Prognostic Value of Fasting vs. Non-Fasting Low Density Lipoprotein Cholesterol Levels on Long-term Mortality: Insight from the National Health and Nutrition Survey III (NHANES-III)
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Prognostic Value of Fasting vs. Non-Fasting Low Density Lipoprotein Cholesterol Levels on Long-term Mortality: Insight from the National Health and Nutrition Survey III (NHANES-III)

Running title: Doran et al.; Fasting vs. non-fasting lipids

Bethany Doran, MD, MPH1; Yu Guo, MA1; Jinfeng Xu, PhD1; Howard Weintraub, MD1; Samia Mora, MD2; David J. Maron, MD3; Sripal Bangalore, MD, MHA1

1New York University School of Medicine, New York, NY; 2Brigham and Women’s Hospital, Boston, MA; 3Stanford University, Stanford, CA

Address for Correspondence:
Sripal Bangalore, MD, MHA, FACC, FAHA, FSCAI
Director of Research, Cardiac Catheterization Laboratory
Director, Cardiovascular Outcomes Group
Associate Professor of Medicine, New York University School of Medicine
New York, NY 10016
Tel: 212-263-3540
Fax: 212-263-3988
E-mail: sripalbangalore@gmail.com

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Abstract

Background—National and international guidelines recommend fasting lipid panel measurement for risk stratification of patients for prevention of cardiovascular (CV) events. Yet, the prognostic value of fasting vs. non-fasting low density lipoprotein cholesterol (LDL-C) is uncertain.

Methods and Results—Patients enrolled in the National Health and Nutrition Survey III (NHANES-III), a nationally representative cross-sectional survey performed between 1988 to 1994, were stratified based on fasting status (≥8 hours or <8 hours) and followed for a mean of 14.0 (±0.22) years. Propensity score matching was used to assemble fasting and non-fasting cohorts with similar baseline characteristics. The risk of outcomes as a function of LDL-C and fasting status was assessed using receiver operating characteristic (ROC) curves and bootstrapping methods. The interaction between fasting status and LDL-C was assessed using Cox proportional hazards modeling. Primary outcome was all-cause mortality. Secondary outcome was CV mortality. One-to-one matching based on propensity score yielded 4,299 pairs of fasting and non-fasting individuals. For the primary outcome, fasting LDL-C yielded similar prognostic value as non-fasting LDL-C [C-statistics=0.59 (95% CI 0.57-0.61) vs. 0.58 (95% CI 0.56-0.60; P=0.73], and LDL-C by fasting status interaction term in the Cox proportional hazard model was not significant (Pinteraction= 0.11). Similar results were seen for the secondary outcome [fasting vs. non-fasting C-statistics=0.62 (95% CI 0.60-0.66) vs. 0.62 (95% CI 0.60-0.66); P=0.96; and Pinteraction=0.34].

Conclusions—Non-fasting LDL-C has similar prognostic value as that of fasting LDL-C. National and international agencies should consider re-evaluating the recommendation that patients fast before obtaining a lipid panel.

Key Words: cholesterol, mortality, fasting
Introduction

Current national and international guidelines on cholesterol management recommend that lipid panel measurement should be performed after an 8-12 hour fast. The reason often stated for obtaining a fasting lipid panel is for greater precision for certain lipid parameters (especially triglycerides), which can be variable, based on time and content of the last meal. From a practical standpoint, it is cumbersome for patients to fast before obtaining a blood draw, and may delay diagnosis and treatment of hyperlipidemia.

Prior data have shown that levels of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) vary little with respect to fasting time, while triglycerides may vary by up to 20-30%. Recently, studies have suggested that non-fasting lipids may be equivalent (and potentially superior) in predicting cardiovascular (CV) outcomes, as the non-fasting state may more accurately reflect the body’s exposure to circulating lipids. Studies have demonstrated no benefit, or even improved risk prediction, with the use of non-fasting as compared to fasting triglycerides. No prior studies have examined the relationship of fasting vs. non-fasting LDL-C and mortality. Our objective was to use the National Health Examination and Nutrition Survey III (NHANES-III), a nationally representative database of the US population, to evaluate the prognostic value of fasting versus non-fasting LDL-C for prediction of all-cause mortality and CV mortality in men and women.

Methods

Study Population

We used the NHANES-III linked to the National Death Index (NDI), a nationally representative civilian cohort of non-institutionalized individuals within the United States. Baseline data was
collected between 1988-1994 using a multistage stratified probability cluster sampling design where certain groups were intentionally oversampled and participant weights were added to reflect the demographics of the 1990 US census. Comprehensive data about the validation and collection of data are available elsewhere. The inclusion criteria for this study were adults 18 years of age and older residing in the United States who had participated in the NHANES III study with data on fasting time. We excluded those in whom LDL-C calculations was not possible due to missing HDL-C, TC, or triglyceride levels and those with triglycerides ≥400 mg/dl in whom the Friedewald equation may not be accurate.

**Data Collection**

Participants were interviewed in their homes and examined in a mobile examination center where blood samples were obtained and physical exam performed. If participants were unable to attend an examination at a center, home exam was performed. Institutional Review Board (IRB) approval and documented consent was obtained from individuals through the Centers for Disease Control and Prevention.

**Laboratory Methods**

Blood samples were collected through venipuncture and shipped on dry ice to the laboratory analyzing the sample. Serum HDL-C, triglyceride, and TC levels were measured enzymatically at Johns Hopkins University Lipoprotein Analytical Laboratory using a Hitachi 704 Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, Indiana). Lipid collection and analyses were standardized to Centers for Disease Control and Prevention criterion. LDL-C was derived using the Friedewald formula \( [\text{LDL-C} = \text{TC} - \text{HDL-C} - \left(\frac{\text{triglycerides}}{5}\right)] \), with prior studies showing excellent correlation between fasting direct and indirect methods of LDL-C measurement, and a 0.97 correlation coefficient between Friedewald and directly measured LDL-C in non-
Variable Definitions

We classified individuals as fasting if they had fasted for at least 8 hours and stratified individuals based on fasting status at the time of phlebotomy. The Adult Treatment Panel III (ATP-III) guidelines define fasting time as 9-12 hours in the US, with new guidelines from the ACC/AHA taskforce recommending obtaining fasting lipids but not specifying the duration of fast.\textsuperscript{1,20} We used 8 hours to define fasting in keeping with recent studies examining lipids\textsuperscript{4,10,21} and to reflect a more conservative fasting definition. Hypertension was defined as systolic blood pressure of $\geq 140$ mmHg, or diastolic blood pressure $\geq 90$ mmHg as per the 2014 evidence based hypertension guidelines.\textsuperscript{22} We defined diabetes as serum glycosylated hemoglobin of $\geq 6.5\%$ as per the World Health Organization’s updated definition of diabetes\textsuperscript{23} or self-reported history of diabetes. We used enlarged waist circumference (defined as $>88$ cm for women and $>102$ cm for men) as a proxy for obesity, as waist circumference has been shown to be more highly correlated with mortality and reflective of central adiposity than BMI.\textsuperscript{24,25}

Outcome Measures

The primary outcome analyzed was mortality from all-causes and the secondary outcome was CV mortality. Data on mortality was obtained using death records from the NDI cross-matched to NHANES-III using probabilistic record matching. \textit{International Classification of Diseases, Ninth Revision} (ICD-9) and ICD-10 codes were recoded as underlying classification of death (UCOD) within the NHANES III-NDI. Deaths from CV related diseases included deaths from ischemic heart disease (I20-I25), heart failure (I50), essential hypertensive heart disease (I11-I13), cerebrovascular disease (I60-I69) and atherosclerosis (I70-I71).
Statistical Analysis

All analyses were performed using SAS software version 9.3 (SAS Institute Inc). We adjusted for the complex, stratified study sampling design using survey weights for examination and interview portions of survey as per the CDC recommendations.26 Sensitivity analyses were performed without using survey weights.

Propensity Score Matching

We used propensity score matching to assemble a cohort of paired participants based on fasting status with similar baseline characteristics. Propensity score was calculated using a non-parsimonious multivariable logistic regression model with fasting status (dichotomized as yes or no) as the dependent variable. CV risk factors were entered into the model as covariables to control for possible confounders (including race, smoking history, prior CVD, cholesterol medication use, diabetes, elevated TC, low HDL-C, hypertension, enlarged waist circumference and low socioeconomic status). Matching was performed using SAS 9.3 and SAS macro (GMATCH) with greedy matching in a 1 to 1 ratio without replacement, with caliper width of 0.2 times the standard deviation of the logit of the propensity scores. The discriminatory power of the fasting and non-fasting LDL-C model was evaluated using the area under the receiver-operator curve (ROC) using the Hosmer-Lemeshow C-statistic. Fasting and non-fasting ROC curves were compared using bootstrapping methods to evaluate for a statistically significant difference. Absolute standardized differences were calculated between the fasting and non-fasting cohort before and after propensity score matching.

We generated Kaplan-Meier curves to assess survival functions in both fasting and non-fasting cohorts. Primary analysis was performed on the matched cohort. The prognostic values of fasting vs. non-fasting LDL-C measurement for primary and secondary outcomes were assessed.
using ROC curves. Sensitivity analysis to assess whether the prognostic significance of fasting vs. non-fasting LDL-C varies by length of fast was performed on the unmatched cohort using different cut-points to define fasting status (<4 vs. $\geq 4$ hours, <8 vs. $\geq 8$ hours, <12 vs. $\geq 12$ hours). We stratified by presence of diabetes to determine whether diabetic status influenced prognostic significance of fasting in unmatched models. Further sensitivity analyses were performed including patients with triglycerides $\geq 400$ mg/dL. We conducted sensitivity analyses at different follow up time cut-points (5, 10, and 15 years) to ensure that the significance of fasting status did not vary by follow-up length. Analyses were also performed to evaluate the influence of fasting vs. non-fasting TC and triglycerides levels on all-cause and CV mortality.

Cox proportional hazard models were used to evaluate the association of LDL-C levels with outcomes after adjustment for potential confounders. Individuals were stratified by tertiles of LDL-C levels (<100 mg/dL [referent], $\geq 100$-130 mg/dL, and $\geq 130$ mg/dL) with the lowest tertile used as the reference group. Secondary analyses were performed using clinical cut points (LDL-C levels <130 mg/dL, $\geq 130$ mg/dL-160 mg/dL, and $\geq 160$ mg/dL). Interaction between fasting status and LDL-C was tested in both primary and secondary outcome models using interaction terms for fasting state and LDL-C tertiles. Two tailed p-values of 0.05 or less were considered statistically significant.

**Results**

Our initial dataset included 20,024 adults. As shown by Figure 1, we excluded those in whom LDL-C calculation was not possible (n=699), those with triglycerides $\geq 400$ mg/dL (n=440) (see sensitivity analyses below), and those in whom fasting time was missing (n=2,723), or final mortality status was missing (n=1). Thus, our final dataset included 16,161 individuals.
representing 172,332,619 adults in the US population.

During a mean follow-up of 14.0 (+/- 0.22) years, there were a total of 3,788 deaths (23.4%) and 1,454 (9.0%) CV deaths. Among the 16,161 individuals 10,023 (62.0%) participants were fasting, and 6,138 (38.0%) individuals were non-fasting at the time of phlebotomy. Prior to propensity score matching there were significant differences in the baseline variables between the two groups (Table 1). Propensity score matching matched 4,299 (42.9% of fasting; 70.0% of non-fasting) individuals with similar propensity scores. Post matching, there were no significant differences between the baseline characteristics of the two groups and the absolute standardized differences were <10% for all matched variables indicating an adequate match.27

All-Cause Mortality

In the unmatched cohort, there was an increased risk of all-cause mortality with increasing LDL-C tertile [HRs 1 (referent), 1.57 (95% CI 1.34-1.83) (2nd tertile), 2.00 (95% CI 1.70-2.33) (3rd tertile), respectively]. Test for interaction between fasting status and all-cause mortality was not significant (Pinteraction = 0.64) indicating lack of association between fasting status and LDL-C with all-cause mortality (eTable 1). Furthermore, the C-statistics for fasting vs. non-fasting groups for predicting all-cause mortality were similar [0.58 (95% CI 0.57-0.60) vs. 0.58 (95% CI 0.56-0.59); P =0.55] (eFigure 1) suggesting similar prognostic value of fasting and non-fasting LDL-C levels. Analyses including individuals with triglycerides of ≥400 mg/dL did not show a significant difference between fasting vs. non-fasting C-statistics [0.58 (95% CI 0.57-0.60) vs. 0.57 (95% CI 0.55-0.59); P=0.34] (eFigure 2). Results were largely similar based on diabetic status: fasting vs. non-fasting C-statistics in non-diabetics were not significantly different [0.59 (95% CI 0.57-0.60) vs. 0.59 (95% CI 0.57-0.61); P=0.79] nor were C-statistics in diabetics [0.51(95% CI 0.46-0.56) vs. 0.51(95% CI 0.46-0.56); P=0.98] (eFigures 3 and 4).
Sensitivity analysis using different cut-point definitions for fasting of <4 hours vs. ≥4 hours [0.58 (95% CI 0.57-0.59) vs. 0.60 (95% CI 0.56-0.64); P=0.37] or for <12 hours vs. ≥12 hours [C-statistics 0.57 (95% CI 0.56-0.59) vs. 0.59 (95% CI 0.57-0.60); P=0.37] (eFigures 5 and 6) showed largely concordant results with using an 8 hour fasting cut-point definition, and did not show significant difference between fasting and non-fasting groups. Sensitivity analysis using different follow up times did not show significant different between fasting and non-fasting groups (data not shown).

Within the propensity score matched cohort, there was increased risk of all-cause mortality by increasing LDL-C tertile [HRs 1 (referent), 1.61 (95% CI 1.25-2.08) (2nd tertile), 2.10 (95% CI 1.70-2.61) (3rd tertile), respectively]. There was no difference between fasting vs. non-fasting LDL-C and all-cause mortality within each tertile of LDL-C (Figure 2). Test for interaction between fasting status and all-cause mortality was not significant (P_{interaction} = 0.11) indicating lack of association between fasting status and LDL-C with all-cause mortality (Table 2). Similarly, the C-statistics for the fasting and non-fasting groups for predicting all-cause mortality were similar [C-statistics 0.59 (95% CI 0.56-0.61) vs. 0.58 (95% CI 0.56-0.60); P=0.73] (Figure 3).

In the unmatched cohort, C-statistics of triglyceride levels in fasting vs. non-fasting groups for predicting all-cause mortality were not significantly different [(C-statistics 0.60 (95% CI 0.59-0.62) vs. 0.61 (95% CI 0.59-0.62); P=0.96, respectively] (eFigure 7). Similarly, C-statistics of TC level in fasting and non-fasting groups for predicting all-cause mortality were not significantly different [(C-statistics 0.60 (95% CI 0.59-0.62) vs. 0.59 (95% CI 0.57-0.61); P=0.31] (eFigure 8).
Cardiovascular Mortality

Outcomes for CV mortality prior to propensity score matching similarly demonstrated increased risk of CV mortality by increasing LDL-C tertile [HRs 1 (referent), 1.82 (95% CI 1.38-2.39) (2nd tertile), 2.94 (95% CI 2.20-3.93) (3rd tertile)]. Test for interaction between fasting status and all-cause mortality was not significant (PInteraction = 0.11) indicating lack of association between fasting status and LDL-C with CV mortality (eTable 1). Fasting vs. non-fasting C-statistics were also similar [0.62 (95% CI 0.59-0.64) vs. 0.62 (95% CI 0.60-0.64); P=0.80] (eFigure 9) suggesting similar prognostic value of fasting and non-fasting LDL-C levels on CV mortality.

Fasting vs. non-fasting C-statistics in non-diabetics were similar [0.62 (95% CI 0.60-0.65) vs. 0.64 (95% CI 0.61-0.67); P=0.42] as well as in diabetics [0.55 (95% CI 0.49-0.61) vs. 0.53 (95% CI 0.47-0.60); P=0.67] (eFigures 10 and 11). Sensitivity analysis including individuals with triglycerides of ≥400 showed largely concordant results with similar prognostic value of fasting and non-fasting LDL-C levels [0.62 (95% CI 0.60-0.64) vs. 0.61 (95% CI 0.58-0.63); P=0.51] (eFigure 12).

Sensitivity analysis using different cut-points for fasting of <4 hours vs. ≥4 hours [0.62 (95% CI 0.60-0.64) vs. 0.65 (95% CI 0.59-0.70); P=0.34] (eFigure 13), or for <12 hours vs. ≥12 hours [0.61 (95% CI 0.59-0.63) vs. 0.63 (95% CI 0.61-0.66); P=0.27] (eFigure 14) showed largely concordant results with an 8 hour fasting cut-point definition, showing similar prognostic value of fasting vs. non-fasting LDL-C. Sensitivity analysis using different follow up time cut-points did not show significant difference between fasting and non-fasting groups (data not shown).

In the propensity score matched cohort, there was increased risk of CV mortality by increasing LDL-C tertile [HR 1 (referent), 1.68 (95% CI 1.13-2.51) (2nd tertile), 3.04 (95% CI
2.00-4.62) (3rd tertile); respectively]. Test for interaction between fasting status and CV mortality remained non-significant ($P_{interaction} = 0.34$) (Table 2) indicating lack of association between fasting status and LDL-C with CV mortality. Similarly, the C-statistics for the fasting and non-fasting groups for predicting CV mortality were similar [0.62 (95% CI 0.60-0.66) vs. 0.62 (95% CI 0.60-0.66); $P=0.96$] (Figure 4) suggesting similar prognostic value of fasting and non-fasting LDL-C.

In the unmatched cohort, C-statistics of triglyceride levels in fasting vs. non-fasting groups predicting CV mortality were not significantly different [(C-statistics 0.62 (95% CI 0.60-0.64) vs. 0.61 (95% CI 0.59-0.64); $P=0.81$, respectively] (Figure 15). The C-statistics of TC levels for CV mortality in fasting and non-fasting groups were similarly not significantly different [C-statistics 0.64 (95% CI 0.62-0.66) vs. 0.63 (95% CI 0.60-0.65); $P=0.49$] (Figure 16).

Sensitivity analyses without using survey weights yielded largely similar results for both primary and secondary outcomes (data not shown).

Discussion

Every year millions of blood samples are drawn across the world for the measurement of lipid panels, in particular LDL-C, with most national and international guidelines recommending a fasting panel for such measurement. The results of this nationally representative cohort study with 16,161 individuals followed for 14.0 years representing >172 million adults in the US population show similar prognostic value of non-fasting LDL-C levels as compared to fasting LDL-C levels for prediction of both all-cause mortality as well as CV mortality, thereby questioning this traditional practice.
Fasting Lipid Panel

The origin of the need for a fasting lipid panel is not entirely clear. It is known that certain lipid parameters, especially triglycerides may be sensitive to fasting status and to the content of the last meal (and in particular high fat loads). As such, fasting panels have been recommended to provide accurate lipid measurements. However there are a number of drawbacks with this approach including the need to reschedule a visit for a separate blood draw if patient is not fasting thereby decreasing compliance and delaying treatment. Moreover, as individuals are in a non-fasting state for the majority of time during the day, obtaining a fasting lipid panel may not accurately reflect post-prandial abnormalities in lipid metabolism and thus obtaining a non-fasting lipid panel may reflect a more relevant physiological state.\textsuperscript{7,8} Obtaining a non-fasting blood sample may also offer the opportunity to assess non-fasting blood glucose which may add accuracy in identifying glucose intolerance.\textsuperscript{28,29}

Recently, several studies have questioned the need for fasting lipid profile, mostly involving the use of non-fasting triglycerides in CV risk assessment. Although the role of triglycerides as an independent CV risk factor is less clear than LDL-C, studies have shown that postprandial triglycerides are similar or possibly even superior to fasting triglycerides in CV risk prediction.\textsuperscript{9,10,30-32} Recent recommendations suggest potentially moving towards non-fasting triglycerides for risk assessment, however further research is needed before definitive recommendations can be made.\textsuperscript{33,34}

Fewer studies have addressed the use of non-fasting LDL-C in risk prediction. Numerous animal, population based, and clinical studies have shown that LDL-C is associated with increased CV mortality,\textsuperscript{35-37} with genetic studies also showing a causative mortality linkage.\textsuperscript{38-40} These studies have traditionally used fasting LDL-C as convention and thus recommendations
made by various agencies such as the ATP-III have generally been for obtaining fasting lipids. However, multiple trials, including the Heart Protection Study and Anglo-Scandinavian Cardiac Outcomes Trial included individuals who were not fasting during the time of phlebotomy when analyzing effects of lipid lowering agents, suggesting that some of the data supporting lipid lowering therapy actually springs from studies involving non-fasting individuals.\textsuperscript{41, 42}

Prior studies examining CV events have demonstrated increased CV risk by LDL-C level for individuals in a non-fasting state,\textsuperscript{21, 43-45} but none have examined long-term mortality outcomes in a representative sample. A recent population based study by Sidhu et al in 2012 showed that in a population based sample, lipid levels by subclass varied little with respect to fasting time, and by less than 10\% for LDL-C.\textsuperscript{4} Other studies have also shown little variation with post-prandial LDL-C levels when compared with fasting levels.\textsuperscript{44, 46} These studies suggest that the variation between fasting and non-fasting LDL-C levels, if any, is small. Our study is the first to show that in a population-based sample, the association between LDL-C with CV and all-cause mortality does not differ by fasting status. Our analyses also suggest that obtaining fasting TC and triglyceride levels do not have improved prognostic significance over that of non-fasting levels.

This provides further evidence that it may be unnecessary to use fasting lipid levels to risk stratify patients. In our primary analyses we excluded patients with a triglyceride level $\geq$400 mg/dl, or roughly $\sim$2\% of the total population. However, the results were largely concordant in a sensitivity analysis after including the above patients. Thus, our results are broadly applicable to all patients undergoing blood draw to assess lipid panel and is applicable to LDL-C measurement as well as triglyceride and TC measurement.
2013 ACC/AHA Guidelines and LDL-C Measurement

The recently published 2013 ACC/AHA guidelines recommend obtaining fasting lipids, however the guidelines do not specify the length of time for fasting, nor cite data to support the need for fasting LDL-C. The guidelines move away from recommending lowering LDL-C to specific targets but recommend moderate to high intensity statin for patients with atherosclerotic CV disease, an intensity of statins that would reduce baseline LDL-C by approximately 40-50% which can easily be assessed with a non-fasting sample.

This has important implications in clinical practice. Requiring patients to fast causes patients increased stress, potential hypoglycemia in patients with diabetes, increased transportation costs, and potentially missed days of work. In addition, the inconvenience of fasting may also delay treatment or diagnosis of hyperlipidemia if patients are unable to fast before clinic visits. Enabling patients to obtain non-fasting lipid profiles would improve patient satisfaction, and potentially avoid delays in detection and treatment of hyperlipidemia while at the same time providing similar prognostic value as that of a non-fasting LDL-C value.

Limitations

The design of this study using data from an existing database limits our ability to prove that fasting and non-fasting lipids have the same prognostic value. In addition, fasting and non-fasting LDL-C were not collected on the same individuals. Moreover, in non-fasting patients data was not available on the composition of patient meals.

Conclusions

In conclusion, the results of this study of 16,161 individuals followed for 14.0 years and representative of the US population fail to show a superior prognostic value of fasting LDL-C
levels when compared with non-fasting LDL-C levels both for the prediction of all-cause mortality as well as CV mortality. Our study suggests that a non-fasting LDL-C measurement offers a more convenient method of phlebotomy while preserving the prognostic value of the test. National and international guideline societies should re-consider the need for fasting LDL-C. Similar results were seen for triglycerides and TC thus questioning the value of obtaining fasting lipid profile.

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Conflict of Interest Disclosures: Dr. Mora reports research grants from Atherotech Diagnostics, AstraZeneca and is on the advisory board for Quest Diagnostics, Cerenis Therapeutics, Genzyme, Lilly. Dr. Bangalore is on the advisory board for Pfizer. The remaining authors have no conflicts to disclose.

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40. Umans-Eckenhausen MA, Sijbrands EJ, Kastelein JJ, Defesche JC. Low-density lipoprotein


Table 1. Baseline Characteristics of Fasting and Non-Fasting LDL-C Cohorts, Pre and Post-Propensity Score Matching

<table>
<thead>
<tr>
<th>Clinical Variables</th>
<th>Pre-match</th>
<th>Post-match</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting (n=10,023)**</td>
<td>Non-fasting (n=6,138)**</td>
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<tr>
<td></td>
<td>Age, mean (SE), y</td>
<td>42.68 (0.43)</td>
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<tr>
<td></td>
<td>Women (%)</td>
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<td></td>
<td>White (%)</td>
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<tr>
<td></td>
<td>Enlarged waist circ (%)</td>
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<td></td>
<td>Smoker (%)</td>
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<td>DM (%)</td>
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<td></td>
<td>HTN (%)</td>
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<tr>
<td></td>
<td>Elevated cholesterol (%)</td>
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<td></td>
<td>Prior CVD (%)</td>
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<tr>
<td></td>
<td>Cholesterol lowering med (%)</td>
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<tr>
<td></td>
<td>Low SES (%)</td>
<td>13.41</td>
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<tr>
<td></td>
<td>LDL-C, mean (SE), mg/dL*</td>
<td>125.04 (0.77)</td>
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<tr>
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<td>Low HDL-C (%)</td>
<td>35.74</td>
</tr>
</tbody>
</table>

Abbreviations: ASD = absolute standardized difference; CVD = cardiovascular disease; med = medication; DM = diabetes mellitus; HDL-C = high density lipoprotein cholesterol; HTN = hypertension; circ = circumference; LDL-C = low density lipoprotein C; SES = socioeconomic status

*To convert to mmol/L, multiply values by 0.0259

**N reported based on unweighted numbers; P-values based on weighted values
<table>
<thead>
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<th>Outcome</th>
<th>Fasting</th>
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<th>P Value</th>
<th>Non-Fasting</th>
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<th>P Value</th>
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<td><strong>All-Cause Mortality</strong></td>
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<td>LDL-C 1st tertile</td>
<td>≤99.60</td>
<td>1 (referent)</td>
<td>&lt;0.001</td>
<td>≤100.73</td>
<td>1 (referent)</td>
<td>0.17</td>
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<td>LDL-C 2nd tertile</td>
<td>99.60-129.68</td>
<td>1.61 (1.25-2.08)</td>
<td>&lt;0.001</td>
<td>100.73-129.22</td>
<td>1.21 (0.92-1.60)</td>
<td>0.17</td>
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<tr>
<td>LDL-C 3rd tertile</td>
<td>129.68-361.40</td>
<td>2.10 (1.70-2.61)</td>
<td>&lt;0.001</td>
<td>129.22-438.00</td>
<td>2.23 (1.76-2.83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C x Fasting Status</td>
<td></td>
<td></td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CV Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C 1st tertile</td>
<td>≤99.60</td>
<td>1 (referent)</td>
<td>0.012</td>
<td>≤100.73</td>
<td>1 (referent)</td>
<td>0.063</td>
</tr>
<tr>
<td>LDL-C 2nd tertile</td>
<td>99.60-129.68</td>
<td>1.68 (1.13-2.51)</td>
<td>0.012</td>
<td>100.73-129.22</td>
<td>1.59 (0.97-2.61)</td>
<td>0.063</td>
</tr>
<tr>
<td>LDL-C 3rd tertile</td>
<td>129.68-361.40</td>
<td>3.04 (2.00-4.62)</td>
<td>&lt;0.001</td>
<td>129.22-438.00</td>
<td>4.00 (2.58-6.19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C x Fasting Status</td>
<td></td>
<td></td>
<td>0.34</td>
<td></td>
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</tr>
</tbody>
</table>

**Abbreviations:** CV = cardiovascular mortality; LDL-C = low density lipoprotein cholesterol

*To convert to mmol/L, multiply values by .0259
Figure Legends:

Figure 1. Participant flowchart.

Figure 2. Kaplan-Meier curve for fasting vs. non-fasting LDL-C levels and all-cause mortality.

Figure 3. Prognostic value of fasting vs. non-fasting LDL-C levels on all-cause mortality in the matched cohort.

Figure 4. Prognostic value of fasting vs. non-fasting LDL-C levels on cardiovascular mortality in the matched cohort.
Figure 1

NHANES III Adult Participants
n=20,024

Excluded (n=3,863)
- Missing lipid data (n=699)
- Triglycerides ≥ 400 (n=440)
- Missing fasting data (n=2,723)
- Missing follow up time (n=1)

Final sample
n=16,161

Non fasting
n=6,138

Fasting
n=10,023
Figure 2
Figure 3

- Fasting: AUC [95% CI] = 0.59 [0.56, 0.61]
- Non-fasting: AUC [95% CI] = 0.58 [0.56, 0.60]

P-value (Fasting Vs. Non-fasting) = 0.73
### Supplemental Table 1 – All Cause and Cardiovascular Mortality by LDL-C Level in Fasting and Non-Fasting Cohorts Prior to Propensity Score Matching

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Fasting</th>
<th></th>
<th></th>
<th>Non-Fasting</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>LDL-C</td>
<td>Hazard Ratio (95% CI)</td>
<td>P Value</td>
<td>LDL-C</td>
<td>Hazard Ratio (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td></td>
<td>(mg/dL)*</td>
<td></td>
<td></td>
<td>(mg/dL)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-Cause Mortality</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LDL-C 1st tertile</td>
<td>≤106.41</td>
<td>1 (referent)</td>
<td></td>
<td>≤104.76</td>
<td>1 (referent)</td>
<td></td>
</tr>
<tr>
<td>(reference)</td>
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<td></td>
</tr>
<tr>
<td>LDL-C 2nd tertile</td>
<td>106.41-138.28</td>
<td>1.57 (1.34-1.83)</td>
<td>&lt;0.001</td>
<td>104.76-137.11</td>
<td>1.28 (1.05-1.55)</td>
<td>0.015</td>
</tr>
<tr>
<td>LDL-C 3rd tertile</td>
<td>138.28-380.00</td>
<td>2.00 (1.70-2.33)</td>
<td>&lt;0.001</td>
<td>137.11-438.00</td>
<td>2.07 (1.67-2.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C x Fasting Status</td>
<td></td>
<td></td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV Mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C 1st tertile</td>
<td>≤106.41</td>
<td>1 (referent)</td>
<td></td>
<td>≤104.76</td>
<td>1 (referent)</td>
<td></td>
</tr>
<tr>
<td>(reference)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C 2nd tertile</td>
<td>106.41-138.28</td>
<td>1.82 (1.38-2.39)</td>
<td>&lt;0.001</td>
<td>104.76-137.11</td>
<td>1.62 (1.15-2.28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C 3rd tertile</td>
<td>138.28-380.00</td>
<td>2.94 (2.20-3.93)</td>
<td>&lt;0.001</td>
<td>137.11-438.00</td>
<td>6.18 (2.21-4.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C x Fasting Status</td>
<td></td>
<td></td>
<td>0.64</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations: CV = cardiovascular; LDL-C = low density lipoprotein cholesterol

*To convert to mmol/L, multiply values by 0.0259
FIGURE LEGENDS

Figure 1 - Prognostic value of fasting vs. non-fasting LDL-C level on all-cause mortality in the unmatched cohort

Figure 2 - Sensitivity analysis: Prognostic value of fasting vs. non-fasting LDL-C level including patients with triglycerides $\geq 400$ mg/dL on all-cause mortality in the unmatched cohort

Figure 3 - Prognostic value of fasting vs. non-fasting LDL-C level on all-cause mortality in patients without diabetes in the unmatched cohort

Figure 4 - Prognostic value of fasting vs. non-fasting LDL-C level on all-cause mortality in diabetic patients in the unmatched cohort

Figure 5 – Sensitivity Analysis: Prognostic value of fasting (<4 hours) vs. non-fasting (≥4 hours) LDL-C level on all-cause mortality in the unmatched cohort

Figure 6 - Sensitivity Analysis: Prognostic value of fasting (<12 hours) vs. non-fasting (≥12 hours) LDL-C level on all-cause mortality in the unmatched cohort

Figure 7 - Prognostic value of fasting vs. non-fasting triglyceride level on all-cause mortality in the unmatched cohort

Figure 8 - Prognostic value of fasting vs. non-fasting total cholesterol level on all-cause mortality in the unmatched cohort

Figure 9 - Prognostic value of fasting vs. non-fasting LDL-C level on cardiovascular mortality in the unmatched cohort

Figure 10 - Prognostic value of fasting vs. non-fasting LDL-C level on cardiovascular mortality in patients without diabetes in the unmatched cohort

Figure 11 - Prognostic value of fasting vs. non-fasting LDL-C level on cardiovascular mortality in diabetic patients in the unmatched cohort

Figure 12 - Sensitivity analysis: Prognostic value of fasting vs. non-fasting LDL-C level including those with triglycerides $\geq 400$ mg/dL on cardiac mortality in the unmatched cohort

Figure 13 - Sensitivity Analysis: Prognostic value of fasting (<4 hours) vs. non-fasting (≥4 hours) LDL-C level on cardiovascular mortality in the unmatched cohort

Figure 14 - Sensitivity Analysis: Prognostic value of fasting (<12 hours) vs. non-fasting (≥12 hours) LDL-C level on cardiovascular mortality in the unmatched cohort

Figure 15 - Prognostic value of fasting vs. non-fasting triglyceride level on cardiovascular mortality in the unmatched cohort

Figure 16 - Prognostic value of fasting vs. non-fasting cholesterol level on cardiovascular mortality in the unmatched cohort
Supplemental Figure 1 – Prognostic value of fasting vs. non-fasting LDL-C level on all-cause mortality in the unmatched cohort.
**Supplemental Figure 2** – Sensitivity analysis: Prognostic value of fasting vs. non-fasting LDL-C level including those with triglycerides ≥400 mg/dL on all-cause mortality in the unmatched cohort
Supplemental Figure 3 – Prognostic value of fasting vs. non-fasting LDL-C level on all-cause mortality in patients without diabetes in the unmatched cohort
Supplemental Figure 4 – Prognostic value of fasting vs. non-fasting LDL-C level on all-cause mortality in diabetic patients in the unmatched cohort
**Supplemental Figure 5** – Sensitivity Analysis: Prognostic value of fasting (<4 hours) vs. non-fasting (≥4 hours) LDL-C level on all-cause mortality in the unmatched cohort

![ROC curve showing sensitivity and specificity for fasting vs. non-fasting LDL-C levels with AUC values and p-value provided.](image)

- Fasting: AUC [95% CI] = 0.58 [0.57, 0.59]
- Non-fasting: AUC [95% CI] = 0.60 [0.56, 0.64]

P-value (Fasting Vs. Non-fasting) = 0.37
Supplemental Figure 6 - Sensitivity Analysis: Prognostic value of fasting (<12 hours) vs. non-fasting (≥12 hours) LDL-C level on all-cause mortality in the unmatched cohort
Supplemental Figure 7 – Prognostic value of fasting vs. non-fasting triglyceride level on all-cause mortality in the unmatched cohort
Supplemental Figure 8 – Prognostic value of fasting vs. non-fasting total cholesterol level on all-cause mortality in the unmatched cohort
Supplemental Figure 9 – Prognostic value of fasting vs. non-fasting LDL-C level on cardiovascular mortality in the unmatched cohort
**Supplemental Figure 10** – Prognostic value of fasting vs. non-fasting LDL-C level on cardiovascular mortality in patients without diabetes in the unmatched cohort.
Supplemental Figure 11 – Prognostic value of fasting vs. non-fasting LDL-C level on cardiovascular mortality in diabetic patients in the unmatched cohort
Supplemental Figure 12 - Sensitivity analysis: Prognostic value of fasting vs. non-fasting LDL-C level including those with triglycerides ≥400 mg/dL on cardiovascular mortality in the unmatched cohort
Supplemental Figure 13 – Sensitivity Analysis: Prognostic value of fasting (<4 hours) vs. non-fasting (≥4 hours) LDL-C level on cardiovascular mortality in the unmatched cohort
Supplemental Figure 14 – Sensitivity Analysis: Prognostic value of fasting (<12 hours) vs. non-fasting (≥12 hours) LDL-C level on cardiovascular mortality in the unmatched cohort
Supplemental Figure 15 – Prognostic value of fasting vs. non-fasting triglyceride level on cardiovascular mortality in the unmatched cohort
Supplemental Figure 16 – Prognostic value of fasting vs. non-fasting total cholesterol level on cardiovascular mortality in the unmatched cohort.