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Original Article

Association between high-density lipoprotein subfractions and low-grade inflammation, insulin resistance, and metabolic syndrome components: The ELSA-Brasil study

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KEYWORDS:
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Metabolic syndrome

BACKGROUND: High-density lipoprotein cholesterol (HDL-C) can be divided into subfractions, which may have variable effects in atherogenesis. The results about the association between HDL-C subfractions and risk factors for cardiovascular disease are mixed.

OBJECTIVE: The objective of this study was to analyze the association between HDL-C subfractions and each metabolic syndrome component, homeostasis model assessment-estimated insulin resistance (HOMA-IR) and C-reactive protein (CRP).

METHODS: Four thousand five hundred thirty-two individuals between 35 and 74 years old without previous manifest cardiovascular disease not using fibrates were enrolled. HDL-C subfractions were separated by vertical ultracentrifugation (vertical auto profile—in mg/dL) into HDL_2-C and HDL_3-C. HDL_2-C/HDL_3-C ratio, HOMA-IR, and high-sensitivity CRP were also included in the analysis.

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RESULTS: Mean age of participants was 51 ± 9 years, and 54.8% were women. In univariate analysis, HDL-C, HDL_2-C, and HDL_3-C were all inversely associated with each of the metabolic syndrome defining factors, HOMA-IR values, and serum CRP. We also observed a negative association between HDL_2-C/HDL_3-C ratio with the variables aforementioned even after adjusting for smoking, alcohol use, physical activity, and HDL-C levels ($P < .01$).

CONCLUSION: HDL-C and its subfractions (HDL_2-C and HDL_3-C) are inversely associated with the defining features of metabolic syndrome, insulin resistance, and systemic inflammation. In addition, the HDL_2-C/HDL_3-C ratio measured by vertical auto profile is significantly associated with the former factors even after comprehensive adjustment for HDL-C and other confounding variables.

Introduction

High-density lipoproteins (HDLs) comprise a group of heterogeneous subfractions, which vary in size, density, shape, lipidome, and proteome.[1] Epidemiological studies have shown an independent inverse association between HDL cholesterol (HDL-C) concentrations and the risk of atherosclerotic cardiovascular disease (ASCVD).[2] However, the independent protective role of the HDL particles has been disputed by Mendelian randomization studies as well as the lack of clinical efficacy of pharmacologic interventions designed to raise HDL-C.[3–8]

Low HDL-C concentrations are a component of atherogenic dyslipidemia that is associated with visceral obesity, insulin resistance, and heightened inflammation. These findings define the metabolic syndrome (MetS), a constellation of risk factors associated with an increased risk of type II diabetes, ASCVD, and death.[9–11] Nevertheless, the role of HDL in atherogenesis is not entirely clear, though it might protect against atherosclerosis by antagonizing oxidation, thrombosis, and inflammation, and potentiating reverse cholesterol transport.[12,13]

Because HDL comprises a heterogeneous group of particles, some of these effects may be related to specific subfractions. One method to analyze these subfractions is to measure their cholesterol by separating the HDL-C into 2 groups: HDL_2-C (carried in larger and less dense particles) and HDL_3-C (carried in smaller and denser particles).[14] There is substantial controversy surrounding the capacity of different HDL subfractions to protect against atherogenesis, with the evidence being decidedly mixed: HDL_3-C was positively [15] and inversely[16] associated with cardiovascular outcomes, as well as HDL_2-C, established as a predictor of ASCVD reduction in some analyses,[17,18] while this finding did not appear in other studies.[15,16] No clear, consistent picture has emerged. Moreover, the exact interplay between such subfractions and parameters of the MetS has not been adequately investigated.

Thus, the objective of the present study was to evaluate the association of HDL-C and its subfractions (HDL_2-C and HDL_3-C) with the defining features of MetS as well as serum markers of inflammation and insulin resistance.

Methods

Sample

Between August 2008 and December 2010, 15,015 men and women were enrolled in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), a prospective longitudinal cohort composed of civil servants aged 35 to 74 years from 6 Brazilian cities, which has previously been described in detail.[19,20] We included those who underwent HDL-C measurement by a conventional method and by the vertical auto profile (VAP) method. All participants were from the São Paulo center. Exclusion criteria for the present analysis were lack of serum measurement of any component of the lipid profile, C-reactive protein (CRP), and insulin; individuals with prior cardiovascular disease (myocardial infarction, stroke, heart failure, and coronary revascularization); and participants using fibrates. We did not exclude persons receiving statin therapy because there was a subgroup analysis excluding this population (Appendix 1), and it showed similar results.

HDL-C and subfractions analysis

Blood collection was obtained from participants after nocturnal fasting. The samples were centrifuged at the sites and stored in tubes at −80°C. Conventional HDL-C concentrations were determined by a nonprecipitated colorimetric method using ADVIA 1200 Siemens equipment.[21] HDL-C and the HDL_2-C and HDL_3-C subfractions were measured by the VAP method (Atherotech), an inverted rate zonal, single vertical spin, density gradient ultracentrifugation technique that simultaneously measures cholesterol concentrations after fraction separation.[14] After separation, cholesterol in subfractions was measured by enzymatic methods. Because of the strong correlation between methods ($P = .95$), we considered HDL-C for those values measured by the VAP rather than conventional testing.

CRP levels

Blood was collected from overnight fasting, and CRP was measured using a high-sensitivity assay by immunochemistry–nephelometry (BN II; Siemens).