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Associations between risk of overall mortality, cause-specific mortality and level of inflammatory factors with extremely low and high high-density lipoprotein cholesterol levels among American adults

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ABSTRACT

Background: The health outcomes associated with extremely low or high plasma concentrations of high-density lipoprotein cholesterol (HDL-C) are not well documented mainly because of the small numbers of participants with such values included in the clinical trials.
Objective: We prospectively investigated the association between extremely low and high HDL-C with: 1) the risk of overall, coronary heart disease (CHD), cerebrovascular and cancer mortality, and, 2) their link with inflammatory factors.
Methods: Analysis was based on subjects ≥18 years old from the National Health and Nutrition Examination Surveys (NHANES). We categorized HDL-C levels as follows: [low HDL-C group ≤30 (extremely low), 30–40 (low), and ≥40 (reference)] [high HDL-C group = 40–80 (reference), 80–100 (high) and ≥100 mg/dl (extremely high). Cox proportional hazard regression models and analysis of covariance accounted for survey design, masked variance and sample weights.
Results: After adjustment for age, race and sex, we found that the very low HDL-C category (<30 mg/dl) had a greater risk of total mortality (risk ratio [RR]: 3.00, 95%CI: 2.20–4.09). RR for CHD and stroke mortality was 2.00 and 2.53, respectively; there was no link between cancer and level of HDL-C ($p = 0.235$). The association between total mortality, CHD and stroke with the level of HDL-C attenuated but remained significant even after adjustment for demographics, dietary, cardiovascular risk factors and treatment for dyslipidemia (all $p < 0.001$). After adjustments, subjects with extremely high HDL-C levels had a higher risk of mortalities (all $p < 0.001$). Mexican-American ethnicity, subjects in the low level of HDL-C (30–40 mg/dl) category had higher risk of mortalities than those with a very low level (all $p < 0.001$). Concentration of C-reactive protein, fibrinogen and white blood count significantly decreased as the level of the HDL-C increased; these findings were robust after adjustment for demographics, dietary, cardiovascular risk factors and treatment for dyslipidemia (all $p < 0.001$); further subjects with extremely high HDL-C levels have a greater levels of inflammatory factors (all $p < 0.001$).
Conclusions: Both extremely low and high HDL-C levels were associated with greater risk of mortalities (total, CHD and stroke) and higher level of inflammatory factors, while there was no link between level of HDL-C and risk of cancer. Moreover, we found evidence of an HDL-C paradox in Mexican-American ethnicity participants.

1. Introduction

An inverse correlation between plasma high density lipoprotein cholesterol (HDL-C) concentrations and cardiovascular (CV) risk has been known for several decades [1]. This link was initially attributed to reverse cholesterol transport via HDL [2]. However, recent trials (e.g. with niacin or cholesteryl ester transfer protein [CETP] inhibitors) failed to demonstrate that raising HDL-C levels decreases vascular events [3]. Furthermore, genetic studies do not support the concept that high HDL-C levels reduce the risk of myocardial infarction (MI) [4]. Clearly, the relationship between HDL-C levels and the risk of CV events is more complex than initially thought.

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Currently, little is known about CV risk in patients with very low HDL-C levels (<30 mg/dl; 0.78 mmol/l) [5,6]. Regarding very high levels of the HDL-C, pharmacological intervention was not beneficial in randomized clinical trials [7]; actually, there was even increased mortality in 1 study [8]. Some genetic variants associated with higher concentrations of HDL-C have also been associated with a high risk of CV events [9–11]. For example, a recent Danish population-based study reported that the association between HDL-C concentrations and all-cause mortality was U-shaped; both extreme high and low concentrations were associated with high all-cause mortality risk [12,13]. However, as the authors mention, more studies are needed especially since they only included white individuals of Danish descent [12]. They also mentioned low statistical power and extreme high HDL-C groups were small [12].

Because only a minority have extremely high HDL-C concentrations [14] they are often grouped together with others with moderately high HDL-C levels [15]. Most observational studies investigating the association between HDL-C and mortality have categorized individuals in larger groups, such as quintiles focusing on low HDL-C levels [12].

We have therefore decided to analyse data from the National Health and Nutrition Examination Surveys (NHANES), which is nationally representative, to test the hypothesis that subjects with either very/extremely low HDL-C (<30 mg/dl) and extremely high HDL-C (>100 mg/dl) have greater risk of all and cause specific mortality as well as higher level of inflammatory factors.

2. Methods

2.1. Population

We used data from participants of the NHANES; these are repeated cross-sectional surveys conducted on an ongoing basis in the USA by the United States National Center for Health Statistics (NCHS). In these surveys, multistage probabilistic sampling strategies are used to select participants, with where appropriate, oversampling of certain segments of the population [16]. Questionnaires administered during home visits recorded demographics, diets and behaviours. Trained staff using mobile examination units collected anthropometric and biomarker data [16,17]. The NCHS research ethics review board approved the underlying protocol. The NHANES survey design, questionnaires and procedures are described elsewhere [18–21].

For the present study, we used NHANES data collected between 1999 and 2010. We have included data from subjects aged ≥18 years in the analyses. The following cardiometabolic risk factors and inflammation markers were included in the study: C-reactive protein (CRP), white blood count (WBC), fibrinogen, alcohol consumption, physical activity, smoking, fasting blood glucose (FBG), glomerular filtration rate (GFR), statin use, body mass index (BMI), triglyceride (TG), HDL-C and low density lipoprotein cholesterol (LDL-C).

For the assessment of height and weight during the physical examination, participants were dressed in underwear, disposable paper gowns and foam slippers. A digital scale was used to measure weight to the nearest 100 g, a fixed stadiometer was used to measure height to the nearest mm. BMI was calculated as weight in kg divided by the square of height in m [22]. A blood sample was drawn from an antecubital vein. Total cholesterol and TGs were measured using enzymatic reactions [22,23]. HDL-C was measured by the direct immunoassay method during 2007–2010, whereas in 1999–2002, the heparin manganese precipitation method was primarily used. The Friedewald equation (LDL-C = TC − [HDL-C + triglycerides/5]) was used to calculate LDL-C for adults whose triglyceride level was less than or equal to 400 mg/dl. Standardization of serum lipid measurements was performed according to the criteria of the CDC's Lipid Standardization Program [22,23]. FBG was measured by a hexokinase method using a Roche/Hitachi 911 Analyzer and Roche Modular P Chemistry Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). CRP and fibrinogen levels were measured with latex enhanced nephelometry (Seattle, USA) and Coagamate XC Plus automated coagulation analyzer (Organon Teknika, Durham, NC), respectively [23]. Creatinine was measured by the Jaffe reaction and standardized by methods described previously [24]. A random urine specimen was collected from participants, and urinary creatinine was measured by the Jaffe rate reaction, urinary albumin was measured by solid-phase fluorescent immunoassay [25]. GFR, (ml/min/1.73 m^2) was estimated using the CKD Epidemiology Collaboration (CKD-EPI) equation [25]. Details on NHANES Laboratory/Medical Technologists Procedures and Anthropometry Procedures are described elsewhere [18–21]. Diabetes defined as a self-reported history of diabetes or FBG ≥126 mg/dl [26]. Smoking status was self-reported and participants classified as current smoker or not. Metabolic equivalent of task (MET) was used to measure the intensity level of physical activity and indicated the rate of energy consumption for a specific activity. A MET is defined as 1 kcal/kg/h that is roughly equal to the energy cost of being at rest [27]. Use of statins, was based on medication inventory. Poverty to income variable is an index for the ratio of family income to poverty. The Department of Health and Human Services' (HHS) poverty guidelines were used as the poverty measure to calculate this index. These guidelines are issued each year, in the Federal Register, for determining financial eligibility for certain federal programs such as Head Start, Supplemental Nutrition Assistance Program (SNAP) (formerly Food Stamp Program), Special Supplemental Nutrition Program for Women, Infants, and Children (WIC), and the National School Lunch Program.

2.2. Mortality

The de-identified and anonymized data of NHANES 1999–2010 participants were linked to longitudinal Medicare and mortality data using the NHANES assigned sequence number. Mortality follow-up data are available from the date of survey participation until December 31, 2011. We examined all-cause mortality, as well as mortality due to coronary heart diseases (CHD) (I00-I09, I11, I13, I20-I51), cancer (C00-C97) and cerebrovascular disease (I60-I69). Cause of death was determined using the 10th revision of the International Classification of Diseases (ICD-10).

2.3. Statistical analysis

All the analyses followed the guidelines set by the Centers for Disease Control and Prevention for analysis of the NHANES dataset, accounting for the masked variance and using their suggested weighting methodology [28]. HDL-C categories were defined into the following categories: low level = 1) "very/extremely low" level HDL-C < 30 mg/dl, 2) "low" level HDL-C 30 ≤ 40 mg/dl, and 3) "reference" level HDL-C ≥ 40 mg/dl. High level = 1) "reference" level HDL-C 50–80 mg/dl, 2) "high" level HDL-C 80–100 mg/dl, 3) "extremely high" level HDL-C ≥ 100 mg/dl. For each category of HDL-C (low vs. high) we have ran separate model with different reference. For the low level HDL-C ≥ 40 mg/dl was considered as a reference. For the high level HDL-C 50–80 mg/dl was considered as a reference.

To assess the association between the HDL-C categories and each outcome, we used a series of Cox regression models: Model 1: adjustment for age, race and sex; Model 2: additional adjustment for SES (income to poverty ratio and education level), alcohol consumption, physical activity, smoking and BMI; Model 3: additional adjustment for diabetes, GFR, statin use, and Model 4: additional adjustment for LDL-C and TG. By applying analysis of co-variance (ANCOVA) we have used the same 4 models for the evaluation of the changes in the inflammatory factors across the HDL-C categories. SAS 9.4 (SAS Institute, Inc.) and R [29] were used for all the analyses. All tests were two-sided; a $p \leq 0.05$ was the level of significance.

3. Results

3.1. Baseline characteristics

We included 25,541 subjects in the study; 48.0% of them were males, 48.8 years was mean age for the overall population (49.2 and 48.4 years for males and females, respectively: $p = 0.025$). With respect to HDL-C, 4.0% participants were in the "extremely low" (<30 mg/dl) HDL-C category; 18.7% in the "low" (30–39.9 mg/dl) and 77.3% were in the ≥40 mg/dl category (reference). Further 88.9% participants were in the 50–80 mg/dL HDL-C category (reference); 9.1% in the 80–100 mg/dl (high), and 2.1% were in the "extremely high" (≥100 mg/dl) category. Table 1 lists the baseline characteristics for the population (stratified by HDL-C category). In "extremely low" level of HDL-C (<30 mg/dl) the majority were males (77.9 vs. 22.1%), whereas in reference level of HDL-C (≥40 mg/dl) females were the higher proportion (58.0 vs. 42.0%, $p < 0.001$, Table 1). For the "extremely high" group (≥100 mg/dl) women consisted the majority compared with men (74.1 vs. 25.9%, $p < 0.001$). With regard to the "low" group of HDL-C, reference level of HDL-C (≥40 mg/dl) mostly consisted of subjects with "more than high school" level of education ("less than high school": 28.2, "completed high school": 23.2, "more than high school": 48.4%, $p < 0.001$), while subjects with lower level of education - "less than high school" - were the majority of subjects in "extremely low" level of HDL-C (<30 mg/dl, $p < 0.001$, Table 1). With regard to high levels of HDL-C categories, subjects were distributed with no particular order. Participants in the "low" (30–39.9 mg/dl) and "extremely low" (<30 mg/dl) HDL-C categories were more likely to be younger, have lower LDL-C levels, and lower consumption of alcohol, whereas they had a higher BMI, GFR, CRP and TG (all $p < 0.001$, Table 1).

3.2. Extremely low and high HDL-C with inflammatory factors

We have calculated adjusted mean of inflammatory factors (CRP, WBC and fibrinogen) across the low level HDL-C categories (Table 2).

Table 1
Demographic characteristics of the population based on the HDL-C categories.

Variables		Low HDL-C (mg/dL) categories			High HDL-C (mg/dl) categories			p
		≥40, Reference	30–39.9, Low	<29.9, Very low	50–79.9, Reference	80–99.9, High	≥100, Extremely high	
Sex	Male (%)	42.0	68.5	77.9	36.1	21.3	25.9	<0.001
	Female (%)	58.0	31.5	22.1	63.9	78.7	74.1	
Race/ethnicity	Mexican-American (%)	21.1	24.8	23.8	20.1	13.7	8.9	<0.001
	Other Hispanic (%)	10.3	11.5	11.7	6.6	3.9	0.9	
	Non-Hispanic White (%)	49.6	50.6	54.0	49.7	55.5	57.6	
	Non-Hispanic Black (%)	19.0	13.2	10.5	19.7	23.4	31.3	
Education	Less than high school (%)	28.2	34.5	39.0	27.2	23.1	23.6	<0.001
	Completed high school (%)	23.2	25.2	25.0	22.5	22.0	21.9	
	More than high school (%)	48.4	40.0	34.9	50.1	54.7	54.5	
Age (years)		47.5 ± 0.2	47.0 ± 0.1	45.9 ± 0.5	47.1 ± 0.2	51.5 ± 0.1	53.2 ± 0.5	<0.001
Income to poverty		2.7 ± 0.02	2.4 ± 0.01	2.3 ± 0.01	2.7 ± 0.01	2.7 ± 0.02	2.8 ± 0.02	<0.001
Alcohol consumption (g/day)		10.1 ± 1.1	8.5 ± 0.8	8.6 ± 0.9	10.2 ± 1.2	10.3 ± 1.1	10.9 ± 0.9	<0.001
BMI (kg/m^2)		27.2 ± 1.7	30.0 ± 2.2	30.3 ± 1.1	27.2 ± 1.8	25.3 ± 2.3	24.1 ± 2.0	<0.001
eGFR (ml/min/1.73m^2)		95.2 ± 0.4	96.4 ± 0.6	98.1 ± 0.8	95.1 ± 0.9	96.3 ± 0.3	98.8 ± 0.9	<0.001
CRP (mg/dl)		0.38 ± 0.02	0.48 ± 0.01	0.51 ± 0.04	0.39 ± 0.01	0.42 ± 0.02	0.49 ± 0.01	<0.001
LDL-C (mg/dl)		225.5 ± 2.6	163.9 ± 3.2	98.2 ± 1.1	212.3 ± 1.0	236.9 ± 2.6	225.2 ± 3.2	<0.001
Triglycerides (mg/dl)		126.5 ± 5.2	216.7 ± 7.4	350.8 ± 6.2	116.2 ± 3.2	94.1 ± 2.9	90.3 ± 5.4	<0.001
Smoking (%)		20.1	21.5	21.9	20.3	21.0	20.9	<0.001

Value expressed as mean ± standard error mean or percent. BMI: body mass index, CRP: C-reactive protein, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, GFR: estimate glomerular filtration rate.

We applied 4 different models with varied confounders. In Model 1 both CRP and WBC level increased as HDL-C levels decreased (both $p < 0.001$, Table 2). For example, from reference group (≥40 mg/dl) level of HDL-C to the extremely low (<30 mg/dl) level of HDL-C, CRP changed from 0.38 to 0.60 mg/dl ($p < 0.001$). We observed the same trend with significant changes for the second, third and fourth models as well (all $p < 0.001$, Table 2). For example, in the fully adjusted model (Model 4) from reference group (≥40 mg/dl) level of HDL-C to extremely low (<30 mg/dl) level of HDL-C (<30 mg/dl), CRP changed from 0.39 to 0.49 mg/dl and WBC varied from 6.8 to 7.2 × 10^9 (both $p < 0.001$, Table 2).

We have run the same analysis for just part of our database with plasma fibrinogen values (n = 5690); changes for fibrinogen across the category of HDL-C were significant for all models (all $p < 0.001$, Table 2). For example, in Model 4, from reference group (≥40 mg/dl) of HDL-C to very low (<30 mg/dl) level of HDL-C, fibrinogen values changed from 354 to 372 mg/dl ($p < 0.001$, Table 2).

With regard to the high HDL-C levels, we have run an ANCOVA with same confounders as for low level HDL-C, Table 2. In Model 1 we have found that the levels of both CRP and WBC were at highest level in the extremely high (≥100 mg/dl) level of the HDL-C (both $p < 0.001$, Table 2). For example, from reference level of HDL-C (50–80 mg/dl) to extremely high (>100 mg/dl) level of the HDL-C, CRP changed from 0.35 to 0.46 mg/dl ($p < 0.001$). We had the same trend with significant changed for the second, third and fourth models as well (all $p < 0.001$ Table 2). For example, in the fully adjusted model (Model 4) from reference level of HDL-C (50–80 mg/dl) to extremely high level (>100 mg/dl) of HDL-C, CRP changed from 0.36 to 0.47 mg/dl and WBC varied from 6.9 to 7.2 × 10^9 as well (both $p < 0.001$, Table 2).

3.3. Extremely low and high HDL-C with total and cause-specific mortality

For each of our interested outcomes we ran 4 models with varied confounders by applying multivariable Cox regression (Table 3). In Model 1, adjusted for age, race and sex, we have found that the lowest category of HDL-C (<30 mg/dl) has greater risk of total mortality (RR: 3.00, 95%CI: 2.20–4.09), CHD (RR = 2.02, 95%CI: 1.49–2.68) and stroke (RR = 1.77, 95%CI: 1.21–2.59, Table 3). The association between HDL-C categories and total, CHD, and stroke mortality remained significant (but diluted) after adjustment for wide range of cofounders in model 4 (all $p < 0.001$, Table 3).

We also ran 4 Cox regression models with same confounders for the high level categories of the HDL-C (Table 3). When we compared the risk of mortalities between reference group (50–80 mg/dl) of HDL-C with high and extremely high level (>100 mg/dl) of the HDL-C, we found neither of the events have significant link with different level of

Table 2
Adjusted mean of inflammatory factors across the category of HDL-C.

Variables		Low HDL-C (mg/dl) categories			p	High HDL-C (mg/dl) categories			p
		≥40, Reference	30–39.9, Low	<30, Extremely low		50–79.9, Reference	80–99.9, High	≥100, Extremely high	
Model 1	CRP	0.38 ± 0.02	0.54 ± 0.01	0.60 ± 0.05	<0.001	0.35 ± 0.01	0.42 ± 0.01	0.46 ± 0.02	<0.001
	WBC	7.0 ± 1.1	7.7 ± 0.9	7.9 ± 0.8	<0.001	6.9 ± 0.8	7.2 ± 0.3	7.4 ± 1.3	<0.001
	Fibrinogen	368 ± 6	389 ± 3	394 ± 8	<0.001	363 ± 5	371 ± 3	3813 ± 7	<0.001
Model 2	CRP	0.40 ± 0.01	0.45 ± 0.05	0.47 ± 0.03	<0.001	0.36 ± 0.01	0.39 ± 0.04	0.43 ± 0.02	<0.001
	WBC	6.8 ± 1.2	7.3 ± 0.7	7.5 ± 1.7	<0.001	6.6 ± 1.0	6.9 ± 0.6	7.1 ± 1.9	<0.001
	Fibrinogen	363.2 ± 4.8	374.8 ± 6.8	377.4 ± 5.5	<0.001	360.1 ± 3.2	370.2 ± 5.9	373.4 ± 4.6	<0.001
Model 3	CRP	0.39 ± 0.04	0.44 ± 0.03	0.46 ± 0.7	<0.001	0.37 ± 0.03	0.40 ± 0.04	0.43 ± 0.8	<0.001
	WBC	6.8 ± 1.1	7.3 ± 1.2	7.6 ± 1.4	<0.001	6.8 ± 0.9	7.1 ± 1.9	7.3 ± 2.1	<0.001
	Fibrinogen	353 ± 9	364 ± 6	371 ± 6	<0.001	355 ± 8	357 ± 6	359 ± 4	<0.001
Model 4	CRP	0.39 ± 0.06	0.45 ± 0.04	0.49 ± 0.01	<0.001	0.36 ± 0.03	0.42 ± 0.03	0.47 ± 0.02	<0.001
	WBC	6.8 ± 1.0	7.1 ± 0.8	7.2 ± 0.9	<0.001	6.9 ± 0.8	7.0 ± 0.9	7.2 ± 0.8	<0.001
	Fibrinogen	354 ± 8	364 ± 8	372 ± 7	<0.001	353 ± 7	355 ± 7	358 ± 5	<0.001

Model 1: adjustment for age, race and sex; Model 2: additional adjustment for SES (poverty to income ratio and education level), alcohol consumption, physical activity, smoking and body mass index; Model 3: additional adjustment for diabetes, estimated glomerular filtration rate, statin use: Model 4: additional adjustment for low density lipoprotein cholesterol and triglycerides. HDL-C: high density lipoprotein cholesterol, CRP: C-reactive protein, WBC: white blood cell.

Table 3
Adjusted Cox regression to examine the association between categories of HDL-C and risk of total and cause specific mortality.

Events	Risk ratio (RR) and 95% confidence interval (95%CI) for each mortality according to HDL-C categories																	
	Low HDL-C categories	Model 1		Model 2		Model 3		Model 4		*High HDL-C categories*	Model 1		Model 2		Model 3		Model 4	
		RR	95%CI	RR	95%CI	RR	95%CI	RR	95%CI		RR	95%CI	RR	95%CI	RR	95%CI	RR	95%CI
Total mortality	Low¥	1.85	1.49–2.30	1.40	1.11–1.78	1.32	1.09–1.56	1.35	1.11–1.89	High*	NS	NS	NS	NS	NS	NS	1.25	1.09–1.49
	Extremely low	3.00	2.20–4.09	2.02	1.48–2.81	1.87	1.31–2.59	2.05	1.34–3.15	Extremely high	NS	NS	NS	NS	NS	NS	1.38	1.11–1.68
CHD	Low	1.40	1.13–1.74	1.21	1.02–1.52	1.29	1.02–1.64	1.32	1.02–1.77	High	NS	NS	1.11	1.03–1.41	1.13	1.04–1.49	1.09	1.02–1.32
	Extremely low	2.02	1.49–2.68	1.56	1.12–2.17	1.65	1.32–2.36	1.72	1.19–2.63	Extremely high	NS	NS	1.23	1.09–1.63	1.36	1.11–1.76	1.34	1.10–1.69
Stroke	Low	1.54	1.23–1.92	1.24	1.02–1.57	1.26	1.09–1.64	1.32	1.14–2.04	High	NS	NS	NS	NS	NS	NS	1.11	1.02–1.32
	Extremely low	1.77	1.21–2.59	1.35	1.11–2.03	1.37	1.22–2.10	1.49	1.25–2.95	Extremely high	NS	NS	NS	NS	NS	NS	1.19	1.12–1.43
Cancer	Low	1.56	1.29–1.88	1.31	1.07–1.60	1.38	1.09–1.74	1.45	1.13–1.88	High	NS	NS	1.36	1.07–1.69	1.30	1.05–1.52	1.20	1.03–1.42
	Extremely low	2.30	1.77–3.00	NS	NS	NS	NS	NS	NS	Extremely high	NS	NS	NS	NS	NS	NS	NS	NS

Model 1: Age, sex and race adjusted; Model 2: additional adjustment for *SES* (poverty to income ratio and education level), alcohol consumption, physical activity, smoking and body mass index; Model 3: additional adjustment for diabetes, *estimate glomerular filtration rate*, statin use; Model 4: additional adjustment for *low density lipoprotein cholesterol* and triglycerides.
CHD: coronary heart disease, *HDL-C: high density lipoprotein cholesterol, NS: not significant.* ¥ = HDL-C ≥ 40 mg/dl considered as reference value. * = HDL-C 50–80 mg/dl considered as reference value. HDL-C categories were defined into the following categories: "very low" HDL-C (<30 mg/dl), "low" HDL-C (30 ≤ 40 mg/dl) and "reference" HDL-C (≥40 mg/dl). For high level of the HDL-C: "reference" HDL-C (50–80 mg/dl), "high" level HDL-C (80–100 mg/dl), "extremely high" level HDL-C (≥100 mg/dl).

the HDL-C in Model 1 (Table 3). However, after more adjustment a significant link was observed. Interestingly all of the events (total, CHD, stroke) have significant association with level of the HDL-C in the fourth model (fully adjusted) for example, subjects in extremely high HDL-C (>100 mg/dl) had 26% higher chance (RR: 1.26, 95% CI: 1.11–1.50, $p < 0.001$) of total mortality compared with reference group (50–80 mg/dl). Subjects in extremely high HDL-C (>100 mg/dl) had 38% higher risk of CHD mortality compared with reference group (50–80 mg/dl) (RR: 1.38, 95%CL: 1.1.1–1.68, $p < 0.001$, Table 3).

When we have run the same models just between Mexican-American participants (age and sex adjusted), the risk for events followed a different trend, suggesting an *HDL-C paradox*. For example, in the age, sex and race adjusted model, subjects in the low HDL-C category (30–40 mg/dl) had higher risk compared with the very low category (<30 mg/dl) for total mortality (2.88 vs 1.78) and CHD (1.79 vs 1.55). In addition, among Mexican-American in age and sex adjusted analysis there was no association between probability of stroke and HDL-C categories ($p > 0.05$).

4. Discussion

By analysing a large and nationally representative sample of American adults, we observed a dose response association between HDL-C levels with total and cause specific mortality that was "J or U-shaped," rather than linear, as traditionally described. Both extreme (low and high) HDL-C levels were associated with high rate of mortality (total, CHD and stroke) and concentration of the inflammatory factors. For the first time we have reported non-significant link between extremely high HDL-C and risk of cancer. Further, we have found an *HDL-paradox* among Mexican-American participants that has been for the first presented based on our analysis of the REasons for Geographic and Racial Differences in Stroke (REGARDS) study [30].

Our results are consistent with those of Tada et al.; in this observational study where HDL-C was measured for any reason [31], 429 participants (from a total of 43,368) had 'extremely low' HDL-C (<15 mg/dl; 0.39 mmol/l); mortality was highest in this group [31]. Another study among Japanese adults (n = 43,407, 40–89 years) assessed the impact of extremely high levels of HDL-C on cause-specific CVD mortality. They found that extremely high levels of HDL-C had an adverse effect on CVD mortality [32].

Low HDL-C levels may be attributed to artefacts (e.g. paraproteins interfering with HDL assays), uncommon genetic causes (e.g. ApoA-I deficiency/mutation) and secondary causes (e.g. androgenic steroids, cancer and rarely, an unusual response to a fibrate) [33]. In the Tada et al. study, most cases of low HDL-C were due to secondary causes and CV mortality only accounted for 10% of deaths [31]. This result is in agreement with our present finding that low HDL-C seems to a better predictor of mortality and higher level of inflammatory markers.

In the present study, similarly to the previous one [30], the prognostic effects of HDL-C in the whole study population were attenuated when we applied model 2 correcting for BMI, and further reduction of the effect was observed with Model 3, which corrects for diabetes. Both high BMI and diabetes are causes of low HDL-C levels [34]. An interesting and relatively new finding of this analysis is the association of lower mortality risk in Mexican-American with HDL-C levels <40 mg/dl, suggesting an '*HDL paradox*'. However, due to the relatively small numbers of participants in the racial subgroups of patients with low HDL-C, one should interpret these results with caution. However, this interesting finding requires further investigation. Racial differences in the ability of HDL-C to predict CVD risk have been reported [35,36]. Variability in outcomes in different racial groups could be attributed to differences in genetic traits relating to HDL-C. Associations of HDL-C, CVD, and genetic variants have been discussed elsewhere [37].

HDL-C particles are heterogeneous and complex macromolecules, carrying many lipid species and proteins as well as microRNAs [31,38]. In fact, there is also a hypothesis that different HDL particles carry

different microRNAs, very typically for them, what might help to identify them and allow for the intervention aimed to increase only those without impaired functionality [39]. This physiological heterogeneity is increased in pathologic conditions, such as inflammation, glycation or oxidative stress, owing to additional quantitative and qualitative molecular changes in HDL-C components, which have been associated with the loss of physiological function and acquiring pathologic dysfunction (i.e. pro-atherosclerotic properties) [31,40]. Further studies focusing not on HDL-C quantity, but especially on HDL quality could provide an opportunity to assess the role of this complex particle, not only in CV disease, but also in other conditions (e.g. with high level of inflammation) [31,39–41].

In the current study there was no link between either extremely high or extremely low levels of HDL-C with cancer risk. This is in accordance with a study that examined 2748 Framingham Heart Study participants and found no conclusive relationship between HDL-C levels and cancer deaths [42]. In contrast, Ko et al. reported that individuals with lower HDL-C levels were independently associated with higher risk of cancer [43]. In the above-mentioned REGARDS analysis very low HDL-C (<30 mg/dl) in women was significantly associated with cancer mortality in a fully adjusted model [30]. This issue still needs to be further investigated, but it seems that HDL-C might be a biomarker/predictor of cancer risk, rather than directly involved in the process of carcinogenesis [44]. However, the risk of cancer with extremely high level of the HDL-C has not been evaluated and our report is the first to do so [30].

Another finding of our study is the greater risk of mortality and higher level of inflammatory factors for the subjects with extremely high (≥100 mg/dl) compared with reference level of HDL-C (50–80 mg/dl). This finding is in line with the Copenhagen general population study; they reported that high HDL-C concentrations were associated with high mortality [12]. However, the authors questioned whether their findings represent a causal role for HDL [12]. A study from Finland has suggested that the increased risk associated with high HDL-C levels in men might be related to increased alcohol intake [45]. However, increased risk was observed in our study even after adjusting for alcohol use. Another possible explanation for the association between extreme high HDL-C and higher mortality is that extreme high concentrations are often due to genetic variants [46]. For example, certain mutations in CETP, ATP-binding cassette subfamily A member 1 (ABCA1), hepatic lipase gene (LIPC), and scavenger receptor class B member 1 (SCARB1), are associated with both high risk of CHD and high concentrations of HDL-C [9–11,46].

Two studies published in 2016 based on routinely collected health data support our findings, as they indicated that the association between HDL-C and mortality is not linear over the entire concentration range of HDL-C [43,47]. In the above-mentioned study by Ko et al., age-standardized all-cause mortality was the lowest in the HDL-C range from 51 to 70 mg/dl in men and from 61 to 70 mg/dl in women, and all-cause mortality increased to 12.1/1000 person-years in men with HDL-C > 90 mg/dl and to 6.8/1000 person-years in women with HDL-C > 90 mg/dl [43]. In another study, Bowe et al. reported a U-shaped association between all-cause mortality and HDL-C in men, with the lowest risk at HDL-C of 30–50 mg/dl [47]. It is worth, however, mentioning that both studies were based on selected populations; the participants had well-obtained goals of lipid lowering therapy, and either focused on men and the association between HDL-C and mortality as modified by kidney function, or excluded individuals with CVD and other comorbidities. Based on the above, results of these studies cannot be treated as representative for the general population [43,47].

Our findings might have some important clinical and public health implications for individuals with extreme high and very low concentrations of HDL-C. First, when HDL-C is used for risk assessment, clinicians should be aware that extremely high or low HDL-C levels might identify a high-risk group for CV events and high level of the inflammatory factors [13]. Our findings on the subject with extremely high HDL-C indicate that the common belief that, the higher the concentration of HDL-C the better, does not hold, as the relationship between HDL-C and CV events was not linear over the entire range of HDL-C concentrations. Secondly, if the associations between extreme high HDL-C and CV events and high level of the inflammatory factors are causal, these findings would add to the uncertainty regarding elevating HDL-C pharmacologically to extreme high concentrations [13]. Some of the developed CETP inhibitors can increase HDL-C to extreme high concentrations (even over 100%) [48] that in the present study were linked with an unfavourable risk. Interestingly, the development of torcetrapib was discontinued as it increased mortality; however, this could be due to off-target effects [8,13]. On the other hand in the Randomized Evaluation of the Effects of Anacetrapib through Lipid Modification (REVEAL) trial with the most potent CETP inhibitor – anacetrapib, the researchers showed that adding anacetrapib to intensive statin therapy reduced the incidence of CV events in high-risk patients [48]. The scale of reduction was similar to other LDL-C lowering drugs, such as statins, and the large increase in HDL cholesterol levels produced by anacetrapib did not appear to have much impact on risk [48]. Taking all the above into account, which is in fact in a line with the recent guidelines, it is no longer recommended to use HDL-C levels while analysing the predicted risk of CV events, as well as the effect of lipid-lowering therapy [49]. It seems therefore, that for all patients with high HDL-C levels, we should always check the functionality, because in high risk patients with high level of inflammation and oxidative stress a large ratio of increased HDL might be simply dysfunctional [50].

4.1. Study strengths and limitations

The obtained data also suggest that there is an alternative explanation, as they might suggest that extreme high HDL-C could in itself be the cause of CV events and unfavourable profile. As our study was observational, it could not determine if the association between extreme high HDL-C levels with CV events and unfavourable profile was causal. The association may be due to unmeasured confounding or reverse causation. Further, inflammatory markers (CRP, fibrinogen and WBC) in our study are of relatively small specificity.

A major strength of our study was the ability to examine a large population-based cohort of individuals living in a similar environment, under the care of the same health care system. Second, we had detailed information on several confounders with known effect on CV events. Although residual confounding cannot be completely ignored.

5. Conclusion

Both the extremely low and high HDL-C levels were associated with greater risk of mortality and higher level of inflammatory factors, while there was no link between level of HDL-C (extremely high or low) and risk of cancer. We also found evidence of an *HDL-C paradox* associated with Mexican-American ethnicity.

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Conflict of interest statement

DPM has given talks and attended conferences sponsored by MSD, AstraZeneca and Libytec. The other authors have no conflict of interest to declare.

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