HDL Cholesterol Subfractions and Risk of Developing Type 2 Diabetes Among Pima Indians

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OBJECTIVE — To examine the relationships between HDL cholesterol subfractions and the incidence of type 2 diabetes and to evaluate potential sex differences in these relationships.

RESEARCH DESIGN AND METHODS — Proportional hazards analyses were performed to examine the relationships between HDL subfractions and the development of type 2 diabetes in Pima Indian women and men. Results were controlled for age, BMI, systolic blood pressure, and 2-h glucose.

RESULTS — Some 54 of 123 women and 25 of 50 men developed type 2 diabetes during a mean follow-up of 10 (2–19) years. For women, in separate models, high levels of total HDL, HDL_{2a}, and HDL_3 were negatively associated with incidence of type 2 diabetes; results were unchanged in models further controlled for fasting insulin level or alcohol consumption. For men, the results were inconsistent and associated with wide confidence intervals; high total HDL and HDL_3 were positively associated with incidence of type 2 diabetes in models further controlled for fasting insulin level, but the risk estimates were attenuated in models further controlled for alcohol consumption.

CONCLUSIONS — High levels of total HDL, HDL_{2a}, and HDL_3 were potential protective factors against type 2 diabetes in women after accounting for alcohol consumption and insulin resistance. High levels of total HDL and HDL_3 were predictive of type 2 diabetes in men; the relationship in men appeared to be due to an association with alcohol consumption. The sex differences in the effects of HDL cholesterol may be related to the effects of sex hormones or lipoproteins.

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Two recent epidemiological studies from different populations (Norwegians and Pima Indians) reported that a high level of HDL cholesterol was a potential protective factor against type 2 diabetes among women, but not among men. These associations persisted in models controlled for age, BMI, blood pressure, glucose, alcohol consumption, physical activity (1), and insulin resistance (2). HDL cholesterol may thus play a direct role in the incidence of type 2 diabetes. However, a residual confounding effect (e.g., by alcohol consumption, physical activity, insulin resistance, abdominal obesity, or sex hormones) may be partially responsible for the relationship between HDL cholesterol and type 2 diabetes in women, and the sex difference may be explained by the different sex hormone effects on HDL metabolism. HDL is a heterogeneous group of particles that can be subdivided based on density into larger and more buoyant HDL_{2b} and HDL_{2a} and smaller, denser HDL_3. Physical activity (3) and alcohol consumption (4) increase both subfractions, but physical activity predominantly affects HDL_2, whereas alcohol consumption predominantly affects HDL_3. Obesity (5) and insulin resistance (6) are associated with low total HDL and HDL_2 and a higher proportion of HDL_3. Exogenous estrogen increases predominantly HDL_2 (7), whereas testosterone may decrease HDL_2 (8). We report here on the relationship between HDL subfractions (HDL_{2b}, HDL_{2a}, and HDL_3) measured in nondiabetic Pima Indians and the subsequent risk of developing type 2 diabetes.

RESEARCH DESIGN AND METHODS

Since 1965, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) has conducted a longitudinal study of type 2 diabetes in the Gila River Indian Community in central Arizona (9). At each participant's biennial examination, blood pressure, height, and weight were measured, and BMI was calculated as weight (in kilograms) divided by the square of height (in meters). Self-reported levels of alcohol consumption were defined as nondrinking, moderate drinking (less than three drinks daily), or heavy drinking (three or more drinks a day, including occasional heavy drinking). A 75-g oral glucose tolerance test was administered after an overnight fast. Type 2 diabetes was diagnosed if the 2-h plasma glucose concentration was ≥11.1 mmol/l (200 mg/dl) (10) or if a glucose concentration ≥11.1 mmol/l was found in the course of routine medical care (11). Plasma insulin concentrations were determined by the Herbert modification of the radioimmunoassay of Berson and Yalow (12). From 1979 to 1982, plasma lipoproteins were measured at fasting for 1,625 participants

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Abbreviations: HRR, hazards rate ratio; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of participants at baseline*

	Women	Men
n	123	50
Age (years)	41 ± 14	42 ± 11
BMI (kg/m²)	32 ± 7	32 ± 7
Systolic blood pressure (mmHg)	117 ± 16	131 ± 21
2-h glucose (mmol/l)	7.1 ± 1.9	6.6 ± 2.0
Fasting insulin (pmol/l)	160 (75–336)	169 (60–360)
Triglycerides (mmol/l)	1.23 (0.70–2.17)	1.49 (0.82–2.61)
Total cholesterol (mmol/l)	4.51 ± 0.85	4.79 ± 0.74
Total HDL (mmol/l)	1.17 (0.83–1.59)	1.14 (0.89–1.62)
HDL_{2b} (mmol/l)	0.15 (0.06–0.37)	0.11 (0.05–0.31)
HDL_{2a} (mmol/l)	0.25 (0.15–0.44)	0.20 (0.10–0.39)
HDL_3 (mmol/l)	0.74 ± 0.15	0.81 ± 0.14
Alcohol consumption		
None	69 (82)	33 (16)
Moderate	26 (31)	48 (23)
Heavy	5 (6)	19 (9)

Data are means ± SD, geometric means (10th–90th percentiles), or % (*n*).

aged ≥15 years. HDL subfractions were measured for the 512 of these participants who had sufficient sera available, of whom 173 (50 men and 123 women) did not have diabetes at baseline and had at least one follow-up examination. Venous blood samples were collected in EDTA after an overnight fast. Plasma was separated after centrifugation at ~700*g* for 15 min at 10°C. A sample was removed for measurement of total cholesterol and triglyceride. Lipoproteins were isolated by using ultracentrifugation procedures previously described (13). Recovery of cholesterol in the lipoprotein fractions isolated by ultracentrifugation averaged 94%. Triglycerides and cholesterol in plasma and isolated lipoproteins were quantified by using an autoanalyzer II (Technicon Instruments, Tarrytown, New York) with the cholesterol extraction method of Rush et al. (14) and the triglyceride enzymatic method of Bucolo and David (15). These assays were standardized with control plasma calibration pools supplied by the Centers for Disease Control and Prevention (CDC) (Atlanta, GA), so that the assays were comparable to those of the Lipid Research Clinics. HDL_{2b}, HDL_{2a}, and HDL_3, the first one being the largest and most buoyant, were isolated from HDL by ultracentrifugation (16). Recovery of total HDL cholesterol in the HDL subfractions isolated by ultracentrifugation averaged 102%. The coefficient of variation for the measurement of control pools was 2.8% for HDL cholesterol. The study was approved by the institutional review board of the NIDDK and by the Gila River Indian Community.

Data were separately analyzed for men and women. Logarithms of total HDL, HDL_{2a}, and HDL_{2b} cholesterol, triglyceride, and fasting insulin were used to normalize the respective distributions. Alcohol consumption was included in the analyses as two dichotomous indicator variables, moderate drinking (yes or no) and heavy drinking (yes or no), which were included together in the models.

The 50 men and 123 women who were selected for these analyses because they had HDL subfraction measurements (and sera available) were compared with the 215 men and 399 women who were included in the previously published analysis (2), for whom no HDL subfraction measurement was made. Men and women with HDL subfraction measurements were older ($P < 0.0001$) than men and women with no measurement, and men with measurements had higher age-adjusted fasting insulin ($P = 0.03$) and glucose levels ($P = 0.02$) than men with no measurement. No difference in total cholesterol, total HDL cholesterol, or triglyceride levels was observed between the two groups of men or women.

The relationship between each baseline lipid measurement and incidence of type 2 diabetes was assessed by Cox's proportional hazards regression analysis (17) in separate models. The hazard rate ratio of developing type 2 diabetes was calculated by comparing the 90th with the 10th percentile of each continuous variable. Interaction terms among predictor variables, evaluated by the likelihood ratio test (18), were not significant. The validity of the proportionality assumption for fixed covariates was determined as suggested by Kalbfleisch and Prentice (19). Proportional hazards models were stratified by 2-h glucose quartiles at baseline because 2-h plasma glucose violated the proportionality assumption.

RESULTS — The characteristics of the 123 women and 50 men at baseline are described in Table 1. During a mean of 10 (range 2–19) years of follow-up, 54 (44%) women and 25 (50%) men developed type 2 diabetes. For women, age (hazards rate ratio [HRR] = 3.3, 95% CI [1.4–7.8]) was a risk factor, as well as 2-h glucose (HRR = 3.6 [1.6–8.0]), systolic blood pressure (HRR = 2.0 [1.0–4.1]), and fasting insulin (HRR = 4.0 [2.1–7.6]), in separate models controlled for age. For men, BMI (HRR = 6.7 [2.3–20]), 2-h glucose (HRR = 5.0 [1.5–17]), and heavy drinking (HRR = 4.7 [1.4–16]) were significant risk factors for type 2 diabetes in separate models controlled for age.

Table 2 presents HRRs and 95% CIs for total HDL and subfractions as risk factors for type 2 diabetes by sex. For women, high levels of total HDL, HDL_{2a}, and HDL_3 were protective factors against type 2 diabetes in separate models controlled for age, BMI, systolic blood pressure, and 2-h glucose; however, the effect of HDL_{2b} was not statistically significant. Further controlling for fasting insulin, alcohol consumption, or total triglyceride level did not modify these results. Excluding women who were pregnant ($n = 2$) or taking oral contraceptives ($n = 4$) or antihypertensive treatment ($n = 9$) also did not modify these results (data not shown).

For men, the relationships between total HDL and subfractions and the incidence of type 2 diabetes was inconsistent and associated with wide confidence intervals. A high level of total HDL cholesterol was positively associated with an increased incidence of type 2 diabetes in a model controlled for age, BMI, systolic blood pressure, and 2-h glucose, but the relationship was only statistically significant when fasting insulin was added to the model. A high level of HDL_3 was a risk factor for diabetes for men in a model controlled for age, BMI, systolic blood pressure, and 2-h glucose. This risk was enhanced when further controlled for fasting insulin, but was not

Table 2— *HRRs and 95% CI for HDL cholesterol subfractions and incidence of type 2 diabetes*

Separate models controlled for	Age	Age, BMI, sBP, and 2-h glucose	Age, BMI, sBP, 2-h glucose and fasting insulin*	Age, BMI, sBP, 2-h glucose and alcohol consumption†
Women				
HDL	0.31 (0.15–0.62)	0.29 (0.13–0.63)	0.32 (0.14–0.73)	0.28 (0.13–0.63)
HDL_{2b}	0.51 (0.23–1.1)	0.48 (0.20–1.2)	0.56 (0.22–1.4)	0.49 (0.20–1.2)
HDL_{2a}	0.32 (0.16–0.63)	0.29 (0.14–0.59)	0.37 (0.18–0.78)	0.30 (0.15–0.60)
HDL_3	0.38 (0.21–0.72)	0.48 (0.25–0.90)	0.46 (0.23–0.92)	0.43 (0.22–0.85)
Men				
HDL	1.1 (0.45–2.6)	3.1 (0.91–11)	6.4 (1.3–31)	1.5 (0.35–6.9)
HDL_{2b}	0.63 (0.23–1.7)	1.2 (0.25–6.2)	1.4 (0.26–7.7)	0.16 (0.01–1.4)
HDL_{2a}	1.1 (0.43–3.1)	2.6 (0.64–10)	4.1 (0.84–20)	1.0 (0.21–4.9)
HDL_3	1.6 (0.64–3.8)	3.8 (1.2–13)	12 (1.8–83)	2.9 (0.69–12)

Data are HRR (95% CI) and are taken from 123 women (54 diabetic patients) and 50 men (25 diabetic patients). Values were obtained from a comparison of the 90th with the 10th percentile using the proportional hazards model. Because of missing values for fasting insulin or alcohol consumption, numbers were *120 women (51 diabetic patients) and 47 men (23 diabetic patients) and †118 women (52 diabetic patients) and 48 men (23 diabetic patients). sBP, systolic blood pressure.

significant in a model controlled for alcohol consumption. HDL_{2a} and HDL_{2b} had no significant effect on the incidence of type 2 diabetes for men.

CONCLUSIONS — In an earlier report (2), we showed that a high level of HDL cholesterol was a potential protective factor for type 2 diabetes among Pima Indian women but not among men, and similar findings were later described in a Norwegian population (1). In a smaller subgroup of the Pima population, we have now found that high levels of total HDL, HDL_{2a}, and HDL_3 were strong potential protectors against type 2 diabetes in women, while high levels of total HDL and HDL_3 were potential risk factors for type 2 diabetes in men in models controlled for age, BMI, systolic blood pressure, 2-h glucose, and fasting insulin. In models that further controlled for alcohol consumption, high levels of total HDL and HDL_3 were less strongly and not significantly predictive of type 2 diabetes in men.

For women, high levels of HDL_{2a} and HDL_3, but not HDL_{2b} (which is the largest, most buoyant, and most cardioprotective subfraction) were potential protectors against type 2 diabetes. However, the subfraction HDL_{2b} corresponds to a very small proportion of HDL among Pima Indians, and HDL_{2a} (which is larger and less dense than HDL_3) had a slightly stronger protective effect in women than did HDL_3. Adjustment for alcohol consumption, which predominantly increases HDL_3 (4), did not affect the relationships between HDL_{2a} and HDL_3 and type 2 diabetes in women. Abdominal obesity (5) and insulin resistance (6) are associated with a decreased concentration of HDL_2 and a higher proportion of HDL_3. However, we controlled for BMI, which has a predictive value for type 2 diabetes similar to that of waist circumference among Pima Indians (20), and for fasting insulin, which is a good estimate of insulin resistance among nondiabetic individuals (21), and HDL_{2a} and HDL_3 were still strong protectors against type 2 diabetes. Data on physical activity, which increases all subfractions, but predominantly HDL_2 (3), were not available for the present analysis. However, the range of physical activity is low among Pima Indian women and is lower than that among men (22). Therefore, physical activity is not likely to be responsible for the relationships with type 2 diabetes found for women.

Other factors not measured in the present study that could be involved in the relationships between HDL subfractions and incidence of type 2 diabetes include dietary and genetic factors and sex hormones. First, sex hormones are more related to HDL_2 than to HDL_3 (7,8), and high HDL_{2a} had a slightly stronger protective effect on type 2 diabetes than HDL_3 did in women. Furthermore, we observed sex differences for the relationships between total HDL (2) and HDL subfractions and incidence of type 2 diabetes; high levels of total HDL and its subfractions were generally protective in women, while they were associated with a high incidence of type 2 diabetes in men. The present finding of an association of high total HDL levels with increased risk for type 2 diabetes in men is at odds with a previous analysis involving a larger group of men (2). Although the subgroup of women did not differ substantially from its original population except for age, the subgroup of men also had higher glucose and insulin levels, and this may partially explain the discrepancies between the present and previous results (2) among men. Furthermore, controlling for alcohol consumption, which predominantly increases HDL_3 (4) and was a risk factor for type 2 diabetes in men in the present analysis, diminished the relationship between total HDL and HDL_3 and type 2 diabetes in men. Moderate to heavy drinking has been reported as a risk factor for type 2 diabetes in men (23) and not in women (23,24) in other populations. The present study suggests that heavy or binge drinking may be a risk factor for diabetes among Pima Indian men. This study also suggests that, if high HDL and HDL_3 levels predicted diabetes in men, this was due to a confounding effect of heavy or binge drinking. However, the analysis of the relationship between HDL subfractions and type 2 diabetes in men was limited by the small number of cases among men. The parameter estimates were unstable and the confidence intervals were wide among men, but not among women, and the present study was therefore inconclusive in men. Finally, it is also possible that high HDL_{2a} and HDL_3 levels directly influence the risk of developing diabetes, which could be mediated by decreasing insulin resistance or affecting insulin secretion.

We suggest that both high levels of HDL_{2a} and HDL_3 are potential protectors against type 2 diabetes in women. The sex difference in the relationship between HDL cholesterol and type 2 diabetes (2) suggests

that sex hormone activity, which may modify both HDL_2 and HDL_3, may be involved in the mechanism. The role of HDL cholesterol subfractions and sex hormone activity on the incidence of type 2 diabetes should be further studied and may explain part of the attenuated sex differences in cardiovascular diseases between diabetic and nondiabetic individuals (25).

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