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Different inverse association of large high-density lipoprotein subclasses with exacerbation of insulin resistance and incidence of type 2 diabetes: The Nagahama study

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ABSTRACT

Aims: In addition to its antiatherogenic action, high-density lipoprotein (HDL) may also have an antidiabetes function. Although the biological actions of small HDL (HDL3) and large HDL (HDL2) subclasses may be different, evidence in support of that hypothesis is lacking. The aim of this study was to clarify the difference in prognostic significance of HDL subclasses for exacerbation of insulin resistance and incidence of type 2 diabetes in the general population.

Methods: Study participants included 8365 community residents 52 ± 13 years of age not taking lipid lowering drugs. Serum HDL cholesterol subclasses and low-density lipoprotein subclasses, were measured by a homogeneous assay. Insulin resistance was assessed by homeostasis model assessment of insulin resistance (HOMA-IR).

Results: Cross-sectional analysis adjusted for possible covariates found that HDL2 cholesterol (HDL2-C) levels were inversely associated with HOMA-IR ($\beta = -0.169$, $p < 0.001$), whereas HDL3-C had the opposite association ($\beta = 0.054$, $p < 0.001$). Similar results were found in an analysis for type 2 diabetes (HDL2-C, odds ratio = 0.96, $p = 0.001$; HDL3-C, odds ratio = 1.04, $p = 0.181$). In a longitudinal analysis with 5.0 years of follow-up, HDL2-C was inversely associated with exacerbation of insulin resistance ($\beta = -0.163$, $p < 0.001$); HDL3-C had the opposite association ($\beta = 0.026$, $p = 0.037$). During follow-up, 205 individuals were newly diagnosed with diabetes, and HDL2-C level was associated with an inverse risk of type 2 diabetes incidence (odds ratio = 0.98, $p = 0.006$).

Conclusions: HDL may have an antidiabetic function; the prognostic value of HDL2-C for diabetes and insulin resistance might be better than that of HDL3-C.

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1. Introduction

High-density lipoprotein (HDL) protects against atherogenesis, and evidence of an association of HDL with reduced risk of diabetes is increasing [1]. A longitudinal cohort analysis in the Framingham Offspring Study [2] found that low HDL cholesterol (HDL-C) was an independent risk factor for 7-year incidence of type 2 diabetes (T2DM). Other studies have reported similar results in the general population, regardless of ethnic differences [3–5], as well as in T2DM patients [6]. The reported predictive value of HDL in those studies was not high, however. Differences in the biological activity of large (HDL2) and small (HDL3) HDL subclasses, may partly account for inconsistency in the strength of the association of HDL with development of T2DM. One small study [7] reported a stronger inverse association with incidence of T2DM for HDL2 than for HDL3. Not only circulating HDL subclasses levels but also HDL particle size was reported to be associated with development of diabetes [8] Another cross-sectional study reported a better association of HDL2-to-HDL3 cholesterol ratio than total HDL-C with insulin resistance [9], warranting further study of HDL class-specificity. Our aim was to clarify the prognostic significance of HDL subclasses for progression of insulin resistance and incidence of T2DM in the general population.

2. Subjects, materials and methods

2.1. Study participants

We analyzed a dataset describing participants in the Nagahama Prospective Cohort for Comprehensive Human Bioscience (the Nagahama Study). Participants in this community-based prospective cohort study were recruited between 2008 and 2010 from the general population of Nagahama City, a rural city of 125,000 inhabitants located in central Japan. Community residents from 30 to 74 years of age, living independently and without physical impairment or dysfunction were eligible. Of the 9804 included participants, nine withdrew consent to participate, and 26 were excluded because genetic analysis showed that they had another ethnic background. Following baseline evaluation, 43 pregnant women, 25 participants on insulin therapy, 191 participants with incomplete data or wide deviation of clinical values required for participation, and 1145 participants taking lipid lowering medications were excluded (Supplementary Table 1). The remaining 8365 participants comprised the study population.

2.2. Follow-up measurements

Participants in the Nagahama cohort were invited to a follow-up assessment 5-years after the baseline evaluation, and 8294 of the original 9769 cohort members participated. After excluding 137 individuals who died and 279 who had moved away from Nagahama City, the follow-up rate was 88.7%. After applying same exclusion criteria as at baseline, 7015 participants remained were included in a longitudinal analysis. Mean follow-up duration was 1814 ± 134 days (5-year interval, $n = 6745$; 6-year interval $n = 256$; and 7-year interval $n = 14$).

2.3. Ethical considerations

All study procedures were approved by the ethics committee of Kyoto University Graduate School of Medicine and by the Nagahama Municipal Review Board. Written informed consent was obtained from all participants.

2.4. Participant characteristics

Clinical and participant data were obtained at the baseline and follow-up evaluations. Triglycerides (Determiner C-TG, Kyowa Medex, Co., Ltd., Tokyo, Japan) and total cholesterol (Determiner C-TC) were assayed in serum samples. Participants with T2DM included those with one or more of the following: use of antihyperglycemic drugs; hemoglobin A1c (HbA1c) $\geq$ 6.5%; fasting ($\geq$4 h after last meal) glucose $\geq$ 126 mg/dl; or occasional glucose $\geq$ 200 mg/dl. Homeostasis model assessment of insulin resistance (HOMA-IR) calculated as (glucose $\times$ insulin)/405 was used as the index of insulin resistance. History of cardiovascular diseases, menopausal status, smoking and drinking habits, and medication use were obtained with a structured, self-administered questionnaire.

2.5. Measurement of lipoprotein subclasses

Lipoprotein subclasses were assayed in a plasma samples stored at −80 °C. To directly measure HDL3-C, triglyceride (TG)-rich lipoproteins and low-density lipoprotein (LDL) were decomposed by sphingomyelinase, and cholesterol released from these lipoproteins were eliminated by cholesterol esterase/oxidase and catalase. HDL3 cholesterol was assayed by a standard peroxidase method after enzymatic treatment with a polyoxyethylene styrenated phenyl ether derivative that specifically reacts with HDL3 (HDL3-EX, Denka Seiken Tokyo, Japan) [10]. HDL2-C levels were then calculated by subtracting HDL3-C from total HDL-C measured using a commercially available assay kit (HDL-EX, Denka Seiken). A strong collinearity between HDL subclasses measured by this method and those measured by an objective standard method using ultracentrifugation has been reported elsewhere [10].

Small-dense LDL cholesterol (sdLDL-C) was assayed using a standard cholesterol assay after enzymatic treatment with polyoxyethylene benzylphenyl ether derivative to eliminate TG-rich lipoproteins and HDL, and with sphingomyelinase, which specifically reacts with large buoyant LDL (lbLDL) (sdLDL-EX, Denka Seiken) [11]. Total LDL cholesterol was measured using a commercially available assay kit (LDL-EX (N), Denka Seiken). lbLDL-C levels were calculated by subtracting sdLDL-C from total LDL-C. The accuracy of this LDL subclass assay method has been reported elsewhere [11].

2.6. Genome-wide association study (GWAS)

Genome-wide single nucleotide polymorphism (SNP) analysis was carried out in a subset of the Nagahama cohort sample using DNA extracted from peripheral blood samples by the