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High-density lipoprotein subfractions and carotid plaque: The Northern Manhattan Study

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ABSTRACT

Objective: The objective of this cross-sectional analysis was to investigate the relation between two major high-density lipoprotein cholesterol (HDL-C) subfractions (HDL2-C and HDL3-C) and carotid plaque in a population based cohort.
Methods: We evaluated 988 stroke-free participants (mean age 66 ± 8 years; 40% men; 66% Hispanic and 34% Non-Hispanic) with available data on HDL subfractions using precipitation method and carotid plaque area and thickness assessed by a high-resolution 2D ultrasound. The associations between HDL-C subfractions and plaque measurements were analyzed by quantile regression.
Results: Plaque was present in 56% of the study population. Among those with plaque, the mean ± SD plaque area was 19.40 ± 20.46 mm^2 and thickness 2.30 ± 4.45 mm. The mean ± SD total HDL-C was 46 ± 14 mg/dl, HDL2-C 14 ± 8 mg/dl, and HDL3-C 32 ± 8 mg/dl. After adjusting for demographics and vascular risk factors, there was an inverse association between HDL3-C and plaque area (per mg/dl: beta = −0.26 at the 75th percentile, $p = 0.001$ and beta = −0.32 at the 90th percentile, $p = 0.02$). A positive association was observed between HDL2-C and plaque thickness (per mg/dl; beta = 0.02 at the 90% percentile, $p = 0.003$). HDL-C was associated with plaque area (per mg/dl: beta = −0.18 at the 90th percentile, $p = 0.01$), but only among Hispanics.
Conclusion: In our cohort we observed an inverse association between HDL3-C and plaque area and a positive association between HDL2-C and plaque thickness. HDL-C subfractions may have different contributions to the risk of vascular disease. More studies are needed to fully elucidate HDL-C anti-atherosclerotic functions in order to improve HDL-based treatments in prevention of vascular disease and stroke.

1. Introduction

Epidemiological studies consistently demonstrate that low levels of high density lipoprotein cholesterol (HDL-C) are associated with increased risk for cardiovascular disease (CVD) and stroke [1,2]. The same inverse association has been demonstrated between HDL-C and carotid atherosclerosis [3], but with less consistency.

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However, recent clinical trials have failed to translate the same epidemiological association into evidence that pharmacological raising of HDL-C prevents CVD morbidity and mortality [4–6]. Such studies reinforce the need to focus on heterogeneity and functionality of HDL-C subclasses, rather than on measurements of total HDL-C, in order to better explain its pleiotropic effects.

Based on its density, HDL-C has two major subfractions, more dense and smaller HDL3-C and less dense and larger HDL2-C cholesterol. High-density lipoprotein subfractions differ in their biochemical properties, vascular metabolism, and biological activity, and their distribution represents a dynamic process that can be altered by the presence of chronic diseases, drug therapies, and lifestyle changes. Decreased risk of CVD has been linked to higher levels of HDL2-C cholesterol, but not consistently, and uncertainty over the value of HDL-C subfractions in vascular risk remains [7,8].

Atherosclerosis is a disease involving a series of inflammatory events. Carotid plaque, as a focal manifestation of atherosclerosis, represents a validated subclinical ultrasonographic marker of cardiovascular disease and stroke [9]. Similar to the association with CVD, the severity and progression of atherosclerosis has been predominantly linked to lower levels of the HDL2-C subfraction [10–12]. However, the benefits of HDL3-C subfraction have also been well documented in the pathogenesis of atherosclerosis [13–15]. The aim of this study was to investigate the relationship between the HDL2-C and HLD3-C subfractions and carotid plaque in a large and predominantly Hispanic cohort comprised of stroke-free individuals.

2. Material and methods

2.1. Study participants

We included 988 stroke-free participants from the Northern Manhattan Study (NOMAS), an ongoing, prospective, population-based study of stroke incidence and vascular risk factors, who had data available on HDL-C, measured by precipitation, subfractions and carotid plaque measured by high-resolution B-mode ultrasound. Details on the subject ascertainment, extensive assessments, and methods used in NOMAS are described elsewhere [16]. Briefly, eligible subjects: a) had never been diagnosed with ischemic stroke; b) were >40 years old; and c) resided in Northern Manhattan for ≥3 months, in a household with a telephone. Subjects were identified by random-digit dialing. The telephone response rate was 91%. Subjects were recruited from the telephone sample to have an in-person baseline interview and assessment. The enrollment response rate was 75%, the overall participation rate was 69%, and a total of 3298 subjects were enrolled. Of the total of 3298 subjects, 54% of subjects had data on the HDL subfractions ($n = 1793$) and carotid ultrasound measurements ($n = 1788$). These two subcohorts overlapped resulting in 30% of the study participants ($n = 988$) having both HDL subfractions and carotid plaque measurements. NOMAS was approved by the Institutional Review Boards of Columbia University Medical Center and the University of Miami, and all participants gave written informed consent.

2.2. Baseline evaluation

Data were collected through interviews with trained research assistants in English or Spanish. Physical and neurological examinations were conducted by study neurologists. Race-ethnicity was based upon self-identification through a series of questions modeled after the US census and conforming to standard definitions outlined by Directive 15 [17]. Standardized questions were adapted from the Behavioral Risk Factor Surveillance System by the Centers for Disease Control regarding hypertension, diabetes, smoking, and cardiac conditions [18]. Blood pressure (BP) was measured with mercury sphygmomanometers and appropriately-sized cuffs. Hypertension was defined as a BP ≥ 140/90 mmHg (based on the average of two measurements during one sitting), the patient's self-reported hypertension, or use of anti-hypertensive medications. Diabetes mellitus was defined by fasting glucose ≥126 mg/dl, the patient's self-reported diabetes, or use of insulin or oral anti-diabetic medication. Body mass index (BMI) was calculated in kg/m^2. Smoking was categorized as never smoking, former smoking, and current (within the past year) smoking. Mild-moderate alcohol use was defined as current drinking of >1 drink per month and ≤2 drinks per day. Physical activity was defined as the frequency and duration of 14 different recreational activities during the 2-week period before the interview, as described previously [16].

2.3. HDL-C, HDL2-C and HDL3-C measurements

Blood samples were drawn after an overnight fast. Plasma levels of cholesterol and triglycerides (TGs) were measured using standardized enzymatic procedures with a Hitachi 705 automated spectrophotometer (Boehringer Mannheim, Mannheim, Germany). HDL-C was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid. The inter-assay coefficient of variation in our laboratory was 3% for HDL-C. Two major HDL subfractions were determined in plasma by sequential precipitation using heparin-manganese and dextran sulfate [19]. In this procedure, apo B-containing lipoproteins are precipitated in the first precipitation reaction using heparin-manganese chloride at final concentrations of 1.26 mg/ml and 0.091 M, respectively. The supernatant (total HDL-C) is removed, an aliquot is saved for analysis, and dextran sulfate (mol wt 15,000; Genzyme, Cambridge, MA) is added to precipitate HDL2-C, which was estimated by subtracting HDL3-C from HDL-C. Following centrifugation, the supernatant (HDL3-C) is removed and analyzed for cholesterol content. The inter-assay coefficients of variation for HDL2-C and HDL3-C assays are 8% and 7%, respectively, at levels of 28 mg/dl (HDL2-C) and 32 mg/dl (HDL3-C).

2.4. Carotid ultrasound

High-resolution B-mode ultrasound (GE LogIQ 700, 9/13-MHz linear-array transducer) was performed by trained and certified sonographers using standardized and validated scanning and reading protocols as described previously [20]. Plaque is defined as a focal wall thickening or protrusion in the lumen more than 50% greater than the surrounding thickness. Carotid plaque area (mm [2]) and thickness (mm) were measured using an automated computerized edge tracking software M'Ath (Paris, France) [21]. Total plaque area (TPA) was a sum of area measured in all plaques within an individual. Maximum plaque thickness was measured for each plaque in an individual at the highest plaque prominence between the lumen–intima and media–adventitia boundaries. The maximum value of maximal thickness measured in all plaques within an individual was used in the analyses [22].

2.5. Statistical analysis

The primary independent variables of interest were HDL2-C, HDL3-C, and total HDL-C, all assessed as continuous measurements in mg/dl. The HDL-C variables were examined in relation to the demographics, anthropometrics, and lifestyle and vascular risk factors, among 988 NOMAS participants. We calculated the means and standard deviations. Due to the non-normal distribution of plaque area and thickness, with 44% of the study population