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HDL Cholesterol and Stroke Risk: The Multi-Ethnic Study of Atherosclerosis

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HDL CHOLESTEROL AND STROKE RISK: THE MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS

By

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Research shows a link between HDL cholesterol and cardiovascular disease. Evidence of the relationship between HDL cholesterol and stroke risk is mixed. Accurate identification of risk factors for stroke is important for public health promotion and disease prevention. Whether measurement of HDL cholesterol content, particle number, or size is a better indicator of stroke risk remains disputed. Furthermore, the degree to which ethnicity is implicated in the emergence of these risk factors is unknown. The current study examined the relationship among HDL cholesterol, particle number, particle size, and ethnicity in predicting stroke. The population was an ethnically diverse cohort of US men and women between the ages of 45 - 84 years enrolled in 2000 - 2002 and followed up through December 2011 in the Multi-Ethnic Study of Atherosclerosis (MESA). Results indicated that HDL-C and number of large HDL particles were negatively associated with stroke outcome. When interactions with race were evaluated, the relationship between both HDL variables and stroke outcome emerged as significant in Blacks, but not other races. We conclude that HDL-Cis a reliable measure of stroke outcome, and the potentially protective role of large HDL particles calls for replication in future samples. Furthermore, the relationship between HDL subfractions and race/ethnicity warrants further study.

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Chapter 1: Introduction

Cholesterol

Cholesterol is a lipid molecule that is biosynthesized by all animal cells, which functions to maintain membrane structural integrity and fluidity and serves as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D (Hanukoglu, 1992). The synthesis of cholesterol begins with the intracellular protein enzyme HMG-CoA reductase, that initiates a 37-step enzymatic biosynthetic process (Mehta, 2013). The cholesterol biosynthetic pathway produces about 70% of total body cholesterol. Cholesterol levels may be influenced by weight, age, sex, diet, exercise, smoking, alcohol use, stress, and genetics (Oklahoma Heart Hospital Physicians, 2014). Since cholesterol itself is insoluble in blood, it is transported in the circulatory system via lipoproteins. These lipoproteins vary in density and have specific cell-targeting signals that direct the lipids they carry to certain tissues in the body. In order of increasing density, these lipoproteins are as follows: chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) (Christie, 2014).

LDL Cholesterol

Colloquially referred to as "the bad cholesterol," LDL particles are the major carriers of cholesterol in the blood. Each molecule contains approximately 1,500 molecules of cholesterol ester and one molecule of apolipoprotein B100. LDL and its receptor are taken up by endocytic vesicles within a cell, which fuses with a lysosome; then the cholesterol esters are hydrolyzed via the enzyme lysosomal acid lipase, and the cholesterol molecules can be used for new membrane synthesis. Synthesis of LDL receptor is regulated by the same transcription factor used to control synthesis of total

cholesterol, SREBP. When cellular cholesterol levels are stable, LDL receptor synthesis is down-regulated; however, when cellular cholesterol levels drop, e.g. when cells are dividing or growing and new cholesterol is needed for the formation of new membranes, more receptors are synthesized. LDL particles are subject to oxidation, taken up by macrophages via scavenger receptors, and result in macrophage foam cells. Very often these cells become trapped in the walls of blood vessels and contribute to early atherosclerotic plaque formation (American Heart Association, 2014a). These plaques affect the development of atherosclerosis and are often the main causes of heart attacks, strokes, and other serious medical problems (Weingartner et al., 2010).

HDL Cholesterol

In contrast, HDL particles, "good cholesterol," primarily function in reverse cholesterol transport (Barter, 1993; Fielding & Fielding, 1995) in which cholesterol in peripheral tissues is transferred via the plasma to the liver for recycling or excretion from the body. High-density lipoprotein cholesterol are the smallest (7.0 – 12 nm in diameter) and densest ($1.063 < d < 1.25$ g/mL) of the plasma lipoproteins (Barter, Kastelein, Nunn, Hobbs, & Future Forum Editorial, 2003). They are composed of a hydrophobic core, which is primarily cholesteryl esters and a small amount of triglyceride and unesterified cholesterol, surrounded by a surface monolayer of phospholipids, unesterified cholesterol, and apolipoproteins. Though this describes the basic structure, human plasma HDL is heterogeneous in terms of size, shape, density, composition, and surface charge. HDL subclasses differ in their ability to promote cholesterol efflux. HDL's ability to remove cholesterol from cells is one key component to its anti-atherogenic functionality (Rye, Bursill, Lambert, Tabet, & Barter, 2009). HDL also inhibits LDL oxidation (Negre-

Salvayre et al., 2006), promotes endothelial repair (Tso et al., 2006), has anti-inflammatory and anti-thrombotic properties (Mineo, Deguchi, Griffin, & Shaul, 2006), and inhibits binding of monocytes, thereby lowering cholesterol (Murphy et al., 2008). The effectiveness of the anti-atherogenic functionality of HDLs may differ based on particle number and/or size (Akinkuolie, Paynter, Padmanabhan, & Mora, 2014).

Through ultracentrifugation, HDLs can be separated into two major subfractions, HDL_2 ($1.063 < d < 1.125$ g/mL) and HDL_3 ($1.125 < d < 1.25$ g/mL). Gradient gel electrophoresis (GGE) breaks HDLs into five distinct subfraction sizes in order of decreasing particle size: HDL_{2b} (mean diameter 10.6 nm), HDL_{2a} (9.2 nm), HDL_{3a} (8.4 nm), HDL_{3b} (8.0 nm), and HDL_{3c} (7.6 nm) (Blanche, Gong, Forte, & Nichols, 1981). Nuclear magnetic resonance (NMR) spectroscopy is an alternative way of quantifying HDL subpopulations in plasma (Ala-Korpela et al., 1994; Otvos, 2000; Otvos, Jeyarajah, Bennett, & Krauss, 1992). The five ranges of subclasses match closely with GGE: 10 – 13 nm, 8.8 – 10 nm, 7.8 – 8.2 nm, and 7.3 – 7.7 nm.

HDL Cholesterol and Cardiovascular disease

Literature supports an inverse relationship between elevated levels of high-density lipoprotein cholesterol concentration (HDL-C) and cardiovascular disease (CVD) (Gordon & Rifkind, 1989). A positive association between low-density lipoprotein (LDL-C) and cardiovascular disease has also been supported (Cromwell & Otvos, 2004). Individuals at risk for developing CVD based on high levels of total cholesterol may be prescribed statins, drugs that inhibit the enzyme HMG-CoA reductase, which plays a role in the production of cholesterol in the liver. Since statins are similar to HMG-CoA on a molecular level, they take the place of HMG-CoA in the enzyme and reduce the rate at

which it produces mevalonate, the next molecule in the cascading process that produces cholesterol and other compounds (Cromwell & Otvos, 2004). This in turn increases LDL receptor synthesis (predominately in the liver) that results in increased clearance of LDL from the blood, thus lowering blood cholesterol. In sum, the pathway for cholesterol synthesis is blocked (Cromwell & Otvos, 2004).

Although evidence shows that statin therapy can lower LDL and raise HDL cholesterol, one in seven statin-treated patients will still experience some CVD-related event within five years (Mora, Glynn, & Ridker, 2013). Trials that look at raising HDL-C have not shown efficacy in reducing cardiovascular disease risk; however it has been suggested that errors in trial design, specific statin agent, or in the subfraction of HDL that is under examination may account for lack of efficacy.

The Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) study enrolled 17,802 asymptomatic men ≥ 50 years and women ≥ 60 years without a previous history of CVD or diabetes mellitus who had LDL-C < 130 mg/dL (considered normal), hsCRP ≥ 2.0 mg/L (a marker of increased inflammation), and triglycerides <500 mg/dL. Cox proportional hazards models were used to compare HDL particle number (HDL-P) to HDL cholesterol concentration (HDL-C). HDL-P was defined as the sum of particle concentrations of the HDL subclasses, quantified based on particle size by using NMR signals (Mora et al., 2013). On-treatment HDL-C was not predictive of residual risk among statin-treated individuals; however, HDL-C was predictive among those taking placebo (Ridker et al., 2010). Baseline HDL size showed no statistically significant association with CVD, but baseline HDL-P did show an association. Post one year of statin therapy, HDL-C, apoA-I, and HDL-P all had

inverse relationships with CVD, but this observation was not found for HDL size. HDL-P was inversely associated with risk and remained significant in almost all models that included HDL-C or HDL size, particularly among rosuvastatin-allocated individuals, suggesting that this relationship is strong and independent (Mora et al., 2013). In conclusion, investigators found that HDL-P may be a better indicator of residual risk, especially among statin-treated individuals.

Mackey and colleagues (2012) examined the relationships among HDL-C, HDL-P, atherosclerosis, and coronary events. While HDL-P was significantly inversely and independently associated with cIMT in women and men, HDL-C was not. HDL-P may be a particularly important subfraction since it is less correlated with metabolic factors like insulin resistance, abdominal obesity, inflammation, and atherogenic lipoproteins. Larger HDL-P may be indicative of greater reverse cholesterol transport capacity and other functional properties, like antioxidant capacity, beyond what can be explained by HDL-C alone (Mora et al., 2013). In other words, associations found between HDL-C and CHD risk might be partially explained by metabolic correlations with atherogenic lipoprotein concentrations, whereas the associations of HDL-P and CHD risk were independent of lipoprotein concentrations (Mackey et al., 2012).

Literature on HDL size is mixed. Although both HDL size and HDL-P have been found to be inversely related with cardiovascular risk, only HDL size has the confounding characteristics of the metabolic syndrome; after adjustment for these metabolic syndrome, including measures of blood pressure, abdominal obesity, dyslipidemia, insulin resistance, and a pro-inflammatory state, the relationship between HDL size and CVD risk often disappears (El Harchaoui et al., 2009). Furthermore, while

HDL size may be indicative of diameter, HDL particle lipid content and function remain undefined. For example, a particle may be classified as small, but this fails to deliver any information regarding the lipid composition or cholesterol efflux capacity (Pascot et al., 2001).

Studies that examine particle size tend to report that high levels of HDL_{3b} (small particles) are associated with CHD risk factors, suggesting that low HDL_{3b} may contribute to decreased coronary heart disease in patients with high HDL cholesterol. However, a greater amount of HDL_{3b} is also associated with high BMI, lower HDL_{2b}, HDL_{2a}, and higher fasting plasma insulin concentrations (Pascot et al., 2001). In one study, participants who developed CHD had lower concentrations of large, very large, and medium HDL subclasses and higher concentrations of small and very small HDL classes. After adjusting for metabolic and lipoprotein variables and other HDL subclasses, higher concentrations of large and small HDL subclasses showed a trend for lower CHD, but the relationship was no longer significant (Akinkuolie et al., 2014). Only medium sized particles were significantly inversely associated with CHD after adjustment. Total HDL-P, however, was independently, inversely associated with incident CHD.

In contrast to these findings on particle size, Mora and colleagues (2009) found that in women, large HDL particles were significantly and inversely associated with CVD (defined as nonfatal myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, nonfatal ischemic stroke, or cardiovascular death), while medium and small particles were not. However, in this prospective study of healthy women, the magnitude of the inverse association of large HDL particles and CVD was similar to that

of apoA-I or HDL cholesterol, suggesting that the use of examining particle size in this study did not prove superior to standard lipid assays (Mora et al., 2009). These studies point to the complex and somewhat confusing findings of HDL size.

HDL Cholesterol and Stroke

The literature on the specific association between HDL cholesterol and stroke risk is mixed. Generally, prospective studies have found higher HDL-C levels are associated with lower stroke risk (Lindenstrom, Boysen, & Nyboe, 1994; Tanne, Yaari, & Goldbourt, 1997), but some studies have found no relation (Shahar et al., 2003; Woodward et al., 2007), or even an increased risk (Bots et al., 2002). Furthermore, HDL-C levels may differ based on sex (Bots et al., 2002; National Heart, 2002) and race/ethnicity (Chirinos et al., 2013; Sprafka, Norsted, Folsom, Burke, & Luepker, 1992). Although many of these studies review ischemic stroke, which accounts for about 87% of all stroke cases, the risk factors for both ischemic and hemorrhagic stroke are the same (American Heart Association, 2014b).

The Northern Manhattan Study (NOMAS) collected baseline fasting lipid blood samples from 2,914 stroke-free community residents. After a mean of 7.5 years follow up, Cox proportional hazard models revealed no significant relationships between baseline HDL or total cholesterol levels and ischemic stroke (Willey et al., 2009). The majority of this sample was Hispanics (53%) living in the same community, pointing to the need for an ethnically diverse sample, spanning various geographical locations to determine if both race/ethnicity and location may influence a detectable relationship between HDL and stroke outcome. Furthermore, Hispanic background may play an important role in influencing general health status and stroke risk.

Total cholesterol levels reported in EUROSTROKE, a collaborative project among European cohort studies on incidence and risk factors of stroke, also were not significantly associated with increased risk of stroke of any type. EUROSTROKE did, however, report differential findings for men and women. In this nested case-control study, pooled analyses showed that in men there was a trend towards lower risk of stroke with increases in HDL cholesterol. However, in women, an increase in HDL cholesterol was associated with a significant increased risk of non-fatal stroke and cerebral infarction (Bots et al., 2002).

Furthermore, researchers from the Women's Health Initiative Observational Study found no significant association between HDL-C and ischemic stroke. However, stroke cases had significantly higher intermediate density lipoprotein (IDL) particles, LDL particles, LDL size, VLDL particles, and VLDL size. HDL size was significantly lower among control subjects versus cases (Berger et al., 2012), whereas total cholesterol was not indicative of stroke risk.

Acute ischemic stroke (AIS) has been associated with lower HDL-C. In a sample of 200 AIS patients and 162 healthy controls, AIS patients had increased amounts of small-sized HDL particles, namely more HDL_{3a}, $_{3b}$, and $_{3c}$, and less HDL_{2b} subclasses. However, after adjustment for traditional non-lipid risk factors, increased small-sized HDL lost its significance as a predictor of AIS. AIS fatalities had significantly higher amounts of small dense LDL (sdLDL), even after adjustment for traditional confounders (Zeljkovic et al., 2010).

Overall, associations between HDL particle concentration, size, and stroke risk are mixed. These inconclusive results suggest the complexity of the HDL particle may be

important to consider when examining the relationship with stroke, and point to the necessity for greater research to elucidate this relationship, inform individuals at risk, and identify targets for treatment.

Role of Race/Ethnicity in HDL Cholesterol

HDL cholesterol levels differ based on race/ethnicity (Haffner et al., 1999). Hispanics tend to have lower levels of HDL-C in comparison to Blacks or non-Hispanic Whites (Burchfiel et al., 1990; Haffner, Stern, Hazuda, Rosenthal, & Knapp, 1986), but they also tend to have higher triglycerides (Haffner et al., 1986). Conversely, Blacks tend to have lower prevalence rates of low HDL-C and high triglycerides, although this does not appear to be protective from CVD (Powell, Thompson, Caspersen, & Kendrick, 1987). Prior to 2011-2012, NHANES had not included any data on Asian Americans (Center for Disease Control and Prevention, 2013), and comparison of the lipid profile of this quickly expanding minority group to other races/ethnicities is lacking. Data indicate that in Hispanics, HDL cholesterol is not a reliable predictor of myocardial infarction, although it is predictive of myocardial infarction in non-Hispanic Whites (Willey et al., 2011). Furthermore, HDL-C does not load reliably onto a factor analysis of the metabolic syndrome in Hispanics (Chirinos et al., 2013; Llabre et al., under review) as it does for other races/ethnicities. Ultimately, this research demonstrates that HDL cholesterol may differentially predict health outcomes for various ethnic groups, highlighting the importance of the current study to address this gap in the literature.

Stroke also disproportionately affects mortality rates for Blacks and Hispanics compared to Whites (Sacco et al., 2001). Compared with Whites in the same community, Blacks had a 2.4-fold increased annual stroke incidence and Caribbean Hispanics had a 2-

fold increased incidence. Although reasons are not entirely clear, other risk factors for stroke, in addition to HDL cholesterol, differ by race/ethnicity. For example, physical inactivity presents greater risk among Hispanics than Whites, atrial fibrillation has a greater impact on stroke outcome in Whites than Blacks, and Blacks have higher rates of hypertension compared to Whites (Sacco et al., 2001). These racial differences are important to consider when examining the relationship between HDL cholesterol and stroke risk, even at a particle-based level.

Proposed Study

Although literature supports the findings that HDL cholesterol is associated with CVD, the evidence demonstrating its relationship to stroke risk is less consistent. Furthermore, given the complex nature of the HDL particle, it is unclear whether cholesterol content, particle number, or size better predicts risk. Additionally, research shows that both total and HDL cholesterol may differentially predict the risk of ischemic stroke in men and women of different races/ethnicities. In the present study, we examined how HDL-C, HDL-P, and size uniquely predicted incident stroke of both types across different races/ethnicities.

Aim 1) Examine the association between HDL-Cand stroke risk.

Hypothesis 1) There will be a significant negative association between HDL-Cand stroke risk.

Aim 2) Examine the association between HDL-P and stroke risk.

Hypothesis 2) There will be a significant negative association between HDL-P and stroke risk.

Aim 3) Examine the association between number of large, medium, and small HDL particles and stroke risk.

Hypothesis 3) There will be a significant negative association between number of large HDL particles and stroke risk.

Aim 4) Examine an effect modification by race/ethnicity on HDL cholesterol and stroke risk.

Chapter 2: Methods

Participants

The Multi-Ethnic Study of Atherosclerosis (MESA) is comprised of 6,814 men and women, between the ages of 45 – 84 enrolled between July 2000 and August 2002 from six field centers across the United States (Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University, and University of California at Los Angeles). This multiethnic population included White, Black, Hispanic, and Chinese participants. Individuals were excluded if they had prior diagnosis of a heart attack, stroke, transient ischemic attack, heart failure, angina, atrial fibrillation, or history of any cardiovascular procedure. Participants who had any existing medical condition that would prevent long-term participation, were pregnant, or weighed over 300 pounds were also excluded from the study. The MESA protocol has been approved by the Institutional Review Boards of all collaborating institutions. The specific protocol for the current study has been approved by the University of Miami Institutional Review Board.

Procedure

Eligibility for participation was initially determined by phone call. Final eligibility was determined in the clinic. At Visit 1, participants arrived at the clinic fasting and completed the following measures: questionnaires, anthropometry, blood pressure, ankle/brachial blood pressure index, electrocardiogram (ECG), coronary calcium determination, carotid ultrasound, arterial wave forms, flow-dependent brachial artery vasodilation, cardiac magnetic resonance imaging, and laboratory measurements. At Visit 2 (beginning in July 2002), Visit 3 (beginning in January 2004), and Visit 4 (beginning in

July 2005), and Visit 5 (between April 2010 – January 2012) selected questionnaires, anthropometry, blood pressure, and laboratory tests were repeated. Participants are contacted every 9 – 12 months to assess clinical morbidity and mortality. Baseline data and stroke outcome at follow-up are used in analyses. MESA was designed to assess predictors of the progression from subclinical to clinical cardiovascular disease in a large-scale, diverse group of adults across the United States. The objectives and design of MESA have been previously described in detail (see Bild et al., 2002).

Lipid measurements

Blood samples were drawn after a 12-hour overnight fast and stored at -70°C. Lipids were measured at Collaborative Studies Clinical Laboratory at Fairview University Medical Center, Minneapolis, Minnesota. Lipids were assayed on thawed ethylenediaminetetraaceticacid plasma within two weeks of sample collection, using Centers for Disease Control Prevention/NHLBI standards. HDL-C was measured using the cholesterol oxidase method (Roche Diagnostics, Indianapolis, Indiana) after precipitation of non–HDL-C with magnesium/dextran (coefficient of variation 2.9%). LDL-C was calculated using the Friedewald equation (Friedewald, Levy, & Fredrickson, 1972). Plasma lipoprotein particle concentrations were measured at LipoScience, Inc. (Raleigh, North Carolina) by nuclear magnetic resonance (NMR) spectroscopy using the LipoProfile-3 algorithm. HDL-P and LDL-P (coefficient of variation < 4%) are the sums of the particle concentrations of their respective subclasses, which are quantified based on particle size using the amplitudes of their lipid methyl group NMR signals, and mean particle sizes are the weighted average of related subclasses (Jeyarajah, Cromwell, & Otvos, 2006; Mackey et al., 2012). Large particles were between 9.4 – 14 nm (μmol/L),

medium particles were between 8.2 – 9.4 nm (μmol/L), and small particles were between 7.3 – 8.2 nm (μmol/L).

Covariates

Relevant covariates that have a known relationship and influence on HDL cholesterol and stroke risk were included. Covariates were as follows: systolic blood pressure; sex (M/F); race/ethnicity (White, Black, Hispanic, Chinese); age (45 – 84 years); educational attainment; medication usage (lipid lowering drugs, hypertensive medication, hypoglycemic medication); BMI (kg/m^2); cigarette status (Current, Former/Never); total cholesterol; triglycerides. Since LDL cholesterol is a component part of total cholesterol, we excluded the need to account for this extra covariate in analyses.

Endpoint

Endpoints were defined as stroke, both fatal and nonfatal. Events were adjudicated for better sensitivity and specificity.

Chapter 3: Statistical Analyses

All analyses were performed using SPSS Version 22.0. All variables were screened for outliers and univariate normality. Distributions were considered non-normal if the absolute value of the skew index was greater than 3.0 and the kurtosis was greater than 10.0 (Kline, 2011). Triglycerides (skew index = 5.86, kurtosis = 90.24) were log transformed to achieve approximate normality. Continuous variables were centered to aid in interpretation of analysis.

Individuals with missing cholesterol data (N = 21) as well as individuals taking anti-coagulant drugs (namely, warfarin) were removed prior to analysis (N = 24), leaving a total sample size of 6,769. Cox proportional hazard regression models were constructed using HDL-C, HDL-P, and particle size as continuous variables with incident stroke events as the outcome over a 9.5 year follow-up period. All covariates were first entered together without any HDL composite measures (Model 1), and three subsequent models were constructed to examine the associations between HDL-C (Model 2), total HDL-P(Model 3), and number of large, medium, and small HDL particles (Model 4) with stroke. Interactions between the HDL cholesterol variables and ethnicity were also examined (Models 5 – 7).

Chapter 4: Results

Sample Characteristics

Participants were an average of 62 years of age (SD = 10.24), and 53% were female. All four race/ethnicities were well represented (38.4% White, 27.8% Black, 22.0% Hispanic/Latino, and 11.9% Chinese American). The majority of individuals were not taking medication that might affect HDL cholesterol level or stroke outcome (16.1% on lipid lowering drugs, 37.2% on hypertension medication, and 8.6% on oral hypoglycemic agents). Cigarette status was grouped into current users (13.0% of people) and former/non-users. Highest level of education completed varied: less than high school (18.0%), high school/GED (18.1%), some college (including technical school certificate and associate's degree) (28.4%), and college and above (including bachelor's degree, graduate, and/or professional school) (35.2%). Stroke events were rare, occurring in 2.6% of the sample (N = 176). The majority of stroke victims experienced an ischemic stroke (2.2%).

There were significant differences in all covariates when grouped by race/ethnicity, except for sex. Blacks had significantly higher systolic blood pressure than all other race/ethnic groups ($F (3, 6769) = 57.45$, $p < .001$). Furthermore, almost half of the sample of Blacks was taking hypertensive medication, which was a significantly larger proportion than other races/ethnicities. Compared to all other races/ethnicities, Hispanics had the highest triglyceride levels ($F (3, 6769) = 107.53$, $p < .001$. Hispanics also had significantly higher total cholesterol levels than Blacks and Chinese Americans ($F (3, 6769) = 18.47$, $p < .001$, but not Whites. Hispanics had significantly lower levels of HDL cholesterol than all other race/ethnic groups ($F (3, 6769) = 40.18$, $p < .001$). Whites

had greater HDL-P than all other races/ethnicities (F (3, 6769) = 31.15, $p < .001$). Hispanics had significantly fewer number of large particles compared to other races/ethnicities (F (3, 6769) = 27.18, $p < .001$). Blacks had significantly fewer number of medium particles compared to other races/ethnicities (F (3, 6769) = 120.87, $p < .001$). Chinese had significantly more small HDL particles compared to other races/ethnicities (F (3, 6769) = 54.68, $p < .001$). Sample descriptives for all covariates are listed in Table 1 for both the total sample and for each race/ethnicity.

Covariate Correlations

HDL-C was strongly, positively correlated with HDL-P ($\rho = .69$), number of large particles ($\rho = .91$), and number of medium particles ($\rho = .45$; ps $< .001$). HDL-C was strongly, negatively correlated with number of small HDL particles ($\rho = -.28$, $p < .001$). All other correlations among continuous covariates are listed in Table 2.

HDL as a Predictor of Stroke Outcome

All models excluded participants taking anticoagulant drugs (i.e., warfarin) and included the following relevant covariates: age, BMI, systolic blood pressure, total cholesterol, triglycerides, race/ethnicity, smoking, medication use (lipid-lowering, hypertension, and hypoglycemic drugs), and education. Model 1 was run without any HDL composite measures. Controlling for all covariates, Model 2 showed that HDL-C was negatively associated with stroke events (Hazard Ratio = .554; 95% CI .311 - .987, $p = .045$). A clinically meaningful increase in risk would be equivalent to .1 mmol/L. These results suggest that for each .1 mmol/L increase in HDL-C, there is a 6% reduction in risk. HDL-P and all particle size variables (μmol/L) were divided by a factor of 10 to aid in interpretation. There was a trend for HDL-P and stroke outcome in the negative

direction in predicting stroke events (HR = .793; 95% CI .613 - 1.026, p = .078) (Model 3). Adjusting for number of medium and number of small HDL particles, the number of large particles was negatively associated with stroke events (HR = .515; 95% CI .278 - .955, p =.035) (Model 4). The full model with HDL-C, HDL-P, and all three HDL sizes adjusting for one another, did not yield significant findings. Results from all four models are listed in Table 3.

Interactions with Race/Ethnicity

Race/ethnicity interactions were tested for all HDL composition predictors. The interaction between HDL-C and race/ethnicity approached significance $\Delta \chi^2$ (3, N = 6769) = 6.960, p = .073. Observed differences occurred between Blacks and the three other race/ethnic groups. The relationship was significant in Blacks (B = -1.379, SE = .490, p <.01), but was not significant in Chinese Americans (B = .907, SE = .799, p = .26), Whites (B = -.416, SE = .380, p = .27), or Hispanics (B = -.353, SE = .496, p = .48).

The interaction between number of large particles and race/ethnicity approached significance $\Delta \chi^2$ (3, N = 6769) = 7.517, p = .057. Again, significant differences occurred between Blacks and other racial/ethnic groups. While the relationship was significant in Blacks (B = -1.639, SE = .548, p < .01), it was not significant in Chinese Americans (B = .924, SE = .848, p = .28), Whites (B = -.370, SE = .415, p = .37), or Hispanics (B = -.513, SE = .554, p = .36). Each race/ethnicity was run as the reference group in separate models to examine the simple effects from the interaction analyses. Results from these analyses are listed in Table 4.

The interaction between HDL-P and race/ethnicity was not significant $\Delta \chi 2$ (3, N = 6769) = 5.245, p = .155. Also, there was no significant interaction between number of

medium $\Delta \chi^2$ (3, N = 6769) = 1.611, p = .657 or number of small particles and race/ethnicity $\Delta \chi^2$ (3, N = 6769) = 2.159, p = .540.

Secondary Analyses: Ischemic Stroke Outcome Only

Given the significant findings for combined stroke outcome, secondary analyses were run to determine if the significant finding was carried primarily by ischemic stroke event. Ischemic stroke events also included "unknown" and "other" responses by the reporter, but excluded any type of hemorrhagic stroke event. All significant findings from the main models were maintained when the event outcome was reduced to ischemic stroke only (N = 147).

Controlling for all covariates, HDL-C was negatively associated with stroke event (HR = .516, 95% CI .273 - .975, p = .042). Again, there was a trend for HDL-Pin the negative direction (HR = .774, 95% CI .584 – 1.027, p = .076). Controlling for medium and small HDL particles, number of large HDL particles was negatively associated with ischemic stroke event (HR = .493, 95% CI .249 - .974, p = .042).

Chapter 5: Discussion

Prior research points to the complexity of the HDL molecule, particularly in its relationship to stroke outcome. In a large, ethnically diverse sample of asymptomatic men and women at baseline, HDL-C and number of large HDL particles were significantly, negatively associated with stroke event after controlling for relevant covariates, which is consistent with prior studies linking stroke outcome and CVD in general to lower HDL-C(Gordon & Rifkind, 1989; Lindenstrom et al., 1994; Tanne et al., 1997) and larger HDL particles (Mora et al., 2009). Above and beyond the contribution to stroke outcome made by these relevant covariates, both HDL-C and number of large HDL particles were negatively associated with stroke event. Although the relationship between stroke outcome and HDL-P did not reach significance, there was a noted trend. This differs slightly from some previous findings, which have supported a significant negative relationship between coronary events and HDL-P (Mackey et al., 2012; Mora et al., 2013). This may be due to the low incidence of stroke in this population, since coronary events include a larger number of cardiovascular problems.

Results were consistent when outcome was reduced to ischemic stroke only, which is logical given that the risk factors for both ischemic and hemorrhagic stroke are similar (American Heart Association, 2014b), and most of the individuals who experienced a stroke in this sample experienced an ischemic stroke (~85%).

Interactions with race/ethnicity approached significance in both HDL–C and HDL large particle number. There were no significant interactions with race/ethnicity in total HDL-P, or number of medium or small HDL particles. This finding suggests that the

relationship between HDL-C and number of large HDL particles may affect stroke outcome differently in Blacks compared to other races.

While this research supports the finding that HDL-C alone is sufficient in prediction of stroke risk, and HDL particle size adds little value, the question remains whether and why these subclasses are important to continue investigating. While HDL-C may be a mere metabolic marker for increased risk for stroke rather than a causal factor (Rader, 2003), the major protein in HDL cholesterol, apolipoprotein A-1, has been found to be directly protective against atherosclerosis in several animal studies (Benoit et al., 1999; Liu, Lawn, Verstuyft, & Rubin, 1994; Miyazaki et al., 1995; Paszty, Maeda, Verstuyft, & Rubin, 1994; Plump, Scott, & Breslow, 1994; Rubin, Krauss, Spangler, Verstuyft, & Clift, 1991; Tangirala et al., 1999). If and how different subclasses differ in their anti-atherogenic functionality, the amount of apolipoprotein A-1, and ultimate efficiency in reverse cholesterol transport, remain important unanswered questions. The pathophysiology of stroke with specific regard to the HDL cholesterol molecule is further complicated by the presence of hypertension and triglycerides, two strong predictors of stroke risk. High blood pressure can damage arteries so they burst or clog more easily, and these subsequent weakened arteries in the brain increase risk for stroke (American Heart Association, 2014b).

Research supports the finding that elevated levels of nonfasting triglycerides are also associated with increased risk of stroke (Freiberg, Tybjaerg-Hansen, Jensen, & Nordestgaard, 2008). These increased triglyceride levels mark the presence of elevated levels of chylomicron and very low-density lipoprotein remnants (Nordestgaard, Benn, Schnohr, & Tybjaerg-Hansen, 2007). These cholesterol-containing, triglyceride-rich

lipoproteins invade the arterial endothelium (Nordestgaard, Tybjaerg-Hansen, & Lewis, 1992; Shaikh et al., 1991) and may get stuck within the subendothelial space (Nordestgaard, 1996; Nordestgaard, Wootton, & Lewis, 1995; Rutledge, Mullick, Gardner, & Goldberg, 2000), potentially leading to the development of atherosclerosis (Kolovou, Anagnostopoulou, Daskalopoulou, Mikhailidis, & Cokkinos, 2005; Zilversmit, 1979).

Accounting for multiple covariates, including hypertension, hypertensive medication, and triglycerides, is one strength of the current study. Additional strengths of the study include its' large-scale, multi-site, longitudinal nature, and inclusion of four different races/ethnicities in addition to multiple validated measures of HDL cholesterol. Few other studies have examined the role of race/ethnicity in the complex relationship between HDL cholesterol and its subfractions with stroke outcome. Furthermore, outcome data was adjudicated.

Some limitations of the study are noted. In general, the incidence of stroke was low, particularly among Chinese-Americans. Future studies should seek to include a larger sample of minority participants. Furthermore, while the follow-up of this study extended to 9.5 years, increasing time duration of follow-up may capture other stroke events that occurred outside the window of follow-up. Additionally, HDL cholesterol may be influenced by a number of factors that were not included in analysis including diet and exercise. We also know that lipid profiles for men and women may differ significantly; however, there was insufficient power to detect differences in the relationship between HDL composite measures and stroke risk in both sexes. Therefore,

we controlled for sex in all analyses in addition to several highly relevant covariates were included in the analyses in order to limit potential confounding.

These results have implications for risk assessment as well as treatment for individuals identified as at-risk for stroke event. More specifically, the way in which race/ethnicity may compound risk for stroke deserves greater attention. Further research is warranted to understand the mechanisms by which HDL functions to influence stroke risk in different races/ethnicities. More research is also needed to elucidate the relationship between HDL-P and stroke outcome, given that this has been identified as a significant predictor of cardiovascular events in other studies.

Summary/Conclusions

This study reinforces the finding that HDL-C provides a reliable, stable measure of stroke outcome. Furthermore, it provides support for the protective role of large HDL particles and calls for replication in future samples. Overall, particle size does not add to risk prediction of stroke above and beyond HDL-C. Given the significant differences in the relationship between HDL-C in Blacks compared to Whites, Hispanics, and Chinese-Americans, further exploration of these racial/ethnic differences is warranted.

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Table 1. Sample descriptives of total sample and broken down by race/ethnicity, written as (Mean (Standard Deviation)).

Variable	Total Sample, N = 6769	Whites, N = 2601	Blacks, N = 1879	Hispanics, N = 1486	Chinese Americans, N = 803
Age (years)	62.14 (10.24)	62.57	62.14	61.25	62.35
BMI (kg/m^2)	28.32 (5.47)	27.70 (5.04)	30.17 (5.87)	29.42 (5.09)	23.98 (3.30)
Seated systolic blood pressure (mmHg)	126.58 (21.50)	123.45 (20.43)	131.69 (21.62)	126.67 (21.93)	124.57 (21.63)
Total cholesterol (mmol/L)	5.02 (.92)	5.06 (.91)	4.90 (.94)	5.12 (.97)	4.98 (.82)
HDL-C(mmol/L)	1.32 (.38)	1.35 (.40)	1.36 (.40)	1.23 (.34)	1.28 (.33)
Triglycerides (mmol/L)	3.40 (2.30)	3.43 (2.33)	2.71 (1.77)	4.06 (2.62)	3.69 (2.19)
HDL-P(total) (µmol/L)	34.04 (6.66)	35.00 (7.04)	33.47 (6.50)	33.23 (6.26)	33.72 (5.88)
Large HDL (9.4-14 nm) (µmol/L)	6.02 (3.46)	6.06 (3.57)	6.45 (3.65)	5.38 (3.04)	6.07 (3.18)
Medium HDL (8.2-9.4 nm) (µmol/L)	13.27 (6.84)	14.98 (7.43)	11.79 (5.98)	13.42 (6.50)	10.93 (5.77)
Small HDL (7.3-8.2 nm) (µmol/L)	14.74 (5.73)	13.97 (5.94)	15.23 (5.49)	14.43 (5.46)	16.72 (5.53)

Variable	Total Sample, N = 6769 N (% of Sample)	Whites, N = 2601	Blacks, N = 1879	Hispanics, N = 1486	Chinese Americans, N = 803
Stroke Outcome	176 (2.6)	67 (2.6)	53 (2.8)	45 (3.0)	11 (1.4)
Sex (Females)	3585 (53.0)	1358 (52.2)	1041 (55.4)	772 (52.0)	414 (51.6)
Cigarette Status (Users)	882 (13.0)	301 (11.6)	336 (17.9)	200 (13.5)	45 (5.6)
Medication Usage					
Lipid Lowering Drugs	1087 (16.1)	472 (18.1)	307 (16.3)	192 (12.9)	116 (14.4)
Hypertensive Meds	2520 (37.2)	860 (33.1)	948 (50.5)	481 (32.4)	231 (28.8)
Hypoglycemic Meds	581 (8.6)	98 (3.8)	220 (11.7)	184 (12.4)	79 (9.8)
Education					
Less than HS	1216 (18.0)	126 (4.8)	228 (12.1)	663 (44.6)	199 (24.8)
HS/GED	1228 (18.1)	437 (16.8)	357 (19.0)	304 (20.5)	130 (16.2)
Some college	1921 (28.4)	740 (28.5)	648 (34.5)	371 (25.0)	162 (20.2)
College and above	2382 (35.2)	1291 (49.6)	632 (33.6)	148 (10.0)	311 (38.7)

Table 2. Correlations among all continuous covariates.

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. HDL-C (mmol/L)	1								
2. HDL -P(µmol/L)	.692**	1							
3. HDL Large Particles (µmol/L)	.909**	.567**	1						
4. HDL Med Particles (µmol/L)	.451**	.628**	.279**	1					
5. HDL Small Particles (µmol/L)	-.284**	.066**	-.279**	-.633**	1				
6. BMI (kg/m2)	-.211**	-.086**	-.226**	-.076**	.128**	1			
7. Systolic Blood Pressure (mmHg)	.005	.074**	.016	-.018	.097**	.154**	1		
8. Total cholesterol (mmol/L)	.192**	.162**	.115**	.106**	-.008	-.008	.036**	1	
9. Triglycerides (mmol/L)	-.374**	.030*	-.343**	-.083**	.272**	.109**	.061**	.288**	1

**$p < .01$, *$p < .05$

Large particles were defined as (9.4-14 nm) (µmol/L), medium as (8.2-9.4 nm) (µmol/L), and small as (7.3-8.2 nm) (µmol/L).

Table 3. Results from survival models with HDL composite measures, adjusted for all covariates.

Variable	B	SE	Exp(B)	95% CI
Model 1				
Age	.056**	.009	1.058	1.039 – 1.077
Sex (M/F)	.147	.160	1.159	.846 – 1.586
Race/Ethnicity:				
Whites	REF			
Blacks	-.016	.224	.984	.634 – 1.528
Hispanics	-.157	.242	.855	.532 – 1.373
Chinese Americans	-.200	.241	.819	.511 – 1.313
Education				
Less than HS	REF			
HS/GED	-.200	.241	.819	.511 – 1.313
Some college	-.016	.224	.984	.634 – 1.528
College and above	-.157	.242	.855	.532 – 1.373
Cigarette status	.685**	.215	1.983	1.302 – 3.022
Medication:				
Lipid Lowering Drugs	-.183	.203	.833	.559 – 1.240
Hypertensive medication	.342*	.170	1.407	1.009 – 1.962
Hypoglycemic medication	.484*	.211	1.623	1.073 – 2.456
BMI (kg/m2)	.018	.016	1.018	.988 – 1.049
Systolic Blood Pressure	.018**	.003	1.018	1.011 – 1.025
Total cholesterol	.077	.088	1.080	.909 – 1.283
Triglycerides	.326*	.159	1.386	1.014 – 1.894
Model 2				
HDL-C	-.591*	.294	.554	.311 – .987
Model 3				
HDL-P	-.232	.132	.793	.613 – 1.026
Model 4				
HDL Large Particles	-.664*	.315	.515	.278 – .955
HDL Medium Particles	-.103	.161	.902	.658 – 1.237
HDL Small Particles	-.135	.186	.874	.607 – 1.259

**$p < .01$, * $p < .05$

Model 1 included all covariates without any HDL composite measures.
Model 2 included all covariates plus HDL-C.
Model 3 included all covariates plus HDL-P.
Model 4 included all covariates plus all three HDL size composites.

Table 4. Simple effects from the race/ethnicity interactions with HDL composite measures.

Interaction	B	Hazard Ratio	95% CI
HDL-C			
Whites	-.416	.660	.314 – 1.388
Blacks	-1.379**	.252	.096 - .657
Hispanics	-.353	.703	.266 – 1.856
Chinese Americans	.907	2.476	.517 – 11.862
HDL Large particles			
Whites	-.370	.691	.306 – 1.559
Blacks	-1.639**	.194	.066 - .569
Hispanics	-.513	.599	.201 – 1.780
Chinese Americans	.924	2.519	.478 – 13.275

Adjusted for all relevant covariates.

**$p < .01$

Betas represent relationship between HDL composite measure and time to stroke event when the corresponding race/ethnicity was entered as the reference group.