123.2)
		30 - < 40 mg/dL	255/4038	80.6 (71.4, 91.0)	81/1283	84.0 (67.8, 104.2)	174/2755	79.1 (68.2, 91.7)	56/1184	60.7 (46.7, 78.9)	199/2854	87.3 (76.0, 100.2)
		>40 mg/dL	843/16965	63.6 (59.5, 68.0)	348/6997	66.1 (59.5, 73.3)	495/9968	61.9 (56.7, 67.6)	426/10683	47.1 (42.6, 52.0)	417/6282	84.2 (76.6, 92.6)
	Overall Event		1156/21751	68.0 (64.2, 72.0)	442/8459	69.5 (63.4, 76.3)	714/13292	67.1 (62.4, 72.1)	494/11982	53.3 (48.8, 58.1)	662/9769	85.7 (79.4, 92.4)
Incident CHD	**HDL Strata**	<30 mg/dL	36/488	177.1 (144.2, 217.5)	8/134	138.0 (84.5, 225.5)	28/354	188.5 (150.3, 236.3)	6/82	154.2 (85.0, 279.7)	30/406	180.8 (145.2, 225.0)
		30 - < 40 mg/dL	182/3075	119.7 (107.6, 133.2)	44/1026	94.3 (76.0, 117.0)	138/2049	131.1 (116.0, 148.2)	26/966	71.0 (54.8, 91.9)	156/2109	139.1 (123.8, 156.4)
		>40 mg/dL	604/14192	81.7 (76.7, 86.9)	268/5932	84.2 (76.4, 92.9)	336/8260	80.0 (73.7, 86.8)	312/9305	59.4 (54.2, 65.2)	292/4887	119.1 (109.4, 129.6)
	Overall Event		822/17755	91.9 (87.2, 96.8)	320/7092	86.8 (79.5, 94.8)	502/10663	94.9 (89.0, 101.3)	344/10353	61.4 (56.3, 66.9)	478/7402	128.9 (120.7, 137.6)

**Crude incidence rates and 95% CI were estimated through modified Poisson regression (Poisson regression with robust standard error estimation)*

Supplementary Table 3. Hazard ratios (HRs) describing the association between HDL-C category and risk of all-cause mortality, mortality from malignant disease and incident CHD.

	HDL Strata	Model 1 Complete Cases	Model 2 Complete Cases	Model 3 Complete Cases	Model 4 Complete Cases	Model 4 MICE
All-cause mortality	<30 mg/dL	1.48 (1.28, 1.73)	1.25 (1.08, 1.46)	1.07 (0.92, 1.25)	0.99 (0.84, 1.16)	1.01 (0.87, 1.18)
	30 - < 40 mg/dL	1.15 (1.06, 1.25)	1.05 (0.96, 1.14)	0.98 (0.90, 1.07)	0.95 (0.87, 1.04)	0.99 (0.90, 1.08)
	> 40 mg/dL	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Cancer-specific mortality	<30 mg/dL	1.35 (1.03, 1.77)	1.19 (0.91, 1.57)	1.10 (0.83, 1.45)	1.02 (0.76, 1.36)	0.95 (0.69, 1.30)
	30 - < 40 mg/dL	1.14 (0.99, 1.32)	1.07 (0.92, 1.24)	1.03 (0.89, 1.20)	1.00 (0.86, 1.17)	0.96 (0.82, 1.13)
	> 40 mg/dL	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Incident CHD	<30 mg/dL	1.39 (0.99, 1.96)	1.10 (0.78, 1.56)	0.95 (0.67, 1.34)	0.90 (0.63, 1.30)	0.83 (0.66, 1.04)
	30 - < 40 mg/dL	1.19 (1.00, 1.41)	1.03 (0.86, 1.22)	0.97 (0.81, 1.15)	0.93 (0.78, 1.12)	0.89 (0.77, 1.03)
	> 40 mg/dL	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)

***Abbreviations:** HDL-C – high density lipoprotein cholesterol; CHD – coronary heart disease; MICE, Multiple Imputation with Chained Equations. Model 1 adjusts for age, race, and sex. Model 2 additionally adjusts for education level, income level, alcohol consumption, physical activity, current smoking, and body mass index (BMI); Model 3 additionally adjusts for diabetes, albumin-to-creatine ratio (ACR), estimated glomerular filtration rate (eGFR), use of statins, use of other lipid-lowering medications (fibrates/niacin), high sensitivity C-reactive protein (hsCRP), and steroid use; Model 4 additionally adjusts for LDL-C and triglycerides.

Supplemental Table 4: Hazard ratios (HRs) describing the association between HDL-C quintile and risk of all-cause mortality, mortality from malignant disease and incident CHD by race.

			Complete Case Model 1*		**Complete Case Model 4***		**MICE Model 4***	
			Black	*White*	*Black*	*White*	*Black*	*White*
All-cause mortality	HDL Quintile	1st 5-38 mg/dL	1.37 (1.16, 1.63)	1.39 (1.20, 1.60)	0.97 (0.82, 1.16)	0.89 (0.76, 1.04)	0.96 (0.81, 1.15)	0.98 (0.85, 1.11)
		2nd 39-45 mg/dL	1.16 (0.98, 1.38)	1.25 (1.07, 1.45)	0.93 (0.78, 1.11)	0.96 (0.82, 1.12)	0.92 (0.76, 1.10)	1.04 (0.91, 1.19)
		3rd 46-53 mg/dL	1.19 (1.01, 1.40)	1.17 (1.01, 1.36)	1.06 (0.90, 1.26)	0.97 (0.83, 1.13)	0.99 (0.83, 1.19)	1.01 (0.88, 1.15)
		4th 54-64 mg/dL	1.06 (0.90, 1.25)	1.06 (0.91, 1.24)	1.05 (0.89, 1.24)	0.95 (0.81, 1.11)	1.02 (0.85, 1.24)	0.96 (0.84, 1.10)
		5th 65-166 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*=0.94		*p*=0.66		*p*=0.79	
Cancer-specific mortality	HDL Quintile	1st 5-38 mg/dL	1.46 (1.06, 2.01)	1.28 (1.01, 1.64)	1.19 (0.86, 1.66)	1.01 (0.77, 1.31)	1.26 (0.91, 1.74)	0.95 (0.75, 1.22)
		2nd 39-45 mg/dL	1.46 (1.08, 1.98)	1.10 (0.85, 1.42)	1.27 (0.93, 1.73)	0.98 (0.75, 1.27)	1.15 (0.83, 1.61)	1.04 (0.82, 1.32)
		3rd 46-53 mg/dL	1.34 (0.99, 1.80)	1.08 (0.84, 1.39)	1.26 (0.93, 1.71)	0.99 (0.76, 1.28)	1.17 (0.83, 1.61)	0.99 (0.78, 1.25)
		4th 54-64 mg/dL	1.14 (0.84, 1.55)	1.01 (0.78, 1.31)	1.10 (0.81, 1.50)	0.95 (0.73, 1.24)	1.15 (0.81, 1.63)	0.95 (0.75, 1.21)
		5th 65-166 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*=0.67		*p*=0.72		*p*=0.76	
Incident CHD	HDL Quintile	1st 5-38 mg/dL	1.22 (0.81, 1.83)	1.84 (1.35, 2.49)	0.77 (0.50, 1.17)	1.03 (0.74, 1.44)	0.81 (0.59 (1.11)	1.30 (1.03, 1.63)
		2nd 39-45 mg/dL	1.59 (1.10, 2.29)	1.62 (1.18, 2.21)	1.12 (0.77, 1.62)	1.09 (0.78, 1.51)	0.99 (0.71, 1.37)	1.28 (1.01, 1.60)
		3rd	1.36 (0.95, 1.96)	1.56 (1.14, 2.12)	1.07 (0.74, 1.55)	1.14 (0.83, 1.57)	1.01 (0.73., 1.40)	1.28 (1.01, 1.60)

		46-53 mg/dL						
		4th 54-64 mg/dL	1.63 (1.15, 2.31)	1.06 (0.75, 1.49)	1.48 (1.04, 2.10)	0.89 (0.63, 1.25)	1.43 (1.01, 2.02)	1.01 (0.79, 1.29)
		5th 65-166 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*=0.009		*p*=0.013		*p*=0.005	

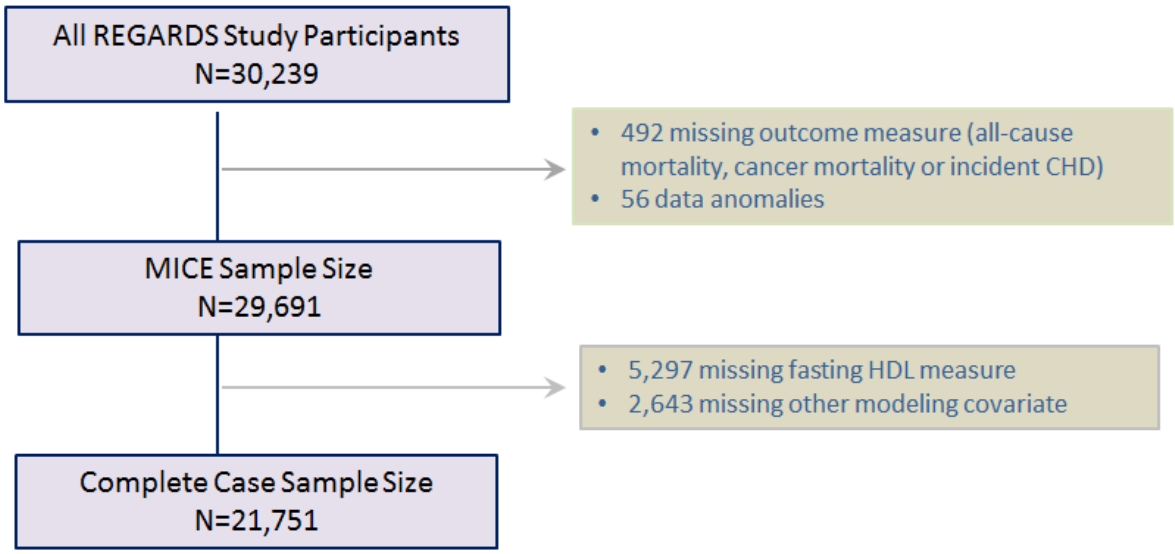
Abbreviations: HDL-C – high density lipoprotein cholesterol; CHD – coronary heart disease; MICE – Multiple Imputation by Chained Equations. *Model 1 estimates are adjusted for age, race and sex. Model 4 estimates are additionally adjusted for education level, income level, alcohol consumption, physical activity, current smoking, body mass index (BMI), diabetes, albumin-to-creatinine ratio (ACR), estimated glomerular filtration rate (eGFR), use of statins, use of other lipid-lowering medications (fibrates/niacin), use of steroids, high sensitivity C-reactive protein (hsCRP), low density lipoprotein cholesterol (LDL-C), and triglycerides.

Supplemental Table 5: Hazard ratios (HRs) describing the association between HDL-C quintile and risk of all-cause mortality, mortality from malignant disease and incident CHD by sex.

			Complete Case Model 1*		**Complete Case Model 4***		**MICE Model 4***	
			Female	*Male*	*Female*	*Male*	*Female*	*Male*
All-cause mortality	HDL Quintile	1st 5-38 mg/dL	1.87 (1.57, 2.24)	1.02 (0.87, 1.20)	1.12 (0.93, 1.35)	0.74 (0.62, 0.88)	1.12 (0.96, 1.30)	0.74 (0.61, 0.90)
		2nd 39-45 mg/dL	1.42 (1.21, 1.66)	0.91 (0.77, 1.08)	1.09 (0.92, 1.28)	0.76 (0.64, 0.91)	1.14 (1.00, 1.30)	0.74 (0.61, 0.90)
		3rd 46-53 mg/dL	1.38 (1.19, 1.59)	0.87 (0.74, 1.04)	1.16 (1.00, 1.35)	0.80 (0.67, 0.95)	1.09 (0.96, 1.23)	0.80 (0.66, 0.97)
		4th 54-64 mg/dL	1.12 (0.97, 1.29)	0.89 (0.74, 1.06)	1.05 (0.90, 1.21)	0.85 (0.71, 1.02)	0.97 (0.85, 1.09)	0.98 (0.80, 1.19)
		5th 65-166 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*<0.0001		*p*=0.003		*p*=0.001	
Cancer-specific mortality	HDL Quintile	1st 5-38 mg/dL	1.95 (1.43, 2.65)	0.94 (0.71, 1.23)	1.46 (1.05, 2.03)	0.79 (0.59, 1.06)	1.34 (1.01, 1.78)	0.61 (0.43, 0.87)
		2nd 39-45 mg/dL	1.74 (1.33, 2.27)	0.80 (0.60, 1.07)	1.51 (1.14, 1.99)	0.74 (0.55, 1.00)	1.47 (1.15, 1.88)	0.56 (0.40, 0.78)
		3rd 46-53 mg/dL	1.38 (1.07, 1.79)	0.84 (0.63, 1.13)	1.26 (0.97, 1.65)	0.82 (0.61, 1.11)	1.21 (0.96, 1.53)	0.68 (0.49, 0.96)
		4th 54-64 mg/dL	1.09 (0.84, 1.41)	0.89 (0.65, 1.22)	1.04 (0.80, 1.35)	0.86 (0.63, 1.18)	1.03 (0.82, 1.29)	0.91 (0.64, 1.29)
		5th 65-166 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*<0.0002		*p*=0.002		*p*=0.002	
Incident CHD	HDL Quintile	1st 5-38 mg/dL	1.33 (0.84, 2.11)	1.32 (0.94, 1.86)	0.71 (0.44, 1.15)	0.84 (0.58, 1.20)	1.17 (0.88, 1.55)	0.92 (0.64, 1.31)
		2nd 39-45 mg/dL	1.85 (1.32, 2.60)	1.21 (0.85, 1.73)	1.22 (0.86, 1.73)	0.89 (0.62, 1.28)	1.46 (1.16, 1.85)	0.72 (0.51, 1.01)
		3rd 46-53 mg/dL	1.93 (1.42, 2.62)	0.98 (0.68, 1.43)	1.43 (1.04, 1.95)	0.79 (0.54, 1.15)	1.44 (1.15, 1.81)	0.74 (0.52, 1.04)
		4th 54-64 mg/dL	1.57 (1.15, 2.13)	0.93 (0.63, 1.39)	1.33 (0.98, 1.82)	0.85 (0.57, 1.27)	1.29 (1.03, 1.61)	0.80 (0.56, 1.15)

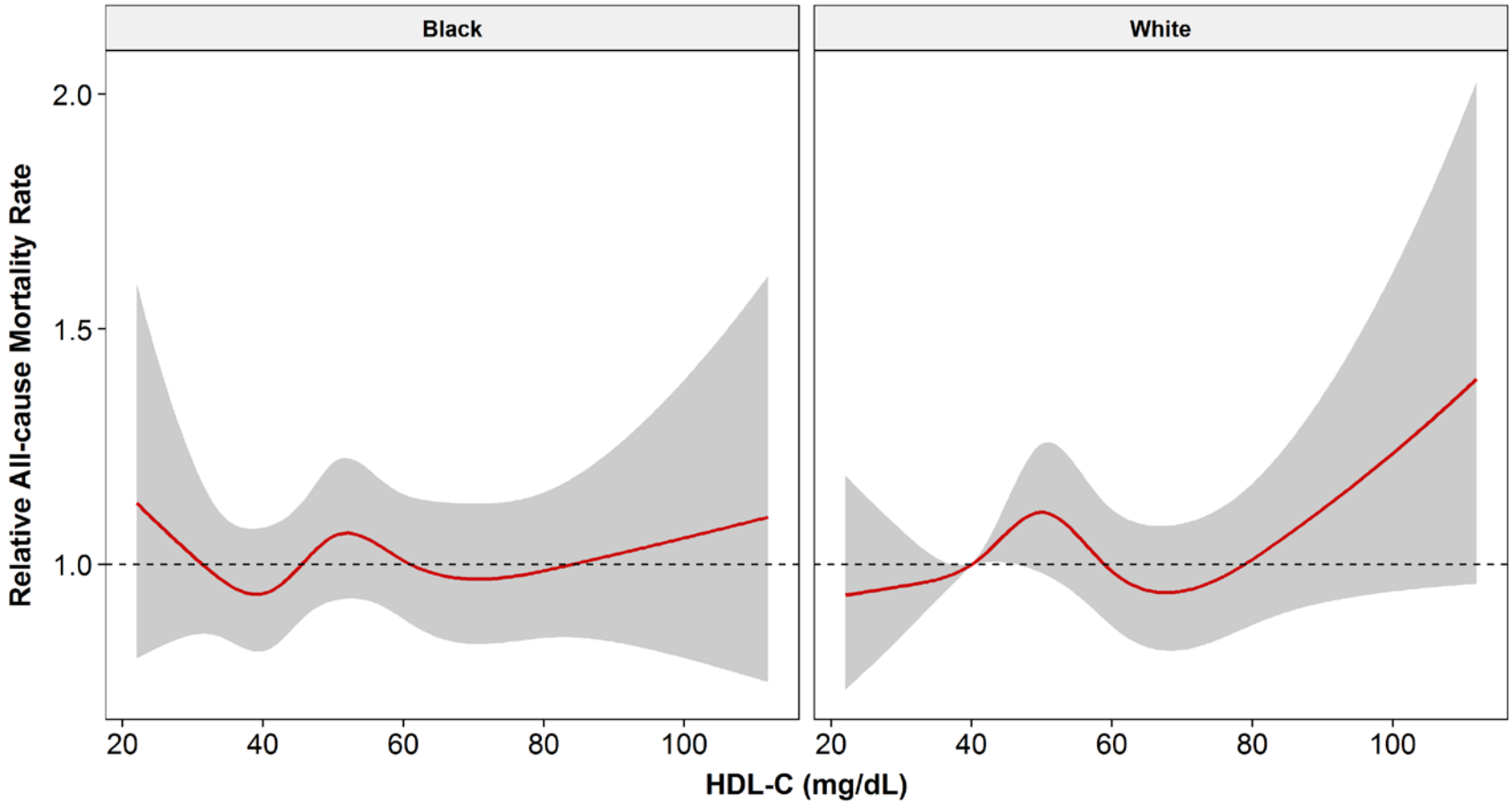
		5th 65-166 mg/dL	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	p for interaction		*p*=0.024		*p*=0.022		*p*=0.22	

Abbreviations: HDL-C – high density lipoprotein cholesterol; CHD – coronary heart disease; MICE – Multiple Imputation by Chained Equations. * Model 1 estimates are adjusted for age, race and sex. Model 4 estimates are additionally adjusted for education level, income level, alcohol consumption, physical activity, current smoking, body mass index (BMI), diabetes, albumin-to-creatinine ratio (ACR), estimated glomerular filtration rate (eGFR), use of statins, use of other lipid-lowering medications (fibrates/niacin), use of steroids, high sensitivity C-reactive protein (hsCRP), low density lipoprotein cholesterol (LDL-C), and triglycerides.

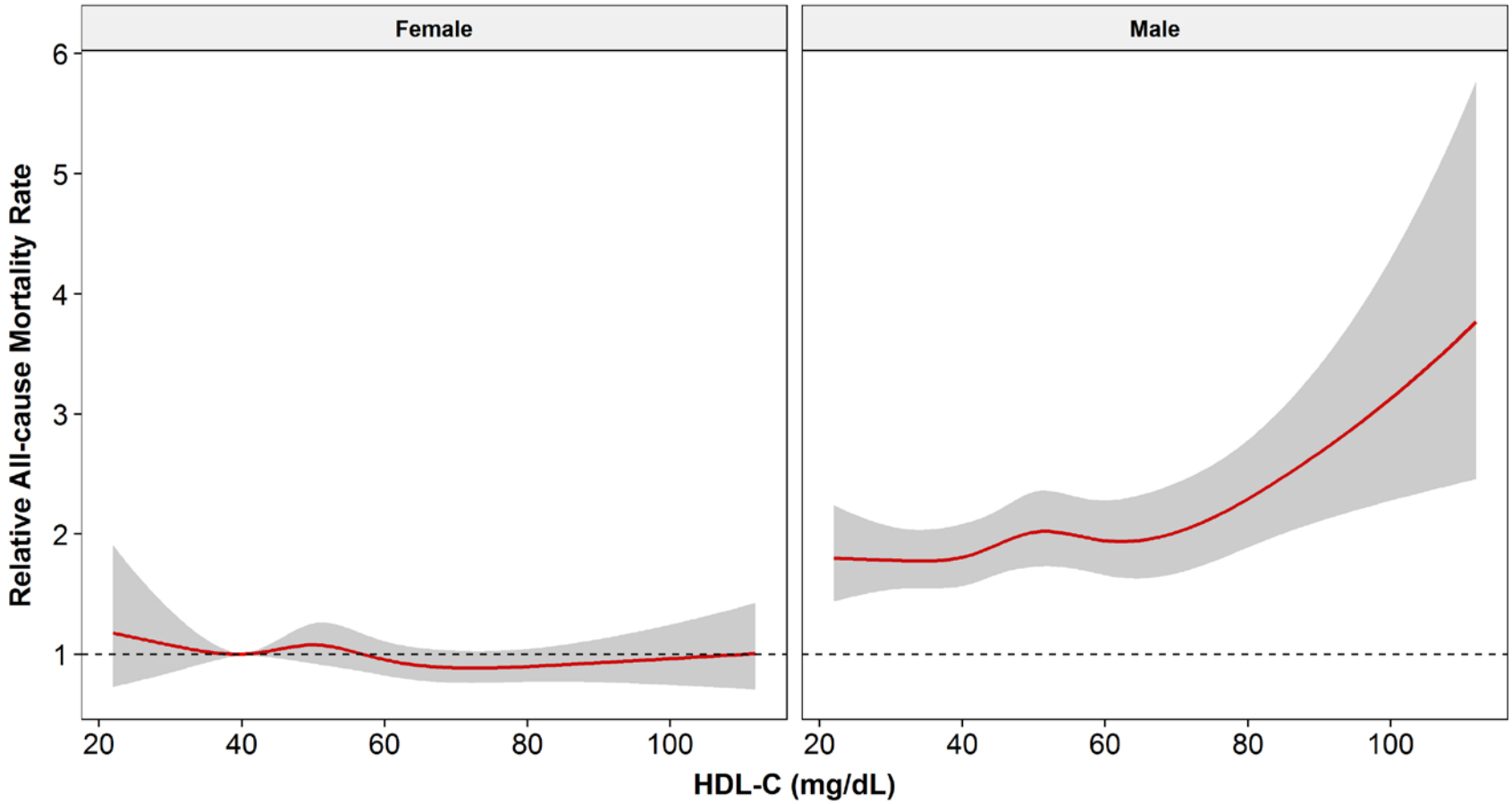


Supplementary Figure 1. The final flow-chart of the sample size.

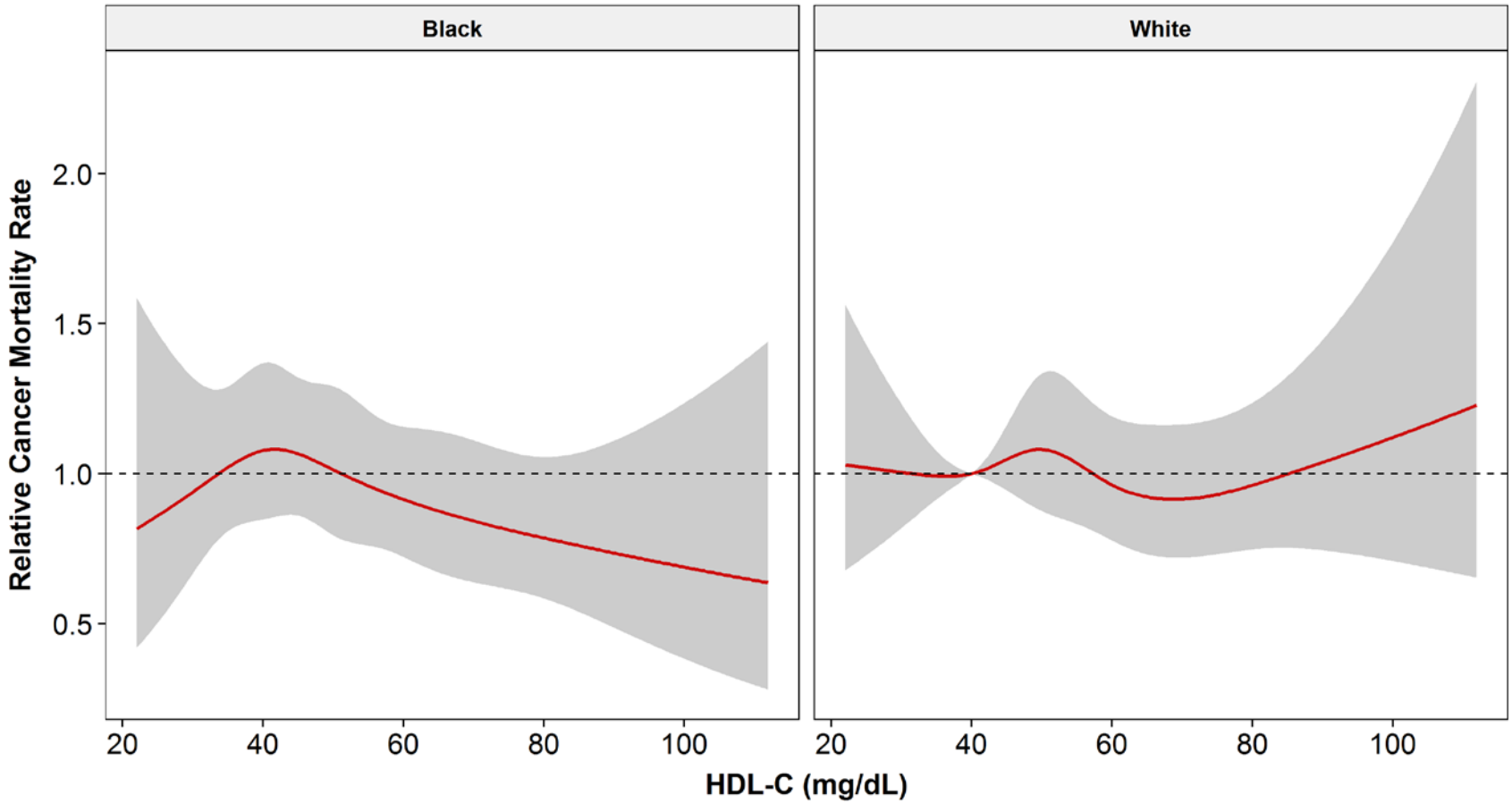
Supplemental Figure 2: Adjusted all-cause mortality ratios using HDL-C restricted cubic splines by race, relative to white participants with HDL-C of 40 mg/dL.



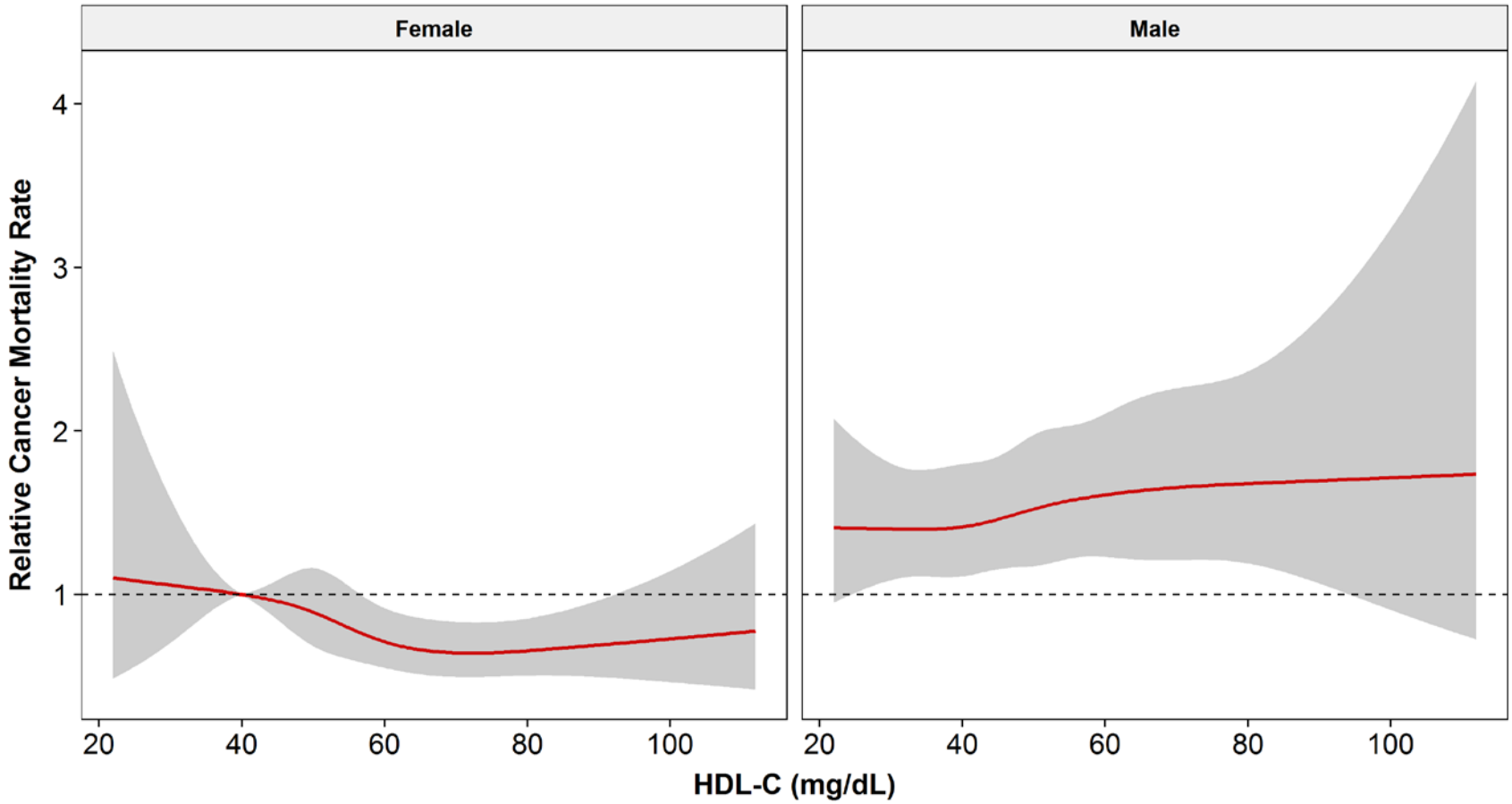
Supplemental Figure 3: Adjusted all-cause mortality ratios using HDL-C restricted cubic splines by sex, relative to female participants with HDL-C of 40 mg/dL.



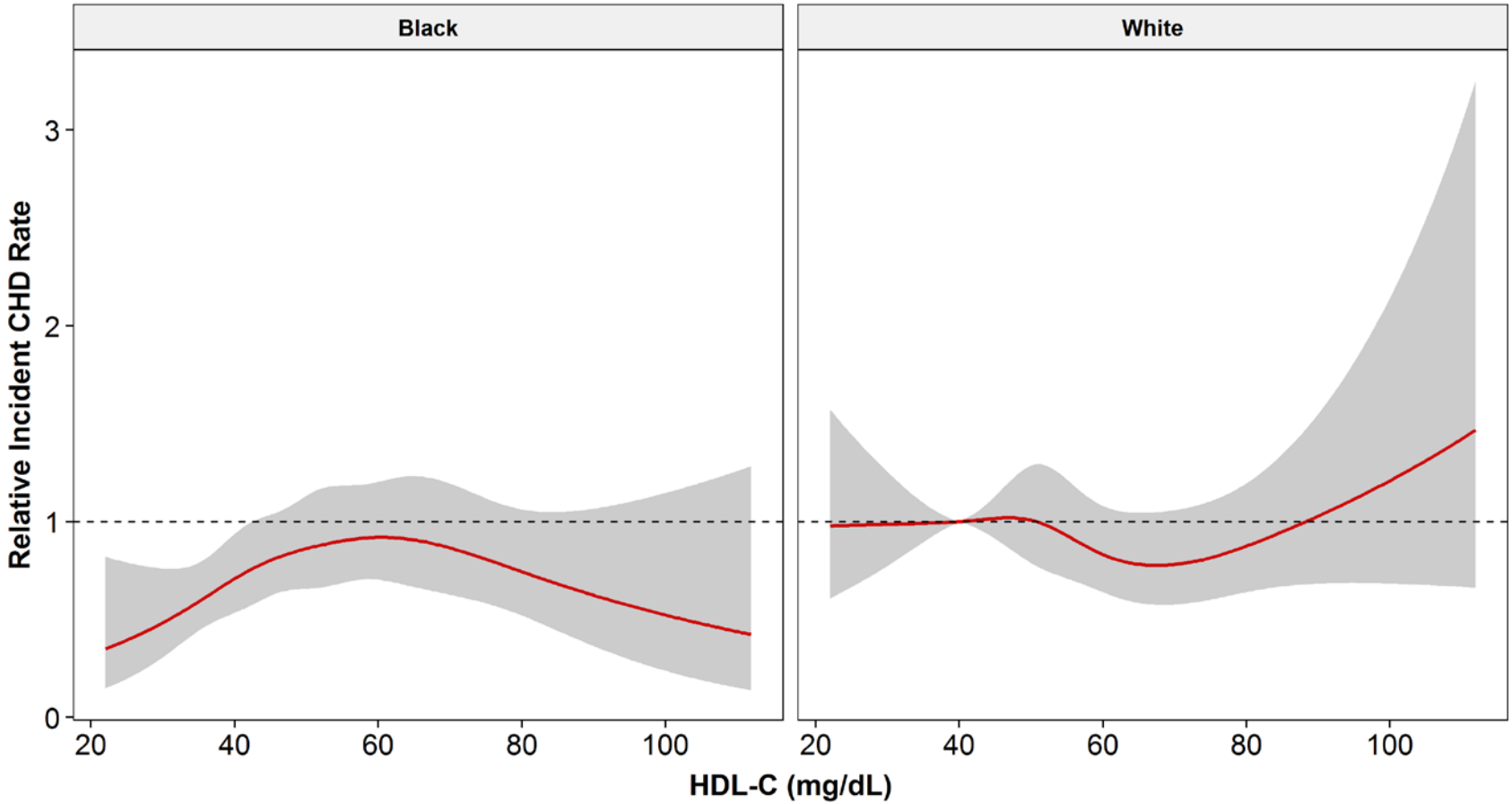
Supplemental Figure 4: Adjusted cancer mortality ratios using HDL-C restricted cubic splines by race, relative to white participants with HDL-C of 40 mg/dL.



Supplemental Figure 5: Adjusted cancer mortality ratios using HDL-C restricted cubic splines by sex, relative to female participants with HDL-C of 40 mg/dL.



Supplemental Figure 6: Adjusted incident CHD ratios using HDL-C restricted cubic splines by race, relative to white participants with HDL-C of 40 mg/dL.



Supplemental Figure 7: Adjusted incident CHD ratios using HDL-C restricted cubic splines by sex, relative to white participants with HDL-C of 40 mg/dL.