Non-HDL Cholesterol as a Predictor of Cardiovascular Disease in Type 2 Diabetes

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OBJECTIVE — To determine whether non-HDL cholesterol, a measure of total cholesterol minus HDL cholesterol, is a predictor of CVD in patients with diabetes.

RESEARCH DESIGN AND METHODS — The Strong Heart Study, a population-based study of CVD and its risk factors in 13 American Indian communities in three geographic areas in the U.S. The baseline examination, conducted between July 1989 and January 1992, consisted of a personal interview, a physical examination, and laboratory tests. Of the 4,549 women and men aged 45–74 years participating in the study, 2,108 had diabetes but no CVD at baseline. Data on fatal and nonfatal CVD were collected during the follow-up period through 31 December 1998 (average 9 years).

RESULTS — Multivariable analyses indicated that non-HDL cholesterol is a strong predictor of CVD in men and women with diabetes and is particularly indicative of coronary events. Hazard ratios for the highest tertile of non-HDL cholesterol in men and women with diabetes (2.23 and 1.80, respectively) were higher than those for either LDL cholesterol or triglycerides alone in both men and women and were higher than the ratio of total/HDL cholesterol in women. The utility of non-HDL cholesterol in predicting CVD extended over a wide range of triglyceride concentrations.

CONCLUSIONS — This study suggests that non-HDL cholesterol index may be particularly useful in predicting CVD risk in patients with diabetes.

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Cardiovascular disease (CVD) is currently the primary cause of morbidity and mortality in patients with diabetes (1–4). Because individuals with diabetes have greatly increased CVD risk compared with nondiabetic individuals (5–9), it is important to identify factors that may increase CVD risk in diabetic patients. In type 2 diabetes, there is a characteristic dyslipidemia consisting of elevated triglycerides, decreased HDL cholesterol, and LDL particles of altered composition (10–12). Previous studies (5–9,13–15) indicate that, in addition to LDL cholesterol level, this dyslipidemia is an important CVD risk factor in individuals with diabetes. Although the CVD risk associated with individual lipoproteins has been examined, it would be valuable to have a measure that reflects the combined risk of all lipoprotein changes observed in diabetes. Some investigators (16–18) have recently suggested that a measure of non-HDL cholesterol, which reflects total cholesterol minus HDL cholesterol (i.e., all apolipoprotein B–containing atherogenic lipoproteins), might be a useful marker of this combined risk. A recent study conducted in a cohort containing both diabetic and nondiabetic individuals showed that non-HDL cholesterol was a somewhat better predictor of CVD than LDL cholesterol (19). Furthermore, the Adult Treatment Panel (ATP-III) of the National Cholesterol Education Program has recommended using non-HDL cholesterol in assessing CVD risk in patients with diabetes (20). However, there have been no population-based studies evaluating the utility of non-HDL cholesterol as a predictor of CVD in patients with diabetes.

The Strong Heart Study is a population-based study of CVD and its risk factors in American Indians. This population has a high prevalence of diabetes, and those with diabetes have a greatly increased risk of CVD (5). Using the data from this population, the purpose of this study is to evaluate the ability of non-HDL cholesterol and individual lipoprotein indicators to predict CVD in patients with diabetes.

RESEARCH DESIGN AND METHODS — The study design, survey methods, and laboratory techniques of the Strong Heart Study have been reported previously in detail (21,22). The baseline examination included American
Indians aged 45–74 years who were residents of the following tribes between July 1989 and January 1992: the Akimel O’odham (Pima), Pee Posh (Marnecopa), and Tohono-Oodham (Papago) tribes of central Arizona who live in the Gila River, Salt River, and Ak-Chin communities; the seven tribes of southwestern Oklahoma (Apache, Caddo, Comanche, Delaware, Fort Sill Apache, Kiowa, and Wichita); and the Ogala and Cheyenne River Sioux in South Dakota and the Spirit Lake Community in the Fort Totten area of North Dakota (23).

The baseline examination consisted of personal interviews, physical examinations, laboratory tests, and 12-lead resting electrocardiograms of 4,549 men and women. Participants were examined in the morning after at least a 12-h overnight fast. After informed consent was obtained, fasting blood samples were collected for measurement of lipid and lipoprotein levels. A 75-g oral glucose tolerance test was administered, and plasma lipoprotein levels. A 75-g oral glucose tolerance test was administered, and plasma lipoprotein levels were measured 2 h after the glucose load (21). All laboratory tests were performed centrally. Standard assays were used to measure plasma glucose (24), cholesterol (24), triglyceride (24), lipid, and lipoprotein levels (25), fibrinogen determinations (26); Hba1c (27), urinary albumin (28), and urinary creatinine levels (29); and LDL particle size (30).

Anthropometric measurements were made with the participant wearing light-weight clothing and no shoes (22). BMI was calculated as weight (in kilograms)/height (in meters) squared. Waist circumference was measured at the level of the umbilicus with the participant in the supine position (31). Participants were considered to have hypertension if systolic blood pressure (SBP) was ≥140 mmHg or diastolic blood pressure (DBP) was ≥90 mmHg or if they were taking antihypertensive medication.

Diabetes was determined by World Health Organization recommendations (32), i.e., treatment with insulin or oral hypoglycemic agents, fasting plasma glucose level ≥126 mg/dl, or 2-h plasma glucose level ≥200 mg/dl after a 75-g glucose tolerance test.

Of the 4,549 participants examined at baseline, for 4,168 patients, diabetes status had been determined, lipoprotein measurements were available, and no CVD was present. Of these individuals, 2,108 had diabetes but no CVD at baseline and were included in this analysis. Total deaths and fatal CVD occurring between baseline and 31 December 1998 were identified through death certificates and tribal and Indian Health Service hospital records and by direct contact of study personnel with the study participants and their families. The process used to confirm CVD deaths has been described previously (21). Primary CVD deaths included myocardial infarction (MI), sudden cardiac death, coronary heart disease (CHD), stroke, congestive heart failure, and other fatal CVD. For nonfatal CVD, medical histories or medical records were reviewed during the second and third examinations to ascertain nonfatal CVD events that had occurred during follow-up. The process used to confirm nonfatal CVD events has been described previously (21). The nonfatal CVD events used in this study were CHD, MI, stroke, and other CVD. All fatal and nonfatal events were reviewed by committee following standard criteria to classify CVD (33).

Data were analyzed using SAS statistical software (Version 8.1; SAS Institute, Cary, NC). Incidence rates for fatal and nonfatal CVD were calculated per 1,000 person-years. Person-years were calculated from the date of the baseline examination to the date of the fatal event, the date of death, or the date of the last known follow-up examination.
Non-HDL cholesterol and CVD in diabetes

Table 2—Adjusted HRs for overall CVD by tertiles of non-HDL cholesterol in diabetic American Indians: the Strong Heart Study

<table>
<thead>
<tr>
<th>Tertiles*</th>
<th>Men</th>
<th></th>
<th>Women</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-HDL cholesterol (mg/dl)</td>
<td>Case/person-year†</td>
<td>HR (95% CI)#</td>
<td>Case/person-year§</td>
<td>HR (95% CI)#</td>
</tr>
<tr>
<td>T-1</td>
<td>57/1,894</td>
<td>1</td>
<td>78/3,404</td>
<td>1</td>
</tr>
<tr>
<td>T-2</td>
<td>56/1,883</td>
<td>1.02 (0.69–1.52)</td>
<td>105/3,344</td>
<td>1.33 (0.96–1.83)</td>
</tr>
<tr>
<td>T-3</td>
<td>89/1,654</td>
<td>2.23 (1.41–3.43)</td>
<td>136/3,123</td>
<td>1.80 (1.32–2.46)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>T-1</td>
<td>89/1,789</td>
<td>1</td>
<td>106/3,314</td>
</tr>
<tr>
<td>T-2</td>
<td>64/1,862</td>
<td>0.76 (0.54–1.07)</td>
<td>121/3,509</td>
<td>1.02 (0.77–1.36)</td>
</tr>
<tr>
<td>T-3</td>
<td>49/1,780</td>
<td>0.59 (0.40–0.88)</td>
<td>92/3,048</td>
<td>0.97 (0.71–1.32)</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>T-1</td>
<td>57/1,905</td>
<td>1</td>
<td>88/3,364</td>
</tr>
<tr>
<td>T-2</td>
<td>70/1,794</td>
<td>1.34 (0.89–1.92)</td>
<td>103/3,327</td>
<td>1.23 (0.91–1.68)</td>
</tr>
<tr>
<td>T-3</td>
<td>73/1,732</td>
<td>1.71 (1.17–2.48)</td>
<td>128/3,180</td>
<td>1.61 (1.19–2.17)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>T-1</td>
<td>56/1,875</td>
<td>1</td>
<td>78/3,428</td>
</tr>
<tr>
<td>T-2</td>
<td>68/1,798</td>
<td>1.40 (0.94–2.07)</td>
<td>110/3,324</td>
<td>1.36 (0.99–1.87)</td>
</tr>
<tr>
<td>T-3</td>
<td>78/1,758</td>
<td>1.39 (1.00–1.98)</td>
<td>131/3,119</td>
<td>1.61 (1.17–2.22)</td>
</tr>
<tr>
<td>Ratio of total/HDL cholesterol</td>
<td>T-1</td>
<td>43/1,937</td>
<td>1</td>
<td>86/3,401</td>
</tr>
<tr>
<td>T-2</td>
<td>68/1,838</td>
<td>1.85 (1.23–2.79)</td>
<td>102/3,313</td>
<td>1.16 (0.85–1.59)</td>
</tr>
<tr>
<td>T-3</td>
<td>91/1,655</td>
<td>2.46 (1.65–3.68)</td>
<td>131/3,157</td>
<td>1.48 (1.09–2.00)</td>
</tr>
<tr>
<td>Total</td>
<td>202/5,431</td>
<td>319/9,871</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


first nonfatal event, or 31 December 1998 in event-free individuals. Subsequent analyses focused on comparisons between diabetic individuals who did and did not develop CVD. ANOVA was used for continuous variables and the chi-square test was used for categorical variables.

Cox multivariate regression models were used to calculate the hazard ratios (HRs) and 95% CIs for tertiles of baseline non-HDL cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and ratio of total to HDL cholesterol to evaluate their independent roles as predictors of fatal and nonfatal CVD by sex, adjusted for confounding variables. Cox models were also used to calculate HRs and 95% CIs for non-HDL cholesterol and other levels to assess the association with incidence of CHD, MI, stroke, and all CVD in patients with diabetes. Kaplan-Meier plots for the proportion of patients with diabetes in whom CVD developed over 9 years of follow-up were estimated using the product-limit method. Statistical significance was defined either as P < 0.05 or 95% CIs for HRs that did not include 1.0. Because of their severely skewed distributions, insulin, triglycerides, and ratio of albumin to creatinine were log-transformed for statistical analyses.

RESULTS—Univariate comparisons of baseline CVD risk factors in patients with diabetes in whom CVD did and did not develop during follow-up are shown in Table 1. Compared with those who did not develop CVD, men and women with diabetes in whom CVD developed were older and had higher HbA1c and SBP. Baseline total cholesterol, LDL cholesterol, triglycerides, VLDL triglycerides, VLDL cholesterol, albumin/creatinine ratio, fibrinogen, non-HDL cholesterol, and total/HDL cholesterol ratio were all significantly higher in patients with diabetes in whom CVD developed than in those with no CVD.

During the 9-year follow-up, CVD developed in 521 of the 2,108 diabetic participants and 145 of the 2,060 nondiabetic participants. The incidence of fatal and nonfatal CVD was 34.1/1,000 person-years (37.2 in men and 32.3 in women) in diabetic subjects and 8.9 in nondiabetic subjects (18.3 in men and 2.0 in women). The highest and middle tertiles of the lipoprotein parameters were compared with the lowest tertile in relation to CVD risk after adjustment for age, BMI, smoking status, study center, SBP, HbA1c, fibrinogen, insulin, and albumin/creatinine ratio (Table 2). Although lipoprotein parameters were all significant predictors of CVD risk in men and women with diabetes (except HDL cholesterol in women), non-HDL cholesterol seemed to be the stronger predictor (except for total/HDL cholesterol ratio in men), with an HR of 2.23 (95% CI 1.41–3.43) in men and an HR of 1.80 (1.32–2.46) in women. A Kaplan-Meier plot by tertiles of non-HDL cholesterol (Fig. 1) shows continuous effects of non-HDL cholesterol on CVD risk in patients with diabetes over the 9-year follow-up period, especially for those in the highest tertile of non-HDL cholesterol.

Analyses of CVD subclasses were conducted in men and women combined. Increasing non-HDL cholesterol concentrations had significant, curvilinear relationships with CVD and CHD risk (P < 0.001) (Fig. 2). Compared with the refer-
ence tertile, after adjustment for covariates (Table 3), non-HDL cholesterol was associated with a higher HR for MI than any of the lipid parameters and was higher than all but total/HDL ratio for CHD. However, there was overlap in the CIs.

Finally, we also compared the predictive value of non-HDL cholesterol for CHD and CVD in participants with triglyceride levels >150 and <150 mg/dl. The predictive value of non-HDL cholesterol was not greater in those with fasting triglyceride levels >150 mg/dl (Table 4). Similar results were observed for those with triglyceride levels ≥200 mg/dl, but the number of events was not adequate for stable estimates (data not shown).

**CONCLUSIONS** — The Adult Treatment Panel III of the National Cholesterol Education Program recently recommended that non-HDL cholesterol be used as a secondary target of therapy in people with triglyceride levels >200 mg/dl, especially those with diabetes or the metabolic syndrome (20). There are several advantages to the non-HDL cholesterol measurement. First, it makes no assumption about the relationship between VLDL cholesterol and triglycerides; in patients with diabetes, this relationship can be altered, leading to falsely low LDL values as calculated by the Friedewald formula, especially in conjunction with elevated triglyceride levels. Second, non-HDL cholesterol includes an assessment of all apolipoprotein B–containing lipoproteins considered to be atherogenic, i.e., VLDL, intermediate-density lipoprotein (IDL), and LDL, and even lipoprotein(a). Finally, non-HDL cholesterol has several practical advantages in a clinical setting, including the ability to be assessed in patients with triglyceride levels >400 mg/dl and in patients who are not fasting (34–41).

Diabetes is associated with greatly increased CVD. Although many factors play a role in the accelerated atherosclerosis observed in diabetes, lipoprotein abnormalities are key contributors. LDL, the main cholesterol-bearing lipoprotein, is a major determinant of atherosclerosis in patients with diabetes. Whereas average LDL concentrations in patients with diabetes may not be higher than those of their nondiabetic counterparts, changes in LDL particle composition, such as density, oxidation potential, and glycation, render even normal LDL levels highly atherogenic (10,42). Other lipoprotein abnormalities in patients with diabetes include changes in triglyceride-rich lipoproteins (43,44). VLDL remnants and IDL accumulate as a result of altered lipoprotein metabolism; both types of particles have been shown to be highly atherogenic. Also, remnant triglyceride-rich lipoproteins can be taken up by macrophages, leading to increased foam cell formation and accelerated atherosclerosis in patients with elevated triglyceride levels (16). Elevated VLDL is also associated with increases in prothrombotic and procoagulant factors. Because of the many lipoprotein abnormalities in diabetes, an easily measured composite indicator may be useful to clinicians who treat patients with diabetes.

The Strong Heart Study cohort is an ideal population to assess the utility of non-HDL cholesterol in predicting CVD in patients with diabetes. This population includes a large number of individuals with type 2 diabetes who have been under continued surveillance since 1989. Our data show that in both men and women with diabetes, non-HDL cholesterol is a strong predictor of CVD; although the CIs were overlapping, HRs are higher than those for either LDL cholesterol or triglycerides alone. When compared with the ratio of total/HDL cholesterol, a compos-
### Table 3: Adjusted HRs for CVD by tertiles of non-HDL cholesterol in American Indians with diabetes: the Strong Heart Study

<table>
<thead>
<tr>
<th>Stratum</th>
<th>Non-HDL cholesterol</th>
<th>CHD</th>
<th>MI</th>
<th>Stroke</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case/Person-year HR (95% CI)</td>
<td>Case/Person-year HR (95% CI)</td>
<td>Case/Person-year HR (95% CI)</td>
<td>Case/Person-year HR (95% CI)</td>
<td>Case/Person-year HR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>706</td>
<td>42/7/203</td>
<td>6/247</td>
<td>9/271</td>
<td>2,108</td>
</tr>
<tr>
<td>Person-year</td>
<td>1</td>
<td>42/7/203</td>
<td>6/247</td>
<td>9/271</td>
<td>2,108</td>
</tr>
<tr>
<td>&lt; 127 mg/dl</td>
<td>714</td>
<td>67/65/274</td>
<td>6/160</td>
<td>8/156</td>
<td>1,006</td>
</tr>
<tr>
<td>127–161 mg/dl</td>
<td>714</td>
<td>67/65/274</td>
<td>6/160</td>
<td>8/156</td>
<td>1,006</td>
</tr>
<tr>
<td>&gt; 161 mg/dl</td>
<td>688</td>
<td>57/51/231</td>
<td>6/155</td>
<td>8/150</td>
<td>957</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>47/52</td>
<td>6/58</td>
<td>8/60</td>
<td>10/60</td>
<td>100</td>
</tr>
<tr>
<td>&lt; 39 mg/dl</td>
<td>777</td>
<td>64/70/268</td>
<td>6/170</td>
<td>8/168</td>
<td>1,176</td>
</tr>
<tr>
<td>39–47 mg/dl</td>
<td>684</td>
<td>60/64/257</td>
<td>6/157</td>
<td>8/155</td>
<td>1,002</td>
</tr>
<tr>
<td>&gt; 47 mg/dl</td>
<td>647</td>
<td>57/52/230</td>
<td>6/155</td>
<td>8/150</td>
<td>953</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>97</td>
<td>7/53</td>
<td>8/53</td>
<td>10/53</td>
<td>100</td>
</tr>
<tr>
<td>&lt; 91 mg/dl</td>
<td>728</td>
<td>57/51/231</td>
<td>6/155</td>
<td>8/150</td>
<td>957</td>
</tr>
<tr>
<td>91–115 mg/dl</td>
<td>683</td>
<td>60/64/257</td>
<td>6/157</td>
<td>8/155</td>
<td>1,002</td>
</tr>
<tr>
<td>&gt; 115 mg/dl</td>
<td>647</td>
<td>57/52/230</td>
<td>6/155</td>
<td>8/150</td>
<td>953</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>111–175 mg/dl</td>
<td>714</td>
<td>67/65/274</td>
<td>6/160</td>
<td>8/156</td>
</tr>
<tr>
<td>&gt; 175 mg/dl</td>
<td>697</td>
<td>60/64/257</td>
<td>6/157</td>
<td>8/155</td>
<td>1,002</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio</td>
<td>&lt; 3.9</td>
<td>702</td>
<td>39/45/173</td>
<td>6/55</td>
<td>8/55</td>
</tr>
<tr>
<td>&gt; 3.9</td>
<td>702</td>
<td>39/45/173</td>
<td>6/55</td>
<td>8/55</td>
<td>100</td>
</tr>
</tbody>
</table>

*Adjusted for sex, age, BMI, smoking status, study center, SBP, HbA1c, fibrinogen, insulin, and albumin to creatinine ratio.

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This report is the first population-based comparison of the utility of non-HDL cholesterol and individual lipoprotein parameters in predicting CVD in patients with diabetes. In the Systolic Hypertension in the Elderly Program (SHEP), a study of elderly, primarily white, nondiabetic individuals, non-HDL cholesterol was assessed along with serum cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol as a predictor of cardiovascular events in 4,736 participants. During an average 4.5 years of follow-up, non-HDL cholesterol was shown to be a predictor of cardiovascular events in multivariate analysis in the total population. Non-HDL cholesterol and LDL cholesterol had similar HRs in individuals with triglyceride levels >400 mg/dl; however, non-HDL cholesterol, but not LDL cholesterol, was an independent predictor of CVD when triglycerides were included in the model (46). In the Gubbio study (47), a sample of 2,963 men and women aged 35–74 with no major CVD was examined in 1983. Risk factors were measured and 6-year incidence was computed for CHD and all cardiovascular events. Multivariate models showed that the relative risk for a difference of 40 mg/dl in non-HDL cholesterol ranged from 1.15 to 1.27. Findings from the Lipid Research Clinics Program Follow-Up Study, in which a total of 4,462 men and women were followed for 19 years, showed that non-HDL cholesterol emerged as a somewhat better predictor of CVD mortality than LDL (19). Our findings of the utility of non-HDL cholesterol as a predictor of CVD in a large cohort of patients with diabetes are of particular interest considering the dyslipidemia that is common in diabetes.

Our analyses provide strong supportive evidence that non-HDL cholesterol may be particularly useful in treating patients with diabetes. However, this analysis is restricted to American Indians, who have the highest rate of diabetes of any ethnic group in the U.S. (5). Ethnic differences in CVD risk factors and cultural/lifestyle practices require that our findings be confirmed in other diabetic populations. Importantly, because there were few cases of CVD in the nondiabetic...
members of this cohort, it was not possible to adequately assess the utility of non-HDL cholesterol in individuals with the metabolic syndrome but without frank diabetes. This important question remains to be answered.

An important issue for interpretation of these results is the impact of a lipoprotein measure on its utility as a predictor of CVD. The intra-individual variation in triglyceride concentrations is much higher than that of HDL or LDL cholesterol. Therefore, it is not clear whether the diminished significance of triglycerides as a predictor is simply due to the fact that an accurate integrated measure of average triglyceride concentrations over a longer period of time cannot be obtained from a single fasting sample. Non-HDL cholesterol would be subjected to some of the same considerations. Although more stable than total triglyceride because a large component is LDL cholesterol, non-HDL cholesterol is also a reflection of VLDL and IDL cholesterol, which fluctuate widely in individuals from day to day, depending on dietary patterns and other metabolic variables. Use of non-HDL cholesterol in the nonfasting state, although feasible, would enhance this variability. Therefore, it is possible that this measure could be of even greater use if an integrated value that reflected days or weeks of combined VLDL, IDL, and LDL cholesterol concentrations could be developed.

In summary, our results show that non-HDL cholesterol is a significant predictor of CVD in diabetic men and women. Because diabetic patients are at high risk for CVD morbidity and mortality, adequate risk assessment and management is imperative. The simple non-HDL cholesterol measurement, which can be conducted in the nonfasting state and can be determined regardless of triglyceride concentration, may be of particular clinical utility. The Adult Treatment Panel (ATP-III) of the National Cholesterol Education Program recommended a therapeutic goal for non-HDL cholesterol of 30 mg/dl higher than the goal for LDL cholesterol; therefore, in patients with diabetes, the goal would be a non-HDL cholesterol target of <130 mg/dl.

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References


1265, 1998
45. Kannel WB: The Framingham Study: its