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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 (0.01–0.060; $P < .001$) 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; $P=0.71$). 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], $OR_{HDL/APO\ A\text{-}I} = 0.14$ [0.04–0.44], and $OR_{HDL/APOA\text{-}II} = 0.12$ [0.04–0.36]; all $P \leq .001$).

Conclusions: Higher HDL-C, as well as higher HDL-C–to–apoA-I and HDL-C–to–apoA-II ratios are strongly and independently related to a lower risk of future type 2 diabetes. **(*J Clin Endocrinol Metab* 98: E1352–E1359, 2013)**

The epidemic of type 2 diabetes mellitus poses a major public health concern worldwide (1, 2). Much attention is currently being paid to the development and validation of diabetes prediction models to optimize guidelines for diabetes prevention (3–5). Many of the proposed diabetes risk scores take account of metabolic syndrome components, including high-density lipoprotein (HDL) cholesterol (HDL-C) (4, 5). Indeed, lower levels of HDL-C confer a higher incidence of type 2 diabetes in various ethnic populations and age groups (6–12), analogous to the cardiovascular protection attributed to HDL (13, 14). In search of better predictors, mechanisms that play a role in the early pathophysiology of type 2 diabetes, independent of established risk factors, are of interest.

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Abbreviations: apo, apolipoprotein; CI, confidence interval; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; IQR, interquartile range; OR, odds ratio; PREVEND Prevention of Renal and Vascular Endstage Disease; UAC, urinary albumin concentration; UAE, urinary albumin excretion.

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 enhancing 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, ie, 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-

cients 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 2 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 χ^2 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, ie, HDL-C, apoA-I, apoA-II, and HDL-C–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 (ie, 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 weight change, calculated as $(\text{weight}_{\text{examination-third}} - \text{weight}_{\text{examination-first}})/(\text{weight}_{\text{examination-first}}) \times 100$ 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 vs 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

Table 1. Baseline Clinical and Laboratory Characteristics of Participants

	Total	Noncases	Incident Cases of Type 2 Diabetes	*P* Value
No. of participants (%)	6820 (100)	6426 (94.2)	394 (5.8)	
Male, n (%)	3247 (47.6)	3021 (47.0)	226 (57.4)	<.001
Age, y	48.8 ± 12.5	48.3 ± 12.4	56.3 ± 10.8	<.001
Family history of diabetes, n (%)	1339 (19.6)	1196 (18.6)	143 (36.3)	<.001
Smoking, n (%)				
Current	2296 (33.7)	2162 (33.6)	134 (34.0)	
Former	2463 (36.1)	2308 (35.9)	155 (39.3)	.22
Never	2061 (30.2)	1956 (30.4)	105 (26.6)	
Alcohol use, n (%)				
≥4 drinks per day	347 (5.1)	326 (5.1)	21 (5.3)	
1–3 drinks per day	1334 (19.6)	1259 (19.6)	75 (19.0)	
2–7 drinks per week	2315 (33.9)	2205 (34.3)	110 (27.9)	.07
1–4 drinks per month	1105 (16.2)	1034 (16.1)	71 (18.0)	
Almost never	1719 (25.2)	1602 (24.9)	117 (29.7)	
Systolic blood pressure, mm Hg	123.7 ± 19.3	122.9 ± 19.0	135.4 ± 20.5	<.001
Diastolic blood pressure, mm Hg	71.5 ± 9.7	71.3 ± 9.6	76.2 ± 9.5	<.001
Hypertension, n (%)	1790 (26.2)	1593 (24.8)	197 (50.0)	<.001
BMI, kg/m^2	26.0 ± 4.2	25.7 ± 4.0	29.5 ± 4.8	<.001
Waist circumference, cm	87.8 ± 12.9	87.2 ± 12.7	98.7 ± 12.3	<.001
Glucose, mmol/L	4.7 ± 0.6	4.7 ± 0.6	5.6 ± 0.7	<.001
IFG, n (%)[a]	689 (10.1)	467 (7.3)	222 (56.3)	<.001
Insulin, mU/L	7.7 (5.4–11.3)	7.5 (5.4–10.9)	8.6 (12.8–19.8)	<.001
HOMA-IR	1.60 (1.08–2.46)	1.55 (1.06–2.33)	3.17 (2.08–5.19)	<.001
Total cholesterol, mmol/L	5.65 ± 1.12	5.63 ± 1.12	6.08 ± 1.12	<.001
HDL-C, mmol/L	1.34 ± 0.40	1.35 ± 0.40	1.11 ± 0.29	<.001
Triglycerides, mmol/L	1.12 (0.82–1.62)	1.10 (0.81–1.57)	1.63 (1.21–2.38)	<.001
apoA-I, g/L	1.40 ± 0.27	1.40 ± 0.28	1.32 ± 0.24	<.001
apoA-II, g/L	0.34 ± .06	0.34 ± 0.06	0.33 ± 0.06	<.001
HDL-C–to–apoA-I ratio	0.96 ± 0.20	0.96 ± 0.21	0.84 ± 0.16	<.001
HDL-C–to–apoA-II ratio	3.96 ± 1.24	4.00 ± 1.25	3.33 ± 0.83	<.001
UAE, mg/24 h	9.2 (6.2–16.4)	9.0 (6.2–15.7)	15.1 (8.1–36.5)	<.001

Data are means ± SD or median (IQR).

[a] IFG, impaired fasting glucose (fasting glucose of 6.1–6.9 mmol/L [110–125 mg/dL]).

web site at http://jcem.endojournals.org.). As shown in Figure 1, we also observed that the risk of diabetes of HDL-C and the ratios of HDL-C to apoA-I and HDL-C to apoA-II did not differ across triglyceride tertiles ($P > .10$ for interactions).

ORs for incident type 2 diabetes mellitus per a 1-SD increase in HDL-C, apoA-I, apoA-II, HDL-C–to–apoA-I ratio, and HDL-C–to–apoA-II ratio with HOMA-IR incorporated in model 4 instead of plasma glucose are shown in Table 3. In multivariable adjusted analysis now controlling for insulin resistance, triglycerides, and other clinical diabetes risk factors, all results remained essentially similar.

Secondary analyses

In secondary analyses, we additionally accounted for 24-hour UAE as an additional covariate (other included confounders are as in model 5) (Table 2). We observed that HDL-C and ratios of HDL-C to apoA-I and apoA-II were

Table 2. Relationships of HDL Variables With the Risk of Developing Type 2 Diabetes Mellitus

	OR (95% CI) per 1-SD Increase									
HDL Variables	Model 1	*P* Value	Model 2	*P* Value	Model 3	*P* value	Model 4	*P* value	Model 5	*P* value
HDL-C	0.44 (0.38–0.51)	<.001	0.54 (0.46–0.63)	<.001	0.55 (0.47–0.64)	<.001	0.63 (0.53–0.74)	<.001	0.74 (0.61–0.88)	.001
apoA-I	0.72 (0.64–0.82)	<.001	0.80 (0.71–0.92)	.001	0.81 (0.71–0.93)	.002	0.89 (0.77–1.03)	.11	0.95 (0.82–1.10)	.50
apoA-II	0.98 (0.87–1.10)	.77	1.03 (0.91–1.16)	.63	1.02 (0.90–1.16)	.71	1.07 (0.92–1.21)	.43	1.01 (0.88–1.16)	.83
HDL-C–to–apoA-I ratio	0.005 (0.002–0.013)	<.001	0.02 (0.007–0.050)	<.001	0.02 (0.01–0.06)	<.001	0.05 (0.02–0.13)	<.001	0.14 (0.04–0.44)	<.001
HDL-C–to–apoA-II ratio	0.006 (0.002–0.015)	<.001	0.02 (0.007–0.044)	<.001	0.03 (0.01–0.06)	<.001	0.05 (0.02–0.12)	<.001	0.12 (0.04–0.36)	<.001

Model 1: adjusted for age and sex. Model 2: model 1 + BMI. Model 3: model 2 + family history of diabetes, hypertension, alcohol use, and smoking. Model 4: model 3 + glucose. Model 5: model 4 + triglycerides.

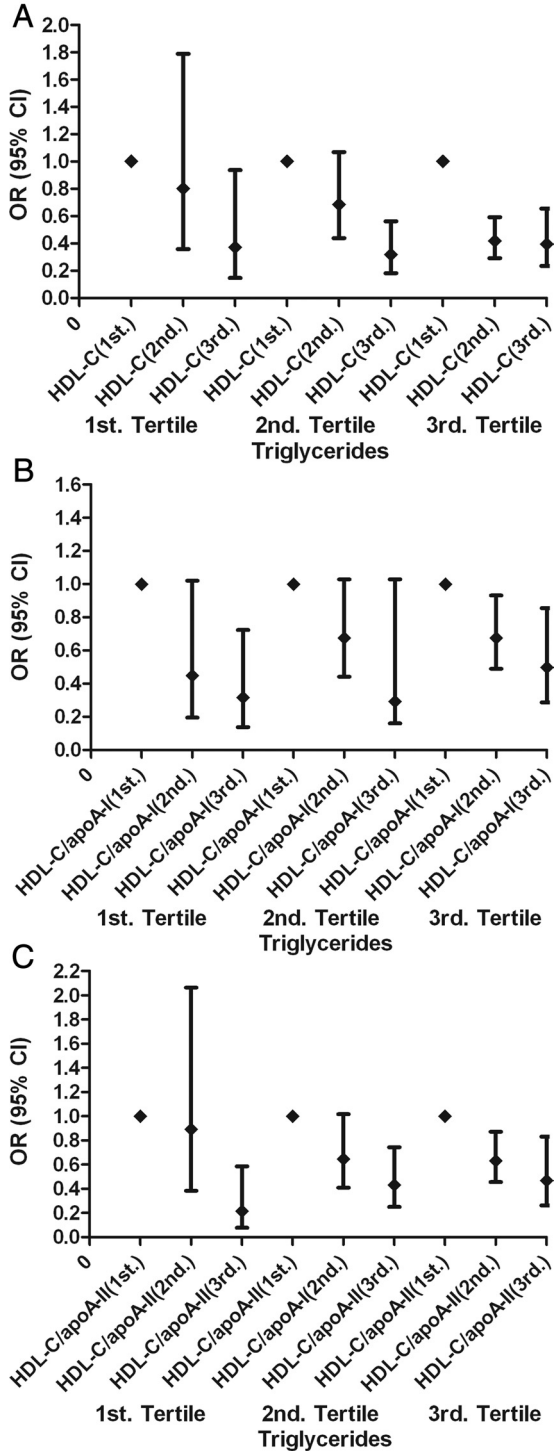

Figure 1. Risk of developing type 2 diabetes mellitus according to tertiles of HDL-C (A), the HDL-C–to–apoA-I ratio (B) and the HDL-C–to–apoA-II ratio (panel C) stratified by triglyceride tertiles. The ORs (95% CI) were calculated by logistic regression models adjusted for age and sex. The individuals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

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 microalbuminuric 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 affected the ORs in model 5 (Supplemental Table 3). In addition, 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 interactions). In sex-stratified analyses, the multivariable-adjusted ORs (adjusted for baseline fasting glucose, triglycerides 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 subsequent 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 associations 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 respective 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).

Discussion

This prospective study in a predominantly Caucasian population 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 components, 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 environmental 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-

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

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

Model A: adjusted for age, sex, BMI, smoking, alcohol use, family history of diabetes, hypertension, and HOMA-IR. Model B: model A + triglycerides.

esis that HDL particles that contain apoA-I rather than apoA-II may influence diabetes development. This result raises the possibility that compositional characteristics of specific HDL particles besides the well established components of the metabolic syndrome may be involved in the pathogenesis of type 2 diabetes mellitus.

Besides increasing age and a positive family history of diabetes, (central) obesity, hypertension, and higher initial plasma glucose are relevant components of the early pathophysiology of type 2 diabetes mellitus (3–5). Thus, it is necessary to take account of these cardiometabolic risk factors to determine the strength of the relationships of incident diabetes with HDL-related variables. Previous reports have already demonstrated that lower HDL-C relates to increased diabetes risk after adjustment for several metabolic syndrome components in predominantly middle-aged Caucasian men (9, 10), in Korean subjects (12), and even in native American children and adolescents (11). In some early surveys, the impact of HDL-C on diabetes incidence was found to be confined to women (7, 8). In the PREVEND cohort, we did not find an interaction between sex and HDL-C on incident diabetes, and the point estimates of HDL-C for diabetes risk were similar for men and women separately. In view of the strong negative relationships of HDL-C and HDL particle size with plasma triglycerides (16, 27, 28, 35, 36), it is also relevant that the impact of the HDL-C–to–apoA-I and the HDL-C–to–apoA-II ratios on diabetes development was not appreciably modified by plasma triglyceride levels. Although not unequivocally established, the impact of apoA-II on HDL remodeling, as well as on the functional properties of HDL, such as the ability to promote cell-derived cholesterol efflux, is likely to be distinct and less pronounced than the multifaceted actions of apoA-I, which are generally considered to be antiatherogenic (26–28, 37). In this context, it is plausible to propose that the lack of association of apoA-II compared with apoA-I with incident diabetes would translate into a differentiated role of these apolipoproteins in their ability to protect against diabetes development. Of further interest, the lowest ORs for incident diabetes were found for the HDL-C–to–apoA-I and the HDL-C–to–apoA-II ratios. Higher ratios of HDL-C compared with those of the major apolipoproteins of HDL provide estimates of more cholesterol loaded and, hence, larger HDL particles (16, 27–29, 38). Therefore, the strong negative relationship of these ratios with diabetes development suggests that larger HDL particles may particularly relate to lower diabetes incidence. In keeping with this supposition and in line with the lack of relationship of diabetes development with apoA-II, HDL particles that contain both apoA-I and apoA-II, ie, LpA-I:A-II particles, are smaller than LpA-I particles, which contain apoA-I only (28). Obviously, the possible diabetes-protective impact of various HDL subfractions including pre-β-HDL and the contribution of specific HDL-associated proteins, such as the antioxidative enzymes, paraoxonase I and lipoprotein-associated phospholipase A_2 (26, 27, 39), needs to be more precisely defined in future human studies. Nevertheless, this large-scale prospective study based on observational data strongly supports previous in vitro and in vivo evidence regarding a potential mechanistic link between HDL particle properties and future development of type 2 diabetes. The present findings also provide a rationale to test the importance of HDL size characteristics on incident diabetes mellitus.

Several methodological aspects and limitations of our study need to be considered. The PREVEND cohort is enriched with microalbuminuric subjects (31). In this cohort, microalbuminuria was shown to predict incident diabetes independently of metabolic syndrome components (40). Nonetheless, the incidence of type 2 diabetes among PREVEND participants during a median follow-up of 7.7 years with January 1, 2007, as census date, ascertained according to 1997 American Diabetes Association criteria, as well as by self-reported physician diagnosis of type 2 diabetes or the use of oral glucose-lowering drugs, roughly agrees with current projections of diabetes prevalence in other established market economies (1, 2, 4, 5). Clearly, it is relevant to take account of microalbuminuria upon establishing the relationship of diabetes develop-

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 UACs (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 A_{1c} was not measured, and oral glucose tolerance tests were not performed. If elevated levels of hemoglobin A_{1c} 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 diabetes development.

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