Role of HDL Cholesterol and Estimates of HDL Particle Composition in Future Development of Type 2 Diabetes in the General Population: The PREVEND Study


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Background and Aims: High-density lipoproteins (HDLs) may directly stimulate β-cell function and glucose metabolism. We determined the relationships of fasting high-density lipoprotein cholesterol (HDL-C), plasma apolipoprotein (apo) A-I and apoA-II, and HDL-C–to–apoA-I and HDL-C–to–apoA-II ratios, as estimates of HDL particle composition, with incident type 2 diabetes mellitus.

Methods: A prospective study was carried out in the Prevention of Renal and Vascular End-Stage Disease (PREVEND) cohort after exclusion of subjects with diabetes at baseline (n = 6820; age, 28–75 years). The association of HDL-related variables with incident type 2 diabetes was determined by multivariate logistic regression analyses.

Results: After a median follow-up of 7.7 years, 394 incident cases of type 2 diabetes mellitus were ascertained (5.8%). After adjustment for age, sex, family history of diabetes, body mass index, hypertension, alcohol, and smoking, odd ratios (ORs) for diabetes were 0.55 (95% confidence interval [CI], 0.47–0.64; P < .001), 0.81 (0.71–0.93; P = .002), 0.02 (0.01–0.06; P < .001), and 0.03 (0.01–0.06; P < .001) per 1-SD increase in HDL-C and apoA-I and in the HDL-C–to–apoA-I and the HDL-C–to–apoA-II ratios, respectively. In contrast, apoA-II was not related to incident diabetes (OR = 1.02; 95% CI, 0.90–1.16; P = .71). The relationships of HDL-C and the ratios of HDL-C to apoA-I and HDL-C to apoA-II remained significant after further adjustment for baseline glucose and triglycerides (OR_{HDL} = 0.74 [95% CI, 0.61–0.88], OR_{HDL/APO A-I} = 0.14 [0.04–0.44], and OR_{HDL/APOA-II} = 0.12 [0.04–0.36]; all P ≤ .001).

The inverse relationship of HDL-C with diabetes development is not surprising in the context of coexisting obesity and disturbances in lipoprotein metabolism in subjects at high risk for diabetes (15–17). Notably, evidence has accumulated recently that HDL may also be directly involved in the pathogenesis of type 2 diabetes mellitus by virtue of its ability to enhance pancreatic β-cell function and glucose uptake in skeletal muscle (18–20). Furthermore, defects in the functional properties of HDL have been shown to result in increased susceptibility of pancreatic β-cells to oxidative stress, apoptosis, islet inflammation, and cholesterol accumulation (20). HDL is able to restore oxidized low-density lipoprotein–induced impairment of insulin processing in vitro, whereas free apolipoprotein (apo) A-I and also reconstituted HDL particles and native HDL of which apo A-I is the most abundant apolipoprotein constituent, have been shown to stimulate insulin secretion by increasing cholesterol efflux out of β-cells (21–23). In agreement with a contributory role of HDL functionality on the maintenance of insulin secretion, both the antioxidative capacity of HDL and the ability of plasma to stimulate cholesterol efflux from cultured fibroblasts have been found to be independent determinants of β-cell function in well-controlled type 2 diabetes mellitus (24). Of further relevance, apoA-I stimulates the AMP-activated protein kinase pathway in myocytes in vitro (25). Reconstituted HDL infusion also stimulates this pathway in skeletal muscle from subjects with type 2 diabetes in vivo (21). HDL could, therefore, lower plasma glucose not only by stimulation of insulin secretion but also by stimulation of glucose uptake via an insulin-independent mechanism (18, 21).

Despite the current focus on the allegedly beneficial effects of HDL on glucose homeostasis, it is still not known whether the major apolipoproteins of HDL, apoA-I and apoA-II, are independently related to incident type 2 diabetes mellitus in the general population. The same is true for HDL particle characteristics. Importantly, apoA-I and apoA-II exert specific effects on HDL functional properties (16, 26), are protein constituents of distinct HDL subfractions, i.e., LpA-I and LpA-I: A-II particles (27, 28), and may have dissimilar potentials in identifying early processes in the development of cardiovascular disease (29, 30) and possibly also in development of type 2 diabetes. For these reasons, it is clinically relevant to discern whether diabetes development is dependent not only on HDL-C but also on plasma levels of apoA-I and apoA-II.

The present study was initiated to determine the strength of associations of incident type 2 diabetes mellitus with HDL-C, plasma apoA-I and apoA-II, and HDL particle composition, as estimated by the ratios of HDL-C to apoA-I and HDL-C to apoA-II. To this end, we performed a prospective study in the population-based Prevention of Renal and Cardiovascular End-Stage Disease (PREVEND) cohort.

Materials and Methods

Study population and design

The PREVEND study was approved by the local medical ethics committee, University Medical Center Groningen, and was performed according to the principles outlined in the Declaration of Helsinki. All participants gave written informed consent. Details on study design, recruitment, and procedures have been reported elsewhere (31).

The study population is based on the PREVEND study, a Dutch cohort drawn from the general population (age ranged between 28 and 75 years) of the city of Groningen, The Netherlands. After exclusion of patients with insulin-treated diabetes mellitus and pregnant women, all subjects with a urinary albumin concentration (UAC) of ≥ 10 mg/L (n = 7768) were invited to participate, of whom 6000 accepted. In addition, 3394 randomly selected subjects with a UAC of < 10 mg/L were invited to participate, of whom 2592 accepted. These 8592 subjects participated in the baseline screening and constitute the actual PREVEND cohort. From this baseline cohort, we first excluded 336 individuals with prevalent cases of diabetes. These patients were defined by either a self-report of physician diagnosis or screening at first visits (1996–1997). Other exclusions were for 285 subjects with no follow-up data or who could not be linked to the pharmacy registry and 807 individuals with nonfasting blood sampling or those using lipid-lowering agents (n = 344), leaving 6820 participants who were free of baseline diabetes for our cohort analysis.

Clinical and laboratory measurements

During 3 rounds of screening from 1997 to 1998 (baseline examination) until January 1, 2007 (third examination), the participants underwent 2 outpatient visits to assess medical history, anthropometry, and cardiovascular and metabolic risk factors, and they had to collect 2 24-hour urine samples. We collected information on use of medications via data from pharmacy registries of all community pharmacies in the city of Groningen (32). Hypertension was defined by self-reported physician diagnosis, use of antihypertensive medication, or blood pressure ≥ 140/90 mm Hg. Total cholesterol and plasma glucose were measured by a dry chemistry method (Eastman Kodak, Rochester, New York). HDL-C was measured by a homogeneous method (direct HDL, Aeroset System; Abbott Laboratories, Abbott Park, Illinois). Serum apoA-I and apoA-II were determined by nephelometry, applying commercially available reagents (apoA-I test kit, code no. OUED and apoA-II test kit, code no. OQBA) for Dade Behring nephelometer II systems (Dade Behring, Marburg, Germany). Fasting insulin was measured with an AxSYM autoanalyzer (Abbott Diagnostics, Amstelveen, The Netherlands). Insulin resistance was assessed based on the homeostasis model assessment for insulin resistance (HOMA-IR), which is calculated by the following formula: [glucose (millimoles per liter) × insulin (milliunits per liter)]/22.5 (33). Triglycerides were measured enzymatically. UAC was determined by nephelometry with a threshold of 2.3 mg/L and intra- and interassay coeffi-
cents of variation of less than 2.2% and less than 2.6%, respectively (Dade Behring). Urinary albumin excretion (UAE) is given as the mean of the 24-hour urine collections. All the technicians were blinded to the participants’ characteristics.

Outcome definition
Incident cases of type 2 diabetes mellitus were ascertained as described previously (4, 34). In brief, type 2 diabetes was ascertained if one or more of the following criteria were met: (1) fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL); (2) random sample plasma glucose ≥11.1 mmol/L (200 mg/dL); (3) self-report of a physician diagnosis; and (4) initiation of glucose-lowering medication use retrieved from a central pharmacy registry (33, 34). We included cases from 3 months after the baseline screening visits.

Statistical analysis
Continuous data were compared by using Student t tests or Mann-Whitney U tests, where applicable. We used χ² tests for the comparison of categorical variables between individuals with and without incident type 2 diabetes. Logistic regression analysis was used to examine the associations of HDL variables, i.e., HDL-C, apoA-I, apoA-II, and HDL–to–apoA-I and HDL–to–apoA-II ratios with the risk of developing type 2 diabetes. Odds ratios (ORs) for type 2 diabetes were calculated per SD change for each HDL variable with 95% confidence intervals (CIs). In model 1, basic adjustment was for age and sex. In model 2, we further adjusted for body mass index (BMI) or waist circumference. In model 3, we additionally adjusted for family history of diabetes, hypertension, alcohol use, and smoking. In models 4 and 5, we further adjusted for fasting glucose and triglycerides plus fasting glucose, respectively. In addition, we also performed analysis in which we adjusted for HOMA-IR as covariate. In model A, we adjusted for those variables in model 3 and for HOMA-IR. In model B, we further adjusted for triglycerides. For HOMA-IR and triglycerides, logarithmic transformation with base 2 (log2) was used.

Given the expected strong negative relationship of HDL-C with triglycerides, a potential confounder in the associations of interest, we also calculated ORs (95% CI) of HDL variables across each tertile of triglycerides. To this end, the associations of diabetes incidence with HDL-C, the HDL-C–to–apoA-I ratio, and the HDL-C–to–apoA-II ratio were ascertained with the lowest HDL variable being used as the reference category in each triglyceride tertile. Subsequently, we calculated interaction terms for HDL-C × sex in each model and performed sex-specific analysis. In view of the enrichment of the PREVEND participants with microalbuminuric subjects (31), we accounted for 24-hour UAE at baseline as another potential confounding factor in the secondary analysis. Next, we repeated regression models by using a weighted method to compensate for baseline enrichment of the PREVEND participants with high UAC (i.e., 10 mg/L or greater). Weight change might confound the associations of HDL-C, the HDL-C–to–apoA-I ratio, and the HDL-C–to–apoA-II ratio with risk of diabetes. Therefore, we calculated absolute and percentage weight change in 4757 participants who underwent follow-up screening at the third examination. As a secondary analysis, we added absolute weight change to a multivariable model including age, sex, family history of diabetes, hypertension, glucose, triglycerides, and HDL-C or the ratios. As another secondary analysis, we added percentage change weight, calculated as (weightexamination-third − weightexamination-first)/(weightexamination-first) ×100 to this multivariate model. For most baseline variables, <1% was missing, whereas this was up to 8% for self-reported variables such as family history of diabetes mellitus. A single imputation and predictive mean matching method was applied for missing data. Two-sided P values <.05 were considered statistically significant. All the statistical analyses were performed using IBM SPSS Statistics 19 and R (version 2.13.1 for Windows; http://cran.r-project.org/).

Results
During median (interquartile range [IQR]) follow-up for 7.7 (7.4–8.0) years, 394 individuals (5.8%) developed new-onset type 2 diabetes mellitus. Baseline clinical and laboratory characteristics of the total cohort and a comparison of individuals who developed new-onset type 2 diabetes vs individuals who remained free of diabetes are shown in Table 1. Individuals with incident type 2 diabetes were older, more likely to be male, more obese, more likely to have a family history of diabetes, and more likely to have hypertension than those who did not develop diabetes (P < .001). Levels of fasting glucose, insulin, HOMA-IR, total cholesterol, triglycerides, and 24-hour UAE were significantly higher in individuals who developed new-onset type 2 diabetes than those without incident diabetes. Concentrations of HDL-C, apoA-I, and apoA-II and the ratios of HDL-C to apoA-I and HDL-C to apoA-II were significantly lower in individuals who developed new-onset type 2 diabetes mellitus than in subjects who did not develop diabetes (P < .001 for all).

HDL variables and risk of type 2 diabetes
ORs (95% CI) for incident type 2 diabetes per 1-SD increase in HDL-C, apoA-I, and apoA-II and the ratios of HDL-C to apoA-I and HDL-C to apoA-II are shown in Table 2. In age- and sex-adjusted analysis, all HDL-related variables were significantly associated with risk of incident type 2 diabetes (P < .001), except for apoA-II (P = .77) (model 1). Further adjustment for BMI, family history of diabetes, hypertension, alcohol use, and smoking did not materially change these associations (models 2 and 3). After further adjustment for baseline fasting glucose and triglycerides, HDL-C, as well as the HDL-C–to–apoA-I and the HDL-C–to–apoA-II ratios, remained independently associated with risk of incident diabetes (models 4 and 5). In the fully adjusted models, the strongest effect size was observed for the HDL-C to apoA-I and HDL-C to apoA-II ratios (ORs of 0.14 and 0.12, respectively; P < .001 for each). Furthermore, the directions and the strengths of the relationships were similar in analyses in which we adjusted for waist circumference instead of BMI (see Supplemental Table 1 published on The Endocrine Society’s Journals Online
shown in Table 3. In multivariable adjusted analysis incorporated in model 4 instead of plasma glucose are ratio, and HDL-C–to–apoA-II ratio with HOMA-IR increase in HDL-C, apoA-I, apoA-II, HDL-C–to–apoA-I smoking. Model 4: model 3

strongly associated with risk of incident type 2 diabetes
after multivariable adjustment for 24-hour UAE, fasting

glucose, and triglycerides plus other clinical diabetes risk
factors (Supplemental Table 2). To account for baseline

enrichment of the PREVEND cohort with microalbumin-
uric subjects, we subsequently repeated the analysis in
model 5 when we weighted for individuals with mean
UAC >10 mg/L. This complex design analysis hardly af-
fected the ORs in model 5 (Supplemental Table 3). In ad-
dition, the associations of HDL-C and the ratios of
HDL-C to apolipoproteins with the risk of incident type 2
diabetes were not modified by sex (P > .10 for interac-
tions). In sex-stratified analyses, the multivariable-ad-
justed ORs (adjusted for baseline fasting glucose, triglyc-

erides and other clinical risk factors) per 1-SD increase of
HDL-C were 0.70 (0.55–0.91, P = .007) and 0.74 (0.57–
0.96, P = .025) in men and women, respectively. In sub-
sequent secondary analyses limited to 4757 participants
who underwent follow-up screening at examination 3,
median (IQR) weight change and percentage weight
change were 2.0 (1.0 to 5.0) kg and 2.3% (1.6% to
6.8%), respectively. Addition of absolute weight change
to model 5 (Table 2) did not materially affect the associ-
ations of HDL-C and the ratios of HDL-C to apo A-I and
apo A-II with the risk of incident type 2 diabetes; the re-
pective ORs were 0.68 (0.54–0.85, P = .001), 0.09
(0.02–0.39, P < .001), and 0.05 (0.01–0.22, P < .001).
The same was true for addition of percentage weight
change to model 5, resulting in respective ORs of 0.68
(0.54–0.85), 0.09 (0.02–0.39), and 0.05 (0.01–0.22).

Figure 1. Risk of developing type 2 diabetes mellitus according to
tertiles of HDL-C (A), the HDL-C–to–apoA-I ratio (B) and the HDL-C–
to–apoA-II ratio (panel C) stratified by triglyceride tertiles. The ORs
(95% CI) were calculated by logistic regression models adjusted for
age and sex. The individuals in the first HDL tertile were considered as
the reference category (P > .10 for interactions).

Discussion

This prospective study in a predominantly Caucasian pop-
ulation shows that the age- and sex-adjusted relationship
of incident type 2 diabetes mellitus with HDL-C is at least
in part independent of other metabolic syndrome compo-
nents, including (central) obesity, hypertension, fasting
plasma glucose, and triglycerides, as well as of a positive
family history of diabetes. Higher HDL-C levels were also
related to a lower risk of diabetes after further adjustment
for alcohol consumption and smoking, important envi-
ronmental factors that govern HDL-C. More strikingly,
incident diabetes was related to plasma apoA-I levels as
well, in marked contrast with the lack of any independent
relationship of diabetes development with plasma apoA-
II. As judged from the respective ORs, the relationship of
incident diabetes with apoA-I was weaker than that with
HDL-C. As a consequence, we observed robust inverse
relationships of incident diabetes with the HDL-C–to–apoA-
I and the HDL-C–to–apoA-II ratio. Of note, the
particularly low ORs are partly a consequence of the fact
that small changes in ratios can reflect relatively large
changes in HDL-C or apolipoprotein differences. Taken

together, the present data are in keeping with the hypoth-

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Table 3. Relationships of HDL Variables With the Risk of Developing Type 2 Diabetes Mellitus After Adjustment for HOMA-IR and Other Clinical Factors

<table>
<thead>
<tr>
<th>HDL Variable</th>
<th>OR (95% CI) per 1-SD Increase</th>
<th>Model A</th>
<th>P Value</th>
<th>Model B</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C</td>
<td>0.66 (0.56–0.77)</td>
<td>&lt;.001</td>
<td>0.76 (0.64–0.89)</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>apoaA-I</td>
<td>0.88 (0.77–1.01)</td>
<td>.08</td>
<td>0.93 (0.82–1.07)</td>
<td>.33</td>
<td></td>
</tr>
<tr>
<td>apoaA-II</td>
<td>1.02 (0.90–1.16)</td>
<td>.71</td>
<td>1.01 (0.88–1.14)</td>
<td>.91</td>
<td></td>
</tr>
<tr>
<td>HDL-C–to–apoA-I ratio</td>
<td>0.07 (0.02–0.19)</td>
<td>&lt;.001</td>
<td>0.16 (0.05–0.51)</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>HDL-C–to–apoA-II ratio</td>
<td>0.07 (0.03–0.18)</td>
<td>&lt;.001</td>
<td>0.14 (0.05–0.41)</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

Model A: adjusted for age, sex, BMI, smoking, alcohol use, family history of diabetes, hypertension, and HOMA-IR. Model B: model A + triglycerides.
ment with HDL. Additional adjustment for 24-hour UAE did not materially change the ORs of any HDL-related variables for incident diabetes. Furthermore, a secondary analysis weighted for the enrichment of the PREVEND cohort with higher UACRs (31) revealed comparable diabetes risk estimates for the HDL-C–to–apoA-I and the HDL-C–to–apoA-II ratio. Hence, a clinically important bias in the interpretation of the current results attributable to overrepresentation of microalbuminuric subjects consequent to the focus of PREVEND on renal disease is unlikely but cannot be excluded. Although early use of metformin is currently recommended for prevention of diabetes in individuals with prediabetes, this approach was not recommended by national guidelines within the time frame of our cohort study. Therefore, overdiagnosis of diabetes due to early metformin use is unlikely. In the PREVEND study, hemoglobin A1c was not measured, and oral glucose tolerance tests were not performed. If elevated levels of hemoglobin A1c or results of oral glucose tolerance tests would serve as diagnostic criteria in addition to elevated fasting glucose (41), the absence of these tests in our study would result in false-negative cases of diabetes, with an underestimation of its incidence. The presence of such false-negative cases could mask an otherwise present association if the underlying association is marginal. If, however, associations are demonstrated, as in our study, the possibility of false-negative cases implies that the association could be stronger than actually observed. Moreover, we only investigated HDL-C, apoA-I, and apoA-II as estimates of the HDL particle composition, and we did not directly measure HDL size. Finally, we had no data on physical activity that might have influenced the extent to which HDL-related parameters associate with diabetes development.

In conclusion, our study demonstrates for the first time that new-onset type 2 diabetes mellitus is related not only to lower HDL-C but also is strongly related to lower HDL-C–to–apoA-I and HDL-C–to–apoA-II ratios, as estimates of HDL particle characteristics. We hypothesize that specific HDL particles may independently affect pathophysiological pathways involved in diabetes development.

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A.A. performed statistical analysis. A.A. and R.P.F.D. researched data and wrote the manuscript. All authors contributed to the discussion and reviewed and edited the manuscript. A.A., S.J.L.B., and R.P.F.D. are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure Summary: The authors have nothing to disclose.

References


