Non-HDL Cholesterol and Apolipoprotein B Predict Cardiovascular Disease Events Among Men with Type 2 Diabetes

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OBJECTIVE — To evaluate the role of non-HDL cholesterol and apolipoprotein (apo)B, markers of all potentially atherogenic lipoproteins, as predictors of cardiovascular disease (CVD) in comparison with LDL cholesterol in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — We prospectively followed 746 diabetic men in the Health Professionals' Follow-up Study who were aged 46–81 years and free of CVD or cancer at the time of blood draw in 1993–1994. During 6 years of follow-up, we ascertained 103 incident CVD cases.

RESULTS — We used Cox proportional hazard modeling to estimate the relative risk (RR) of CVD. After adjustment for age, BMI, and other lifestyle risk factors, the multivariate RR of CVD (the highest versus the lowest quartile) was 2.34 (95% CI 1.26–4.32) for non-HDL cholesterol, 2.31 (1.23–4.35) for apoB, and 1.74 (0.99–3.06) for LDL cholesterol. Comparisons of nested models indicate that non-HDL cholesterol, but not apoB, adds significantly to the prediction of CVD risk beyond LDL cholesterol. The area under the receiver operating characteristic curve was 0.685, 0.691, 0.695, and 0.722 for the CVD risk-prediction model with LDL cholesterol, apoB, non-HDL cholesterol, and total cholesterol–to–HDL cholesterol ratio (or the non-HDL–to–HDL cholesterol ratio), respectively.

CONCLUSIONS — Non-HDL cholesterol and apoB are more potent predictors of CVD incidence among diabetic men than LDL cholesterol. Statistically, the ratio of total to HDL cholesterol is the best predictor of CVD in this cohort of diabetic men.

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Dyslipidemia is common in diabetes and may contribute significantly to the excess risk of cardiovascular disease (CVD) among patients with type 2 diabetes (1). The dyslipidemia in type 2 diabetic patients is characterized by elevated triglyceride and triglyceride-rich lipoproteins (including very-low-density lipoprotein [VLDL] and intermediate-density lipoprotein [IDL]), decreased HDL cholesterol, and small dense LDL particles (1). Some investigators have suggested that non-HDL cholesterol (LDL + IDL + VLDL cholesterol) may be superior to LDL cholesterol alone as a predictor of CVD among diabetic patients, largely because cholesterol-enriched VLDL and IDL have been shown to be atherogenic in addition to LDL, and the total cholesterol in LDL, IDL, and VLDL may confer a greater CVD risk than LDL cholesterol alone (1–3). The recent National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) has acknowledged the importance of the atherogenic role of non-HDL cholesterol in diabetes and recommends it as the secondary target of cholesterol-lowering therapy in individuals with type 2 diabetes after the primary target of LDL cholesterol (4).

Because there is one apolipoprotein (apo)B molecule per particle of LDL, IDL, and VLDL, total apoB levels highly correlate with non-HDL cholesterol levels (5). However, unlike non-HDL cholesterol, which provides the total cholesterol content of LDL, IDL, and VLDL, apoB reflects the total particle number in these lipoproteins (2). Whether total apoB could be a better measure than LDL cholesterol in predicting CVD risk in diabetic patients, who often have elevated apoB levels, is unclear.

Although growing evidence suggests that non-HDL cholesterol and total apoB are stronger predictors of CVD than LDL cholesterol alone in the general population (6,7), epidemiological data among diabetic individuals are limited. We therefore conducted a prospective study to compare the predictive value of non-HDL cholesterol and apoB with LDL cholesterol in diabetic men from the Health Professionals' Follow-up Study.

RESEARCH DESIGN AND METHODS

— The Health Professionals' Follow-up Study is a prospective cohort study designed to study etiologies of heart disease, cancer, and other major chronic diseases in 51,529 U.S. male health professionals (dentists, veterinarians, pharmacists, optometrists, osteopathic physicians, and podiatrists) aged 40–75 years at study baseline in 1986 (8). Lifestyle factors and health outcomes

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Abbreviations: apo, apolipoprotein; CHD, coronary heart disease; CVD, cardiovascular disease; IDL, intermediate-density lipoprotein; ROC, receiver-operating characteristic; VLDL, very-low-density lipoprotein.

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

have been obtained by questionnaires every 2 years. Dietary information using a food frequency questionnaire has been collected every 4 years. Between 1993 and 1994, 18,159 study participants provided blood samples. Among those with blood samples, 1,000 were diagnosed with definite type 2 diabetes at baseline or during follow-up until 1998 (with the vast majority being diagnosed before or within 2 years after blood collection). The present study included 746 diabetic men who did not report a diagnosis of angina pectoris, myocardial infarction, coronary bypass surgery, or stroke on any of the biennial questionnaires before blood collection.

Based on the diagnostic criteria proposed by the National Diabetes Data Group (9), a diagnosis of diabetes was established when at least one of following criteria was reported on a supplementary questionnaire sent to all men who reported a diagnosis of diabetes on any biennial follow-up questionnaire: *1*) one or more classic symptoms (excessive thirst, polyuria, weight loss, hunger, or coma) plus a fasting plasma glucose concentration of ≥140 mg/dl or a random plasma glucose concentration of ≥200 mg/dl; *2*) at least two elevated plasma glucose concentrations on different occasions (fasting ≥140 mg/dl and/or random ≥200 mg/dl and/or ≥200 mg/dl after 2 h or more on oral glucose tolerance testing) in the absence of symptoms; or *3*) treatment with hypoglycemic medication (insulin or oral hypoglycemic agents). We used the National Diabetes Data Group criteria to define diabetes because our cases were diagnosed before the release of the American Diabetes Association criteria (10). Men with type 1 diabetes were excluded. A validation study in a subsample of the Health Professionals' Follow-up Study demonstrated that our supplementary questionnaire is highly reliable in confirming diabetes diagnosis (11). Among a random sample of 71 men classified by our criteria as having type 2 diabetes according to the information reported on the supplementary questionnaire, medical records were available for 59. A physician blinded to the information reported on the questionnaire reviewed the records. The diagnosis of type 2 diabetes was confirmed in 57 (97%) of the 59 men.

Ascertainment of CVD

The primary outcome of CVD consisted of fatal coronary heart disease (CHD), nonfatal myocardial infarction, fatal stroke, nonfatal stroke, coronary artery bypass, or angioplasty. We asked all men who reported a new diagnosis of nonfatal myocardial infarction or stroke on any biennial follow-up questionnaire to confirm the report and to provide permission to review medical records. Study physicians blinded to questionnaire information for the study participants reviewed the records. Myocardial infarction was confirmed using World Health Organization criteria, requiring symptoms plus either diagnostic electrocardiographic changes or elevated cardiac enzymes (12). Stroke was confirmed using the criteria of the National Survey of Stroke, which require a constellation of neurological deficits, sudden or rapid in onset, lasting at least 24 h or until death (13). Deaths were reported by next of kin, coworkers, or postal authorities or ascertained by a search for nonrespondents using the National Death Index; following up for mortality using these procedures exceeded 98% in a similar cohort study (14).

Laboratory procedures

We sent a phlebotomy kit (including blood tubes, needles, a tourniquet, etc.) and instructions to men willing to provide blood specimens. The participants made arrangements for the blood to be drawn. Blood specimens were collected in three 10-ml liquid EDTA blood tubes, placed on ice packs stored in Styrofoam containers, and returned by overnight mail in a frozen water bottle. After arrival, the blood samples were centrifuged and stored in liquid nitrogen until laboratory analysis. Ninety-seven percent of samples arrived within 26 h of phlebotomy. We requested information on the date and time of the blood sample drawing and the time elapsed since the last meal to identify fasting subjects (>8 h). Quality control samples were routinely frozen along with study samples to monitor long-term stability of plasma samples collected and stored under this protocol. Frozen plasma aliquots from study subjects were selected for simultaneous analysis in 2002 and were analyzed in random order to reduce systematic bias and interassay variation.

The concentrations of total cholesterol, HDL cholesterol, and triglycerides were simultaneously measured on the Hitachi 911 analyzer using reagents and calibrates from Roche Diagnostics (Indianapolis, IN); coefficients of variation for these measurements were <1.8%. LDL cholesterol concentrations were measured by a homogenous direct method from Genzyme (Cambridge, MA). (The LDL cholesterol concentrations calculated using the Friedewald formula were similar to those of the actual measured LDL cholesterol concentrations.) The day-to-day variability at LDL cholesterol concentrations of 90, 106, and 129 mg/dl was <3.1%. ApoB concentrations were measured via an immunonephelometric assay using reagents and calibrators from Wako (Wako Chemicals, Richmond, VA) with a day-to-day variability of <5%. HbA_{1c} concentrations were determined based on turbidimetric immunoinhibition using hemolyzed whole blood or packed red cells. The day-to-day variability at HbA_{1c} concentrations of 5.5 and 9.1% was 1.9 and 3.0%, respectively. We calculated non-HDL cholesterol as the difference between total and HDL cholesterol.

Assessment of lifestyle factors

Participants provided information biennially on their body weight, cigarette smoking, aspirin use, and physical activity. If the weight was missing we used the weight reported on the preceding questionnaire instead. Self-reports of body weight have been shown to be highly correlated with technician-measured weights ($r = 0.96$) in a parallel cohort of female health professionals (15).

We calculated BMI as the ratio of weight (in kilograms) to height (in meters squared), the latter being assessed in 1986 only. Physical activity (MET hours per week) was calculated using the reported time spent on various activities, weighting each activity by intensity level. History of high blood pressure was determined from self-reports preceding the blood collection. Family history of CHD was reported in 1986. Alcohol intake was estimated with a dietary questionnaire in 1994. Participants also provided information biennially on whether they were regularly using cholesterol-lowering drugs.

Statistical analysis

Each participant contributed follow-up time from the reported date of blood draw to the date of first event of CVD or death or 1 December 2000. We first calculated

Table 1—*CVD risk factors at baseline in diabetic men who did and did not develop CVD during follow-up*

	Diabetic men with incident CVD	Diabetic men without CVD events	*P* for difference in mean
n	103	643	
Age (years)	65.3 ± 7.11 (66.0)	62.7 ± 8.58 (63.0)	<0.001
BMI (kg/m^2)*	27.6 ± 3.90 (27.3)	27.9 ± 4.41 (27.1)	0.45
Family history of MI (%)	13.6	12.4	0.74
Physical activity (MET hours/week)*	31.9 ± 35.6 (20.7)	29.3 ± 33.2 (18.5)	0.46
Current smoker (%)	7.77	5.91	0.47
Alcohol consumption (g/day)*	7.68 ± 13.5 (1.90)	9.54 ± 14.5 (3.00)	0.23
Aspirin use (%)	46.6	37.5	0.08
History of hypertension (%)	61.2	44.8	0.002
Fasting (≥8 h) (%)	52.4	54.9	0.64
HbA_{1c} (%)	7.60 ± 1.68 (7.14)	7.29 ± 1.57 (6.88)	0.06
Receiving cholesterol-lowering medications (%)	10.7	8.7	0.52
Total cholesterol (mg/dl)	220 ± 38.4 (215)	209 ± 40.8 (207)	0.02
LDL cholesterol (mg/dl)	135 ± 34.9 (136)	125 ± 36.6 (126)	0.02
HDL cholesterol (mg/dl)	38.3 ± 8.96 (36.9)	41.2 ± 11.3 (39.6)	0.003
Non-HDL cholesterol (mg/dl)†	181 ± 36.0 (176)	168 ± 39.4 (167)	0.002
ApoB (mg/dl)	111 ± 22.3 (110)	104 ± 24.6 (103)	0.01
Triglycerides (mg/dl)	211 ± 102 (193)	187 ± 100 (168)	0.03
Fasting triglycerides (mg/dl)	213 ± 94.1 (192)	177 ± 93.6 (158)	0.01
Ratio of total to HDL cholesterol	5.94 ± 1.29 (5.88)	5.34 ± 1.41 (5.26)	<0.001

Data are means ± SD (median). *For BMI, $n = 98$ case and 617 control subjects; for physical activity, $n = 102$ case and 628 control subjects; for alcohol, $n = 99$ case and 600 control subjects. †Non-HDL cholesterol = total cholesterol − HDL cholesterol. MI, myocardial infarction.

means ± SDs, medians, and proportions of potential CVD risk factors at baseline for 746 diabetic men who did and did not develop CVD during follow-up. Student's *t* tests and χ^2 tests were used for comparisons of the means and the proportions. We divided the distributions of the lipid parameters into quartiles, and quartile-specific relative risks (RRs) of CVD were estimated from Cox proportional hazards models stratified on the 5-year age categories. In multivariate models, we adjusted for conventional CVD risk factors including BMI, family history of myocardial infarction, physical activity, cigarette smoking, alcohol consumption, fasting status (≥8 h), hypertension at baseline, aspirin use, and HbA_{1c}. All of these variables were included in the models as binary indicator variables. To reduce confounding due to lipid-lowering treatment, we further restricted the analyses to those who were not receiving cholesterol-lowering medications. Tests for trend were conducted by modeling the median of categories of each exposure as a continuous variable. Likelihood ratio tests were used for comparisons of nested models. We also calculated the area under the receiver-operating characteristic (ROC) curve to compare the models' ability at discriminating between subjects who do and those who do not experience CVD events (16). The greater the area under the ROC curve, the better the predictivity of the model. Generally, an area under the ROC of 0.5 suggests no discrimination, whereas a maximal ROC of 1 suggests outstanding discrimination. All *P* values were two sided. All analyses were performed with SAS version 8.12 software (SAS Institute, Cary, NC).

RESULTS — In this cohort of 746 diabetic men, 19.2% reported using insulin. In addition, 39.3% reported taking sulfonylureas and 16.1% taking other diabetes medications (metformin, thiazolidinediones, and other medications). The distributions of potential CVD risk factors at baseline among the diabetic men who did and did not develop CVD during follow-up are presented in Table 1. Overall, diabetic men who developed CVD (18 myocardial infarction, 15 stroke, and 70 coronary procedures) were older and tended to have a history of hypertension compared with those who did not develop CVD. Compared with those who did not develop CVD, diabetic men with incident CVD had higher concentrations of non-HDL cholesterol, apoB, LDL cholesterol, total cholesterol, triglycerides, and higher ratio of total to HDL cholesterol and lower concentrations of HDL cholesterol. Distributions of other covariates were not statistically significant different between the two groups.

During the 6 years of follow-up, 103 CVD events occurred in 746 diabetic men. All lipid parameters were strongly positively associated with CVD risk in men with type 2 diabetes, except for HDL cholesterol, which was inversely associated with CVD risk (Table 2). In age-adjusted Cox proportional hazard models, the RRs (the highest quartile versus the lowest quartile) for non-HDL cholesterol (RR 2.25, 95% CI 1.24–4.08) and apoB (2.31, 1.25–4.27) were higher than those for LDL cholesterol, low HDL cholesterol, total cholesterol, and triglycerides but were lower than those for the ratio of total to HDL cholesterol and fasting triglycerides. After adjusting for BMI, family history of myocardial infarction, physical activity, cigarette smoking, alcohol consumption, fasting status, history of hypertension, aspirin use, and HbA_{1c}, the associations of these lipid parameters with CVD risk did not appreciably change (Table 2). Further analyses restricted to

Table 2—*RRs of CVD in diabetic men according to quartiles of blood lipid levels at baseline*

	Median (range)	Cases (*n*)	Age-adjusted RR (95% CI)	Multivariate RR (95% CI)*
Total cholesterol				
Q1	165 (66.0–181)	18	1.0	1.0
Q2	196 (182–208)	25	1.44 (0.79–2.64)	1.40 (0.75–2.60)
Q3	222 (209–237)	28	1.67 (0.92–3.03)	1.75 (0.96–3.21)
Q4	256 (238–347)	32	1.85 (1.04–3.30)	2.02 (1.11–3.69)
P for trend	—	—	0.03	0.02
LDL cholesterol				
Q1	83.4 (11.5–102)	21	1.0	1.0
Q2	116 (103–126)	17	0.84 (0.44–1.59)	0.82 (0.43–1.56)
Q3	138 (127–148)	31	1.47 (0.85–2.56)	1.45 (0.82–2.54)
Q4	166 (149–260)	34	1.63 (0.94–2.81)	1.74 (0.99–3.06)
P for trend	—	—	0.03	0.02
HDL cholesterol				
Q1	30.1 (14.1–33.3)	35	1.0	1.0
Q2	36.2 (33.4–39.1)	27	0.74 (0.45–1.23)	0.71 (0.42–1.19)
Q3	42.1 (39.2–46.8)	21	0.53 (0.31–0.92)	0.55 (0.31–0.96)
Q4	52.7 (46.9–97.4)	20	0.51 (0.29–0.88)	0.57 (0.32–1.04)
P for trend	—	—	0.01	0.05
Non-HDL cholesterol				
Q1	127 (51.9–143)	16	1.0	1.0
Q2	156 (144–167)	24	1.49 (0.79–2.81)	1.40 (0.73–2.68)
Q3	179 (168–194)	29	1.85 (1.00–3.40)	1.85 (0.99–3.45)
Q4	215 (195–317)	34	2.25 (1.24–4.08)	2.34 (1.26–4.32)
P for trend	—	—	0.005	0.004
ApoB				
Q1	78.1 (33.0–87.8)	15	1.0	1.0
Q2	95.6 (87.9–102)	24	1.61 (0.85–3.08)	1.58 (0.82–3.04)
Q3	111 (103–119)	32	2.22 (1.20–4.10)	2.13 (1.14–3.98)
Q4	132 (119–199)	32	2.31 (1.25–4.27)	2.31 (1.23–4.35)
P for trend	—	—	0.005	0.006
Triglycerides				
Q1	86.0 (37.0–111)	18	1.0	1.0
Q2	141 (112–170)	23	1.23 (0.66–2.28)	1.24 (0.67–2.33)
Q3	207 (171–249)	30	1.65 (0.92–2.96)	1.63 (0.89–3.01)
Q4	314 (250–877)	32	1.99 (1.11–3.55)	1.97 (1.08–3.61)
P for trend	—	—	0.01	0.02
Fasting triglycerides				
Q1	79.0 (37.0–107)	7	1.0	1.0
Q2	131 (108–164)	9	1.74 (0.67–4.49)	1.81 (0.68–4.84)
Q3	191 (171–241)	20	3.02 (1.26–7.24)	3.48 (1.36–8.91)
Q4	294 (242–517)	18	3.45 (1.43–8.31)	3.73 (1.45–9.60)
P for trend	—	—	0.002	0.014
Ratio of total to HDL cholesterol				
Q1	3.86 (2.02–4.40)	11	1.0	1.0
Q2	4.85 (4.41–5.31)	27	2.32 (1.15–4.68)	2.23 (1.09–4.53)
Q3	5.82 (5.32–6.25)	23	2.15 (1.05–4.41)	1.94 (0.92–4.06)
Q4	7.01 (6.26–11.5)	42	4.56 (2.34–8.88)	4.26 (2.14–8.48)
P for trend	—	—	<0.001	<0.001

*Controlled for age (in 5-year categories), BMI (<23, 23–24, 25–27, 28–30, ≥31 kg/m^2, and missing), family history of myocardial infarction (yes or no), physical activity (in quartiles), cigarette smoking (never, past, or current), alcohol consumption (0, 0.1–4.9, 5.0–9.9, 10.0–14.9, and ≥15.0 g/day), fasting status (yes or no), history of hypertension (yes or no), aspirin use (yes or no), and HbA_{1c} (quartiles).

diabetic men not receiving cholesterol-lowering medications yielded similar results. Among those who did not receive cholesterol-lowing medications, the multivariate RR comparing the highest versus lowest quartile was 2.23 (95% CI 1.13–4.40) for non-HDL cholesterol, 2.34 (1.19–4.61) for apoB, 1.64 (0.87–3.09) for LDL cholesterol, 0.53 (0.28–0.99) for HDL cholesterol, 1.85 (0.95–3.59) for total cholesterol, 1.97 (1.03–3.72) for triglycerides, 3.32 (1.23–8.98) for fasting

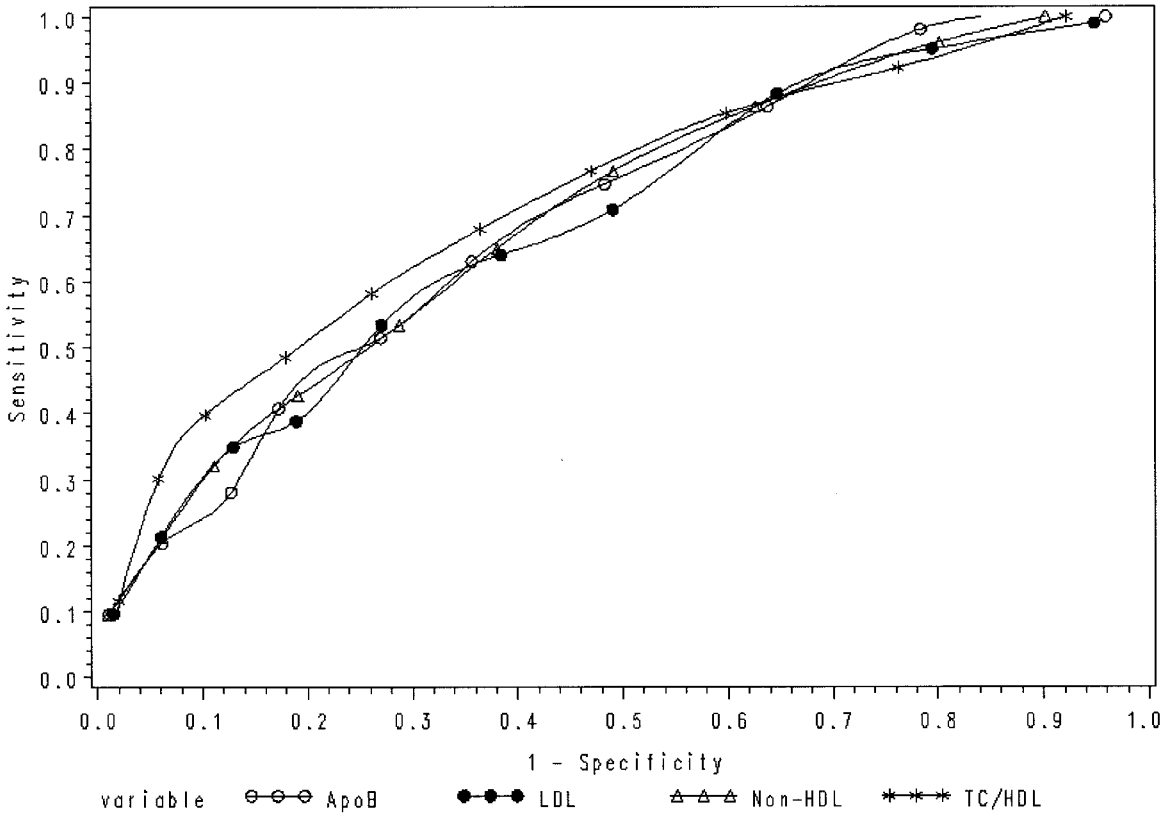

Figure 1—*ROC curves for LDL cholesterol, apoB, non-HDL cholesterol, and the total cholesterol–to–HDL cholesterol ratio deciles. The area under the ROC curve was 0.685, 0.691, 0.695, and 0.722 for the CVD risk-prediction model with LDL cholesterol, apoB, non-HDL cholesterol, and the ratio of total to HDL cholesterol, respectively. The CVD risk prediction model included age (in 5-year categories), BMI (<23, 23–24, 25–27, 28–30, ≥31 kg/m^2, and missing), family history of myocardial infarction (yes or no), physical activity (quartiles), cigarette smoking (never, past, or current), alcohol consumption (0, 0.1–4.9, 5.0–9.9, 10.0–14.9, and ≥15.0 g/day), fasting status (yes or no), history of hypertension (yes or no), aspirin use (yes or no), and HbA_{1c} (quartiles), and LDL cholesterol, apoB, non-HDL cholesterol, or the ratio of total to HDL cholesterol.*

triglycerides, and 4.09 (1.96–8.51) for the total cholesterol–to–HDL cholesterol ratio.

To determine whether the predictive value of non-HDL cholesterol and apoB was independent of LDL cholesterol, we conducted log likelihood ratio tests by comparing two nested models, one model with LDL and other CVD risk factors and the other model with LDL and other CVD risk factors plus non-HDL cholesterol or apoB. The test results indicate that non-HDL cholesterol, but not apoB, adds significantly to the prediction of CVD risk beyond LDL cholesterol and other CVD risk factors in the multivariate model (χ^2 for adding non-HDL cholesterol = 6.90, $P = 0.01$; χ^2 for adding apoB = 0.80, $P = 0.37$).

We further compared the predictive value of non-HDL cholesterol or apoB among diabetic individuals with triglycerides levels <200 and ≥200 mg/dl. The associations of non-HDL cholesterol with CVD risk or apoB with CVD risk were stronger among diabetic men with lower triglyceride levels than those with higher triglyceride levels. However, tests for interaction were not statistically significant ($P > 0.05$) (data not shown).

We also plotted the ROC curves for LDL cholesterol, apoB, non-HDL cholesterol, and the total/HDL cholesterol deciles (Fig. 1). The area under the ROC curve for the CVD risk-prediction model with non-HDL cholesterol or apoB was larger than that for LDL cholesterol, indicating a better risk prediction for non-HDL cholesterol and apoB than for LDL cholesterol. The area under the ROC curve was largest for the total cholesterol–to–HDL cholesterol ratio, indicating the model with this ratio was superior to the other CVD risk-prediction models. The area with the ROC curve for the total cholesterol–to–HDL cholesterol ratio was also larger than that for the ratio of LDL to HDL cholesterol (0.697).

CONCLUSIONS — In this prospective cohort of 746 diabetic men aged 46–81 years with no previous history of CVD at the beginning of this study (time of blood draw), higher levels of non-HDL cholesterol and apoB at baseline were associated with a significantly increased future risk of CVD independent of BMI and other lifestyle CVD risk factors. Non-HDL cholesterol and apoB appeared to be stronger predictors of CVD risk than LDL cholesterol in patients with type 2 diabetes. The joint effect of total cholesterol and HDL cholesterol (the total cholesterol–to–HDL cholesterol ratio) was most strongly associated with CVD risk.

Non-HDL cholesterol has also been shown to be a strong predictor of CVD risk in patients with type 2 diabetes in two other studies. In the Strong Heart Study cohort (17), non-HDL cholesterol was a strong predictor of CVD in 2,108 American Indian men and women aged 45–74 years with diabetes during an average of 9

years of follow-up. The hazard ratios for the highest tertile of non-HDL cholesterol in diabetic men and women (2.23 and 1.80, respectively) were higher than those for either LDL cholesterol or triglycerides alone, although the CIs were overlapping. In a Finnish cohort study (18) of 1,059 middle-aged men and women with type 2 diabetes, higher levels of non-HDL cholesterol, as well as low HDL cholesterol and triglyceride levels, were each independently associated with a twofold increase in the risk of CHD morbidity or mortality during 7 years of follow-up. The association between LDL and CHD morbidity was weaker, and LDL cholesterol was not a significant predictor of CHD mortality. However, in the study, no direct comparison was made between LDL cholesterol and non-HDL cholesterol for the predictive values of CHD. Our findings that non-HDL cholesterol significantly predicts CVD risk beyond LDL cholesterol in diabetic men provide strong evidence supporting that non-HDL cholesterol could be a useful marker in CVD risk assessment in diabetic patients. Although several studies indicate that elevated apoB predicts an RR for CHD more than LDL cholesterol in the general population (19), to our knowledge there have been no previous population studies evaluating the value of apoB in predicting CVD risk in diabetic individuals.

Non-HDL cholesterol or apoB may be superior to LDL cholesterol in diabetic patients for several reasons (2,3). First, diabetes is often concomitant with many lipid and lipoprotein abnormalities characterized mainly by elevated VLDL, IDL, and chylomicrons, all of which are potentially atherogenic. The use of only LDL cholesterol ignores the significant contribution of atherogenic VLDL and IDL cholesterol to CVD. Second, the LDL cholesterol level is usually calculated from the Friedewald formula based on the measurement of total cholesterol, HDL cholesterol, and triglycerides (though in our study it was measured directly) (20). However, the Friedewald formula requires a fasting triglyceride level <400 mg/dl in order to accurately calculate LDL cholesterol. Because of elevated triglyceride levels in diabetic patients, the calculated LDL cholesterol level is likely to be unreliable. On the other hand, non-HDL cholesterol can be easily calculated from the total and HDL cholesterol levels without the limitation of hypertriglyceridemia, thus also eliminating the cost for additional lipid measurements. Furthermore, neither non-HDL cholesterol nor apoB measurements require fasting samples. In clinical practice, non-HDL cholesterol level can be used as a reasonable surrogate for apoB because of their high correlation and similar predictive role. Non-HDL cholesterol provides an even simpler and more familiar approach to quantify all atherogenic lipoproteins, although the methodology for measuring apoB is improving and becoming more widely available (2).

Consistent with previous studies of largely nondiabetic populations (21–25), the ratio of total to HDL cholesterol is the strongest lipid predictor for CVD in our diabetic cohort. This is not surprising because the ratio involves a joint or synergistic effect of two variables that have opposite biological effects. Of note, the total cholesterol–to–HDL cholesterol ratio is mathematically equivalent to the ratio of non-HDL to HDL cholesterol.

One limitation of our study was the relatively small sample size, particularly for fasting samples. For example, in the analyses comparing lipid parameters for predicting CVD risk, the RR comparing the highest versus the lowest quartiles of triglycerides was lower than the corresponding RR for non-HDL or apoB but was higher when limited to those who were fasting. The analyses of fasting samples should be interpreted with caution because the reference group (the lowest quartile) for triglycerides in the fasting sample had only seven CVD events, which could lead to unstable estimates. Also, we were unable to examine CHD and stroke separately but used a single outcome that combined the two. Nonetheless, the analyses with and without stroke as an end point yielded essentially the same results. Another issue deserving attention is that the diagnostic criteria for type 2 diabetes used in this study were changed in 1997 so that a lower fasting glucose level (≥126 mg/dl) would now be considered the diagnostic cutoff point. However, we believe that our results would apply to most diabetic patients because only a small fraction of diabetes cases would meet the newer American Diabetes Association criteria but not the National Diabetes Data Group criteria (fasting glucose between 126 and 140 mg/dl).

In conclusion, our data show that non-HDL cholesterol and apoB are more potent predictors of CVD incidence than LDL cholesterol among diabetic men. The potential value of using non-HDL cholesterol or apoB instead of LDL cholesterol as a primary target for cholesterol-lowing therapy in diabetic patients needs to be investigated further.

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References

1. Garg A, Grundy SM: Management of dyslipidemia in NIDDM. *Diabetes Care* 13: 153–169, 1990
2. Grundy SM: Low-density lipoprotein, non-high-density lipoprotein, and apolipoprotein B as targets of lipid-lowering therapy. *Circulation* 106:2526–2529, 2002
3. Hsia SH: Non-HDL cholesterol: into the spotlight. *Diabetes Care* 26:240–242, 2003
4. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 285:2486–2497, 2001
5. Abate N, Vega GL, Grundy SM: Variability in cholesterol content and physical properties of lipoproteins containing apolipoprotein B-100. *Atherosclerosis* 104:159–171, 1993
6. Frost PH, Davis BR, Burlando AJ, Curb JD, Guthrie GP Jr, Isaacsohn JL, Wassertheil-Smoller S, Wilson AC, Stamler J: Serum lipids and incidence of coronary heart disease: findings from the Systolic Hypertension in the Elderly Program (SHEP). *Circulation* 94:2381–2388, 1996
7. Cui Y, Blumenthal RS, Flaws JA, Whiteman MK, Langenberg P, Bachorik PS, Bush TL: Non-high-density lipoprotein cholesterol level as a predictor of cardiovascular disease mortality. *Arch Intern Med* 161:1413–1419, 2001
8. Rimm EB, Giovannucci EL, Willett WC, Colditz GA, Ascherio A, Rosner B, Stampfer MJ: Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet* 338:464–468, 1991
9. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039–1057, 1979
10. 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* 20:1183–1197, 1997
11. Hu FB, Leitzmann MF, Stampfer MJ, Colditz GA, Willett WC, Rimm EB: Physical activity and television watching in relation to risk for type 2 diabetes mellitus in men. *Arch Intern Med* 161:1542–1548, 2001
12. Rose G, Blackburn H: *Cardiovascular Survey Methods*. Geneva, World Health Organization, 1982 (monogr. no. 58)
13. Walker AE, Robins M, Weinfeld FD: The National Survey of Stroke: clinical findings. *Stroke* 12 (Supp. 1):I13–I44, 1981
14. Rich-Edwards JW, Corsano KA, Stampfer MJ: Test of the National Death Index. *Am J Epidemiol* 119:837–839, 1984
15. Willett W, Stampfer MJ, Bain C, Lipnick R, Speizer FE, Rosner B, Cramer D, Hennekens CH: Cigarette smoking, relative weight, and menopause. *Am J Epidemiol* 117:651–658, 1983
16. Hosmer DW, Lemeshow S: *Applied Logistic Regression*. New York, John Wiley & Sons, 2000
17. Lu W, Resnick HE, Jablonski KA, Jones KL, Jain AK, Howard WJ, Robbins DC, Howard BV: Non-HDL cholesterol as a predictor of cardiovascular disease in type 2 diabetes: the Strong Heart Study. *Diabetes Care* 26:16–23, 2003
18. Lehto S, Ronnemaa T, Haffner SM, Pyorala K, Kallio V, Laakso M: Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM. *Diabetes* 46:1354–1359, 1997
19. Sniderman AD: Counterpoint: to (measure apo) B or not to (measure apo) B: a critique of modern medical decision-making. *Clin Chem* 43:1310–1314, 1997
20. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502, 1972
21. Kinosian B, Glick H, Garland G: Cholesterol and coronary heart disease: predicting risks by levels and ratios. *Ann Intern Med* 121:641–647, 1994
22. Kinosian B, Glick H, Preiss L, Puder KL: Cholesterol and coronary heart disease: predicting risks in men by changes in levels and ratios. *J Investig Med Oct* 43:443–450, 1995
23. Kannel WB, Wilson PW: Efficacy of lipid profiles in prediction of coronary disease. *Am Heart J* 124:768–774, 1992
24. Hong MK, Romm PA, Reagan K, Green CE, Rackley CE: Usefulness of the total cholesterol to high-density lipoprotein cholesterol ratio in predicting angiographic coronary artery disease in women. *Am J Cardiol* 68:1646–1650, 1991
25. Criqui MH, Golomb BA: Epidemiologic aspects of lipid abnormalities. *Am J Med* 105:48S–57S, 1998