Apolipoprotein B is not superior to non-high-density lipoprotein cholesterol for dyslipidemic classification of glycated hemoglobin-defined diabetic patients

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Abstract

Low-density lipoprotein (LDL) cholesterol (LDL-C) always underestimates the true cholesterol burden in diabetic patients. We aimed to explore the impact of the inclusion of apolipoprotein B (apoB) or non-high-density lipoprotein (HDL) cholesterol (non-HDL-C), which are alternative markers of LDL-related risk, results in a better classification of glycated hemoglobin (HbA1c)-defined diabetic patients into different dyslipidemic phenotypes.

We used data from the nationwide China Health and Nutrition Survey 2009 in which standardized HbA1c was measured. The prevalence of abnormal LDL using non-HDL-cholesterol (74.1%) was similar to the prevalence rate using LDL-C (75.2%), whereas the prevalence was relatively lower when using apoB (69.6%). In normotriglycerideremic HbA1c-defined diabetic patients, apoB and non-HDL-C were not superior to LDL-C in detecting abnormal LDL. However, in hypertriglycerideremic patients, apoB and non-HDL-C were superior to LDL-C for the detection of abnormal lipid levels, but apoB was not superior to non-HDL-C in detecting abnormal LDL in hypertriglycerideremic participants.

Both apoB and non-HDL-C identify high-risk dyslipidemic phenotypes that are not detected by LDL-C in hypertriglycerideremic HbA1c-defined diabetic patients, with the superiority of non-HDL-C over apoB.

Abbreviations: ACC = American College of Cardiology Foundation, ADA = American Diabetes Association, ApoB = apolipoprotein B, BP = blood pressure, CHNS = China Health and Nutrition Survey, CVD = cardiovascular disease, IDL = intermediate-density lipoprotein, LDL-C = low-density lipoprotein cholesterol, NECP = National Cholesterol Education Program, Non-HDL-C = non-high-density lipoprotein cholesterol, TC = total cholesterol, TG = triglyceride, VLDL = very-low-density lipoprotein, WC = waist circumference.

Keywords: apolipoprotein B, diabetes, HbA1c, non-HDL-C

1. Introduction

Diabetes mellitus is associated with an increased risk of cardiovascular disease (CVD). Dyslipidemia, that frequently occurs in diabetic patients, is a major modifiable risk factor for the accelerated development of CVD. Low-density lipoprotein cholesterol (LDL-C) has been considered a major marker for accelerated development of CVD. Low-density lipoprotein cholesterol for dyslipidemic characteristic dyslipidemic profile consisting of hypertriglycerideremia and elevated triglyceride (TG)-rich lipoproteins (including very-low-density lipoprotein [VLDL] and intermediate-density lipoprotein [IDL]), and small dense LDL particles. LDL-C cannot cover the whole burden of cholesterol. Thus, LDL-C definition underestimates the true cholesterol burden in such individuals, as evidenced by the residual CVD risk in the face of aggressive cholesterol-lowering therapies to achieve the desirable LDL-C levels. This resulted in a recommendation by the Adult Treatment Panel (ATP) III of the National Cholesterol Education Program (NECP) to determine non-high-density lipoprotein cholesterol (non-HDL-C) and apolipoprotein B (apoB) levels. Both represent the cholesterol content of all atherogenic lipoproteins (LDL, VLDL, and IDL cholesterol), and can function as alternative therapeutic targets in hypertriglycerideremic or diabetic patients. Studies that compare apoB and non-HDL-C for the identification of dyslipidemic phenotypes among glucose-defined diabetic patients have reported that apoB is superior to non-HDL-C in the detection of high-risk dyslipidemic phenotypes. More recently, glycated hemoglobin (HbA1c) has been endorsed as an alternative diagnostic criterion for diabetes. To our knowledge, there are limited data on dyslipidemic phenotypes while incorporating apoB or non-HDL-C among HbA1c-defined diabetic patients. Hence, we aimed to explore the impact of the inclusion of apoB and non-HDL-C on the classification of HbA1c-defined diabetic patients into different dyslipidemic phenotypes.

2. Methods

2.1. Study population

We used data from the China Health and Nutrition Survey (CHNS) for our analysis. Full details of the study have been
described.\[8\] Briefly, CHNS examinations were conducted in 1989, 1991, 1993, 1997, 2000, 2004, 2006, and 2009. For each round, a stratified, multistage, random cluster process was employed to draw study sample from each of the 9 provinces (Liaoning, Heilongjiang, Jiangsu, Shandong, Henan, Hebei, Hunan, Guangxi, and Guizhou). These 9 provinces cover approximately 36% of China’s population and vary significantly in terms of geography, economic development, and health status. Written informed consent was obtained from each participant, and the study was approved by the institutional review committees of the University of North Carolina at Chapel Hill, the National Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, and the China-Japan Friendship Hospital, Ministry of Health. The study protocol followed the ethical guidelines of the 1975 Declaration of Helsinki and the study protocol had previously been approved by the institution’s ethics committee on research on humans.

Since fasting blood samples were initially collected in 2009, this study examined data from CHNS 2009. All participants were asked to complete a structured questionnaire on history of current and previous illness and medical treatment. A total of 10,038 adult respondents were surveyed at the 2009 examination. However, 1423 did not give blood, 402 were not fasting before blood collection, and 62 were pregnant, resulting in a total of 8151 individuals with fasting blood samples. Exclusion criteria included participants with extreme TG (>500 mg/dL) or HDL-C (>100 mg/dL) values, and no information on total cholesterol (TC), TG, LDL-C, HDL-C, apoB or Hba1c. Hba1c was used to diagnose diabetes; therefore, participants with anemia (hemoglobin <13 g/dL in men and <12 g/dL in women) and chronic kidney disease (estimated glomerular filtration rate <15 mL/min per 1.73 m²) were excluded. In addition, participants using lipid-lowering medication were also excluded. The remaining 7761 participants were included in the present analysis.

2.2. Measurements

Weight, height, waist circumference (WC), and blood pressure (BP) were measured following standardized protocols from the World Health Organization. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). WC was measured with an inelastic tape at the midpoint located between the bottom of the rib cage and the top of the iliac crest at the end of exhalation. Seated systolic/diastolic BP was measured using mercury manometers by trained technicians in triplicate after a 10-minute rest of the participant. The 3 BP readings were averaged.

2.3. Biochemical measurements

Blood was collected after overnight fasting of >8 hours. All samples were analyzed in a national central laboratory in Beijing, China, with strict quality control. Fasting plasma glucose (FPG) was measured by the GOD-PAP method (Randox Laboratories Ltd, UK). All lipids (TC, TG, LDL-C, and HDL-C) were measured by Hitachi 7600 automated analyzer (Hitachi Inc., Tokyo, Japan). TC, LDL-C, and HDL-C were also measured enzymatically (Kyowa, Japan). Non-HDL-C was calculated as TC minus HDL-C. TG was measured by the GPO-PAP method (Kyowa, Japan). ApoB was measured by the immunoturbidimetric method (Randox Laboratories Ltd, UK).

2.4. Definitions

According to the 2015 American Diabetes Association (ADA) criteria,\[7\] diabetes is defined as Hba1c ≥6.5%.

According to the current NECP/Adult Treatment Panel (ATP III) guidelines,\[6\] elevated TG is defined as ≥150 mg/dL.

According to the ADA and the American College of Cardiology Foundation (ACC),\[9\] elevated apoB is defined as ≥90 mg/dL; and elevated non-HDL-C as ≥130 mg/dL.

2.5. Statistical analysis

All statistical analyses were performed using SPSS software (version 12.0 for Windows; SPSS, Chicago, IL). Continuous variables were presented as medians and interquartile ranges (IQRs) due to their skewed distribution. Categorical variables were presented as numbers (percentages). Of the 7761 participants, 637 participants were classified as Hba1c-defined diabetes. The patients were divided into 4 exclusive groups based on the presence or absence of TG ≥150 mg/dL and apoB ≥90 mg/dL. To compare, participants were also categorized based on TG and non-HDL-C cut-off points with levels of 150 mg/dL for TG and 130 mg/dL for non-HDL-C. LDL-C values corresponding to the apoB or non-HDL-C cut-off concentrations are not known, therefore, a value of 100 mg/dL for LDL-C was chosen to identify patients with dyslipidemic phenotypes in consensus with the report from the ADA/ACC panel.\[9\] Participants were also categorized into 4 phenotypes based on the conventional approach of using TG and LDL-C levels. For all continuous variables, Kruskal–Wallis analysis of median test was used followed by the Mann–Whitney U test for pairwise comparisons. Chi-square test was performed to assess differences in proportions across groups. The kappa (κ) statistic was calculated to test for an agreement between apoB- and non-HDL-C-based identification of dyslipidemic phenotypes. Values for κ can be between 0 and 1, with a value of ≥0.75 meaning strong agreement, whereas with a value of <0.40 indicating poor agreement. Significance was accepted at a 2-tailed P value of <0.05.

3. Results

Using the conventional classification, 8.0% of participants were identified as normal, 16.8% as hypertriglyceridemic with normal LDL-C levels, 38.0% with normal TG and increased LDL-C levels, and 37.2% as hypertriglyceridemic with increased LDL-C levels (Fig. 1A). Hence, 75.2% had abnormal LDL, as demonstrated by increased LDL-C. Using TG and apoB to identify dyslipidemic phenotypes, 16.3% of participants were identified as normal, 14.3% as hypertriglyceridemic with normal apoB levels, 29.7% with normal TG and increased apoB levels, and 39.9% as hypertriglyceridemic with increased apoB levels (Fig. 1B). In total, 69.6% of the Hba1c-defined diabetic participants had abnormal LDL, as evidenced by increased apoB. The corresponding results using TG and non-HDL-C to classify dyslipidemic phenotypes are shown in Figure 1C: 17.4% were normal, 8.5% were hypertriglyceridemic with normal non-HDL-C levels, 28.6% with normal TG and increased non-HDL-C, and 45.5% were hypertriglyceridemic with increased non-HDL-C. Thus, 74.1% had abnormal LDL, as demonstrated by increased non-HDL-C. Thus, somewhat similar proportions of the cohort were identified as abnormal LDL by the conventional LDL-C-based approach and the non-HDL-C-based approach. ApoB was not superior to non-HDL-C or LDL-C in detecting abnormal LDL.
Moderate agreement existed for LDL-C- and apoB-based diagnoses of abnormal LDL ($\kappa = 0.65; 95\% \text{ CI} 0.59\text{–}0.72$). Discordant classifications were seen for 9.7% of participants who had an LDL-C $\geq 100 \text{mg/dL}$ and apoB $< 90 \text{mg/dL}$ and for 4.1% who had an apoB $\geq 90 \text{mg/dL}$ and LDL-C $< 100 \text{mg/dL}$. For comparison, patients were also categorized based on LDL-C and non-HDL-C. Again, moderate agreement existed for LDL-C- and non-HDL-C-based diagnoses of abnormal LDL ($\kappa = 0.47; 95\% \text{ CI} 0.39\text{–}0.54$). Discordant classifications were seen for 10.7% of participants who had an LDL-C $\geq 100 \text{mg/dL}$ and non-HDL-C $< 130 \text{mg/dL}$ and for 9.6% who had a non-HDL-C $\geq 130 \text{mg/dL}$ and LDL-C $< 100 \text{mg/dL}$.

Since TG levels have a strong effect on the associations of apoB or non-HDL-C with LDL-C,[10] we evaluated the discordance between classifications based on LDL-C and apoB or based on LDL-C and non-HDL-C according to TG levels (TG $< 150 \text{mg/dL}$ and TG $\geq 150 \text{mg/dL}$) (Fig. 2). In participants with TG $< 150 \text{mg/dL}$, 55 of the 293 participants considered as abnormal LDL according to LDL-C fell into the normo-apoB phenotype, whereas only 2 of the 293 participants considered as abnormal LDL according to apoB fell into the normo-LDL-C phenotype (Fig. 2A). In participants with increased TG, 7 of the 344 participants considered as abnormal LDL according to LDL-C fell into the normo-apoB phenotype, whereas 24 of the 344 participants considered to have abnormal LDL according to apoB fell into the normo-LDL-C phenotype. The corresponding results using LDL-C and non-HDL-C to classify dyslipidemic phenotypes are shown in Figure 2B. Non-HDL-C detected fewer participants with abnormal LDL than LDL-C in the normotriglyceridemic subgroup: 61 of the 293 participants considered as abnormal LDL according to LDL-C fell into the normo-non-HDL-C phenotype, whereas only 1 of the 293 participants considered as abnormal LDL according to non-HDL-C fell into the normo-LDL-C phenotype. However, in the hypertriglyceridemic state, 7 of the 344 participants considered as abnormal LDL according to LDL-C fell into the normo-non-HDL-C phenotype, whereas 60 of the 344 participants considered as abnormal LDL according to non-HDL-C fell into the normo-LDL-C phenotype. Hence, apoB was not superior to non-HDL-C in detecting abnormal LDL in hypertriglyceridemic participants, which was further supported by the results shown in Figure 3: 36 ($41\% \div 5 = 36$) more participants considered as abnormal LDL according to non-HDL-C fell into the normo-apoB phenotype.

Although most CVD risk profiles were comparable between participants with hyper-non-HDL-C-normo-apoB and participants with hyper-apoB-normo-non-HDL-C, participants with hyper-non-HDL-C-normo-apoB had higher levels of uric acid, alanine aminotransferase, TC, TG, non-HDL-C, and lower levels of HDL-C (Table 1).
4. Discussion

To our knowledge, this is the first study to explore dyslipidemic phenotypes while incorporating apoB or non-HDL-C in HbA1c-defined diabetic patients. In the present study, although roughly equivalent proportions of abnormal LDL were identified by LDL-C- and non-HDL-C-based approach, the discordance of prevalence between these 2 methods was approximately 20.3%.

As shown in Figure 2, our results indicate that apoB and non-HDL-C are superior to LDL-C in identifying abnormal LDL in hypertriglyceridemic HbA1c-defined diabetic patients. A possible explanation for this finding could be that in patients with hypertriglyceridermia, TG molecules from VLDL are exchanged by cholesterol esters from LDL, resulting in TG-enriched LDL particles and cholesterol-enriched VLDL particles. Hence, apoB and non-HDL-C give a better estimation of true LDL particle number than LDL-C in hypertriglyceridemia patients.

Most studies, in which diabetes is diagnosed by glucose, have favored apoB over non-HDL-C for identifying LDL-related dyslipidemic phenotypes. However, these studies did not assess the discordance between classifications based on these LDL-related measures, but this was done according to TG levels. The present report, in which diabetes is diagnosed by HbA1c, showed a relevant stratified analysis. We provided evidence of the superiority of apoB or non-HDL-C over LDL-C in the identification of LDL-related dyslipidemic phenotypes in hypertriglyceridemic patients. Moreover, our present report indicated that non-HDL-C gives a better identification of patients at risk than apoB in hypertriglyceridemic patients. Although explanations for these differences remain unknown, it is probably related to differences in the relation of glucose and HbA1c to insulin resistance or insulin secretion as insulin plays an important role in lipid metabolism. Furthermore, glycation of apoB may also contribute to these differences. The greater susceptibility of small-dense-LDL to glycation is likely to contribute to the raised levels of circulating glycated-apoB and therefore decreased plasma apoB levels.

In the present study, measurements of non-HDL-C identified a subgroup of normolipidemic and hypertriglyceridemic patients with increased non-HDL-C levels. The identification of the dyslipidemic phenotype with increased non-HDL-C levels is noteworthy as the core lipid composition of LDL is altered in a proatherogenic direction. Accumulating the results of prospective studies indicated that non-HDL-C is a stronger predictor of CVD than LDL-C. Our data suggested that the prevalence of dyslipidemia using non-HDL-C is similar to the prevalence using LDL-C, indicating that non-HDL-C may still be a useful marker for dyslipidemia in HbA1c-defined diabetic patients. Moreover, both apoB and non-HDL-C identify additional high-risk dyslipidemic phenotypes that were not detected by LDL-C-based conventional approach in hypertriglyceridermic patients. There-

![Figure 3. Venn diagram for a visual display of how the 3 parameters (triglyceride, apolipoprotein B, and non-HDL- cholesterol) cluster together. non-HDL=non-high-density lipoprotein.](image)
fore, the present study supports the current guidelines that recommend apoB or non-HDL-C as alternative targets of therapy to LDL-C for the management of dyslipidemias in individuals with diabetes or high TG levels.\(^5,^9\) However, the guidelines do not comment that non-HDL-C is preferred over apoB. Our results that apoB is not superior to non-HDL-C in the dyslipidemic classification of hypertriglyceridemic HbA1c-defined diabetic patients together with the logistical advantages of non-HDL-C (a cost-free test) may result in a preference of non-HDL-C by a clinician in hypertriglyceridemic HbA1c-defined diabetic patients. Our previous work has also shown that apoB is not superior to non-HDL-C in correlating with insulin resistance.\(^18\)

The present study has several limitations. First, lipoproteins and apolipoproteins were not measured by the more sophisticated method of nuclear magnetic resonance spectroscopy. However, increasing evidence suggests that the association of coronary artery calcification with nuclear magnetic resonance-measured lipoproteins is comparable to that with lipids measured by standard methods.\(^19,^20\) Second, we studied a cohort of HbA1c-defined Chinese diabetic participants, thus, the results may not be generalizable to other racial or ethnic patients. Third, the cross-sectional nature of this study makes it difficult to draw conclusions on causality between different lipid phenotypes and the relative CVD risk in HbA1c-defined diabetic patients. Nevertheless, the analysis of the dyslipidemic classification based on LDL-related measures was not influenced by this particular study design. At last, the cut-offs used for LDL-C, apoB, and non-HDL-C, which were reaffirmed by the ATPIII update,\(^20\) as well as by the ADA/ACC,\(^9\) do not correspond to the same percentile distribution of patients. However, the discordance of prevalence defined according to concentrations of LDL-C compared to apoB, or non-HDL-C was common,\(^11,^21\) regardless of the cut-offs for LDL-C, apoB, and non-HDL-C that were used.

In conclusion, use of apoB and non-HDL-C did not result in a better classification of high-risk dyslipidemia phenotypes than LDL-C in normotriglyceridemic HbA1c-defined diabetic patients into high-risk dyslipidemia phenotypes. However, both apoB and non-HDL-C identified high-risk dyslipidemia phenotypes not detected by LDL-C in hypertriglyceridemic HbA1c-defined diabetic patients, with non-HDL-C being superior to apoB. Therefore, our results support the use of non-HDL-C for diagnostic and even therapeutic purposes in hypertriglyceridemic HbA1c-defined diabetic patients.

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