Differential Association Between HDL Subclasses and the Development of Type 2 Diabetes in a Prospective Study of Japanese Americans

You-Cheol Hwang,[1,2] Tomoshige Hayashi,[3] Wilfred Y. Fujimoto,[4] Steven E. Kahn,[4,5] Donna L. Leonetti,[6] Marguerite J. McNeely,[4†] and Edward J. Boyko[1,4]

OBJECTIVE

Recent studies have suggested that HDL cholesterol is inversely associated with the development of type 2 diabetes. However, little is known about the association between different HDL subclasses and the risk for future type 2 diabetes.

RESEARCH DESIGN AND METHODS

The study enrolled 406 Japanese Americans (51% male) without diabetes, aged 34–75 years. Oral glucose tolerance tests were performed to determine type 2 diabetes status at baseline, 2.5 years, 5 years, and 10 years after enrollment. HDL2, HDL3, total HDL cholesterol, and visceral adipose tissue (VAT) area by computed tomography were measured at baseline.

RESULTS

In univariate analysis, total HDL and HDL2 cholesterol were inversely associated with the incidence of type 2 diabetes, but HDL3 cholesterol was not. In multivariate analysis, total HDL cholesterol (odds ratio per 1-SD increment, 0.72 [95% CI 0.52–0.995], *P* = 0.047) and HDL2 cholesterol (odds ratio per 1-SD increment, 0.64 [95% CI 0.44–0.93], *P* = 0.018) were inversely associated with the risk for type 2 diabetes independent of age, sex, BMI, waist circumference, family history of diabetes, lifestyle factors, systolic blood pressure, lipid-lowering medication use, triglyceride level, HOMA-insulin resistance, and 2-h glucose; however, HDL3 cholesterol was not associated with diabetes risk. The association between diabetes risk and total HDL and HDL2 cholesterol became insignificant after adjustment for VAT area.

CONCLUSIONS

Subjects with higher HDL2 cholesterol were at lower risk for incident type 2 diabetes, but this association was confounded by and not independent of VAT. Higher HDL3 cholesterol was not associated with diabetes risk.

[1]Seattle Epidemiologic Research and Information Center, VA Puget Sound Health Care System, Seattle, WA
[2]Division of Endocrinology and Metabolism, Department of Medicine, Kyung Hee University School of Medicine, Kyung Hee University Hospital at Gangdong, Seoul, Korea
[3]Department of Preventive Medicine and Environmental Health, Graduate School of Medicine, Osaka City University, Osaka, Japan
[4]Department of Medicine, University of Washington School of Medicine, Seattle, WA
[5]Hospital and Specialty Medicine Service, VA Puget Sound Health Care System, Seattle, WA
[6]Department of Anthropology, University of Washington, Seattle, WA

Corresponding author: Edward J. Boyko, eboyko@uw.edu.

†Deceased.

Numerous epidemiological studies from multiple countries and different ethnic groups have consistently shown that HDL cholesterol is strongly and inversely associated with the future development of cardiovascular events in diverse clinical conditions (1–4). However, plasma HDL particles are not homogeneous. Rather, they comprise a family of different particles that vary by size, density, apolipoprotein

composition, and lipid content. In particular, apolipoprotein A-I is the structural apolipoprotein in HDL particles and constitutes ~70% of the apolipoprotein content of HDL particles (5,6). Ultracentrifugation can be used to separate HDL particles into two main subclasses by density: a small, dense, and relatively cholesterol-poor form classified as HDL3 (1.125–1.21 g/mL) and a large, light, and relatively cholesterol-rich form classified as HDL2 (1.062–1.125 g/mL) (7).

Accumulating evidence suggests that the risk for cardiovascular disease (CVD) might be different according to HDL subclasses, and most cross-sectional and prospective studies have suggested that HDL2 may be more protective than HDL3 (8–10). Recent studies have demonstrated that HDL directly modulates glucose metabolism (11,12). Accordingly, epidemiological studies have shown that HDL cholesterol is associated with a lower risk of type 2 diabetes in various ethnic and age-groups (13–15).

To date, however, little is known about the associations between different HDL subclasses and the risk for future type 2 diabetes. Therefore, the aim of this study was to determine the differential association between HDL subclasses, that is HDL2 and HDL3 cholesterol, and future development of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study Subjects
The study population consisted of Japanese American men and women enrolled in the Japanese American Community Diabetes Study, a cohort of second- (Nisei) and third-generation (Sansei) Japanese Americans of 100% Japanese ancestry. A detailed description of the selection and recruitment of the study subjects has been published previously (16). In brief, study participants were selected as volunteers from a community-wide comprehensive mailing list and telephone directory that included nearly 95% of the Japanese American population in King County, WA. Among 658 subjects in the original cohort, 166 were excluded because they had diabetes at baseline, and thus, 492 subjects without diabetes at baseline were available for this analysis. Subjects were followed up at 2.5 years (Nisei men

only), 5–6 years, and then again at 10–11 years after a baseline examination, and finally, 406 subjects (208 men, 198 women) without diabetes, aged 34–75 years, were enrolled in this study. Of the 86 subjects who did not complete the 10-year follow-up examination, 30 died and 56 were lost to follow-up. The study received approval from the University of Washington Human Subjects Division, and written informed consent was obtained from all subjects.

Clinical and Laboratory Examination
All evaluations were performed at the General Clinical Research Center, University of Washington, Seattle, WA. A complete physical examination was performed at baseline. A standardized questionnaire was used to determine personal medical history and lifestyle factors that possibly affect HDL cholesterol level, including cigarette smoking, alcohol consumption, and physical activity. Smoking was classified into three groups (current smoker, past smoker, and never smoker). Previous meta-analysis of prospective observational studies suggested that moderate alcohol consumption (6–48 g/day) reduces the risk of type 2 diabetes, and thus, we used this criterion to define moderate alcohol consumption (17). The Paffenbarger physical activity index questionnaire was used to determine physical activity level (usual kilocalories expended weekly) (18), and regular physical activity was defined as more than moderate-intensity physical activity.

BMI was calculated as weight in kilograms divided by the square of the height in meters. Waist circumference was measured at the level of umbilicus. Blood pressure was measured with a mercury sphygmomanometer with the subject in a recumbent position. Average blood pressure was calculated from the second and third of three consecutive measurements. Visceral adipose tissue (VAT) area at the level of the umbilicus was measured at baseline using computed tomography.

Biochemical measurements were performed at the time of sample collection using fresh samples as reported previously (19). All blood samples were obtained after an overnight fast of 10 h. Plasma glucose was measured by the hexokinase method using an autoanalyzer (Department of Laboratory Medicine,

University of Washington). Plasma insulin was measured by radioimmunoassay (Immunoassay Core, Diabetes Endocrinology Research Center, University of Washington). The HOMA-insulin resistance (IR) based on fasting glucose and insulin concentration was used to estimate insulin sensitivity (20). Lipids and lipoproteins measurements were performed according to modified procedures of the Lipid Research Clinics and apolipoproteins A-I and B by radioimmunoassays (Northwest Lipid Research Laboratory, Seattle, WA). Total HDL cholesterol was determined in the supernatant after precipitation of apolipoprotein B–containing lipoproteins with dextran sulfate. A second precipitation with high-molecular-weight dextran sulfate was performed on the supernatant containing HDL to separate the HDL2 and HDL3 subclasses (21).

Diabetes status was determined by 75-g oral glucose tolerance tests (OGTTs) that were performed at baseline and at 2.5 years (Nisei men only), 5–6 years, and 10–11 years after the enrollment. In this study, type 2 diabetes was defined by the presence of one of the following: 1) fasting glucose level $\geq$ 7.0 mmol/L; 2) treatment involving oral hypoglycemic agents or insulin therapy; or 3) 2-h postglucose load $\geq$11.1 mmol/L (22).

Statistical Analyses
Data are expressed as means (SD) for continuous measures or as proportions for categorical variables. Differences between groups were tested by the Student t test or Mann-Whitney U test for continuous variables and the χ^2 test or Fisher exact test for categorical variables. Pearson correlation coefficients were performed to determine the associations between the independent variables. Multiple logistic regression analysis with backward selection was used to determine whether plasma total HDL cholesterol and HDL cholesterol subclasses were independently associated with incident type 2 diabetes. Odds ratios (ORs) with 95% CIs were calculated for independent variables included in logistic models, with a 1-SD increment used for OR calculations for continuous measurements. The presence of interaction was assessed in multivariate models by testing the significance of first-order interaction terms. Presence of nonlinearity was assessed by insertion of a quadratic transformation of the main

exposures of interests into models that contained the linear term. All statistical analyses were performed using PASW 18.0 software (IBM Corp., Armonk, NY). A *P* value of <0.05 was considered significant.

RESULTS

Table 1 reports the baseline characteristics of study subjects. Mean age was 51.6 years, and approximately half of subjects were women. In terms of personal history possibly affecting plasma HDL cholesterol level, 18.0% of subjects were moderate alcohol drinkers, 13.1% were current smokers, and 23.6% performed regular physical activity of more than moderate intensity. In 8.9% (36 of 406) of subjects, total HDL cholesterol levels were <1.04 mmol/L, and HDL2 cholesterol comprised 25.0% of total HDL cholesterol.

During 10 years of follow-up, the status of type 2 diabetes could be assessed in 406 subjects, with 91 subjects developing diabetes, leading to a cumulative incidence of 22.4%. In univariate analysis, well-known risk factors for type 2 diabetes, including age, BMI, waist circumference, family history of diabetes, blood pressure, plasma glucose levels, and HOMA-IR, showed positive associations with the development of type 2 diabetes. The lipid parameters of total cholesterol, LDL cholesterol, triglycerides, non-HDL cholesterol, and apolipoprotein B levels were significantly and positively associated with type 2 diabetes risk. In contrast, total HDL cholesterol (OR per 1-SD increment, 0.68 [95% CI 0.52–0.88], *P* = 0.004) and HDL2 cholesterol (OR per 1-SD increment, 0.57 [95% CI 0.42–0.77], *P* < 0.001) were inversely associated with type 2 diabetes incidence. No significant association was observed between HDL3 cholesterol (OR per 1-SD increment, 0.96 [95% CI 0.76–1.21]; *P* = 0.70) or apolipoprotein A-I (OR per 1-SD increment, 0.97 [95% CI 0.73–1.29]; *P* = 0.81) and type 2 diabetes risk (Table 2). Other factors possibly affecting plasma HDL cholesterol level or diabetes risk, including alcohol consumption, smoking, and physical activity, did not show any association with the development of type 2 diabetes in this analysis. Lipid-lowering medications were also not associated with type 2 diabetes risk.

In multivariate analysis, the model starting points for the backward elimination algorithm included age, sex, BMI, waist circumference, family history of diabetes, systolic blood pressure, current smoking, alcohol consumption, regular physical activity, lipid-lowering medications, triglyceride, HOMA-IR, and 2-h plasma glucose based on univariate analysis, prior knowledge for diabetes risk, and confounding structure (Supplementary Table 1). Total HDL cholesterol (OR per 1-SD increment, 0.72 [95% CI 0.52–0.995]; *P* = 0.047) and HDL2 cholesterol (OR per 1-SD increment, 0.64 [95% CI 0.44–0.93]; *P* = 0.018) were inversely and independently associated with the risk of diabetes. However, HDL3 cholesterol was not significantly associated with risk for incident type 2 diabetes in univariate (Table 2) or multivariate models (OR per 1-SD increment, 0.90 [95% CI 0.67–1.19]; *P* = 0.44) (Table 3). After further adjustment for VAT in the models in Table 3,

Table 1—Baseline characteristics

	Total (*n* = 406)	Men (*n* = 208)	Women (*n* = 198)	*P*
Age (years)	51.6 (11.8)	51.4 (11.4)	51.8 (12.2)	0.74
BMI (kg/m^2)	24.2 (3.3)	25.4 (3.1)	22.9 (3.1)	<0.001
Waist circumference (cm)*	82.0 (10.5)	88.4 (8.0)	75.3 (8.4)	<0.001
VAT area (cm^2)	76.2 (40.9–108.7)	57.1 (30.9–90.1)	91.3 (58.5–124.7)	<0.001
Family history of diabetes, *n* (%)	145 (35.7)	68 (32.7)	77 (38.9)	0.19
Moderate alcohol consumption, *n* (%)	73 (18.0)	57 (27.4)	16 (8.1)	<0.001
Current smoking, *n* (%)	53 (13.1)	29 (13.9)	24 (12.1)	0.59
Regular physical activity, *n* (%)	96 (23.6)	70 (33.7)	26 (13.1)	<0.001
Lipid-lowering medication, *n* (%)	6 (1.7)	4 (1.9)	2 (1.0)	0.69
Systolic blood pressure (mmHg)	128.3 (16.9)	131.2 (16.8)	125.2 (16.7)	<0.001
Diastolic blood pressure (mmHg)	76.7 (9.4)	79.2 (8.9)	74.1 (9.3)	<0.001
Fasting plasma glucose (mmol/L)	5.16 (0.57)	5.31 (0.59)	5.00 (0.50)	<0.001
2-h plasma glucose (mmol/L)	7.25 (1.68)	7.23 (1.72)	7.27 (1.65)	0.82
HOMA-IR	2.67 (1.91–3.70)	2.61 (1.89–3.56)	2.73 (1.96–3.87)	0.27
Total cholesterol (mmol/L)	5.84 (1.05)	5.95 (1.06)	5.73 (1.04)	0.040
LDL cholesterol (mmol/L)	3.63 (0.93)	3.81 (0.97)	3.44 (0.85)	<0.001
Triglycerides (mmol/L)	1.25 (0.86–1.77)	1.45 (1.03–2.13)	1.04 (0.73–1.46)	<0.001
Total HDL cholesterol (mmol/L)	1.51 (0.44)	1.33 (0.34)	1.70 (0.45)	<0.001
HDL2 cholesterol (mmol/L)	0.42 (0.32)	0.28 (0.21)	0.57 (0.35)	<0.001
HDL3 cholesterol (mmol/L)	1.09 (0.17)	1.05 (0.17)	1.13 (0.17)	<0.001
Non-HDL cholesterol (mmol/L)	4.33 (1.14)	4.61 (1.10)	4.03 (1.10)	<0.001
ApoB (g/L)†	1.10 (0.27)	1.11 (0.23)	1.10 (0.29)	0.86
ApoA-I (g/L)†	1.54 (0.31)	1.37 (0.25)	1.62 (0.30)	<0.001
Total HDL cholesterol–to–apoA-I ratio†	0.40 (0.08)	0.38 (0.07)	0.41 (0.09)	0.007

Data are expressed as mean (SD), median (interquartile range), or as *n* (%). Moderate alcohol consumption was defined as 6–48 g/day of ethanol. Regular physical activity was defined as more than moderate physical activity. Apo, apolipoprotein. *n = 401. †n = 292.

Table 2—Univariate logistic regression analysis of incident diabetes in relation to clinical and laboratory measurements

	OR per 1-SD increment (95% CI)	P
Age	1.86 (1.44–2.41)	<0.001
Men	1.08 (0.68–1.73)	0.74
BMI	1.61 (1.28–2.04)	<0.001
Waist circumference	1.57 (1.24–2.00)	<0.001
VAT area	2.14 (1.67–2.74)	<0.001
Family history of diabetes	2.37 (1.47–3.81)	<0.001
Moderate alcohol consumption	0.97 (0.52–1.78)	0.91
Current smoking	1.15 (0.58–2.25)	0.69
Regular physical activity	0.75 (0.42–1.33)	0.33
Lipid-lowering medication	3.55 (0.70–17.87)	0.13
Systolic blood pressure	1.78 (1.40–2.26)	<0.001
Diastolic blood pressure	1.57 (1.23–2.00)	<0.001
Fasting plasma glucose	2.87 (2.14–3.84)	<0.001
2-h plasma glucose	4.46 (3.14–6.33)	<0.001
HOMA-IR	1.76 (1.40–2.22)	<0.001
Total cholesterol	1.46 (1.16–1.85)	0.001
LDL cholesterol	1.38 (1.09–1.74)	0.007
Triglycerides	1.46 (1.15–1.86)	0.002
Total HDL cholesterol	0.68 (0.52–0.88)	0.004
HDL2 cholesterol	0.57 (0.42–0.77)	<0.001
HDL3 cholesterol	0.96 (0.76–1.21)	0.70
Non-HDL cholesterol	1.62 (1.28–2.06)	<0.001
ApoB	1.66 (1.24–2.22)	0.001
ApoA-I	0.97 (0.73–1.29)	0.81
Total HDL cholesterol–to–apoA-I ratio	0.53 (0.36–0.77)	0.001

Apo, apolipoprotein.

the associations between diabetes risk and total HDL cholesterol and HDL2 cholesterol became insignificant (OR per 1-SD increment, 0.84 [95% CI 0.58–1.2], and 0.74 [0.49–1.11], respectively). Quadratic transformations of HDL cholesterol and the HDL subclasses when inserted into the models in Tables 3 to assess presence of nonlinearity were not statistically significant.

Finally, we determined whether the HDL cholesterol–to–apolipoprotein A-I ratio, a marker of HDL particle size, was associated with the risk for type 2 diabetes. The ratio was inversely and independently associated with type 2 diabetes incidence after the adjustment for the aforementioned risk factors (OR per 1-SD increment, 0.58 [95% CI 0.38–0.88]; P = 0.010; Supplementary Table 2). Furthermore, the quadratic transformation of the HDL cholesterol–to–apolipoprotein A-I ratio when inserted into the model (Supplementary Table 2), was statistically significant (P = 0.019), indicating that the association between this ratio and diabetes odds was nonlinear. The OR for the HDL cholesterol–to–apolipoprotein A-I ratio in this model for a 1-SD increment was 0.30 (95% CI 0.12–0.76; P = 0.011).

CONCLUSIONS

In the current prospective study performed in Japanese American men and women, total HDL cholesterol and HDL2 cholesterol were inversely associated with future development of type 2 diabetes determined by OGTT, and this association was independent not only of lifestyle factors potentially affecting HDL cholesterol level but also of well-established risk factors for type 2 diabetes during 10 years of follow-up. However, HDL3 cholesterol did not show any association with future diabetes risk. Furthermore, the HDL cholesterol–to–apolipoprotein A-I ratio was inversely associated with the risk of type 2 diabetes in a nonlinear fashion, with a greater risk reduction seen with increasing values for this ratio.

In addition to cardioprotection, many epidemiological studies have shown that HDL cholesterol is inversely associated with incident type 2 diabetes (13–15). The individual roles of HDL subclasses

Table 3—Multivariate logistic regression analysis of incident diabetes in relation to total HDL cholesterol and HDL cholesterol subclasses

	Total HDL cholesterol		HDL2 cholesterol		HDL3 cholesterol	
	OR per 1-SD increment (95% CI)	P	OR per 1-SD increment (95% CI)	P	OR per 1-SD increment (95% CI)	P
Age	1.80 (1.29–2.50)	<0.001	1.75 (1.26–2.43)	0.001	1.80 (1.30–2.51)	<0.001
Family history of diabetes	2.08 (1.16–3.73)	0.014	2.12 (1.18–3.82)	0.012	2.02 (1.14–3.60)	0.017
2-h plasma glucose	4.00 (2.72–5.88)	<0.001	4.01 (2.72–5.90)	<0.001	3.94 (2.69–5.76)	<0.001
HOMA-IR	1.55 (1.17–2.04)	0.002	1.51 (1.14–1.99)	0.004	1.64 (1.25–2.15)	<0.001
Total HDL cholesterol	0.72 (0.52–0.995)	0.047	Not included in the model		Not included in the model	
HDL2 cholesterol	Not included in the model		0.64 (0.44–0.93)	0.018	Not included in the model	
HDL3 cholesterol	Not included in the model		Not included in the model		0.90 (0.67–1.19)	0.44

Age, sex, BMI, waist circumference, family history of diabetes, systolic blood pressure, current smoking, alcohol consumption, regular physical activity, lipid-lowering medications, triglyceride, HOMA-IR, 2-h plasma glucose, and total HDL cholesterol, HDL2 cholesterol, or HDL3 cholesterol were included in each model.

in type 2 diabetes pathogenesis, though, are largely unknown. To the best of our knowledge, only one study has investigated the relationship between HDL subclasses and type 2 diabetes incidence (23). This study, conducted among Pima Indians, estimated associations between total HDL, HDL_{2a}, HDL_{2b} and HDL_3 with risk of type 2 diabetes in 123 women and 50 men during 10 years of follow-up. Results varied by sex. Among women, total HDL, HDL_{2a}, and HDL_3 were inversely associated with type 2 diabetes risk. Among men, the opposite association was seen for total HDL and HDL_3, with both lipid measurements positively correlated with type 2 diabetes risk. However, these associations were not significant after further adjustment for alcohol consumption. Therefore, the authors concluded that the association between HDL or HDL subclasses and incident type 2 diabetes in men was largely dependent on alcohol consumption (23). The results in Pima women agree with our observation of an inverse association between HDL2 cholesterol and diabetes risk but differ regarding the HDL3 cholesterol result, because we did not observe an association between HDL3 cholesterol and type 2 diabetes risk. The result in Pima men regarding HDL_3 also differs from our univariate analysis, where we found no association between HDL3 cholesterol and type 2 diabetes risk. The Pima investigators, though, did not specifically examine HDL subclass–to–total HDL cholesterol ratios in association with type 2 diabetes risk. Potential explanations for these discrepancies include the differences in ethnic groups under study, the limited power of the Pima analysis in men that included only 50 subjects, and the definition of diabetes based on a 2-h glucose value ≥11.1 mmol/L in a 75-g OGTT or routine medical care. Nonuse of the fasting plasma glucose to identify diabetes may have resulted in misclassification of subjects with diabetes at baseline because as subjects without diabetes and also of subjects achieving the primary end point, thereby potentially producing bias that might explain the differences with our results.

In our study, although higher total HDL cholesterol was associated with a lower diabetes risk, this risk for diabetes differed according to different HDL cholesterol subclasses. That is, although subjects with higher HDL2 cholesterol were associated with a lower risk for incident type 2 diabetes, HDL3 cholesterol did not show any association with future diabetes risk, and thus, the association between total HDL cholesterol and lower diabetes risk appears to mainly arise from a higher HDL2 cholesterol level (Table 3). Therefore, the measurement of HDL subclasses in a clinical setting may provide additional information over simple measurement of total HDL cholesterol to assess future diabetes risk in addition to the risk for future development of CVD. These differences also might be of potential importance in furthering our understanding of the pathogenesis of type 2 diabetes in relation to HDL and its subclasses. Similarly, previous studies suggested that the HDL cholesterol–to–apolipoprotein A-I ratio reflects the core-to-surface ratio, or the particle size of HDL particles (24,25). This ratio was lower in subjects with metabolic syndrome compared with subjects without metabolic syndrome. This result suggests that subjects with metabolic syndrome have smaller and denser HDL particles than those without metabolic syndrome (25). In addition, subjects with impaired fasting glucose who had a higher HDL cholesterol–to–apolipoprotein A-I ratio showed favorable metabolic profiles and a lower risk for incident type 2 diabetes (15). In agreement with previous studies, the ratio was an independent negative predictor for incident type 2 diabetes in this study (OR per 1-SD increment, 0.58 [95% CI 0.38–0.88], P = 0.010; Supplementary Table 2).

The associations between diabetes risk and both total HDL and HDL2 cholesterol became insignificant after adjustment of multivariate models for VAT at baseline. Previous research in this cohort has demonstrated an association between HDL cholesterol concentrations and the size of the visceral fat depot, the latter being a known risk factor for type 2 diabetes (26,27). Whether total HDL cholesterol and HDL2 cholesterol serve only as a correlate of visceral adipose depot size or have other effects will require further study.

To date, numerous basic studies have investigated the mechanism by which HDL regulates glucose metabolism, but the mechanisms by which HDL may protect against incident type 2 diabetes in humans is still elusive. However, previous studies have demonstrated that an intravenous infusion of reconstituted HDL in humans decreased plasma glucose and increased plasma insulin and HOMA estimated β-cell function more than placebo (12). In addition, infusion of reconstituted HDL altered fasting-induced fatty acid turnover and oxidation (28), which have been implicated as key players in the regulation of glucose metabolism (29). HDL particles also have known anti-inflammatory properties (11), which may reduce the systemic inflammatory state implicated in the pathogenesis of insulin resistance and progression to type 2 diabetes (30).

Our study has some limitations. First, our results, which were obtained in a 100% Japanese American cohort, may not be generalizable to other ethnic groups, and there may be a possibility that HDL subclasses have different associations with incident type 2 diabetes among different ethnic groups. Second, it was suggested that methodological differences to measure HDL subclasses may partly explain the inconsistent results between HDL subclasses and CVD in epidemiological studies and clinical trials (5–10). In this study, we used a differential precipitation method to measure HDL subclasses; however, good agreements have been reported between HDL subclasses measured by dextran precipitation and preparative ultracentrifugation especially for HDL2 (31) and a novel homogeneous assay for HDL3 (32). Lastly, although loss to follow-up occurred during the 10-year span of the study, it was at an acceptably low level (17%).

In summary, our results suggest that total HDL cholesterol is associated with a lower risk for incident type 2 diabetes in Japanese Americans during 10 years of follow-up and that this association may be largely attributable to the HDL2 cholesterol subclass, but not by HDL3 cholesterol. These data provide prospective evidence for a potential benefit of the HDL2 subclass on glucose metabolism in this population, possibly mediated by direct and indirect (anti-inflammatory) effects, as shown by others.

References

1. Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 1986;256:2835–2838
2. Turner RC, Millns H, Neil HA, et al. Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). BMJ 1998;316:823–828
3. Olsson AG, Schwartz GG, Szarek M, et al. High-density lipoprotein, but not low-density lipoprotein cholesterol levels influence short-term prognosis after acute coronary syndrome: results from the MIRACL trial. Eur Heart J 2005;26:890–896
4. Boekholdt SM, Arsenault BJ, Hovingh GK, et al. Levels and changes of HDL cholesterol and apolipoprotein A-I in relation to risk of cardiovascular events among statin-treated patients: a meta-analysis. Circulation 2013;128:1504–1512
5. Movva R, Rader DJ. Laboratory assessment of HDL heterogeneity and function. Clin Chem 2008;54:788–800
6. Asztalos BF, Tani M, Schaefer EJ. Metabolic and functional relevance of HDL subspecies. Curr Opin Lipidol 2011;22:176–185

7. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 1955;34:1345–1353
8. Krauss RM. Lipoprotein subfractions and cardiovascular disease risk. Curr Opin Lipidol 2010;21:305–311
9. Pirillo A, Norata GD, Catapano AL. High-density lipoprotein subfractions–what the clinicians need to know. Cardiology 2013;124:116–125
10. Morgan J, Carey C, Lincoff A, Capuzzi D. High-density lipoprotein subfractions and risk of coronary artery disease. Curr Atheroscler Rep 2004;6:359–365
11. Gordon SM, Hofmann S, Askew DS, Davidson WS. High density lipoprotein: it's not just about lipid transport anymore. Trends Endocrinol Metab 2011;22:9–15
12. Drew BG, Duffy SJ, Formosa MF, et al. High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation 2009;119:2103–2111
13. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes? JAMA 1990;263:2893–2898
14. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med 2007;167:1068–1074
15. Hwang YC, Ahn HY, Park SW, Park CY. Association of HDL-C and apolipoprotein A-I with the risk of type 2 diabetes in subjects with impaired fasting glucose. Eur J Endocrinol 2014;171:137–142
16. Fujimoto WY, Leonetti DL, Kinyoun JL, Shuman WP, Stolov WC, Wahl PW. Prevalence of complications among second-generation Japanese-American men with diabetes, impaired glucose tolerance, or normal glucose tolerance. Diabetes 1987;36:730–739
17. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 2005;28:719–725
18. Paffenbarger RS Jr, Wing AL, Hyde RT. Physical activity as an index of heart attack risk in college alumni. Am J Epidemiol 1978;108:161–175
19. Bergstrom RW, Leonetti DL, Newell-Morris LL, Shuman WP, Wahl PW, Fujimoto WY. Association of plasma triglyceride and C-peptide with coronary heart disease in Japanese-American men with a high prevalence of glucose intolerance. Diabetologia 1990;33:489–496
20. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis

model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–419
21. Warnick GR. Enzymatic methods for quantification of lipoprotein lipids. Methods Enzymol 1986;129:101–123
22. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–1197
23. Fagot-Campagna A, Knowler WC, Narayan KM, Hanson RL, Saaddine J, Howard BV. HDL cholesterol subfractions and risk of developing type 2 diabetes among Pima Indians. Diabetes Care 1999;22:271–274
24. Miwa K, Yoshida N, Nakagawa K, Inoue H. High-density lipoprotein particles are large in patients with variant angina. Cardiovasc Res 1998;37:729–737
25. Garin MC, Kalix B, Morabia A, James RW. Small, dense lipoprotein particles and reduced paraoxonase-1 in patients with the metabolic syndrome. J Clin Endocrinol Metab 2005;90:2264–2269
26. Boyko EJ, Leonetti DL, Bergstrom RW, Newell-Morris L, Fujimoto WY. Visceral adiposity, fasting plasma insulin, and lipid and lipoprotein levels in Japanese Americans. Int J Obes Relat Metab Disord 1996;20:801–808
27. Wander PL, Boyko EJ, Leonetti DL, McNeely MJ, Kahn SE, Fujimoto WY. Change in visceral adiposity independently predicts a greater risk of developing type 2 diabetes over 10 years in Japanese Americans. Diabetes Care 2013;36:289–293
28. Drew BG, Carey AL, Natoli AK, et al. Reconstituted high-density lipoprotein infusion modulates fatty acid metabolism in patients with type 2 diabetes mellitus. J Lipid Res 2011;52:572–581
29. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 2003;46:3–19
30. Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, Shoelson SE; TINSAL-T2D (Targeting Inflammation Using Salsalate in Type 2 Diabetes) Study Team. The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med 2010;152:346–357
31. Gidez LI, Miller GJ, Burstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. J Lipid Res 1982;23:1206–1223
32. Ashmaig ME, Gupta S, McConnell JP, Warnick GR. Validation of a novel homogeneous assay for of HDL3-C measurement. Clin Chim Acta 2013;425:37–41