Association of remnant cholesterol and non-high density lipoprotein cholesterol with risk of cardiovascular mortality among US general population

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ABSTRACT

Background: There are strong association between remnant cholesterol (RC)/non-high density lipoprotein cholesterol (NHDL-C) and increase cardiovascular (CV) risk. The aim of present study was to investigate the association between target lipid parameters (RC and NHDL-C) and the risk of CV mortality in general population.

Methods: Data set from an open database—National Health and Nutrition Examination Surveys (NHANES) 2003–2014 were extracted (n = 14992). Kaplan-Meier, multivariable COX regression, and restricted cubic spline (RCS) parameters.

Results: Compared to the lowest quartile, RC (adjusted hazard ratio [HR] = 1.63 95%CI 1.05–2.52, P for trend = 0.037) and triglycerides (TG: Model 3: HR = 1.69 95%CI 1.10–2.60, P for trend = 0.049) in the highest quartile were independently associated with the increased cardiovascular mortality, while NHDL-C and apolipoprotein B (ApoB) in adjusted models did not show association (P for trend > 0.05). In addition, RCS regression demonstrated that RC (P for nonlinearity = 0.011) and TG (P for nonlinearity = 0.010) levels had a similar J-shape association with CV mortality. Threshold effect analysis showed that when RC ≤ 29.3 mg/dL, the level of RC and CV mortality risk were positively correlated.

Conclusions: Our findings suggest high RC levels are associated with an increased risk of CV mortality, which support that the integration of TG-rich lipoproteins parameters in risk assessment might optimize the identification and management of selected population.

1. Introduction

Due to the overall social development and population aging, cardiovascular disease (CVD) is the most common cause of death worldwide in the general population. Epidemiological studies demonstrated that CVD accounted for over 17 million deaths worldwide yearly [1, 2, 3]. The prevalence of CVD and disease burden are rising over years [4, 5].

Lipids are essential in the development of atherosclerosis (AS). Serum lipid profile reflects the overall cardiometabolic health and significantly relates to CVDs [6, 7]. The lipid subfractions have gained increasing attention as more valid measures of atherogenicity. As the key factor in the pathogenesis and perpetuation of ASCVD, the treatment toward the atherogenic cholesterol—low-density lipoprotein cholesterol (LDL-C) lowering has been established to reduce certain CVD risks [8, 9, 10]. However, studies showed that LDL-C does not account for the total risk of ASCVD [11], while other forms of dyslipidemia also contribute to the increased risk.

Several corresponding lipid parameters have been shown to further improve management and/or reflect the overlooked residual risk of LDL-C. Previous studies indicated that TRL and their remnants may contribute significantly to residual cardiovascular risk in patients on optimized lipid-lowering therapy [12, 13]. Among, remnant cholesterol (RC) and non-high-density lipoprotein cholesterol (NHDL-C) share overlapped
characteristics, and both parameters demonstrated certain associations with TG-rich lipoproteins (TRL) which have a different pathophysiological characteristic to LDL-C.

As the cholesterol content of a subset of the TRL, RC mainly accounts for the very low-density lipoproteins (VLDL) and intermediate-density lipoproteins (IDL) cholesterol. RC is highly correlated with an increased risk of major cardiovascular events regardless of LDL-C levels and statin-treatment [14, 15, 16]. It can be assessed using several methods, including immunoaffinity assays, nuclear magnetic resonance spectroscopy, and calculations-based on plasma triglyceride concentrations.

In addition, NHDL-C specifies the amount of cholesterol associated with TRLs without high-density lipoprotein cholesterol (HDL-C) which being small and dense molecules. While NHDL-C is one of the major targets in assessing CVD risk and is the secondary target of therapy in individuals with hypertriglyceridemia, studies also suggested that NHDL-C has a superior predictive value to LDL-C in estimating the risk of major cardiovascular events [17, 18, 19, 20].

Despite the shared promising values of RC and NHDL-C in risk and/or prognostic evaluation in broad patient cohorts, most of these studies have focused on high-risk populations and the secondary complementary marker to LDL-C [21, 22, 23]. Furthermore, the different roles between RC and NHDL-C in CVD remained unclear.

Given the existing evidence, the current study aimed to examine the association of these lipid markers with the cardiovascular risk and to explore the different effects of NHDL-C and RC in the general US population.

Table 1. Baseline characteristics.

| Variable                              | All (n = 14992) | Cardiovascular Death (n = 254) | Survival (n = 14738) | P value |
|---------------------------------------|----------------|--------------------------------|---------------------|---------|
| Age, years                            | 48.1 (19.2)    | 71.8 (12.1)                    | 47.7 (19.1)         | <0.001  |
| Male, %                               | 7428 (49.5%)   | 166 (65.4%)                    | 7262 (49.3%)        | <0.001  |
| Body mass index, kg/m2                | 28.6 (6.8)     | 28.3 (6.2)                     | 28.6 (6.8)          | 0.001   |
| Education level, %                    |                |                                |                     |         |
| Below high school                     | 4066 (26.7%)   | 103 (40.6%)                    | 3903 (26.5%)        | <0.001  |
| High school                           | 3452 (23.0%)   | 65 (25.6%)                     | 3387 (23.0%)        |         |
| Above high school                     | 7534 (50.3%)   | 86 (33.9%)                     | 7448 (50.5%)        |         |
| Race/ethnicity, %                     |                |                                |                     | <0.001  |
| Mexican American                      | 2529 (16.9%)   | 27 (10.6%)                     | 2502 (17.0%)        |         |
| Other Hispanic                        | 1255 (8.4%)    | 11 (4.3%)                      | 1244 (8.4%)         |         |
| Non-Hispanic White                    | 6830 (45.6%)   | 164 (64.6%)                    | 6666 (45.2%)        |         |
| Non-Hispanic Black                    | 3140 (20.9%)   | 44 (17.3%)                     | 3096 (21.0%)        |         |
| Other race                            | 1238 (8.3%)    | 8 (3.1%)                       | 1230 (8.3%)         |         |
| Smoker, %                             | 6789 (45.3%)   | 150 (59.1%)                    | 6639 (45.0%)        | <0.001  |
| Alcohol user, %                       | 10649 (71.0%)  | 167 (65.7%)                    | 10482 (71.1%)       | <0.001  |
| Lipid-lowering drugs, %               | 2540 (16.9%)   | 91 (35.8%)                     | 2449 (16.6%)        | <0.001  |
| TG, mg/dL                             | 121.1 (66.8)   | 145.8 (70.1)                   | 120.6 (66.7)        | <0.001  |
| TC, mg/dL                             | 191.3 (40.9)   | 190.3 (46.2)                   | 191.1 (40.8)        | <0.001  |
| HDL-C, mg/dL                          | 54.1 (15.6)    | 51.9 (15.3)                    | 54.1 (15.6)         | <0.001  |
| LDL-C, mg/dL                          | 113.0 (35.7)   | 111.4 (39.2)                   | 113.0 (35.6)        | <0.001  |
| RC, mg/dL                             | 24.2 (13.4)    | 29.1 (14.0)                    | 24.1 (13.3)         | <0.001  |
| NHDL-C, mg/dL                         | 137.2 (40.2)   | 140.5 (44.5)                   | 137.1 (40.1)        | <0.001  |
| ApoB, mg/dL                           | 92.1 (25.0)    | 98.0 (27.7)                    | 92.0 (24.9)         | <0.001  |
| Comorbid illness, %                   |                |                                |                     |         |
| Diabetes mellitus, %                  | 1654 (11.0%)   | 76 (29.9%)                     | 1578 (10.7%)        | <0.001  |
| Hypertension, %                       | 5162 (34.4%)   | 160 (63.0%)                    | 5002 (33.9%)        | <0.001  |
| Congestive heart failure              | 478 (3.2%)     | 53 (20.9%)                     | 425 (2.9%)          |         |
| Coronary heart disease                | 594 (4.0%)     | 52 (20.5%)                     | 542 (3.7%)          | <0.001  |
| Stroke                                | 562 (3.7%)     | 35 (13.8%)                     | 527 (3.6%)          | <0.001  |

Data are presented as mean (SD) or n (%).

TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; ApoB, apolipoprotein B; RC, remnant cholesterol; NHDL-C, non-high density lipoprotein cholesterol.
2. Method and material

2.1. Study population and design

This retrospective cohort study used publicly available data from National Health and Nutrition Examination Survey (NHANES) conducted by the US National Center for Health Statistics (Centers for Disease Control and Prevention, Atlanta, GA, USA). Authors did not involve in the collection and production of the database. The detailed survey design, methods, and data are available on the NHANES website [24] and were in accordance with the "Declaration of Helsinki". The protocols for NHANES were approved by the National Center for Health Statistics of the Centers for Disease Control and Prevention Institutional Review Board, and informed consent was obtained from all participants.

From NHANES 2003–2014 including 6 survey cycles (apolipoprotein B [ApoB] only in the NHANES 2005–2014), there were 14992 participants with available lipid profiles enrolled for the analysis. Inclusion flow chart is shown in Figure 1.

2.2. Demographic characteristics and covariates

Baseline demographic variables including age, gender, education levels (below high school, high school, above high school), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, other race), body weight, and height were collected from the household interview. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m2). The prevalence of the comorbidity (hypertension, diabetes, congestive heart failure, coronary heart disease, and stroke) was recorded by a standardized medical condition questionnaire which administered by trained interviewers.

Information on smoking, alcohol use, medication (including lipid-lowering agents), and history of comorbidities had been obtained from the physical examination and associated questionnaire. Smoking habit was identified as someone who smoked ≥100 cigarettes in their lifetime. Alcohol user was defined as those who drank at least 12 alcohol drinks in any one year.

2.3. Lipid profiles

Specimens from subjects who fasted for at least 8.5 h but less than 24 h were assayed for the lipid profile. Total cholesterol (TC), triglyceride (TG), HDL-C, and ApoB level were collected. Across included NHANES cycles, TC and TG were measured using enzymatic assays, and HDL-C and apoB were measured using immunoassays. LDL-C is calculated from measured values of TC, TG, and HDL-C according to the Friedewald calculation. Level of RC was calculated as TC minus LDL-C minus HDL-C [17, 25]. Level of NHDL-C was calculated as the difference between TC and HDL-C [14, 16]. Detailed instructions are discussed in each corresponding NHANES cycle Laboratory Procedures Manual https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx.

2.4. Mortality ascertainment

With mean follow-up time of 5.5 years, the primary outcome was cardiovascular (CV) mortality which identified using variable for the
leading cause of death. In this study, we defined death resulting from heart disease or cerebrovascular diseases as CV mortality. All-cause mortality and CVD mortality were defined according to the Tenth Version of the International Classification of Diseases (ICD-10). Information on follow-up time and mortality status were ascertained via using person months from the National Death Index to date of death or the end of the mortality period (December 31, 2015) [26]. Secondary outcome of all-cause mortality was acquired accordingly.

2.5. Statistical analysis

Continuous variables accorded with normal distribution are presented as means (standard deviations, SDs); otherwise, they are presented as medians with interquartile ranges (IQRs). Categorical variables are presented as numbers (%). Multiple imputations were applied for the missing cycle of ApoB. RC and NHDL-C were calculated and compared in each of the upper three quartiles to the lowest quartile. The shape of the relationship between lipid parameters and cardiovascular mortality was explored using the restricted cubic spline regression model and used ANOVA to test for nonlinearity. If nonlinearity was detected, we used the segmented regression to fit the piecewise-linear relationship between lipid parameters and CV mortality risk and to calculate the threshold inflection point using a recursive algorithm. Stratified Cox regression models were used to perform subgroup analyses. The significance of interaction (p-interaction) was tested using the likelihood ratio test. Hazard ratios (HR) (95% confidence intervals (CI)) for risk of endpoints were presented.

The cumulative survival rate was calculated by Kaplan-Meier method, and log-rank test was used for comparison between groups. Cox proportional hazards regression was performed, to analyze CV mortality according to the increasing values and quartiles of RC and NHDL-C. We evaluated the associations of lipids with endpoint using multivariable linear regression models analyzed by the following three adjustment models: model 1 was unadjusted; model 2 was adjusted for basic epidemic characteristic including age and sex; model 3 was further adjusted for the significant baseline characteristic of ethnicity, education level, smoker, alcohol user, BMI, lipid-lowering drugs, diabetes mellitus, hypertension, coronary heart disease, congestive heart failure, and stroke.

The statistical analyses were performed by SPSS (version 24.0; IBM) and R software (version 3.6.0; The R Foundation for Statistical Computing). Two-sided P < 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

Table 1 showed the characteristics of the study population. Study included 14992 participants with 49.5% of male and a mean age of 48.1 ± 19.2 years old. There were 16.9% of participants who received lipid-lowering drugs. The mean RC level in the overall population was 24.2 mg/dL, TG was 121.1 mg/dL, NHDL-C was 137.2 mg/dL, and ApoB was 92.1 mg/dL.

Based on the primary endpoint—CV mortality, participants were divided into death group (254, 1.69%) and survival group (14738, 98.31%). Comparing these two groups, significances were shown in age, sex, BMI, education level, ethnicity, cigarette smoke, alcohol use, lipid-lowering drug, lipid profiles (including TG, TC, LDL-C, HDL-C, ApoB, RC, and NHDL-C), and the comorbidity (All P < 0.05).

3.2. Survival analysis

Kaplan-Meier survival curve showed that the survival rate of RC in the highest quartile (Q4) was lower than in the other three quartile groups, and the difference between groups was statistically significant (Log-rank test P < 0.0001, Figure 2A). TG showed a similar result as RC with statistical significance between groups (P < 0.0001, Figure 2B). On the other hand, the survival rate of patients with NHDL-C (P = 0.77, Figure 2C) and ApoB (P = 0.1, Figure 2D) did not show significance between quartiles.

3.3. Prognostic value

Furthermore, univariate COX regression analysis showed that the risk of CV mortality of RC was significantly different between quartiles (P < 0.001); compared to Q1, the Q4 group had a 3-fold higher cardiovascular mortality risk (unadjusted HR = 3.23; 95%CI 2.13–4.91, P < 0.001, Table 2); while NHDL-C did not show significance in overall and quartile comparison (All P > 0.05, Table 2). In addition, age, sex, education level, ethnicity, cigarette smoke, alcohol, creatinine,
triglyceride, lipid-lowering drugs, and comorbidity (including diabetes mellitus, hypertension, coronary heart disease, congestive heart failure, and stroke) were also associated with the risk of CV mortality (All P < 0.05).

Further analysis using multivariate Cox proportional hazards regression (Table 3), the highest quartile (Q4) of RC remained statistically significant compared to Q1 after adjustment (Model 3: HR = 1.63, 95%CI 1.05–2.52, P = 0.037). Also, across the increasing quartiles of RC showed a significant trend regardless of the adjustment models (All P for trend < 0.05).

TG also demonstrated a similar significant trend for cardiovascular mortality risk across the quartiles (Model 3, Q1 vs Q4: HR = 1.69 95%CI 1.10–2.60, P = 0.049). On the other hand, ApoB only showed significance in Q4 in the unadjusted model. NHDL-C did not show statistically significant in both univariate and multivariate COX regression, P for trend across the quartiles were all > 0.05.

### Table 3. Multivariate COX regression analysis for the prediction of cardiovascular mortality.

|            | Model 1 | Pt | Model 2 | Pt | Model 3 | Pt |
|------------|---------|----|---------|----|---------|----|
| RC         |         |    |         |    |         |    |
| HR (95% CI)| <0.001  |    | 0.012   |    | 0.037   |    |
| Q1         | 1       |    | 1       |    | 1       |    |
| Q2         | 2.12 (1.36,3.31)** |    | 1.35 (0.87,2.12) |    | 1.41 (0.90,2.21) |    |
| Q3         | 2.08 (1.33,3.24)** |    | 1.12 (0.72,1.75) |    | 1.11 (0.70,1.74) |    |
| Q4         | 3.23 (2.13,4.91)** |    | 1.73 (1.14,2.64)* |    | 1.63 (1.05,2.52)* |    |
| TG         | <0.001  |    | 0.016   |    | 0.049   |    |
| Q1         | 1       |    | 1       |    | 1       |    |
| Q2         | 2.04 (1.31,1.71)** |    | 1.31 (0.84,2.04) |    | 1.40 (0.89,2.19) |    |
| Q3         | 2.10 (1.35,3.25)** |    | 1.16 (0.75,1.79) |    | 1.19 (0.76,1.86) |    |
| Q4         | 3.17 (2.09,4.79)** |    | 1.73 (1.14,2.62)* |    | 1.69 (1.10,2.60)* |    |
| NHDL       | 0.766   |    | 0.724   |    | 0.404   |    |
| Q1         | 1       |    | 1       |    | 1       |    |
| Q2         | 0.97 (0.68,1.39) |    | 0.92 (0.65,1.31) |    | 1.07 (0.74,1.52) |    |
| Q3         | 0.93 (0.65,1.33) |    | 0.88 (0.61,1.26) |    | 1.12 (0.77,1.62) |    |
| Q4         | 1.11 (0.79,1.56) |    | 1.06 (0.75,1.51) |    | 1.32 (0.92,1.88) |    |
| ApoB       | 0.102   |    | 0.431   |    | 0.209   |    |
| Q1         | 1       |    | 1       |    | 1       |    |
| Q2         | 1.03 (0.71,1.51) |    | 0.88 (0.61,1.29) |    | 0.94 (0.64,1.37) |    |
| Q3         | 1.04 (0.72,1.51) |    | 0.82 (0.56,1.19) |    | 1.01 (0.69,1.48) |    |
| Q4         | 1.43 (1.01,2.03)* |    | 1.06 (0.75,1.51) |    | 1.31 (0.91,1.88) |    |

HR, Hazard ratio; 95%CI, 95% confidence interval; Pt, P for trend; RC, remnant cholesterol; TG, triglyceride; NHDL-C, non-high density lipoprotein cholesterol; ApoB, apolipoprotein B.

Model 1: unadjusted.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, ethnicity, education level, diabetes mellitus, hypertension, smoker, alcohol user, BMI, coronary heart disease, congestive heart failure and stroke, lipid-lowering drugs.

RC (mg/dL): Q1 <14.85, Q2 14.85–20.96, Q3 20.96–30.13, Q4 >30.13.

TG (mg/dL): Q1 <73.0, Q2 73.0–104.0, Q3 104.0–152.0, Q4 >152.0.

NHDL (mg/dL): Q1 <42.92, Q2 42.92–51.82, Q3 51.82–61.87, Q4 >61.87.

ApoB (mg/dL): Q1 <74.0, Q2 74.0–90.0, Q3 90.0–108.0, Q4 >108.0.

### Table 4. Multivariate COX regression analysis for the prediction of all-cause mortality and cardiovascular mortality.

|                     | RC per 5 mg/dL increase | RC ≥15.5 mg/dL | NHDL per 5 mg/dL increase | NHDL ≥130 mg/dL |
|---------------------|-------------------------|----------------|---------------------------|-----------------|
|                     | HR (95%CI) P            | HR (95%CI) P   | HR (95%CI) P               | HR (95%CI) P    |

All-cause mortality

Model 1 1.06 (1.04, 1.07) <0.001 1.63 (1.43, 1.87) <0.001 0.99 (0.99, 1.00) 0.046 0.84 (0.75, 0.93) 0.001
Model 2 1.00 (0.98, 1.02) 0.998 0.93 (0.84, 1.07) 0.355 0.99 (0.98, 0.99) <0.001 0.74 (0.67, 0.83) <0.001
Model 3 0.99 (0.97, 1.01) 0.398 0.94 (0.82, 1.08) 0.400 0.99 (0.98, 1.00) 0.003 0.79 (0.71, 0.89) <0.001

Cardiovascular mortality

Model 1 1.11 (1.07, 1.15) <0.001 2.33 (1.63, 3.33) <0.001 1.01 (0.99, 1.02) 0.382 0.99 (0.77, 1.26) 0.920
Model 2 1.07 (1.02, 1.11) 0.003 1.35 (0.94, 1.93) 0.103 1.00 (0.99, 1.02) 0.636 0.93 (0.73, 1.20) 0.589
Model 3 1.05 (1.01, 1.10) 0.025 1.34 (0.93, 1.94) 0.114 1.01 (1.00, 1.03) 0.073 1.13 (0.87, 1.47) 0.355

Model 1: unadjusted.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, ethnicity, education level, diabetes mellitus, hypertension, smoker, alcohol user, BMI, coronary heart disease, congestive heart failure, stroke, and lipid-lowering drugs.
Further analysis employing both increasing value and the cut-off values based on previous research [9], the increasing value of RC showed significant association with cardiovascular mortality (Model 3: HR = 1.05, 95%CI 1.01–1.10, \( P = 0.025 \), Table 4) but no significance in the cut-off model (\( \geq 15.5 \text{ mg/dL} \)). NHDL-C did not show significance in CVD mortality, while significantly associated with all-cause mortality after adjustment (Cut-off \( \geq 130 \text{ mg/dL} \), Model 3: HR = 0.79, 95% CI 0.71–0.89, \( P < 0.001 \), Table 4).

### 3.4. Restricted cubic spline

To further evaluate the linearity between the target lipid parameter and the risk of CV mortality, restricted cubic spline model showed a nonlinear J-shaped association in RC (P for non-linearity <0.001, Figure 3A). The inflection point of RC was 29.3 mg/dL (P for log likelihood ratio = 0.003). On the left of the inflection point, the risk of cardiovascular increased with increasing RC (HR = 1.05, 95%CI 1.02–1.08, \( P < 0.001 \); Table 5). On the right side of the inflection point, the fluctuation was not significant (HR = 1.00, 95%CI 0.99–1.02, \( P > 0.05 \); Table 5). Furthermore, TG also showed a similar nonlinear J-shaped association (P for non-linearity = 0.01, Figure 3B).

Although ApoB and NHDL-C did not show significant in trend, ApoB (P for non-linearity = 0.001, Figure 3C) and NHDL-C (P for non-linearity = 0.349, Figure 3D) showed a linear association in RCS.

### 3.5. Result for secondary endpoint of all-cause mortality

The association of the lipid parameters above with the secondary endpoint—all-cause mortality were also explored (Table 6). RC, TG, NHDL-C, and ApoB were all shown significance in the univariate Cox regression analysis. Results of K-M survival analysis, Cox regression analysis, and RCS suggested that both ApoB and NHDL-C showed a U-shape association with all-cause mortality (Figures 4 and 5, Table 7). To be noted, TG and RC remained a similar trend and pattern in the analysis.

### 3.6. Subgroup analysis of CVD mortality risk prediction

While the above results showed the association with RC and cardiovascular mortality and association with NHDL-C and all-cause mortality,
subgroup analysis incorporating the Framingham risk score (FRS) did not show added risk prediction value across the increasing quartiles (P > 0.05, Tables 8, 9, and 10).

Furthermore, association between the lipids and CVD mortality in the subgroup patients who have been taking lipid-lowering therapy were analyzed. Although the overall trend did not show significance, multivariate regression analysis showed that TG (Model 3, Q1 vs Q4: HR = 2.74, 95%CI 1.06–7.11) and RC (Model 3, Q1 vs Q4: HR = 3.01, 95%CI 1.16–7.80) in the highest quartile remained significant among the models (Table 11).
synthesis to circulation. The pathophysiology of increasing TG overlapped with various metabolic cardiovascular risk conditions [36, 37, 38] such as obesity, diabetes, metabolic syndrome, etc. As in the current study, researchers have focused on the potential role of TG, TRLs, and remnants on cardiovascular risk. The J-shaped association we observed between TGs and CV mortality was consistent with results from some major studies [39].

The mechanisms regulating plasma triglyceride, TRL, and TRL-residue levels involve complex pathways. There is evidence regard to the involvement of triglyceride-rich remnants in development of cardiovascular disease. Because TRL cholesterol (TRL-C) levels change proportionally to residue concentrations, assessing TRL-C provides an approximation of circulating residue levels. Current data emphasizes the distinct value of remnants in CVD and complements a certain knowledge gap for exploring reliable biomarkers in clinical practice. However, it is not an accurate or specific biomarker and must be used with caution. Further prognostic cohort studies should be conducted to determine whether this line of investigation translates into new and effective paradigms for lipid management.

In summary, our findings are consistent with previous studies and support the role of these parameters including TG and RC as risk factors for CVD risk. Thus, measurement of remnant cholesterol levels may be helpful for CVD risk assessment in clinical practice. With the emerging and novel lipid lowering therapies including statins, proprotein convertase subtilisin/kexin type 9 inhibitors, antisense inhibitors of Apolipoprotein (a), microsomal triglyceride transfer protein inhibitors, etc., will likely disturb the precise assessment in these patients. Levels of RC and NHDL-C are relatively accessible parameters. Incorporate these lipid parameters reflecting various aspects of lipometabolism, especially in VLDL and IDL, might provide a better assessment and optimal management target for high CVD risk patients. Results of future prospective cohort studies regarding the measurement of each lipid component and their participating pathway in ASCVD should be further revisited and explored.

4.1. Limitation

There are certain limitations of our study. This study was a community-based retrospective study with a relatively large sample size from representative US general population, but further generalization of the results should be validated in other study settings. Due to the nature of study design, the level of LDL-C was calculated with an indirect method which might lead to a certain level of bias in the analysis of RC. In addition, study included partial participants (16.9%) who received lipid-lowering agents. Although results showed there was significance in the lipid-lowering subgroup which indicated the residual risk, improvement of the methodology and/or selection of population might be needed for precluding bias. Finally, although the regression models included a broad set of covariates, other confounding factors such as eating habits may also have played a role.

Figure 4. Kaplan-Meier survival curve between different quartile of the lipid parameters in all-cause mortality. A: Remnant Cholesterol; B: Triglyceride; C: Non-High Density Lipoprotein Cholesterol; D: Apolipoprotein B.
Figure 5. Association between the lipid parameters and all-cause mortality. Adjusted hazard ratio of all-cause mortality from a restricted cubic spline logistic regression model with knots at the 5th, 35th, 65th, and 95th percentiles. Adjusted for age, sex, ethnicity, education level, diabetes mellitus, hypertension, smoker, alcohol user, BMI, coronary heart disease, congestive heart failure and stroke, lipid-lowering drugs. The solid line and marked area represent the log-transformed hazard ratios and corresponding 95% confidence intervals. A: Remnant Cholesterol; B: Triglyceride; C: Non-High Density Lipoprotein Cholesterol; D: Apolipoprotein B.

Table 7. Multivariate COX regression analysis for the prediction of all-cause mortality.

|     | Model 1     | Pt  | Model 2     | Pt  | Model 3     | Pt  |
|-----|-------------|-----|-------------|-----|-------------|-----|
|     | HR (95% CI) |     | HR (95% CI) |     | HR (95% CI) |     |
| RC  |             |     |             |     |             |     |
| Q1  | 1           | <0.001 | 1           | 0.597 | 1           | 0.304 |
| Q2  | 1.46 (1.24,1.73)** | 0.94 (0.80,1.12) | 0.97 (0.82,1.15) |
| Q3  | 1.65 (1.40,1.95)** | 0.90 (0.76,1.06) | 0.90 (0.76,1.06) |
| Q4  | 1.74 (1.48,2.05)** | 0.93 (0.79,1.09) | 0.88 (0.74,1.04) |
| TG  |             |     |             |     |             |     |
| Q1  | 1           | <0.001 | 1           | 0.406 | 1           | 0.186 |
| Q2  | 1.54 (1.30,1.81)** | 0.99 (0.84,1.18) | 1.04 (0.88,1.23) |
| Q3  | 1.63 (1.38,1.91)** | 0.89 (0.76,1.05) | 0.91 (0.77,1.07) |
| Q4  | 1.75 (1.49,2.06)** | 0.94 (0.80,1.10) | 0.90 (0.76,1.07) |
| NHDL|             |     |             |     |             |     |
| Q1  | 0.007       | <0.001 | 1           | 1    | 0.009       | 0.001 |
| Q2  | 0.88 (0.76,1.02) | 0.79 (0.68,0.91)** | 0.86 (0.75,1.00) |
| Q3  | 0.77 (0.66,0.89) | 0.64 (0.55,0.74)** | 0.77 (0.66,0.90)* |
| Q4  | 0.87 (0.75,1.00) | 0.69 (0.59,0.80)** | 0.84 (0.72,0.97)* |
| ApoB |             |     |             |     |             |     |
| Q1  | 0.213       | <0.001 | 1           | 1    | 0.001       | 0.001 |
| Q2  | 1.03 (0.89,1.20) | 0.85 (0.73,0.98)* | 0.87 (0.74,1.01) |
| Q3  | 0.91 (0.78,1.06) | 0.68 (0.58,0.79)** | 0.75 (0.64,0.88)** |
| Q4  | 1.05 (0.91,1.22) | 0.73 (0.63,0.85)** | 0.78 (0.67,0.91)** |
Table 8. Discrimination and reclassification after update FRS Model.

| Endpoint level | FRS Model | Update Model | C-index | P value | IDI (95%CI) | P value | NRI (95%CI) | P value |
|----------------|-----------|--------------|---------|---------|-------------|---------|-------------|---------|
| CV mortality   | age, sex, blood pressure, smoking, LDL, and HDL | age, sex, blood pressure, smoking, and RC | 0.863 vs 0.864 | 0.507 | 0.001 (-0.006, 0.006) | 0.931 | 0.057 (-0.048, 0.151) | 0.554 |
|                | age, sex, blood pressure, smoking, TC, and HDL | age, sex, blood pressure, smoking, and RC | 0.864 vs 0.864 | 0.603 | 0.001 (-0.006, 0.006) | 0.911 | 0.038 (-0.089, 0.145) | 0.653 |
| All-cause      | age, sex, blood pressure, smoking, LDL, and HDL | age, sex, blood pressure, smoking, and HDL | 0.832 vs 0.831 | 0.200 | 0.002 (-0.002, 0.005) | 0.455 | 0.006 (-0.041, 0.145) | 0.733 |
| mortality      | age, sex, blood pressure, smoking, TC, and HDL | age, sex, blood pressure, smoking, and HDL | 0.831 vs 0.831 | 0.386 | 0.002 (-0.001, 0.005) | 0.257 | 0.009 (-0.033, 0.044) | 0.614 |

IDI, integrated discrimination improvement; NRI, net reclassification improvement; CI, confidence interval.

Table 9. Subgroup analysis of the association with RC and cardiovascular mortality.

| Variables        | Subgroups | N   | Q1 OR (95%CI) | Q2 OR (95%CI) | Q3 OR (95%CI) | Q4 OR (95%CI) | p-t  | p-int  |
|------------------|-----------|-----|---------------|---------------|---------------|---------------|------|--------|
| Age              | <45       | 6995 | 1.00 (Ref.)   | 1.30 (0.18, 9.55) | 2.43 (0.41, 14.29) | 1.92 (0.29, 12.65) | 0.770 | 0.708  |
|                  | 45–69     | 5413 | 1.00 (Ref.)   | 1.27 (0.51, 3.17) | 1.23 (0.50, 3.00) | 1.60 (0.68, 3.75) | 0.662 |        |
|                  | >69       | 2584 | 1.00 (Ref.)   | 1.52 (0.88, 2.61) | 1.07 (0.62, 1.86) | 1.70 (1.00, 2.91) | 0.061 |        |
| Sex              | Male      | 7428 | 1.00 (Ref.)   | 1.60 (0.92, 2.80) | 1.18 (0.67, 2.07) | 1.80 (1.05, 3.08) | 0.067 | 0.856  |
|                  | Female    | 7564 | 1.00 (Ref.)   | 1.13 (0.53, 2.43) | 1.00 (0.46, 2.14) | 1.49 (0.70, 3.17) | 0.482 |        |
| Lipid-lowering   | Yes       | 2540 | 1.00 (Ref.)   | 2.48 (0.93, 6.62) | 1.66 (0.62, 4.43) | 2.74 (1.06, 7.11) | 0.061 | 0.856  |
| drugs            | No        | 12452| 1.00 (Ref.)   | 1.22 (0.73, 2.04) | 1.04 (0.62, 1.74) | 1.40 (0.85, 2.33) | 0.410 |        |
| Hypertension     | Yes       | 5162 | 1.00 (Ref.)   | 1.20 (0.68, 2.13) | 1.07 (0.61, 1.87) | 1.73 (1.02, 2.93) | 0.051 | 0.574  |
|                  | No        | 9830 | 1.00 (Ref.)   | 1.74 (0.82, 3.70) | 1.23 (0.57, 2.69) | 1.57 (0.73, 3.39) | 0.391 |        |
| Diabetes         | Yes       | 1654 | 1.00 (Ref.)   | 1.70 (0.61, 4.70) | 1.51 (0.56, 4.09) | 2.15 (0.82, 5.64) | 0.364 | 0.958  |
|                  | No        | 13338| 1.00 (Ref.)   | 1.37 (0.83, 2.27) | 1.06 (0.63, 1.77) | 1.56 (0.95, 2.56) | 0.141 |        |
| FRS              | Low risk  | 11145| 1.00 (Ref.)   | 1.33 (0.69, 2.55) | 0.76 (0.37, 1.54) | 1.23 (0.60, 2.51) | 0.310 | 0.647  |
|                  | Moderate risk | 2682 | 1.00 (Ref.)   | 1.28 (0.59, 2.79) | 1.29 (0.60, 2.75) | 1.53 (0.71, 3.29) | 0.745 |        |
|                  | High risk | 1165 | 1.00 (Ref.)   | 1.98 (0.57, 6.87) | 1.74 (0.51, 5.92) | 3.00 (0.91, 9.92) | 0.080 |        |

Analyses were adjusted for covariates age, sex, ethnicity, education level, diabetes mellitus, hypertension, smoker, alcohol user, BMI, coronary heart disease, congestive heart failure and stroke, lipid-lowering drugs.

Table 10. Subgroup analysis of the association with NHDL-C and all-cause mortality.

| Variables        | Subgroups | N   | Q1 OR (95%CI) | Q2 OR (95%CI) | Q3 OR (95%CI) | Q4 OR (95%CI) | p-t  | p-int  |
|------------------|-----------|-----|---------------|---------------|---------------|---------------|------|--------|
| Age              | <45       | 6995 | 1.00 (Ref.)   | 0.95 (0.56, 1.63) | 0.97 (0.55, 1.69) | 1.12 (0.65, 1.93) | 0.944 | 0.002  |
|                  | 45–69     | 5413 | 1.00 (Ref.)   | 0.73 (0.55, 0.97) | 0.59 (0.44, 0.97) | 0.64 (0.48, 0.84) | 0.002 |        |
|                  | >69       | 2584 | 1.00 (Ref.)   | 0.88 (0.73, 1.06) | 0.80 (0.66, 0.97) | 0.81 (0.67, 0.98) | 0.091 |        |
| Sex              | Male      | 7428 | 1.00 (Ref.)   | 0.85 (0.71, 1.03) | 0.71 (0.58, 0.87) | 0.75 (0.61, 0.91) | 0.004 | 0.933  |
|                  | Female    | 7564 | 1.00 (Ref.)   | 0.87 (0.69, 1.02) | 0.80 (0.63, 1.02) | 0.83 (0.66, 1.05) | 0.304 |        |
| Lipid-lowering   | Yes       | 2540 | 1.00 (Ref.)   | 0.85 (0.66, 1.10) | 0.97 (0.74, 1.28) | 1.16 (0.87, 1.55) | 0.227 | 0.002  |
| drugs            | No        | 12452| 1.00 (Ref.)   | 0.83 (0.69, 0.99) | 0.63 (0.52, 0.76) | 0.67 (0.56, 0.80) | <0.001|        |
| Hypertension     | Yes       | 5162 | 1.00 (Ref.)   | 0.89 (0.74, 1.08) | 0.86 (0.71, 1.04) | 0.83 (0.68, 1.01) | 0.255 | 0.089  |
|                  | No        | 9830 | 1.00 (Ref.)   | 0.78 (0.62, 0.99) | 0.57 (0.44, 0.74) | 0.69 (0.55, 0.87) | <0.001|        |
5. Conclusion

Our study demonstrated that levels of the target lipid parameters (RC and NHDL-C) are associated with the outcomes in US general population. RC displayed a positive correlation and J-shaped relationship with cardiovascular mortality, while NHDL-C only showed an association in all-cause mortality. Study further supported that considering these TRL parameters in clinical practice could help optimize risk management. Further large-scale prospective studies are required to confirm the prognostic effect and underlying mechanism.

Table 11. Multivariate COX regression analysis for the prediction of cardiovascular mortality in patients with lipid-lowering therapy.

| Variables | Subgroups  | N    | Q1 OR (95%CI) | Q2 OR (95%CI) | Q3 OR (95%CI) | Q4 OR (95%CI) | p-t | p-int |
|-----------|------------|------|---------------|---------------|---------------|---------------|------|-------|
| Diabetes  | Yes        | 1654 | 1.00 (Ref.)   | 0.88 (0.67, 1.16) | 0.92 (0.68, 1.25) | 0.96 (0.71, 1.30) | 0.819 | 0.152 |
|           | No         | 13338| 1.00 (Ref.)   | 0.84 (0.70, 0.99) | 0.67 (0.56, 0.81) | 0.71 (0.59, 0.84) | <0.001|       |
| FRS       | Low risk   | 11145| 1.00 (Ref.)   | 0.73 (0.59, 0.91) | 0.64 (0.50, 0.82) | 0.62 (0.48, 0.80) | <0.001| 0.055 |
|           | Moderate risk | 2682 | 1.00 (Ref.)   | 0.90 (0.70, 1.16) | 0.71 (0.53, 0.95) | 0.79 (0.59, 1.07) | 0.127 |       |
|           | High risk  | 1165 | 1.00 (Ref.)   | 1.29 (0.88, 1.91) | 1.20 (0.81, 1.76) | 1.23 (0.84, 1.82) | 0.630 |       |

Analyses were adjusted for covariates age, sex, ethnicity, education level, diabetes mellitus, hypertension, smoker, alcohol user, BMI, coronary heart disease, congestive heart failure, stroke, and lipid-lowering drugs when they were not the strata variables. HR, hazard ratio; CI, confidence interval; Q: quartile; Ref., reference; p-t, p for trend; p-int, p for interaction. FRS, Framingham risk score; Low risk (<10%), moderate risk (10–20%), high risk (>20%). All risks calculated using FRS.

Declarations

Author contribution statement

Iokfai Cheang; Xinli Li: Conceived and designed the experiments; Wrote the paper.
Xu Zhu: Analyzed and interpreted the data; Wrote the paper.
Xinyi Lu; Shi Shi: Analyzed and interpreted the data.
Yuan Tang; Xin Yue; Shengen Liao: Contributed reagents, materials, analysis tools or data.
Wenning Yao; Yanli Zhou; Haifeng Zhang; Yanxiu Li: Conceived and designed the experiments.

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Study used publicly available data from National Health and Nutrition Examination Survey (NHANES). Data will be made available on request.

Declaration of interest's statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

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