Spring 12-2019

Cholesterol Efflux Capacity And Its Association With Metabolic Syndrome In A Multi-Ethnic Population (Dallas Heart Study): A Cross-Sectional Study

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Presented to the Faculty of The University of Texas

School of Public Health

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF PUBLIC HEALTH

THE UNIVERSITY OF TEXAS
SCHOOL OF PUBLIC HEALTH
Houston, Texas
December, 2019

ACKNOWLEDGEMENTS

This work was made possible thanks to the excellent mentorship of Dr. Anand Rohatgi, Dr. Bijal Balasubramanian, and Dr. Ian Neeland. Their guidance led to the completion of this work.

This work was supported in part by grant UL1TR001105 from the National Center for Advancing Translational Science, National Institutes of Health.

CHOLESTEROL EFFLUX CAPACITY AND ITS ASSOCIATION WITH METABOLIC SYNDROME IN A MULTI-ETHNIC POPULATION (DALLAS HEART STUDY): A CROSS-SECTIONAL STUDY

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Metabolic syndrome (MetS) is a multi-component risk factor for cardiovascular disease (CVD) and type 2 diabetes. MetS has been found to be associated with increased risk of incident CVD, cardiovascular morbidity and mortality, and prevalence atherosclerosis. Cholesterol efflux capacity (CEC) is a measure of the functional property of high-density lipoprotein cholesterol (HDL-C). In addition, it characterizes the ability of HDL-C to accept cholesterol from extra-hepatic cells in the periphery to the liver and has been shown in clinical studies to be inversely associated with atherosclerosis cardiovascular disease (ASCVD) and incident cardiovascular disease (CVD) . Low HDL-C is one of the components of MetS and it is important to understand how the functionality of HDL captured through CEC is affected in MetS. The aim of this study was to evaluate the association between CEC and MetS in a multi-ethnic population. In addition, the results obtained based on the labeled cholesterol used in the efflux assay were compared for similarities and differences.

A cross-sectional study was performed using data obtained from participants at the entry into Dallas Heart Study phase 2 (DHS 2). DHS 2 is a subset of participants from DHS

1, a multiethnic probability-based cohort study of Dallas County residents supplemented by recruitment of participants' spouses or significant others. Multivariate regression analyses were performed to assess the relationship between CEC and MetS.

A total of 2942 participants were included in the study. The mean age was 49.4 years. A total of 40% of the participants were men and 52% were non-Hispanic Black. CEC measured using radiolabeled cholesterol was found to be inversely associated with MetS in the unadjusted model (odds ratio per 1-SD 0.86; 95%CI 0.80 – 0.93; *P*=0.0002). This finding remained significant after adjusting for demographics, modifiable risk factors, lipids, post-menopausal status, and history of cardiovascular disease. CEC measured using fluorescent-labeled cholesterol was not significantly associated with MetS in the unadjusted model, but significant after adjusting for lipids (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol) (adjusted odds ratio per 1-SD 0.82; 95%CI 0.73 – 0.93; *P*=0.0013).

There was an inverse relationship between cholesterol efflux capacity, irrespective of the labeled cholesterol used in the efflux assay, and metabolic syndrome. With the observed association between cholesterol efflux capacity and metabolic syndrome, cholesterol efflux capacity can serve as a marker to predict metabolic syndrome and to understand the functionality of HDL-C in metabolic syndrome, ultimately allowing for early detection and intervention in reducing the risk of cardiovascular disease.

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BACKGROUND

Epidemiology of Metabolic Syndrome and Obesity

Metabolic syndrome (MetS) is a cluster of risk factors that include elevated plasma triglyceride, blood pressure, fasting blood glucose, and waist circumference in addition to a reduced high-density lipoprotein cholesterol (HDL-C). Based on the 2007 to 2014 National Health and Nutrition Examination Survey (NHANES) survey, 34.3% of Americans had MetS; however, the prevalence of MetS was unequally distributed among the ethnic groups. MetS was lower among non-Hispanic Black males than White males and Mexican-Americans males while the prevalence was higher among Mexican-Americans females than White and Black females.[1] Also, the prevalence of MetS has been found to increase with age. Metabolic syndrome was found to be present in 19.3% of people 20 to 39 years of age, 37.7% of people 40 to 59 years of age, and 54.9% of people ≥60 years of age.[1]

Obesity is a major driver of MetS[2] and its overall age-adjusted prevalence among US adults aged ≥20 years was 39.6% (37.9% of males and 41.1% of females).[1] The prevalence of obesity is higher among non-Hispanic Black females than among non-Hispanic Black males and also higher among Hispanic females than among Hispanic males.[1] From the Behavior Risk Factor Surveillance System (BRFSS) 2014-2016 data, the prevalence of obesity was found to be higher among Hispanic adults and non-Hispanic Black adults than White adults.[1] Although the prevalence of MetS increases with age, the prevalence of obesity decreases with age. The prevalence of obesity among middle-aged adults (40-59 years) was found to be 40.2% and 37.0% among older adults (≥60 years).[1]

Metabolic Syndrome and Cardiovascular Disease

MetS is a multicomponent risk factor for Cardiovascular Disease (CVD) and type 2 diabetes. In several population-based studies (Dallas Heart Study, Framingham, Danish, Hoorn, Atherosclerosis Risk in Communities) across the world involving participants who were free of CVD and diabetes at baseline, MetS was found to be associated with increased risk of incident CVD, cardiovascular morbidity and mortality, and prevalence atherosclerosis.[3-7] The role of MetS with and without central obesity on incident ischemic heart disease was examined among participants enrolled in Singapore Cardiovascular Cohort Study that were free of CVD at baseline and followed for an average of 9.6 years. MetS either with or without central obesity was found to increase the risk of ischemic heart disease.[8]

Cholesterol Efflux Capacity

Cholesterol efflux capacity (CEC) characterizes the ability of HDL-C to accept cholesterol from extra-hepatic cells in the periphery, including macrophage-derived foam cells in arterial atherosclerotic plaque, to the liver for excretion into the bile as either a free cholesterol or bile acids and this pathway is an important early step in anti-atherogenic reverse cholesterol transport.[9,10] There are several pathways that have been shown to mediate cholesterol efflux. These pathways are scavenger receptor class B type 1 (SR-B1), ATP binding cassette transporter G1 (ABCG1), ATP binding cassette transporter A1 (ABCA1), and aqueous diffusion.[11-13] Among these pathways, ABCA1 pathway has been shown to play a major role in the maintenance of a normal cholesterol level in tissues. This has been demonstrated by the observed accumulation of large amounts of cholesterol in macrophages

in mouse models with knocked out ABCA1 gene and in individuals with ABCA1 genetic mutations (Tangier disease).[14,15]

Cholesterol Efflux Capacity and Metabolic Syndrome

In the assessment of how the functionality of HDL-C captured through CEC is affected in MetS, results from studies have been contradictory in the relationship between CEC and MetS. In a study that comprised of patients with personal history of dyslipidemia referred to a hospital in Paris, France, the association between clinical and biological features of MetS and CEC was investigated. Individual criterion of MetS was associated with CEC and there was a statistically significant inverse relation between increased number of MetS criteria and CEC independent of other traditional CVD risk factors.[16] Among patients with untreated MetS, ABCA1 mediated cholesterol efflux was higher, but with no difference in ABCG1 mediated cholesterol efflux when compared to a gender and age matched healthy controls.[17] Another study done in the Netherlands that involved participants free of clinical manifestation of CVD, CEC was slightly higher in participants with MetS compared to participants without MetS, but this difference was not significant after adjusting for age, sex, and diabetes status.[18]

In a case-control study, Borja et al explored HDL-apolipoprotein A-I exchange (HAE), a measure of HDL function and a key step in reverse cholesterol transport, and ABCA1-specific CEC in MetS patients without diabetes and CVD and normolipidemic control subjects. HAE and ABCA1-specific CEC were significantly reduced in patients with MetS compared to the normolipidemic age and sex matched control subjects.[19] In a cross-sectional study by Annema et al that examined the association of CEC and MetS in a high

cardiometabolic risk population of Caucasian origin from the CODAM (Cohort on Diabetes and Atherosclerosis Maastricht) cohort. CEC was significantly reduced in patient with MetS compared with patient without MetS.[20] CEC was also found to be negatively related to MetS and this relationship remained significant after adjusting for clinical covariates like age, sex, current smoking, alcohol consumption, CVD, glucose lowering drugs, lipid modifying drugs, and antihypertensive medication.[20] CEC has also been found to be decreased in women with polycystic ovarian syndrome compared to health women controls and reduced in all women with MetS compared to those without MetS.[21]

CEC Measurement Methods and Association with Cardiovascular Events, Risk Factors, and HDL-C

Many studies involving CEC generally use radiolabeled cholesterol efflux assay as the standard protocol to measure cholesterol efflux, but this method is not ideal to develop a high-throughput assay to assess efflux in a population study involving large number of serums.[22] An alternative method of efflux measurement is to substitute the radiolabeled cholesterol for a fluorescent-labeled cholesterol, which has been shown to provide an efficient, rapid, and high-throughput assay to measure efflux in a large number of serums from a population study.[22] In addition, fluorescent-labeled cholesterol efflux was found to be more sensitive for determining ABCA1-mediated efflux than radiolabeled cholesterol efflux as ABCA1-mediated efflux has been shown to play a major role in maintenance of a normal cholesterol levels in tissues.[22] In a study that compared cholesterol efflux using fluorescent-labeled cholesterol efflux assay with that of radiolabeled cholesterol efflux assay, cholesterol efflux measured by fluorescent-labeled cholesterol efflux assay did not significantly correlate

with HDL-C while cholesterol efflux measured by radiolabeled cholesterol efflux assay correlated significantly with HDL-C (r2 = 0.6, *P*<0.0001), although both efflux methods were significantly correlated with each other.[22]

In clinical studies, both CEC measured by fluorescent-labeled cholesterol and radiolabeled cholesterol efflux assays have shown consistent findings in their association with cardiovascular events, but there are differences in their association with HDL-C and other risk factors. Studies from Dallas Heart Study and Guangdong Coronary Artery Disease Cohorts used fluorescent-labeled cholesterol efflux assay to measure CEC and showed a minimal correlation between CEC and HDL-C (reported correlation coefficients between 0.07 and 0.3).[23,24] EPIC-Norfolk and other studies have used radiolabeled cholesterol efflux assay to measure CEC and have shown strong correlation between CEC and HDL-C (reported correlation coefficients between 0.1 and 0.8).[25-30]

Knowledge Gaps and Public Health Significance

There are contradictory results regarding the association between CEC and MetS from several studies and no study has explored the association in a multi-ethnic population. DHS is a multi-ethnic population cohort study that allows for evaluation of clinical phenotypes, outcomes, and cardiovascular events in a multi-ethnic population. In addition, current studies examining the association between CEC and MetS have used efflux determined by radiolabeled cholesterol efflux assay and no study has examined this association using efflux determined by fluorescent-labeled cholesterol efflux assay. MetS is a multi-component risk factor for cardiovascular disease (CVD) and CEC may serve as a novel

biomarker to provide early detection of cardiometabolic risks and modification of CEC through developed therapies may help improve these risks.

Aims

The aim of this study was to perform a cross-sectional study involving a multi-ethnic population to evaluate the association between CEC and MetS. In addition, the results obtained based on the labeled cholesterol used in the efflux assay were compared for similarities and differences.

METHODS

Study Design and Study Population

The study design is a cross-sectional study involving Dallas Heart Study phase 2 (DHS2) participants. DHS2 is a longitudinal follow-up study of a subset of participants who completed the Dallas Heart Study phase 1 (DHS1), a multiethnic probability-based sample of Dallas County adults enrolled between 2000 and 2002 that was weighted to include approximately 50% Blacks/African Americans. Recruitment procedures and study design have been reported previously.[31] A second comprehensive clinical study assessment with repeat data collection was done in participants from DHS1 who volunteered between September 2007 and December 2009. In addition to DHS1 participants, the DHS2 cohort was supplemented by recruitment of participants' spouses or significant others. The assessments done included an extensive health survey, laboratory testing and imaging studies during their visit to the University of Texas Southwestern Medical Center. Data and samples were collected under the oversight of the institutional review board of the University of Texas Southwestern Medical Center. Participants with history of malignancies, history of End Stage

Renal Disease (ESRD), and Human Immunodeficiency Virus (HIV) were excluded from this study. This study protocol was determined to qualify for exempt status according to 45 CFR 46.101 (b) by the Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston.

Assessment of Demographics, Anthropometric Variables, and Covariates

Age, sex, race/ethnicity, post-menopausal status, anti-hypertensive medication use, statin use, smoking status (current or past smoker), drinking status (current or past drinker) and alcohol consumption (grams/week) were self-reported. Height and weight were measured using a standard physician's scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured to the nearest centimeter at the level of the umbilicus. Physical activity was measured using Actical (Philips Respironics, Bend, Oregon) physical activity monitor that the participants wore on their wrist for 7 days and the monitors were set to record bodily movement, which was quantified as an activity count (AC) per minute and moderate to vigorous activity (AC >1500 per minute) were recorded.[32] Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were obtained by an average of the third through fifth measurements of blood pressures. Fasting concentration of glucose, insulin, and glycated hemoglobin (HgbA1c) were determined from venous blood samples. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using [fasting insulin (μIU/ml) × fasting glucose (mmol/liter) /22.5]. Plasma lipids measurements have been described previously.[31] History of diabetes was defined by a fasting glucose level ≥126 mg/dL, non-fasting glucose of >200 mg/dL, HbA1c ≥ 6.5% or self-reported history of diabetes in addition to the use of

any glucose lowering medication. History of CVD was defined as self-reported or adjudicated myocardial infarction, congestive heart failure (CHF)/CHF hospitalization, stroke, transient ischemic attack, peripheral revascularization, unstable angina, atrial fibrillation, CABG surgery, percutaneous coronary intervention, and other vascular events.

Assessment of Cholesterol Efflux Capacity and Metabolic Syndrome

CEC was assessed in vitro using both radiolabeled cholesterol efflux assay and fluorescent-labeled cholesterol efflux assay by measuring the efflux of labeled cholesterol from J774 macrophages to apolipoprotein B (ApoB)–depleted plasma from study participants. These assays evaluate cholesterol efflux as mediated by multiple transports and passive diffusion, although fluorescent-labeled cholesterol efflux assay has been shown to be more sensitive for ATP-binding cassette transporter A1 (ABCA1)-mediated efflux.[22] Individual efflux values were normalized to values obtained with a pool of 2% apoB-depleted plasma from selected controls which make the efflux values not to have a specific unit. The details of both measurement methods have been described previously.[22,33] MetS, according to the Adult Treatment Panel (ATP) III criteria, was defined as having any 3 or more of the following criteria: waist circumference >102 cm in men or >89 cm in women; triglycerides ≥150 mg/dL; HDL-C <40 mg/dL in men or <50 mg/dL in women; blood pressure ≥130/≥85 mmHg; and fasting blood glucose (FBG) ≥100 mg/dL.[34]

Statistical Analysis

CEC was described as both continuous and categorical (based on quartiles) variables. MetS was described as categorical variables based on yes/no status and increasing number of MetS components (MetS0 = participants without any MetS component; MetS1 = participants with 1 MetS component; MetS2 = participants with 2 MetS components; MetS3 = participants with 3 MetS components; and MetS4-5 = participants with 4 or 5 MetS components).

Demographic and clinical variables were compared across MetS categorical variables using Kruskal-Wallis test for continuous variables and $\chi 2$ test for categorical variables. Demographic and clinical variables were compared across increasing quartiles of both CEC measured by fluorescent-labeled cholesterol efflux assay and CEC measured by radiolabeled cholesterol efflux assay using Jonckheere–Terpstra trend test for continuous and categorical variables. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as means with standard deviations for normally distributed variables and medians with interquartile ranges for skewed variables.

Multivariate logistic regression analyses were performed to assess the association between CEC (continuous and quartiles) and MetS status. Multivariate ordinal logistic regression analyses using a generalized link function were performed to assess the association between CEC (continuous) and increasing component of MetS. Multivariate linear regression analyses were performed to assess the association between CEC (continuous) and the individual components of MetS (waist circumference, SBP, DBP,

triglycerides, HDL-C, and FBG). Covariates were adjusted in five models for logistic regression analyses. Model 1 was unadjusted. Model 2 adjusted for demographics (age, sex, and race/ethnicity). Model 3 adjusted for modifiable risk factors (physical activity, smoking status, and drinking status), in addition to the variables adjusted for in model 2. Model 4 adjusted for lipids (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol), in addition to the variables adjusted for in model 3. Model 5 adjusted for post-menopausal status, in addition to the variables adjusted for in model 4. Model 6 adjusted for history of CVD, in addition to the variables adjusted for in model 5. For linear regression analyses, covariates were adjusted for in one model. Model 1 was unadjusted while model 2 adjusted for demographics (age, sex, and race/ethnicity) and modifiable risk factors (physical activity, smoking status, and drinking status). Non-normally distributed continuous variables were log-transformed prior to use in regression analysis. Test for interaction was performed to identify effect modification of other covariates such as low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), gender, ethnicity, obesity, history of CVD, and history of DM in the relationship between CEC and MetS. Stratified analysis was presented to evaluate the association within each stratum of the interacting variables with significant interaction.

Standardized odds ratios (OR) with 95% confidence intervals (CI) were reported for the multivariable binomial and ordinal logistic regression models. Standardized regression coefficients (Std β) with 95% confidence intervals (CI) were also reported for linear regression models. The standardized measures of association corresponded to the impact of 1-SD increase in the independent variable on the variability of the dependent variable. Two-

sided P values <0.05 was considered statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Summary of Demographic and Clinical Variables in Study Participants

A total of 2942 participants were included in the study. The mean age was 49.4 years. A total of 40% of the participants were men and 52% were non-Hispanic Black (Table 1). For MetS components, mean BMI and waist circumference were 31.1 kg/m^2 and 97.2 cm respectively; median fasting blood glucose was 94 mg/dL; mean SBP and DBP were 133 mmHg and 81 mmHg respectively; mean HDL-C was 53 mg/dL; and median triglyceride was 102 mg/dL (Table 1).

Clinical and Biological Variables across MetS Status and Increasing Number of MetS Components

Individuals with MetS were more likely to be older, obese, and females (Table 2). A higher proportion of Hispanic had MetS compared with non-Hispanic (16% and 14%, *P*=0.0410; 28% and 32%, *P*=0.0133, respectively). Individuals with MetS had higher BMI, waist circumference, FBG, HgbA1c, insulin, HOMA-IR, SBP, DBP, triglyceride, VLDL-C, and lower HDL-C compared to individuals without MetS (Table 2 and Fig. 1). There was no significant difference in CEC measured by fluorescent-labeled efflux assay in individuals with MetS compared to those without MetS however, individuals without MetS had a significantly higher CEC measured by radiolabeled cholesterol efflux assay compared to individuals with MetS (absolute difference 0.03; *P*=0.0002). In addition, physical activity was significantly reduced in individuals with MetS compared with those without MetS

(absolute difference -10.69; $P<0.0001$). The prevalence of CVD, diabetes mellitus, antihypertensive drug use, and stain use were higher among individuals with MetS compared to individuals without MetS. Interestingly, the median alcohol consumption was higher among individuals without MetS compared to individuals with MetS (19.1 grams/week and 7.5 grams/week, respectively; $P<0.0001$) (Table 2). Individuals who had at least one component of MetS were similar to those with MetS (Table 2); however, these results were more pronounced among individuals who had between 4-5 MetS components. Individuals with no component of MetS had the highest cholesterol efflux capacity measured by radiolabeled cholesterol efflux assay and individuals with one component of MetS reported higher alcohol consumption compared to individuals in other categories (see Appendix A). For the components of MetS, FBG, waist circumference, SBP, DBP, and triglyceride increased across increasing number of MetS components while HDL-C decreased across increasing number of MetS components (See Appendix A).

Clinical and Biological Variables across Quartiles of Cholesterol Efflux Capacity

The proportion of non-Hispanic Black participants was significantly higher in the lowest quartile of CEC measured by fluorescent-labeled cholesterol efflux assay compared to the highest quartile (56% and 42%, respectively; $P=0.0018$) (Table 3). Similar result was seen in CEC measured by radiolabeled cholesterol efflux assay (54% and 46%, respectively; $P=0.0003$) (Table 4). The proportion of Hispanic participants was significantly higher in the highest quartile of CEC measured by fluorescent-labeled cholesterol efflux assay compared to Hispanics in the lowest quartile (17.14% and 11.47 %, respectively; $P=0.0062$) (Table 3). In addition, the proportion of non-Hispanic White participants was significantly higher in the

highest quartile CEC measured by radiolabeled efflux assay compared to the lowest quartile (29% and 36%, respectively; *P*=0.0007) (Table 4). Individuals in the lowest quartile of CEC measured by fluorescent-labeled cholesterol efflux assay had higher BMI, waist circumference, insulin and HOMA-IR compared to individuals in the highest quartile while individuals in the lowest quartile of CEC measured by radiolabeled cholesterol efflux assay had higher BMI, waist circumference, and insulin compared to individuals in the highest quartile (Tables 3 and 4). Individuals in the highest quartile of both CEC measured by fluorescent-labeled cholesterol efflux and CEC measured by radiolabeled cholesterol efflux assays had higher HDL-C, LDL-C, VLDL-C, triglyceride, and median alcohol consumption (gram/week) compared to individuals in the lowest quartile (Tables 3 and 4).

Association between Cholesterol Efflux Capacity and MetS

In multivariate logistic regression analyses, there was a significant inverse relationship between CEC measured by radiolabeled cholesterol efflux assay and MetS in the unadjusted model (OR 0.86; 95%CI 0.80 – 0.93; *P*=0.0002) (Table 6). This finding remained significant after adjusting for demographics (age, sex, and ethnicity), modifiable risk factors (physical activity, smoking status, and drinking status), lipids (LDL-C and VLDL-C), post-menopausal status, and history of cardiovascular disease. Also, the association was strengthened after adjusting for LDL-C and VLDL-C (adjusted OR, aOR 0.71; 95%CI 0.62 – 0.80) (Table 6). For CEC measured by fluorescent-labeled cholesterol efflux assay, no significant association was found with MetS in the unadjusted model, but significant inverse relationship was found after adjusting for demographics (age, sex, and ethnicity), modifiable risk factors (physical activity, smoking status, and drinking status), LDL-C, and VLDL-C

(aOR, 0.82; 95%CI 0.73 – 0.93; *P*=0.0013) (Table 5). Similar findings were seen when CEC was assessed as quartiles. A significant inverse association between quartiles of CEC measured by radiolabeled cholesterol efflux assay and MetS was observed, which was again strengthened after adjusting for LDL-C and VLDL-C in model 4 (aOR for second vs. first quartile of CEC 0.50; 95%CI 0.36 – 0.71; *P*<0.0001 and aOR for fourth vs. first quartile of CEC 0.38; 95%CI 0.27 – 0.54; *P*<0.0001) (Table 8). Similar findings were observed in the association between CEC measured by fluorescent-labeled cholesterol efflux assay and MetS in model 4 (aOR for second vs. first quartile of CEC 0.81; 95%CI 0.58 – 1.13; *P*=0.2177 and aOR for fourth vs. first quartile of CEC 0.54; 95%CI 0.38 – 0.76; *P*= 0.0005) (Table 7).

Association between Cholesterol Efflux Capacity and Increasing Number of MetS Components

MetS participants were further categorized based on the number of MetS components and the association between CEC measured by fluorescent-labeled cholesterol efflux assay and MetS was assessed using multivariate ordinal logistic regression. A significant progressive decrease in aOR was observed among participants with increasing number of MetS components compared with MetS0, reference category, after adjusting for LDL-C and VLDL-C (aOR for MetS3 vs. MetS0 0.78; 95%CI 0.64 – 0.96; *P*=0.0159 and aOR for MetS4-5 vs. MetS0 0.64; 95%CI 0.50 – 0.82; *P*=0.0003) (Table 9 and Fig. 2). Similar trends were observed in the association between CEC measured by radiolabeled efflux assay and MetS after adjusting for LDL-C and VLDL-C (aOR for MetS2 vs. MetS0 0.77; 95%CI 0.64 – 0.92; *P*=0.0037 and aOR for MetS4-5 vs. MetS0 0.46; 95%CI 0.36 – 0.60; *P*<0.0001) (Table 10 and Fig. 3).

Association between Cholesterol Efflux Capacity and Individual MetS Components

In multivariate linear regression analyses, CEC measured by fluorescent-labeled efflux assay was inversely associated with waist circumference (Adjusted Std β = -0.071; 95%CI -0.108 to -0.035; *P*=0.0001) and directly associated with log TG (Adjusted Std β = 0.107; 95%CI 0.071 to 0.143; *P*<0.0001) and HDL-C (Adjusted Std β = 0.140; 95%CI 0.103 to 0.176; *P*<0.0001) (Table 11). These findings remained significant after adjusting for demographics and modifiable risk factors. A significant inverse association was found between CEC measured by radiolabeled cholesterol efflux assay and waist circumference (Adjusted Std β = -0.117; 95%CI -0.154 to -0.080; P < 0.0001) (Table 11). CEC measured by radiolabeled cholesterol efflux assay was significantly associated with log TG (Adjusted Std β = 0.095; 95%CI 0.058 to 0.132; *P*< 0.0001), HDL-C (Adjusted Std β = 0.350; 95%CI 0.315 to 0.384; *P*<0.0001), and FBG (Adjusted Std β = 0.050; 95%CI 0.013 to 0.088; *P*=0.0078) (Table 11). These associations remained significant after adjusting for demographics and modifiable risk factors. CEC radiolabeled was not significantly associated with SBP and DBP while CEC fluorescent was not significantly associated with SBD, DBP, and FBG.

Interaction between Cholesterol Efflux Capacity and Covariates in its Relationship with MetS

There was no significant interaction between CEC measured by radiolabeled efflux assay and other covariates such as LDL-C, VLDL-C, gender, ethnicity, obesity, history of CVD, and history of DM in its relationship with MetS (See Appendix C). There was significant interaction between CEC measured by fluorescent efflux assay and history of

CVD in its relationship with MetS (*P* for interaction = 0.03) (See Appendix B). The association between CEC measured by fluorescent-cholesterol efflux assay and MetS was preserved among those without a hx of CVD but was attenuated among those with a history of CVD (Hx CVD: aOR 1.25; 95%CI 0.72 to 2.16; *P*=0.4284; no hx CVD: aOR 0.79; 95%CI 0.69 to 0.90; *P*=0.0001) (See Appendix D).

DISCUSSION

This cross-sectional study assessed the relationship between cholesterol efflux capacity, assessed by measuring the efflux of labeled cholesterol from J774 macrophages to apolipoprotein B–depleted plasma using both fluorescent-labeled and radiolabeled cholesterol efflux assays, and metabolic syndrome in a multi-ethnic cohort. Cholesterol efflux capacity was found to be inversely associated with metabolic syndrome, regardless of efflux assay. In addition, there was a significant progressive reduction in cholesterol efflux capacity associated with increased number of metabolic syndrome components. Furthermore, cholesterol efflux capacity was found to be positively associated with individual components of metabolic syndrome such as triglyceride, HDL-C, fasting blood glucose, and negatively associated with waist circumference. These findings contradicted the findings from the study by Dullaart et al that assessed the ability of plasma from metabolic syndrome subjects to promote cholesterol efflux capacity out of a cultured human fibroblast using the radiolabeled efflux assay. In their study that involved a total of 170 participants (76 with metabolic syndrome and 94 without metabolic syndrome), they concluded that the ability of plasma to promote cholesterol efflux out of fibroblast that express abundant ABCA1 is not impaired in

individuals with metabolic syndrome despite the presence of low HDL-C.[18] The findings from this current study also contradicted the findings from Alenezi et al involving 59 subjects (22 with metabolic syndrome) that showed that cholesterol efflux capacity using radiolabeled cholesterol efflux assay from fibroblasts to plasma of patients with metabolic syndrome was not defective.[35] The two referenced studies above have similarities as they both used fibroblasts and radiolabeled cholesterol efflux assay in determining cholesterol efflux capacity and also involved a small sample size. Contrary to the results from these two studies, Gall et al demonstrated that cholesterol efflux measured by fluorescent-labeled cholesterol efflux assay from human THP-1 macrophages was reduced in 307 individuals with metabolic syndrome independent of age, LDL-C, lipid-lowering therapy, smoking status, and alcohol consumption.[16] The findings from the study by Gall et al were consistent with the findings from this current study, which also used macrophages.

Despite the varying correlations between cholesterol efflux capacity, based on the methods of efflux assay, and HDL-C, the associations with metabolic syndrome were similar especially after adjusting for VLDL-C. There was strengthening of the association between cholesterol efflux capacity and metabolic syndrome after adjusting for VLDL-C, which may be partly explained by the fact that triglyceride, one of the components of metabolic syndrome, is the main component of VLDL-C and highly correlated with VLDL-C.[35] VLDL-C in this case served as a negative confounder and biased the measure of association of cholesterol efflux capacity and metabolic syndrome towards the null. The prevalence of metabolic syndrome has been found to be unevenly distributed among ethnic groups[1] however, in this current study there was no difference in the association between cholesterol

efflux capacity and metabolic syndrome among ethnic groups and this was also the case for gender.

Several limitations of this study are worth mentioning. First, this is a cross-sectional analysis of a large multi-ethnic cohort and the race and ethnic distribution of the study sample with oversampling of Blacks do not reflect the general population, which limits the generalizability of this study. As expected in observation studies, temporality and causality cannot be properly assessed and this is the case in this cross-sectional study. Furthermore, there may be sampling bias as the data used for the study was from an existing database with voluntary participants who may be different from the general population in terms of health status and other important factors. In addition, the effect of multiple testing on statistical significance of measure of associations was not accounted for. Lastly, the use of lipid lowering, glucose lowering, and anti-hypertensive medications were not adjusted for in the multivariable regression analyses, which may be potential confounders. It is worth mentioning that the sample size for this study was large as compared to previous studies, which provided statistical power to avoid type II error and allowed for further exploration of the association of cholesterol efflux capacity and increasing number of metabolic syndrome components. In addition, this study included a multi-ethnic cohort and measured cholesterol efflux capacity using two different assays.

CONCLUSION

This cross-sectional study demonstrated an inverse relationship between cholesterol efflux capacity and metabolic syndrome in a multi-ethnic population. Cholesterol efflux capacity was also found to be positively correlated with individual components of metabolic syndrome such as HDL-C, triglyceride, fasting blood glucose, and negatively correlated with waist circumference. These findings were consistent regardless of the labeled cholesterol used in the efflux assay and remained significant after adjusting for demographics, modifiable risk factors, lipids, post-menopausal status, and history of cardiovascular disease. However, the associations were strengthened after adjustment for VLDL-C.

Metabolic syndrome is a multicomponent risk factor for cardiovascular disease and it has been found to be associated with increased risk of incident cardiovascular disease, cardiovascular morbidity and mortality, and prevalence atherosclerosis.[3-7] Low HDL-C is one of the components of metabolic syndrome and it is important to understand how the function of HDL-C is affected in this syndrome. With the observed association between cholesterol efflux capacity and metabolic syndrome, cholesterol efflux capacity can serve as a marker to predict metabolic syndrome and to understand the functionality of HDL-C in metabolic syndrome, ultimately allowing for early detection and intervention in reducing the risk of cardiovascular disease.

It is important to replicate these findings in longitudinal studies to address the issue of temporality. Giving that this is the only study till date that assessed the association between cholesterol efflux capacity measured by fluorescent-labeled cholesterol efflux assay and

metabolic syndrome, additional studies are necessary to replicate the findings from this study.

Table 1: Overall Demographic and Clinical Variables among Participants

	All Participants (N = 2942)
Age (year)	49.4 ± 11.1
Male (%)	40
Post-menopausal, Female (%)	32
Race/Ethnicity (%)	
Non-Hispanic Black	52
Non-Hispanic White	30
Hispanic	15
History of CVD (%)	6
History of DM (%)	16
Antihypertensive drugs (%)	35
Statin (%)	17
Ever Smoked (%)	23
Alcohol Consumption (grams/week)*	15.0 (2.1, 58.5)
Alcohol Consumption Status (%)	
Current Drinker	70
Recent Abstainer	20
Lifetime Abstainer	9
Obesity (%)	49
BMI (kg/m^2)	31.1 ± 7.5
Waist Circumference (cm)	97.2 ± 16.8
Hip Circumference (cm)	109.2 ± 15.5
Waist-to-hip Ratio	0.9 ± 0.1
FBG (mg/dL)*	94 (87, 103)
Insulin (uIU/mL)*	12.5 (8.2, 19.3)
HgbA1c (%)	5.8 ± 1.15
HOMA-IR (Glucose*Insulin / 22.5*18)*	3.0 (1.9, 4.9)
Physical Activity (Moderate/Vigorous)*	28.5 (13.9, 52.6)
SBP (mmHg)	132 ± 20
DBP (mmHg)	81 ± 9
Cholesterol Efflux Capacity (measured by florescent labeled cholesterol)	0.85 ± 0.24
Cholesterol Efflux Capacity (measured by radiolabeled cholesterol)	0.94 ± 0.18
TC (mg/dL)	192 ± 39.62
TG (mg/dL)*	102 (73, 145)
VLDL-C (mg/dL)*	20 (15, 29)
LDL-C (mg/dL)	115 ± 36
HDL-C (mg/dL)	53 ± 15

Data reported as mean ± SD, median (interquartile range), or percentage. CVD, cardiovascular disease; DM, diabetes mellitus; BMI, body mass index; FBG, fasting blood glucose; HgbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC; total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. *Non-normally distributed variable.

Table 2: Clinical and Biological Variables in Participants According to Metabolic Syndrome Status

	No MetS (N = 1823)	MetS (N = 1119)	ǂP Value (Two-sided)
Age (year)	47.7 ± 11.1	52.1 ± 10.7	**<0.0001**
Male (%)	42	38	**0.0301**
Post-menopausal, Female (%)	27	40	**<0.0001**
Race/Ethnicity (%)			
Non-Hispanic Black	51	54	0.1596
Non-Hispanic White	32	28	**0.0133**
Hispanic	14	16	**0.0410**
History of CVD (%)	5	14	**<0.0001**
History of DM (%)	5	33	**<0.0001**
Antihypertensive drugs (%)	22	57	**<0.0001**
Statin (%)	11	26	**<0.0001**
Ever Smoked (%)	25	21	**0.0055**
Alcohol Consumption (grams/week)*	19.1 (3.0, 68.3)	7.5 (1.5, 45.5)	**<0.0001**
Alcohol Consumption Status (%)			
Current Drinker	74	64	**<0.0001**
Recent Abstainer	17.85	24.01	**<0.0001**
Lifetime Abstainer	8	12	**<0.0001**
Obesity (%)	32	76	**<0.0001**
BMI (kg/m^2)	28.5 ± 6.3	35.4 ± 7.3	**<0.0001**
Waist Circumference (cm)	90.5 ± 14.1	108.0 ± 15.0	**<0.0001**
Hip Circumference (cm)	104.4 ± 13.3	117.2 ± 15.6	**<0.0001**
Waist-to-hip Ratio	0.9 ± 0.1	0.9 ± 0.1	**<0.0001**
FBG (mg/dL)*	91 (85, 96)	103 (94, 119)	**<0.0001**
Insulin (uIU/mL)*	9.9 (6.85, 14.21)	18.8 (13.1, 27.4)	**<0.0001**
HgbA1c (%)	5.5 ± 0.8	6.2 ± 1.5	**<0.0001**
HOMA-IR (Glucose*Insulin / 22.5*18)*	2.2 (1.5, 3.3)	5.1 (3.3, 8.2)	**<0.0001**
Physical Activity (Moderate/Vigorous)*	32.8 (16.7, 59.8)	22.1 (10.1, 42.2)	**<0.0001**
SBP (mmHg)	129 ± 20	139 ± 20	**<0.0001**
DBP (mmHg)	80 ± 9	83 ± 9	**<0.0001**
Cholesterol Efflux Capacity (measured by fluorescent-labeled cholesterol)	0.85 ± 0.23	0.84 ± 0.25	0.0849
Cholesterol Efflux Capacity (measured by radiolabeled cholesterol)	0.95 ± 0.17	0.92 ± 0.18	**0.0002**
TC (mg/dL)	192 ± 38	193 ± 42	0.9554
TG (mg/dL)*	87 (65, 116)	138 (98, 190)	**<0.0001**
VLDL-C (mg/dL)*	17 (13, 23)	27 (20, 38)	**<0.0001**
LDL-C (mg/dL)	115 ± 34	116 ± 38	0.7974
HDL-C (mg/dL)	57 ± 15	46 ± 12	**<0.0001**

Data reported as mean ± SD, median (interquartile range), or percentage. CVD, cardiovascular disease; DM, diabetes mellitus; BMI, body mass index; FBG, fasting blood glucose; HgbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC; total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. *Non-normally distributed variable. Bolded values indicate statistical significance. ǂTest for intergroup differences using Kruskal-Wallis test for continuous variables and $\chi 2$ for categorical variables.

Table 3: Clinical and Biological Variables in Participants across Increasing Quartile of Cholesterol Efflux Capacity Measured by Fluorescent-Labeled Cholesterol

	Cholesterol Efflux Capacity (Measured by Fluorescent-Labeled Cholesterol)				‡P Value for Trend (Two-sided)
	Q1 (N=706)	Q2 (N=707)	Q3 (N=707)	Q4 (N=706)	
Age (year)	50.0 ± 10.7	48.6 ± 11.3	48.5 ± 11.1	50.4 ± 11.0	**0.0022**
Male (%)	41	39	39	43	0.5834
Post-menopausal, Female (%)	33	31	32	31	0.5280
Race/Ethnicity (%)					
Non-Hispanic Black	56	53	51	42	**0.0018**
Non-Hispanic White	30	29	31	33	0.2510
Hispanic	11	15	15	17	**0.0062**
History of CVD (%)	10	8	8	8	0.1918
History of DM (%)	16	15	16	16	0.8531
Antihypertensive drugs (%)	40	34	34	34	**0.0303**
Statin (%)	19	15	17	16	0.2512
Ever Smoked (%)	23	23	22	24	0.6897
Alcohol Consumption (grams/week)*	15.0 (2.1, 58.5)	7.5 (1.5, 45.0)	15.0 (2.1, 58.5)	22.5 (3.0, 91.0)	**<0.0001**
Alcohol Consumption Status (%)					
Current Drinker	67	67	74	74	**0.0012**
Recent Abstainer	23	21	17	19	**0.0060**
Lifetime Abstainer	9	11	9	8	0.1972
Obesity (%)	54	49	50	43	**<0.0001**
BMI (kg/m^2)	31.8 ± 7.6	31.3 ± 7.4	31.2 ± 7.4	30.0 ± 7.2	**<0.0001**
Waist Circumference (cm)	99.0 ± 16.8	97.3 ± 16.4	97.0 ± 17.3	95.1 ± 16.2	**0.0002**
Hip Circumference (cm)	110.7 ± 15.3	109.6 ± 15.4	109.2 ± 15.8	107.0 ± 14.8	**<0.0001**
Waist-to-hip Ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.5461
FBG (mg/dL)*	95 (88, 103)	93 (86, 101)	94 (87, 103)	94 (87, 103)	0.2484
Insulin (uIU/mL)*	13.1 (8.5, 19.8)	12.6 (8.36, 19.96)	12.8 (8.5, 20.3)	11.3 (7.8, 17.3)	**0.0003**
HgbA1c (%)	5.8 ± 1.1	5.7 ± 1.1	5.7 ± 1.1	5.8 ± 1.3	0.3196
HOMA-IR (Glucose*Insulin / 22.5*18)*	3.1 (1.9, 5.0)	2.9 (1.9, 4.9)	3.0 (1.9, 5.2)	2.7 (1.7, 4.5)	**0.0113**
Physical Activity (Moderate/Vigorous)*	27.8 (14.0, 50.0)	28.0 (14.0, 50.7)	28.5 (14.4, 55.0)	28.4 (13.1, 53.5)	0.7666
SBP (mmHg)	133 ± 20.4	132 ± 20	132 ± 20	133 ± 21	0.2908
DBP (mmHg)	81 ± 10	81 ± 9	67 ± 10	81 ± 9	0.8193
Cholesterol Efflux Capacity	0.56 ± 0.11	0.77 ± 0.05	0.92 ± 0.04	1.15 ± 0.16	**<0.0001**
TC (mg/dL)	185 ± 38	187 ± 37	195 ± 40	202 ± 39	**<0.0001**
TG (mg/dL)*	98 (71, 132)	98 (70, 136)	102 (74, 145)	110 (79, 160)	**<0.0001**
VLDL-C (mg/dL)*	20 (14, 26)	20 (14, 27)	20 (15, 29)	22 (16, 32)	**<0.0001**
LDL-C (mg/dL)	113 ± 34	112 ± 35	118 ± 36	119 ± 37	**0.0003**
HDL-C (mg/dL)	50 ± 14	52 ± 14	53 ± 16	55 ± 16	**<0.0001**

Data reported as mean ± SD, median (interquartile range), or percentage. MetS indicates metabolic syndrome. MetS0 = participants without any MetS component. MetS1 = participants with 1 MetS component. MetS2 = participants with 2 MetS component. MetS3 = participants with 3 MetS component. MetS4-5 = participants with 4 or 5 MetS component. Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4,

fourth quartile. CVD, cardiovascular disease; DM, diabetes mellitus; BMI, body mass index; FBG, fasting blood glucose; HgbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC; total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. *Non-normally distributed variable. Bolded values indicate statistical significance. ǂTest for intergroup differences performed using Jonckheere–Terpstra trend test.

Table 4: Clinical and Biological Variables in Participants across Increasing Quartile of Cholesterol Efflux Capacity Measured by Radiolabeled Cholesterol

	Cholesterol Efflux Capacity (Measured by Radiolabeled Cholesterol)				**ǂP Value for Trend (Two-sided)**
	Q1 (N=696)	**Q2 (N=697)**	**Q3 (N=697)**	**Q4 (N=696)**	
Age (year)	50.0 ± 11.40	48.9 ± 11.2	49.9 ± 10.9	50.5 ± 10.5	**<0.0001**
Male (%)	44	40	41	36	**0.0041**
Post-menopausal, Female (%)	26	31	33	37	**<0.0001**
Race/Ethnicity (%)					
Non-Hispanic Black	54	56	50	46	**0.0003**
Non-Hispanic White	29	27	31	36	**0.0007**
Hispanic	14	14	16	15	0.4458
History of CVD (%)	10	6	7	9	0.9267
History of DM (%)	14	15	17	16	0.2347
Antihypertensive drugs (%)	37	35	33	35	0.3747
Statin (%)	17	16	16	17	0.9097
Ever Smoked (%)	23	23	23	24	0.6673
Alcohol Consumption (grams/week)*	7.5 (1.5, 45.5)	7.5 (1.5, 45.5)	15.0 (2.1, 58.5)	21.0 (3.0, 91.0)	**<0.0001**
Alcohol Consumption Status (%)					
Current Drinker	69	69	71	74	**0.0225**
Recent Abstainer	21	20	20	19	0.3630
Lifetime Abstainer	10	11	9	7	**0.0209**
Obesity (%)	56	51	47	41	**<0.0001**
BMI (kg/m^2)	32.3 ± 8.0	31.6 ± 7.5	30.6 ± 7.0	29.6 ± 6.9	**<0.0001**
Waist Circumference (cm)	99.5 ± 17.3	97.7 ± 17.2	96.5 ± 15.8	94.3 ± 16.1	**<0.0001**
Hip Circumference (cm)	110.9 ± 16.3	109.9 ± 16.0	108.3 ± 14.6	106.9 ± 14.2	**<0.0001**
Waist-to-hip Ratio	0.90 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	**0.0038**
FBG (mg/dL)*	94 (87, 102)	93 (86, 102)	94 (87, 105)	94 (87, 102)	0.1886
Insulin (uIU/mL)*	13.5 (8.8, 21.6)	12.6 (8.6, 19.0)	12.3 (8.1, 18.5)	11.3 (7.7, 17.3)	**<0.0001**
HgbA1c (%)	5.7 ± 1.0	5.8 ± 1.0	5.8 ± 1.2	5.8 ± 1.3	0.0530
HOMA-IR (Glucose*Insulin / 22.5*18)*	3.3 (1.9, 5.4)	2.9 (1.9, 4.7)	2.9 (1.9, 4.9)	2.7 (1.7, 4.5)	**0.0007**
Physical Activity (Moderate/Vigorous)*	28.1 (13.4, 50.7)	27.6 (13.7, 52.7)	28.0 (13.9, 51.9)	30.2 (14.5, 53.8)	0.7222
SBP (mmHg)	131 ± 20	132 ± 20	133 ± 20	133 ± 21	0.5984
DBP (mmHg)	81 ± 9	81 ± 9	81 ± 9	81 ± 10	0.9364
Cholesterol Efflux Capacity	0.72±0.10	0.88±0.03	0.99±0.03	1.16±0.11	**<0.0001**
TC (mg/dL)	178 ± 37	189 ± 36	197 ± 38	205 ± 40	**<0.0001**
TG (mg/dL)*	96 (70, 133)	100 (72, 137)	106 (76, 156)	106 (76, 152)	**<0.0001**
VLDL-C (mg/dL)*	19 (14, 27)	20 (14, 27)	21 (15, 31)	21 (15, 30)	**0.0001**
LDL-C (mg/dL)	110 ± 33	116 ± 34	118 ± 36	119 ± 38	**<0.0001**

HDL-C (mg/dL)	46 ± 13	51 ± 12	54 ± 13	60 ± 18	**<0.0001**

Data reported as mean ± SD, median (interquartile range), or percentage. MetS indicates metabolic syndrome. MetS0 = participants without any MetS component. MetS1 = participants with 1 MetS component. MetS2 = participants with 2 MetS component. MetS3 = participants with 3 MetS component. MetS4-5 = participants with 4 or 5 MetS component. Q1, first quartile; Q2, second quartile; Q3, third quartile; Q4, fourth quartile. CVD, cardiovascular disease; DM, diabetes mellitus; BMI, body mass index; FBG, fasting blood glucose; HgbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC; total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. *Non-normally distributed variable. Bolded values indicate statistical significance. ǂTest for intergroup differences performed using Jonckheere–Terpstra trend test.

Table 5: Results of Multivariate Binomial Logistic Regression Analyses for the Relationship between Cholesterol Efflux Capacity Measured by Fluorescent-Labeled Cholesterol and Metabolic Syndrome

Model	OR	95% CI	P value
1	0.95	0.88 to 1.03	0.2295
2	0.94	0.87 to 1.02	0.1358
3	0.93	0.84 to 1.04	0.2118
4	**0.82**	**0.73 to 0.93**	**0.0013**
5	**0.82**	**0.73 to 0.93**	**0.0013**
6	**0.82**	**0.73 to 0.93**	**0.0015**

OR = odds ratio Model 1 = unadjusted OR; Model 2 = adjusted for demographics (age, sex, and ethnicity); Model 3 = adjusted for variables in model 2 + physical activity, smoking status, and drinking status; Model 4 = adjusted for variables in model 3 + lipids (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol); Model 5 = adjusted for variables in model 4 + menopausal status; model 6 = adjusted for variables in model 5 + cardiovascular disease history. No MetS as reference group. Bolded values indicate statistical significance.

Table 6: Results of Multivariate Binomial Logistic Regression Analyses for the Relationship between Cholesterol Efflux Capacity Measured by Radiolabeled Cholesterol and Metabolic Syndrome

Model	OR	95% CI	P value
1	**0.86**	**0.80 to 0.93**	**0.0002**
2	**0.82**	**0.75 to 0.89**	**<0.0001**
3	**0.85**	**0.76 to 0.95**	**0.0035**
4	**0.71**	**0.62 to 0.80**	**<0.0001**
5	**0.71**	**0.62 to 0.80**	**<0.0001**
6	**0.70**	**0.62 to 0.80**	**<0.0001**

OR = odds ratio. Model 1 = unadjusted OR; Model 2 = adjusted for demographics (age, sex, and ethnicity); Model 3 = adjusted for variables in model 2 + physical activity, smoking status, and drinking status; Model 4 = adjusted for variables in model 3 + lipids (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol); Model 5 = adjusted for variables in model 4 + menopausal status; model 6 = adjusted for variables in model 5 + cardiovascular disease history. No MetS as reference group. Bolded values indicate statistical significance.

Table 7: Results of Multivariate Binomial Logistic Regression Analyses for the Relationship between Quartiles of Cholesterol Efflux Capacity Measured by Fluorescent-Labeled Cholesterol and Metabolic Syndrome

Model	Quartiles	OR	95% CI	P value
1	**Q2**	**0.79**	**0.64 to 0.98**	**0.0317**
	Q3	0.85	0.69 to 1.05	0.1364
	Q4	**0.79**	**0.64 to 0.98**	**0.0290**
2	Q2	0.81	0.65 to 1.01	0.0618

	Q3	0.89	0.71 to 1.11	0.2925
	Q4	**0.76**	**0.61 to 0.95**	**0.0139**
3	Q2	0.84	0.62 to 1.14	0.2658
	Q3	**0.73**	**0.54 to 0.98**	**0.0382**
	Q4	0.78	0.58 to 1.06	0.1169
4	Q2	0.81	0.58 to 1.13	0.2134
	Q3	**0.65**	**0.46 to 0.90**	**0.0105**
	Q4	**0.54**	**0.38 to 0.76**	**0.0005**
5	Q2	0.81	0.58 to 1.13	0.2177
	Q3	**0.65**	**0.47 to 0.91**	**0.0113**
	Q4	**0.54**	**0.38 to 0.76**	**0.0005**
6	Q2	0.81	0.58 to 1.14	0.2307
	Q3	**0.65**	**0.47 to 0.91**	**0.0120**
	Q4	**0.54**	**0.39 to 0.77**	**0.0006**

OR = odds ratio. Model 1 = unadjusted OR; Model 2 = adjusted for demographics (age, sex, and ethnicity); Model 3 = adjusted for variables in model 2 + physical activity, smoking status, and drinking status; Model 4 = adjusted for variables in model 3 + lipids (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol); Model 5 = adjusted for variables in model 4 + menopausal status; model 6 = adjusted for variables in model 5 + cardiovascular disease history. No MetS and Q1, first quartile of cholesterol efflux capacity, as reference groups. Q2, second quartile; Q3, third quartile; and Q4, fourth quartile. Bolded values indicate statistical significance.

Table 8: Results of Multivariate Binomial Logistic Regression Analyses for the Relationship between Quartiles of Cholesterol Efflux Capacity Measured by Radiolabeled Cholesterol and Metabolic Syndrome

Model	Quartiles	OR	95% CI	P value
1	**Q2**	**0.65**	**0.52 to 0.81**	**<0.0001**
	Q3	**0.70**	**0.57 to 0.87**	**0.0012**
	Q4	**0.64**	**0.52 to 0.79**	**<0.0001**
2	**Q2**	**0.60**	**0.48 to 0.75**	**<0.0001**
	Q3	**0.63**	**0.50 to 0.78**	**<0.0001**
	Q4	**0.56**	**0.45 to 0.71**	**<0.0001**
3	**Q2**	**0.60**	**0.44 to 0.81**	**0.0011**
	Q3	**0.69**	**0.51 to 0.93**	**0.0162**
	Q4	**0.60**	**0.44 to 0.81**	**0.0009**
4	**Q2**	**0.50**	**0.36 to 0.71**	**<0.0001**
	Q3	**0.50**	**0.36 to 0.71**	**<0.0001**
	Q4	**0.38**	**0.27 to 0.54**	**<0.0001**
5	**Q2**	**0.50**	**0.36 to 0.71**	**<0.0001**
	Q3	**0.50**	**0.36 to 0.71**	**<0.0001**
	Q4	**0.38**	**0.27 to 0.54**	**<0.0001**
6	**Q2**	**0.52**	**0.37 to 0.73**	**0.0002**
	Q3	**0.51**	**0.36 to 0.72**	**0.0001**
	Q4	**0.38**	**0.27 to 0.53**	**<0.0001**

OR = odds ratio. Model 1 = unadjusted OR; Model 2 = adjusted for demographics (age, sex, and ethnicity); Model 3 = adjusted for variables in model 2 + physical activity, smoking status, and drinking status; Model 4 = adjusted for variables in model 3 + lipids (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol); Model 5 = adjusted for variables in model 4 + menopausal status; model 6 = adjusted for variables in model 5 + cardiovascular disease history. No MetS and Q1, first quartile of cholesterol efflux capacity, as reference groups. Q2, second quartile; Q3, third quartile; and Q4, fourth quartile. Bolded values indicate statistical significance.

Table 9: Results of Multivariate Ordinal Logistic Regression Analyses for the Relationship between Cholesterol Efflux Capacity Measured by Fluorescent-Labeled Cholesterol and Increasing Number of Metabolic Syndrome Components

Model	MetS1			MetS2			MetS3			MetS4-5		
	OR	P value	95% CI	OR	P value	95% CI	OR	P value	95% CI	OR	P value	95% CI
1	0.92	0.1457	0.81 to 1.03	0.93	0.2136	0.83 to 1.04	0.91	0.1223	0.80 to 1.03	0.89	0.1033	0.77 to 1.03
2	0.91	0.1149	0.80 to 1.02	0.92	0.1776	0.82 to 1.04	0.89	0.0772	0.78 to 1.01	0.86	0.0513	0.74 to 1.00
3	1.00	0.9315	0.84 to 1.17	1.00	0.9954	0.85 to 1.18	0.96	0.6370	0.80 to 1.14	0.86	0.1697	0.70 to 1.07
4	0.93	0.4128	0.78 to 1.11	0.90	0.2364	0.75 to 1.07	**0.78**	**0.0159**	**0.64 to 0.96**	**0.64**	**0.0003**	**0.50 to 0.82**
5	0.93	0.4203	0.78 to 1.11	0.90	0.2285	0.75 to 1.07	**0.78**	**0.0145**	**0.64 to 0.95**	**0.64**	**0.0003**	**0.50 to 0.82**
6	0.93	0.4246	0.79 to 1.11	0.90	0.2378	0.75 to 1.07	**0.78**	**0.0157**	**0.64 to 0.96**	**0.64**	**0.0003**	**0.50 to 0.82**

MetS indicates metabolic syndrome. MetS0 = participants without any MetS component. MetS1 = participants with 1 MetS component. MetS2 = participants with 2 MetS component. MetS3 = participants with 3 MetS component. MetS4-5 = participants with 4 or 5 MetS component. OR = odds ratio. Model 1 = unadjusted OR; Model 2 = adjusted for demographics (age, sex, and ethnicity); Model 3 = adjusted for variables in model 2 + physical activity, smoking status, and drinking status; Model 4 = adjusted for variables in model 3 + lipids (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol); Model 5 = adjusted for variables in model 4 + menopausal status; model 6 = adjusted for variables in model 5 + cardiovascular disease history. MetS0 as reference group. Bolded values indicate statistical significance.

Table 10: Results of Multivariate Ordinal Logistic Regression Analyses for the Relationship between Cholesterol Efflux Capacity Measured by Radiolabeled Cholesterol and Increasing Number of Metabolic Syndrome Components

Model	MetS1			MetS2			MetS3			MetS4-5		
	OR	P value	95% CI	OR	P value	95% CI	OR	P value	95% CI	OR	P value	95% CI
1	0.95	0.4312	0.85 to 1.07	**0.88**	**0.0324**	**0.78 to 0.99**	**0.75**	**<0.0001**	**0.66 to 0.85**	**0.83**	**0.0130**	**0.72 to 0.96**
2	0.91	0.1259	0.81 to 1.03	**0.83**	**0.0029**	**0.74 to 0.94**	**0.69**	**<0.0001**	**0.61 to 0.79**	**0.75**	**0.0002**	**0.65 to 0.88**
3	0.93	0.3695	0.79 to 1.09	0.88	0.1220	0.74 to 1.04	**0.82**	**0.0251**	**0.68 to 0.98**	**0.72**	**0.0029**	**0.58 to 0.90**
4	0.85	0.0737	0.72 to 1.02	**0.77**	**0.0037**	**0.64 to 0.92**	**0.62**	**<0.0001**	**0.50 to 0.76**	**0.46**	**<0.0001**	**0.36 to 0.60**
5	0.86	0.0826	0.72 to 1.02	**0.77**	**0.0041**	**0.64 to 0.92**	**0.62**	**<0.0001**	**0.50 to 0.76**	**0.47**	**<0.0001**	**0.36 to 0.60**
6	0.85	0.0735	0.71 to 1.02	**0.76**	**0.0026**	**0.63 to 0.91**	**0.61**	**<0.0001**	**0.50 to 0.75**	**0.46**	**<0.0001**	**0.36 to 0.59**

MetS indicates metabolic syndrome. MetS0 = participants without any MetS component. MetS1 = participants with 1 MetS component. MetS2 = participants with 2 MetS component. MetS3 = participants with 3 MetS component. MetS4-5 = participants with 4 or 5 MetS component. OR = odds ratio. Model 1 = unadjusted OR; Model 2 = adjusted for demographics (age, sex, and ethnicity); Model 3 = adjusted for variables in model 2 + physical activity, smoking status, and drinking status; Model 4 = adjusted for variables in model 3 + lipids (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol); Model 5 = adjusted for variables in model 4 + menopausal status; model 6 = adjusted for variables in model 5 + cardiovascular disease history. MetS0 as reference group. Bolded values indicate statistical significance.

Table 11: Results of Multivariate Linear Regression Analyses for the Relationship between Cholesterol Efflux Capacity Measured by Fluorescent-Labeled Cholesterol and Individual Components of Metabolic Syndrome

	Std β	95% CI	P value
Waist Circumference			
Model 1	**-0.071**	**-0.108 to -0.035**	**0.0001**
Model 2	**-0.055**	**-0.103 to -0.007**	**0.0238**
SBP			
Model 1	0.016	-0.020 to 0.053	0.3837
Model 2	0.009	-0.036 to 0.055	0.6873
DBP			
Model 1	0.006	-0.031 to 0.043	0.7466
Model 2	0.019	-0.030 to 0.068	0.4534
logTG			
Model 1	**0.107**	**0.071 to 0.143**	**<0.0001**
Model 2	**0.088**	**0.040 to 0.136**	**0.0003**
HDL-C			
Model 1	**0.140**	**0.103 to 0.176**	**<0.0001**
Model 2	**0.124**	**0.076 to 0.173**	**<0.0001**
FBG			
Model 1	0.031	-0.006 to 0.068	0.0975
Model 2	0.029	-0.018 to 0.075	0.2273

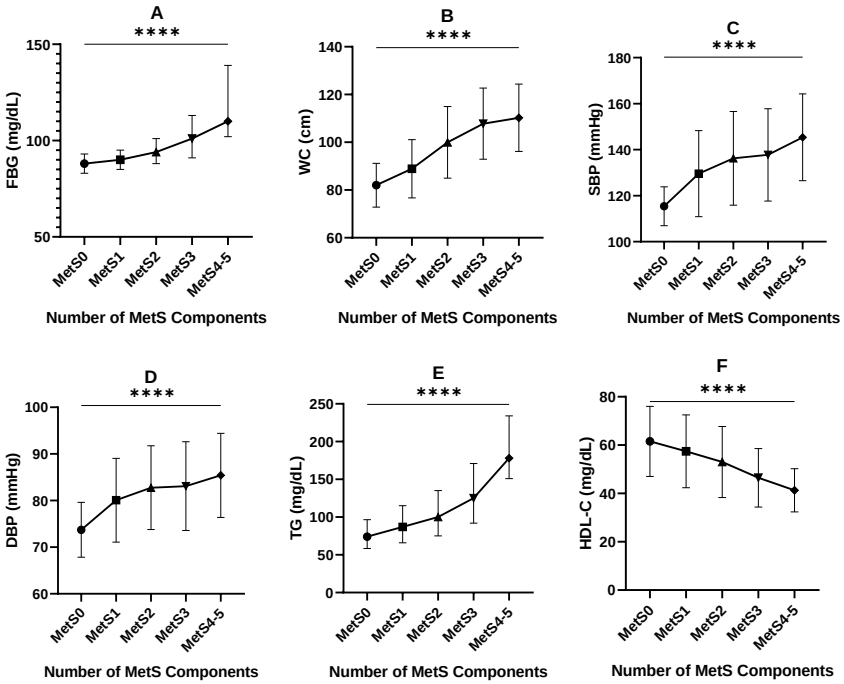
Std β = standardized regression coefficient. Model 1 = unadjusted standardized beta; Model 2 = adjusted for demographics (age, sex, and ethnicity); and Model 2 = adjusted for demographics (age, sex, and ethnicity) and modifiable risk factors (physical activity, smoking status, and drinking status). Bolded values indicate statistical significance.

Table 12: Results of Multivariate Linear Regression Analyses for the Relationship between Cholesterol Efflux Capacity Measured by Radiolabeled Cholesterol and Individual Components of Metabolic Syndrome

	Std β	95% CI	P value
Waist Circumference			
Model 1	**-0.117**	**-0.154 to -0.080**	**<0.0001**
Model 2	**-0.098**	**-0.145 to -0.050**	**<0.0001**
SBP			
Model 1	0.027	-0.010 to 0.064	0.1504
Model 2	0.023	-0.022 to 0.068	0.3182
DBP			
Model 1	-0.005	-0.042 to 0.033	0.8120
Model 2	0.027	-0.022 to 0.076	0.2800
logTG			
Model 1	**0.095**	**0.058 to 0.132**	**<0.0001**
Model 2	**0.097**	**0.049 to 0.145**	**<0.0001**
HDL-C			
Model 1	**0.350**	**0.315 to 0.384**	**<0.0001**
Model 2	**0.357**	**0.312 to 0.403**	**<0.0001**
FBG			
Model 1	**0.050**	**0.013 to 0.088**	**0.0078**
Model 2	**0.077**	**0.030 to 0.123**	**0.0013**

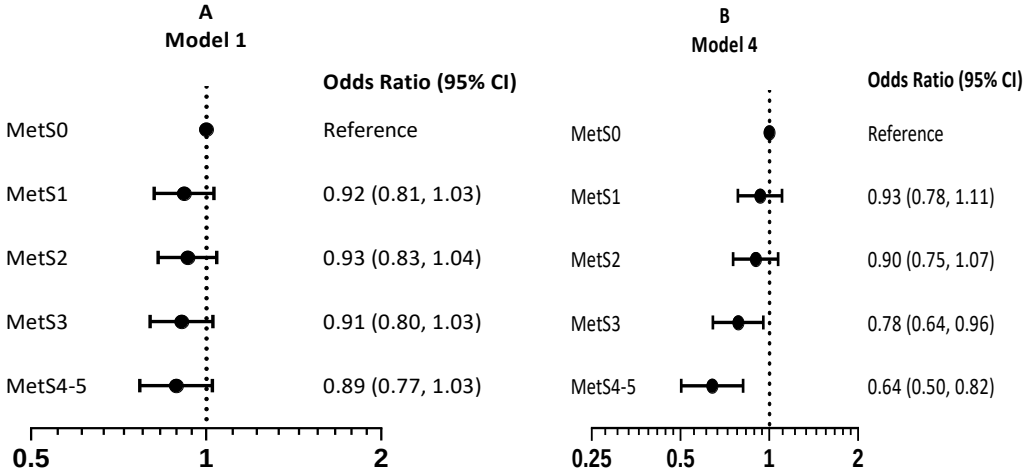
Std β = standardized regression coefficient. Model 1 = unadjusted standardized beta; Model 2 = adjusted for demographics (age, sex, and ethnicity); and Model 2 = adjusted for demographics (age, sex, and ethnicity) and modifiable risk factors (physical activity, smoking status, and drinking status). Bolded values indicate statistical significance.

Figure 1: Individual Components of Metabolic Syndrome across Increasing Number of Metabolic Syndrome Components



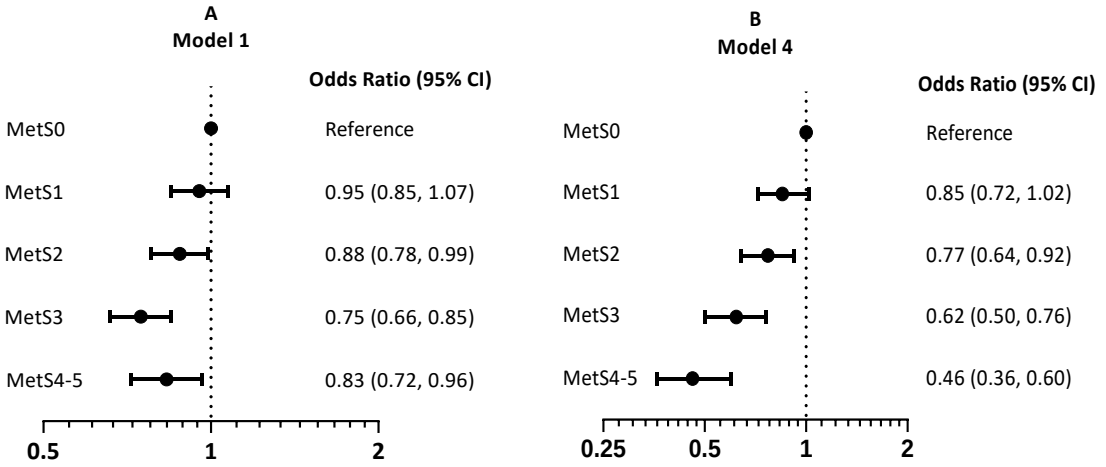
FBG = Fasting blood glucose, WC = waist circumference, SBP = systolic blood pressure, DBP = diastolic blood pressure, TG = triglyceride, and HDL-C = high-density lipoprotein. Mean ± SD reported for WC, SBP, DBP, and HDL-C. Median (interquartile range) reported for FBG. MetS indicates metabolic syndrome. MetS0 = participants without any MetS component. MetS1 = participants with 1 MetS component. MetS2 = participants with 2 MetS component. MetS3 = participants with 3 MetS component. MetS4-5 = participants with 4 or 5 MetS component. Statistical significance was determined by performing two-tailed, Jonckheere–Terpstra trend test (**** $P < 0.0001$).

Figure 2: Relationship between Cholesterol Efflux Capacity Measured by Fluorescent-Labeled Cholesterol and Increasing Number of Metabolic Syndrome Components



Model 1 = unadjusted OR. Model 4 = adjusted for demographics (age, sex, and ethnicity) + modifiable risk factors (physical activity, smoking status, and drinking status) + lipid (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol). MetS0 = participants without any MetS component. MetS1 = participants with 1 MetS component. MetS2 = participants with 2 MetS component. MetS3 = participants with 3 MetS component. MetS4-5 = participants with 4 or 5 MetS component. MetS indicates metabolic syndrome. MetS0 as reference group.

Figure 3: Relationship between Cholesterol Efflux Capacity Measured by Radiolabeled Cholesterol and Increasing Number of Metabolic Syndrome Components



Model 1 = unadjusted OR. Model 4 = adjusted for demographics (age, sex, and ethnicity) + modifiable risk factors (physical activity, smoking status, and drinking status) + lipid (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol). MetS0 = participants without any MetS component. MetS1 = participants with 1 MetS component. MetS2 = participants with 2 MetS component. MetS3 = participants with 3 MetS component. MetS4-5 = participants with 4 or 5 MetS component. MetS indicates metabolic syndrome. MetS0 as reference group.

APPENDICES

Appendix A: Clinical and Biological Variables among Participants According to Increasing Number of Metabolic Syndrome Components

	Number of MetS Criteria					ǂP Value for Trend (Two-sided)
	MetS0 (N = 460)	MetS1 (N = 707)	MetS2 (N = 792)	MetS3 (N = 575)	MetS4-5 (N = 340)	
Age (year)	44.4 ± 10.7	49.2 ± 11.0	50.2 ± 10.9	50.9 ± 10.9	52.6 ± 10.1	**<0.0001**
Male (%)	42	42	40	40	35	**0.0352**
Post-menopausal, Female (%)	21	30	33	36	43	**<.0001**
Race/Ethnicity (%)						
Non-Hispanic Black	44	51	56	56	49	**0.0039**
Non-Hispanic White	39	33	28	25	28	**<.0001**
Hispanic	15	13	13	17	19	**0.0103**
History of CVD (%)	2	7	9	13	13	**<0.0001**
History of DM (%)	1	4	14	27	45	**<0.0001**
Antihypertensive drugs (%)	11	25	39	51	56	**<0.0001**
Statin (%)	9	14	16	24	24	**<0.0001**
Ever Smoked (%)	23	28	22	22	22	0.0565
Alcohol Consumption (grams/week)*	19.5 (3.0, 58.5)	22.5 (3.0, 91.0)	9.0 (1.5, 45.5)	7.5 (1.5, 58.5)	10.6 (1.5, 58.5)	**0.0006**
Alcohol Consumption Status (%)						
Current Drinker	77	74	71	66	60	**<0.0001**
Recent Abstainer	17	17	22	22	25	**<0.0001**
Lifetime Abstainer	6	10	8	12	15	**<0.0001**
Obesity (%)	7	28	56	78	82	**<0.0001**
BMI (kg/m^2)	25.0 ± 3.4	27.7 ± 5.4	32.3 ± 7.0	35.4 ± 7.3	36.2 ± 6.6	**<0.0001**
Waist Circumference (cm)	82.0 ± 9.1	88.9 ± 12.2	100.0 ± 15.0	107.8 ± 14.9	110.3 ± 14.1	**<0.0001**
Hip Circumference (cm)	97.9 ± 7.8	102.8 ± 12.0	111.6 ± 15.0	117.3 ± 15.8	118.3 ± 14.5	**<0.0001**
Waist-to-hip Ratio	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	**<0.0001**
FBG (mg/dL)*	88 (83, 93)	90 (85, 95)	94 (88, 101)	101 (91, 113)	110 (102, 139)	**<0.0001**
Insulin (uIU/mL)*	7.7 (5.4, 10.3)	9.6 (6.8, 13.4)	13.4 (9.3, 18.6)	17.9 (12.8, 26.2)	21.4 (14.5, 30.4)	**<0.0001**
HgbA1c (%)	5.3 ± 0.3	5.4 ± 0.5	5.8 ± 1.1	6.1 ± 1.3	6.6 ± 1.7	**<0.0001**
HOMA-IR (Glucose*Insulin / 22.5*18)*	1.6 (1.1, 2.3)	2.2 (1.5, 3.1)	3.2 (2.2, 4.5)	4.8 (3.2, 7.3)	6.4 (4.3, 10.1)	**<0.0001**
Physical Activity (Moderate/Vigorous)*	39.5 (21.0, 65.0)	30.2 (16.1, 55.6)	28.0 (13.4, 54.0)	23.4 (11.1, 43.6)	21.9 (9.8, 41.1)	**<0.0001**
SBP (mmHg)	115 ± 8	130 ± 19	136 ± 20	138 ± 20	145 ± 19	**<0.0001**
DBP (mmHg)	74 ± 6	80 ± 9	83 ± 9	83 ± 10	85 ± 9	**<0.0001**
Cholesterol Efflux Capacity (Fluorescent method)	0.87 ± 0.23	0.85 ± 0.23	0.85 ± 0.23	0.84 ± 0.26	0.84 ± 0.26	0.0588
Cholesterol Efflux Capacity (Radiolabeled method)	0.96 ± 0.18	0.95 ± 0.17	0.94 ± 0.17	0.91 ± 0.18	0.93 ± 0.17	**<0.0001**
TC (mg/dL)	186 ± 34	192 ± 38	193 ± 39	194 ± 43	198 ± 43	**0.0005**
TG (mg/dL)*	74 (59, 97)	87 (66, 115)	100 (75, 135)	125 (92, 171)	178 (151, 234)	**<0.0001**
VLDL-C (mg/dL)*	15 (12, 19)	17 (13, 23)	20 (15, 27)	25 (18, 34)	36 (30, 46)	**<0.0001**
LDL-C (mg/dL)	109 ± 32	115 ± 34	117 ± 35	119 ± 38	116 ± 41	**<0.0001**

HDL-C (mg/dL)	62 ± 14	57 ± 15	53 ± 15	46 ± 12	41 ± 9	**<0.0001**

Data reported as mean ± SD, median (interquartile range), or percentage. MetS0 = participants without any MetS component. MetS1 = participants with 1 MetS component. MetS2 = participants with 2 MetS component. MetS3 = participants with 3 MetS component. MetS4-5 = participants with 4 or 5 MetS component. CVD, cardiovascular disease; DM, diabetes mellitus; BMI, body mass index; FBG, fasting blood glucose; HgbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC; total cholesterol; TG, triglyceride; VLDL-C, very low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. *Non-normally distributed variable. Bolded values indicate statistical significance. ǂTest for intergroup differences performed using Jonckheere–Terpstra trend test.

Appendix B: Results of Test for Interaction between Cholesterol Efflux Capacity Measured by Fluorescent-Labeled Cholesterol and Other Covariates in its Relationship with Metabolic Syndrome

	P value‡
CEC x LDL-C	0.3216
CEC x VLDL-C	0.5754
CEC x Male	0.0601
CEC x Black	0.9613
CEC x History of CVD	**0.0304**
CEC x History of DM	0.2856

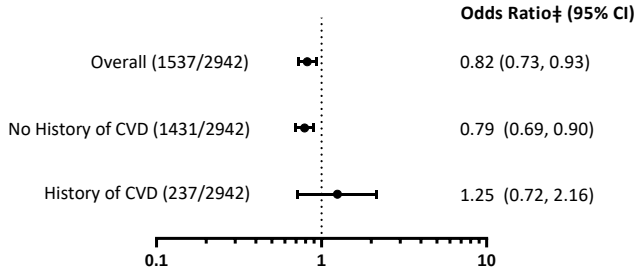
CEC, cholesterol efflux capacity measured by fluorescent-labeled cholesterol. LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; CVD, cardiovascular disease; and DM, diabetes mellitus. ‡Adjusted for demographics (age, sex, and ethnicity), modifiable risk factors (physical activity, smoking status, and drinking status), lipids (LDL-C and VLDL-C), post-menopausal status, and history of CVD.

Appendix C: Results of Test for Interaction between Cholesterol Efflux Capacity Measured by Radiolabeled Cholesterol and Other Covariates in its Relationship with Metabolic Syndrome

	P value‡
CEC x LDL-C	0.2248
CEC x VLDL-C	0.3354
CEC x Male	0.1168
CEC x Black	0.7511
CEC x History of CVD	0.7107
CEC x History of DM	0.9740

CEC, cholesterol efflux capacity measured by radiolabeled cholesterol. LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; CVD, cardiovascular disease; and DM, diabetes mellitus. ‡Adjusted for demographics (age, sex, and ethnicity), modifiable risk factors (physical activity, smoking status, and drinking status), lipids (LDL-C and VLDL-C), post-menopausal status, and history of CVD.

Appendix D: Relationship between Cholesterol Efflux Capacity Measured by Fluorescent-Labeled Cholesterol and Metabolic Syndrome within Cardiovascular History Strata



Odds ratio reported separately for participants with and without history of cardiovascular. Bolded values indicate statistical significance. ‡Odds ratio adjusted for demographics (age, sex, and ethnicity), modifiable risk factors (physical activity, smoking status, and drinking status), lipids (low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol), post-menopausal status, and history of CVD.

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