Atherosclerosis 231 (2013) 246–251

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Impact of inflammatory biomarkers on relation of high density lipoprotein-cholesterol with incident coronary heart disease: Cardiovascular Health Study

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ARTICLE INFO

Article history:
Received 28 May 2013
Received in revised form
4 August 2013
Accepted 28 August 2013
Available online 5 September 2013

Keywords:
High density lipoprotein
Inflammation
C-reactive protein
Coronary heart disease

ABSTRACT

Background: Inflammatory factors and low HDL-C relate to CHD risk, but whether inflammation attenuates any protective association of high HDL-C is unknown.
Objective: Investigate inflammatory markers' individual and collective impact on the association of HDL-C with incident coronary heart disease (CHD).
Methods: In 3888 older adults without known cardiovascular disease (CVD), we examined if the inflammatory markers C-reactive protein (CRP), interleukin-6 (IL-6), and lipoprotein-associated phospholipase A2 (Lp-PLA_2) modify the relation of HDL-C with CHD. HDL-C, CRP, IL-6, and Lp-PLA_2 values were grouped as using gender-specific tertiles. Also, an inflammation index of *z*-score sums for CRP, IL-6, and Lp-PLA_2 was categorized into tertiles. We calculated CHD incidence for each HDL-C/inflammation group and performed Cox regression, adjusted for standard CVD risk factors and triglycerides to examine the relationship of combined HDL-C-inflammation groups with incident events.
Results: CHD incidence (per 1000 person years) was higher for higher levels of CRP, IL-6, and the index, and lower for higher levels of HDL-C. Compared to high HDL-C/low-inflammation categories (referent), adjusted HRs for incident CHD were increased for those with high HDL-C and high CRP (HR = 1.50, $p < 0.01$) or highest IL-6 tertile (HR = 1.40, $p < 0.05$), but not with highest Lp-PLA_2 tertile. Higher CHD incidence was similarly seen for those with intermediate or low HDL-C accompanied by high CRP, high IL-6, or a high inflammatory index.
Conclusion: The protective relation of high HDL-C for incident CHD appears to be attenuated by greater inflammation.

High-density lipoprotein cholesterol (HDL-C) has been shown to be associated with protection from coronary heart disease (CHD) through possible mechanisms of reverse cholesterol transport and reduction of low-density lipoprotein oxidative stress [1,2]. Increased inflammation has a prominent role in the atherosclerotic process. C-reactive protein (CRP), interleukin-6, and other inflammatory markers have been shown to be independently related to progression of atherosclerosis and CHD [3–7]. Lipoprotein-associated phospholipase A2 (Lp-PLA_2), a proatherogenic inflammatory marker, has also been shown to be associated with risk for CHD, independent of traditional cardiovascular risk factors [8]. We recently demonstrated in US adults from the cross-sectional National Health and Nutrition Examination Survey that CRP levels >3 mg/L are associated with a greater prevalence of CHD at all levels of HDL-C [9].

While low HDL-C is related to future risk of CHD and cardiovascular disease (CVD), it is unknown if the relationship of HDL-C levels with incident CHD and CVD is influenced by inflammation

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http://dx.doi.org/10.1016/j.atherosclerosis.2013.08.036

[10]. Accordingly, we investigated the influence of inflammatory markers CRP, IL-6 and Lp-PLA_2, individually and collectively, on the association of HDL-C with incident CHD and CVD in older individuals without CVD at baseline from the Cardiovascular Health Study (CHS).

1. Methods

The CHS is a prospective National Institutes of Health-sponsored study focused on identifying CVD risk factors and outcomes in an older community-dwelling sample recruited from Health Care Financing Administration Medicare eligibility lists and from other household members in four US geographic regions (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania). Initial enrollment during 1989–1990 included 5201 participants. In 1992–1993 a second cohort of 687 African–American participants was recruited, bringing the total sample size to 5888. Baseline data were collected from standardized questionnaires, a structured physical examination, and fasting blood analyses.

The methodology and design of CHS have been previously reported [11,12]. Our study included subjects with baseline values for age, gender, race, family history of 'heart attack', smoking history, diabetes, use of lipid and hypertension medication, blood pressure, waist size, triglycerides, HDL-C, low density lipoprotein cholesterol (LDL-C), and the inflammatory biomarkers CRP, IL-6, and Lp-PLA_2, but who were free of known cardiovascular disease at baseline.

1.1. Laboratory methods

HDL-C and LDL-C were analyzed from aliquots (0.5 ml) of EDTA plasma which were stored at −80 °C at the CHS central laboratory and shipped on dry ice to LipMed, Inc. for NMR lipoprotein subclass analysis. CRP was measured from stored frozen serum samples using a validated high-sensitivity enzyme-linked-immunosorbent-assay (analytical coefficient of variation 8.9%) [13]. IL-6 was measured from stored frozen serum samples using a commercial assay (Quantikine HS Human IL-6 Immunoassay, R&D Systems, Minneapolis, MN). Lp-PLA_2 mass was measured at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT) using the PLAC™ Test (Diadexus, San Francisco, CA): analytical coefficient of variation was 6.3%. Triglycerides were measured by enzymatic methods on an Olympus Demand System (Olympus Corp., Lake Success, NY).

1.2. Variable definitions and categories

Baseline HDL-C, CRP, IL-6, and Lp-PLA_2 values were categorized into gender-specific tertiles. An inflammatory index was calculated based on the conversion of each individual's corresponding three inflammation values into *z*-score values using gender specific values. The sums of each individual's three inflammatory *z*-scores were then categorized into tertile sub-groups. Additionally, baseline HDL-C values were categorized into ≥60 mg/dl (high), 40–59 mg/dl (intermediate), and <40 mg/dl (low) sub-groups based on guidelines from the National Cholesterol Education Program Adult Treatment Panel III [14]. Similarly, baseline CRP values were categorized into <1 mg/L (low CRP), 1–3 mg/L (intermediate CRP), and >3 mg/L (high CRP) categories based on previous recommendations for healthcare professionals [15].

Diabetes mellitus was defined as having a fasting glucose level ≥6.99 mmol/L (126 mg/dl), taking oral hypoglycemic medication, or self-reported use of insulin. Baseline CVD was defined as a history of angina, stroke, MI, congestive heart failure, coronary artery angioplasty, coronary artery bypass grafting (CABG), or peripheral vascular disease (PVD) as determined from the participant's self-report and/or medical records. Subsequent CVD events (angina, MI, claudication/PVD, and congestive heart failure), as well as the cause of death, were adjudicated by the CHS Cardiac Events Committee, using standardized criteria. The primary outcome of our study's analysis is incident CHD defined as angina, myocardial infarction, coronary artery angioplasty, coronary bypass surgery, or coronary heart disease death; we also conducted secondary analyses involving incident CVD as defined above. For those without an event, we defined patient's time-to-survival as time-to-death (from non-CVD causes), time-to-last follow up, or time-to-the end of the study.

1.3. Statistical analysis

For patients with and without incident CHD, we compared baseline laboratory values and past cardiovascular history using the chi-square test for categorical variables and Student's *t*-test for continuous variables. Incident CHD event rates were calculated according to tertile HDL-C categories paired with respective CRP, IL-6, Lp-PLA_2, and inflammatory index tertile categories per 1000 person years. Cox proportional hazards regression was used to examine the hazard ratios (HRs) and corresponding 95% confidence intervals for CHD events using the highest HDL-C/lowest inflammation group as a reference. Models were adjusted for age, sex, African American race, recruitment site, family history of 'heart attack', low-density lipoprotein cholesterol (LDL-C), hypertension medication, systolic and diastolic blood pressure, lipid-lowering medication, current or past smoking status, waist size, diabetes, and triglycerides. We further evaluated unadjusted and adjusted multiplicative interaction terms between HDL-C groups and each inflammatory factor group for CHD incidence. Additionally, we used previously described guideline cut-offs for HDL-C and CRP to calculate adjusted hazard ratio values.

Separately, we normalized values of HDL, CRP, IL-6, and Lp-PLA_2 using log scale. Adjusted Cox regression analysis calculated hazard ratios utilizing standardized continuous measures of HDL and each respective inflammation marker or the created inflammatory index for CHD incidence. We further evaluated interaction terms between HDL-C and each inflammatory factor for CHD incidence. Finally, using the same HDL-C/inflammation groups, we evaluated adjusted models using the broader outcome of incident total CVD events. SAS statistical software (version 9.1.3; SAS, Cary, NC) was used for all statistical analyses [16].

2. Results

2.1. Study population

We identified 4426 adults with no baseline CVD among the 5888 CHS cohort participants. An additional 538 individuals were excluded due to incomplete baseline data, leaving 3888 individuals with complete data for our analysis. Those developing incident CHD had lower HDL-C and higher LDL-C, diabetes prevalence, systolic blood pressure, waist size, triglycerides, and inflammatory biomarkers as compared to the non-CHD incident group at baseline ($p < 0.05$ for LDL-C, $p < 0.01$ for hs-CRP and Lp-PLA_2, and $p < 0.001$ for all others) (Table 1). Of the 3888 included individuals, 27.1% had a family history of heart attack or stroke, 52.2% were either a past or current smoker, and 13.1% had diabetes. Females had higher HDL-C levels and lower inflammatory factors of IL-6 and Lp-PLA_2 compared to males (all $p < 0.001$, data not shown). For those with incident CHD, median (interquartile range) values of CRP, IL-6 and Lp-PLA_2 were 1.96 mg/L (1.00–3.42), 1.68 pg/ml (1.24–2.55), and 332.23 ng/ml (265.99–411.66) respectively. For those without