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HDL anti-oxidant function associates with LDL level in young adults

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ABSTRACT

Objectives: The primary objective was to evaluate predictors of HDL anti-oxidant function in young adults.
Background: High-density lipoprotein (HDL) cholesterol is considered a protective factor for cardiovascular disease (CVD). However, increased levels are not always associated with decreased cardiovascular risk. A better understanding of the importance of HDL functionality and how it affects CVD risk is needed.
Methods: Fifty non-Hispanic white subjects from the Testing Responses on Youth (TROY) study were randomly selected to investigate whether differences in HDL anti-oxidant function are associated with traditional cardiovascular risk factors, including carotid intima media thickness (CIMT), arterial stiffness and other inflammatory/metabolic parameters. HDL anti-oxidant capacity was evaluated by assessing its ability to inhibit low-density lipoprotein (LDL) cholesterol oxidation by air using a DCF-based fluorescent assay and expressed as a HDL oxidant index (HOI). The associations between HOI and other variables were assessed using both linear and logistic regression.
Results: Eleven subjects (25%) had an HOI ≥ 1, indicating a pro-oxidant HDL. Age, LDL, high sensitivity C-reactive protein (hsCRP), and paraoxonase activity (PON1), but not HDL, were all associated with HOI level in univariate linear regression models. In multivariate models that mutually adjusted for these variables, LDL remained the strongest predictor of HOI (0.13 increase in HOI per 1 SD increase in LDL, 95% CI 0.04, 0.22).
Atherogenic index of plasma, pulse pressure, homocysteine, glucose, insulin, CIMT and measurements of arterial stiffness were not associated with HOI in this population.
Conclusions: These results suggest LDL, hsCRP and DBP might predict HDL anti-oxidant function at an early age.

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1. Introduction

High-density lipoprotein (HDL) cholesterol is a well characterized protective factor for cardiovascular disease (CVD) [1]. However, increased HDL levels are not always associated with decreased risk [2]. There is a growing need to better understand the importance of HDL functional status and how this may affect CVD risk [3].

HDL is thought to decrease CVD risk by virtue of its anti-oxidant, anti-inflammatory and reverse cholesterol transport functions [4–6]. HDL promotes reverse cholesterol transport by facilitating the efflux of cholesterol from cells such as macrophages [7]. A recent study showed that the cholesterol efflux capacity correlated negatively with the likelihood of angiographically-defined coronary artery disease even after adjustment for traditional CVD risk factors including HDL cholesterol level [8]. In addition, the same group showed that HDL anti-oxidant function was significantly impaired in subjects with acute coronary syndromes as compared with healthy subjects or those with stable coronary artery disease [7]. The authors used an HDL inflammatory index (HII), which reflected the ability of HDL to mitigate oxidation of low-density lipoprotein [7]. Higher HII indicated a smaller antioxidant capacity and resulted in a better predictor of acute coronary syndrome than HDL level alone [7,9].

HII was also associated with other CVD risk factors such as body mass index (BMI), HDL, triglycerides and baseline high sensitivity C-reactive protein (hsCRP) level. Interestingly, there was no correlation between HII and HDL-mediated cholesterol efflux capacity in the latter study [7] suggesting that different functional aspects of HDL can associate with different cardiovascular endpoints [7,8]. In addition, we have reported that exposure to environmental factors such as air pollutants can affect HDL anti-oxidant and anti-inflammatory capacities with different kinetics [10,11].

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To date, several techniques have been developed to evaluate HDL function and have been used in older individuals presenting with pre-existing cardiovascular disease [7–9,12]. However, the relationship between HDL function and early stages of cardiovascular disease has not been investigated, especially in young subjects. In this study, we aimed to investigate the relationship between HDL function, CVD risk factors and carotid intima-media thickness (CIMT) in a group of healthy college students. We assessed HDL anti-oxidant capacity using a DCF-based cell free fluorescent assay that evaluated the ability of HDL to inhibit oxidation of low-density lipoprotein cholesterol (LDL) by air. We used an HDL Oxidant Index (HOI) to express the HDL anti-oxidant capacity and evaluated this metric with respect to traditional CVD risk factors, including CIMT, arterial stiffness, blood pressure, BMI, cholesterol levels, triglycerides, hsCRP, homocysteine, glucose and insulin levels.

2. Methods

2.1. Study design

The Testing Responses on Youth (TROY) study consists of 861 college students recruited from USC in 2007–2009 and has been described in detail elsewhere [13]. For the current study, a subset of 50 randomly selected non-Hispanic white subjects who consented to collection of a serum sample were selected from the TROY population in order to investigate whether differences in HDL anti-oxidant function are associated with traditional cardiovascular risk factors, including CIMT and arterial stiffness as well as other inflammatory/metabolic parameters.

Participants attended a study visit during which CIMT, arterial stiffness, systolic (SBP) and diastolic (DBP) blood pressure, heart rate, height, and weight were measured. CIMT, arterial stiffness, heart rate, and blood pressure were assessed by a single physician-imaging specialist from the USC Atherosclerosis Research Unit Core Imaging and Reading Center. Several self-administered questionnaires were completed during or prior to the office visit to gather information about health and socio-demographic characteristics. These included three separate questions to assess family history of heart disease. Participants were asked if their biological mothers and fathers ever had any of the following: stroke, heart failure, or heart attack. Because frequencies of affirmative responses were low, family history of heart disease was considered to be positive if an individual responded yes to any of these three. Two additional questions were also asked about biological parents regarding medication use against high blood pressure and medication use to lower cholesterol or lipids. Participants provided a 12-h fasting blood sample for lipid and biomarker analyses following completion of health testing.

The study protocol was approved by the institutional review board for human studies at the University of Southern California, and written consent was provided by the study subjects.

2.2. Health measurements

High-resolution B-mode ultrasound images of the right common carotid artery were obtained with a portable Biosound MyLab 25 ultrasound system attached to a 10-MHz linear array transducer and read by a single physician-imaging specialist as described previously (Patents 2005, 2006, 2011) [14–16]. Three metrics of arterial stiffness were calculated: carotid distensibility, carotid stiffness index beta (β), and Young's elastic module (YEM) as described elsewhere [17–19]. Systolic (P_s) and diastolic (P_d) blood pressures and heart rate were measured immediately after the ultrasound examination by standard techniques after the subject was recumbent for at least 10 min. Blood pressure was measured three times in 1-min intervals, using an OMRON blood pressure monitor with automatic cuff inflation and deflation. Heart rate was measured using a three lead electrocardiogram as part of the Biosound MyLab 25 ultrasound system. Subject standing height was measured in stocking feet to the nearest centimeter using a metal measuring tape placed perpendicularly to the floor through the use of a construction-type bubble level and a measurement block to properly align head orientation. Weight was measured to the nearest pound with a medical-grade scale calibrated prior to each day's testing using pre-determined calibration weights.

2.3. Biologic measurements

Plasma and serum were divided into one ml samples and stored at −80° C until analyzed. One ml of plasma from each subject was used to measure total cholesterol, triglyceride, and HDL cholesterol levels using an enzymatic method in conformance with the Standardization Program of the National Centers for Disease Control and Prevention. LDL-C was calculated using the Friedwald formula [14].

Insulin and hsCRP were measured by a solid-phase chemiluminescent immunometric assay and homocysteine was measured by a competitive chemiluminescent immunoassay using the Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics, Malvern, PA). The sensitivities of the assays are 2 μIU/ml, 0.02 mg/dL, and 1.2 μmol/L. The inter-assay coefficients of variation were 4.2% and 2.9% at 10.0 and 47.8 μIU/ml, respectively, for insulin; 6.6%, 6.2% and 8.3% at 1.64, 7.83 and 88 mg/dL, respectively, for CRP; 13.1% and 9.8% at 12.1 and 20.3 μmol/L, respectively, for homocysteine.

Glucose was measured by a standard procedure using the Vitros Chemistry System. The analysis is based on the glucose oxidase-catalyzed reaction of glucose with molecular oxygen, followed by a second reaction that produces a highly colored red dye. The intensity of the color is proportional to the amount of glucose in the sample.

HDL was isolated from serum samples using a precipitation-based method and HOI was measured using a DCF-based fluorescent assay as described [10,12]. Prior to each experiment, 1 ml of 0.1 M NaOH was added to 250 μl of stock dichlorofluorescein diacetate (DCF-DA) and incubated at room temperature while protected from light for 30 min. The reaction was stopped by neutralizing the solution with 8.75 ml of 0.1 M phosphate buffered saline (PBS), resulting in the conversion of DCF-DA to dihydrodichlorofluorescein (DCFH). Upon oxidation, DCFH transforms into DCF. We evaluated HDL anti-oxidant capacity by assessing its ability to inhibit LDL oxidation by air, measured by DCF fluorescence. The change in fluorescence intensity is the result of the oxidation of DCFH induced by free radicals generated in the oxidation of human LDL in the absence or presence of the test HDL. 12.5 μl of human LDL (50 μg LDL cholesterol/ml) was mixed with 12.5 μl of test human HDL (50 μg HDL cholesterol/ml), and 75 μl of Tris–HCL buffer (pH7.4) in black, flat bottom polystyrene microtiter plates and incubated at 37 °C for 60 min. 25 μl of DCFH solution (50 μg/ml) was added to each well, mixed, and incubated at 37 °C for 2 h. Fluorescence intensity was determined with a plate reader (SynergyMx, BioTek, Vermont, USA) at an excitation wavelength of 485 nm and emission wavelength of 530 nm. A sensitivity level slit width of 9 nm was used for excitation and emission. This assay has shown to have a coefficient of variation of less than 10% between different plates and different days, as far as 2 months apart, with the use of two concentrations of HDL (Supplementary Figure S1).

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