**Clinical Investigation and Reports**

**Lipids and Lipoproteins Predicting Coronary Heart Disease Mortality and Morbidity in Patients With Non–Insulin-Dependent Diabetes**

Markku Laakso, MD; Seppo Lehto, MD; Ilkka Penttilä, MD; Kalevi Pyörälä, MD

*Background.* The aim of this study was to investigate the association of lipoprotein fractions with the future risk of coronary heart disease (CHD) in patients with non–insulin-dependent diabetes (NIDDM).

*Methods and Results.* At baseline, lipoprotein fractions were determined in 313 diabetic patients with NIDDM (153 men and 160 women), and these patients were followed up for 7 years with respect to CHD events (CHD death or all CHD events including CHD death or nonfatal myocardial infarction). Altogether, 56 NIDDM patients (28 men and 28 women) died from CHD and 25 had a nonfatal myocardial infarction (17 men and 8 women) during the follow-up. NIDDM patients having these CHD events during the follow-up had higher levels of total and very-low-density lipoprotein (VLDL) triglycerides and VLDL cholesterol and lower levels of high-density lipoprotein (HDL) and HDL2 cholesterol than those without CHD events. The risk for CHD death was fourfold and for all CHD events, twofold higher among diabetics with low HDL cholesterol (<0.9 mmol/L) than among diabetics with HDL cholesterol ≥0.9 mmol/L. High triglyceride level (>2.3 mmol/L) was associated with a twofold increase in the risk of CHD events. In multiple logistic regression analyses, HDL was inversely associated with CHD events and VLDL triglycerides with CHD events in NIDDM patients with low HDL cholesterol level (≤1.12 mmol/L).

*Conclusions.* Our 7-year follow-up study gives evidence that low HDL and HDL2 cholesterol, high VLDL cholesterol, and high total and VLDL triglycerides are powerful risk indicators for CHD events in patients with NIDDM. (Circulation. 1993;88[part 1]:1421-1430.)

**KEY WORDS**

- diabetes mellitus
- insulin
- lipids
- lipoproteins
- cholesterol

Subjects with non–insulin-dependent diabetes (NIDDM) are at increased risk of developing all manifestations of atherosclerotic vascular disease. The most important of these manifestations, coronary heart disease (CHD), is the leading cause of death among patients with NIDDM. Several studies have indicated that mortality and morbidity rates of CHD are 2 to 4 times higher among diabetics than among nondiabetics. A congregation of various cardiovascular risk factors is frequently found in patients with NIDDM, including elevated blood pressure, obesity, insulin resistance, and dyslipidemia, including high serum total and very-low-density lipoprotein (VLDL) triglyceride levels, low serum high-density lipoprotein (HDL) cholesterol, and apolipoprotein A1 levels.

Although much of the excess CHD risk in diabetic patients can be accounted for by high prevalence of these risk factors, a significant proportion of it remains unexplained. However, classic risk factors (high levels of total cholesterol, smoking, and hypertension) retain the same adverse impact on the development of atherosclerotic vascular disease in diabetic patients as in nondiabetic subjects.

In addition to high total and low-density lipoprotein (LDL) cholesterol levels, the potential of other lipids and lipoproteins to modulate the risk for atherosclerosis has recently gained a good deal of attention because high total and VLDL triglyceride and low HDL cholesterol concentrations have been associated with insulin resistance measured by the glucose clamp technique not only in nondiabetic subjects but also in patients with NIDDM. It has been proposed that these lipoprotein changes associated with insulin resistance could increase the risk for CHD. No prospective population-based studies are, however, available in which the association of lipoprotein fractions with the risk of CHD had been studied in patients with NIDDM. To investigate this important issue, we correlated the levels of lipids and lipoproteins with the future risk of CHD events in a 7-year, prospective, population-based study including a large number of patients with NIDDM.

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**Methods**

All diabetic patients in Finland who need drug therapy receive it free of charge according to the Sickness Insurance Act. The Social Insurance Institution maintains a central register of diabetic subjects receiving drug reimbursement. Based on this register, we identified all diabetic patients, aged 45 to 64 years, who were...


<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>153</td>
<td>160</td>
</tr>
<tr>
<td>Age, y</td>
<td>56±1</td>
<td>58±1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.7±0.3</td>
<td>29.4±0.4</td>
</tr>
<tr>
<td>Duration of diabetes, y</td>
<td>9.1±0.4</td>
<td>9.2±0.3</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>62.1</td>
<td>69.4</td>
</tr>
<tr>
<td>Alcohol Intake, g/wk</td>
<td>53±7</td>
<td>5±2</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>26.8</td>
<td>6.3</td>
</tr>
<tr>
<td>Regular physical activity, %</td>
<td>35.9</td>
<td>26.9</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>10.9±0.3</td>
<td>12.6±0.4</td>
</tr>
<tr>
<td>Glycosylated hemoglobin A₁, %</td>
<td>9.6±0.2</td>
<td>10.0±0.2</td>
</tr>
<tr>
<td>Treatment for diabetes, %</td>
<td>Diet 31.4</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>Oral drugs</td>
<td>47.7</td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td>20.9</td>
</tr>
</tbody>
</table>

TABLE 1. Baseline Characteristics of Patients With Non–Insulin-Dependent Diabetes

born and living in the Kuopio University Hospital district, East Finland. The main purpose of this procedure was to obtain a population for a study that would compare the prevalence of CHD and its risk factors in patients with NIDDM living in East and West Finland.16 The formation of this patient population consisting of 510 diabetic subjects who participated in this study from Kuopio University Hospital district, East Finland (participation rate, 83%) has been described in detail previously.16 In a sample of 313 NIDDM patients (153 men and 160 women), lipoprotein fractions were also determined. For this substudy, NIDDM patients were selected as follows: all 88 patients treated with diet (48 men and 40 women), a random sample of 140 patients drawn from 358 patients treated with oral drugs (56 men and 50 women with sulfonylureas, 3 men and 2 women with metformin, 14 men and 15 women with a combination of sulfonylureas and metformin), and all 85 patients treated with insulin (32 men and 53 women) whose plasma C-peptide concentration was at least 0.20 nmol/L 6 minutes after 1 mg of intravenous glucagon. A cutoff point of 0.20 nmol/L was used because postglucagon C-peptide values below this limit have been shown to be associated with the occurrence of ketoacidosis in insulin-treated diabetic subjects.17 None of the diabetic patients classified as having NIDDM according to the WHO criteria18 and included in the final study population had a history of ketoacidosis. Furthermore, none of the subjects received hypolipidemic drug therapy or had any renal, hepatic, or thyroid disease affecting glucose or lipid metabolism.

Baseline clinical characteristics of the study population by sex are presented in Table 1. NIDDM patients were middle-aged, relatively obese, and in poor metabolic control, and their mean duration of diabetes was 9 years.

Study Program and Methods at Baseline Examination

The study program in 1982 to 1983 included an interview on the history of smoking, alcohol intake, physical activity, and the use of drugs. Blood pressure was measured in the sitting position after a 5-minute rest with a mercury sphygmomanometer and read to the nearest 2 mm Hg. A patient was classified as having hypertension if he/she was receiving drug treatment for hypertension or if his/her systolic blood pressure was at least 160 mm Hg or diastolic blood pressure was at least 95 mm Hg. Alcohol intake was determined according to a patient's estimate of the average number of drinks of beer, wine, or spirits consumed per week (transformed to absolute alcohol in grams per week). Exercise level was divided into two categories: (1) little or no physical exercise during leisure or work and (2) regular physical exercise during leisure time (eg, walking, bicycling, jogging, or swimming for at least 30 minutes at least twice a week) and/or heavy physical activity at work (eg, work in heavy industry, farming, or lumberjacking).

Serum lipids and lipoproteins were determined from fresh serum samples drawn after a 12-hour overnight fast. Lipoprotein fractionation was performed by using ultracentrifugation and selective precipitation with minor modifications19 of the method of Havel et al.20 All spinnings were done at 10°C with a Kontron TGA-65 ultracentrifuge. Serum samples were centrifuged at d=1.006 (105 000g for 18 hours). VLDL fraction (d<1.006) was recovered as the top fraction. Total HDL was determined directly after precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride. This method correlates closely with the results obtained by ultracentrifugation.19 LDL cholesterol (d=1.006 to 1.063, including intermediate-density lipoprotein) was calculated by subtracting HDL cholesterol from the bottom fraction of ultracentrifuged serum (d>1.006). The HDL₃ and HDL₄ subfractions were separated by running the total HDL fraction at d=1.25 (105 000g for 40 hours), and the top and bottom fractions were isolated by the tube-slicing technique. The cholesterol and triglycerides from the whole serum and from lipoprotein fractions were assayed by automated enzymatic methods (Boehringer Mannheim GmbH, Mannheim, Germany). In each series, two commercial control sera and our own serum pool were run with patients' samples as quality controls. On the average, the mean day-to-day variation in HDL cholesterol measurements was 3.3% and the daily variation was 0.95%. The plasma C-peptide response to glucagon was determined according to the method of Faber and Binder.21 C-peptide was measured by radioimmunoassay (antibody M 1230, Novo, Denmark). Glycosylated hemoglobin A₁ (GHbA₁) was determined by commercial affinity chromatography (Quick-Sep Fast Hemoglobin Test System, Isolab Inc., Akron, Ohio) after incubation in 0.9% saline solution for 12 hours. Fasting plasma glucose was determined by the glucose oxidase method (Boehringer, Germany).

Collection of Follow-up Data

In 1990, a postal questionnaire containing questions about hospitalization because of acute chest pain and symptoms suggestive of stroke were sent to every surviving participant of the original study. All medical records of those subjects who died between baseline examination and December 31, 1989, or who reported in the questionnaire that they had been admitted to the hospital on the basis of chest pain symptoms between
the baseline examination and December 31, 1989, were reviewed. The WHO criteria for verified and possible myocardial infarction (MI) based on chest pain symptoms, ECG changes, and enzyme determinations were used in the ascertainment of the diagnosis of MI. Copies of death certificates of those patients who had died were obtained from the files of the Central Statistical Office of Finland. In the final classification of the causes of death, hospital records and autopsy records were also used, if available. The mortality data included in the present article are mortality from CHD (International Classification of Diseases 9, Codes 410-414).

Statistical Analysis

The results are expressed as mean±SEM. The differences between the groups were assessed by the χ² test, Student’s two-tailed t test for independent samples, ANOVA, and ANCOVA. Correlations were calculated by Pearson’s correlation coefficients. The association of cardiovascular risk factors and CHD was studied by applying univariate and multivariate logistic regression analyses. Age-adjusted odds ratios and their 95% confidence intervals were calculated according to Mantel and Haenzel. In statistical analyses, CHD events were classified as follows: (1) CHD death and (2) all CHD events (CHD death or definite or possible nonfatal MI). Logarithmic transformation of VLDL cholesterol and total and VLDL triglycerides were performed because of skewed distribution in all statistical analyses including these variables.

Statistical analyses were first performed separately for men and women. Because the results were essentially similar in both men and women, results are given for both sexes combined.

Approval of Ethics Committee

This study was approved by the Ethics Committee of the Kuopio University Central Hospital.

Results

During the 7-year follow-up (mean follow-up was 7.2 years in men and women), altogether, 126 NIDDM patients died (62 men and 64 women). CHD was the cause of death in 56 patients (28 men and 28 women) and other cardiovascular diseases in 38 patients (20 men and 18 women). Thirty-two deaths (14 men and 18 women) occurred from noncardiovascular causes. Altogether, 81 NIDDM patients (45 men and 36 women) had a serious CHD event during the follow-up (CHD death or definite or possible nonfatal MI). Occurrence of serious CHD events among NIDDM patients was equally distributed with respect to the mode of treatment (diet, 21.6%; sulfonylureas, 26.4%; metformin, 20.0%; combination of sulfonylureas and metformin, 27.6%; and insulin, 29.4%).

Fig 1 summarizes the levels of total cholesterol and cholesterol fractions in those diabetic patients who died of CHD and in those who did not (bars on the left-hand side) and in those diabetic patients who had a serious CHD event (all CHD) during the follow-up compared with those who did not (bars on the right-hand side). Total cholesterol was somewhat higher in those who died of CHD than in those who did not (7.33±0.26 vs 6.95±0.12 mmol/L, P=NS) or who had a serious CHD death.
event during the follow-up than those who did not (7.23±0.20 vs 6.94±0.13 mmol/L, P=NS), but these differences were not statistically significant. Similarly, no differences in LDL cholesterol levels were observed between these groups (CHD death: nonsurvivors vs others, 4.25±0.14 vs 4.38±0.07 mmol/L, P=NS; all CHD events: with CHD event vs without CHD event, 4.36±0.13 vs 4.36±0.07 mmol/L, P=NS). HDL cholesterol (CHD death: 1.01±0.04 vs 1.20±0.02 mmol/L, P<.001; all CHD events: 1.06±0.03 vs 1.20±0.02 mmol/L, P<.001) and LDL cholesterols levels (CHD death: 0.62±0.04 vs 0.78±0.02 mmol/L, P<.001; all CHD events: 0.66±0.03 vs 0.79±0.02 mmol/L, P=.02) were lower in patients having had a CHD event than in those who did not, but no differences in HDL₄ cholesterol levels were observed between these patient groups (CHD death: 0.39±0.02 vs 0.41±0.01 mmol/L, P=NS; all CHD events: 0.40±0.01 vs 0.41±0.01 mmol/L, P=NS). VLDL cholesterol levels were higher in patients with CHD events than in those without CHD events (CHD death: 2.07±0.28 vs 1.37±0.10 mmol/L, P<.01; all CHD events: 1.81±0.20 vs 1.38±0.11 mmol/L, P<.05).

Levels of total triglyceride and triglyceride levels in various lipoprotein fractions with respect to CHD events are shown in Fig 2. Total triglycerides were higher in those with CHD events than in those without CHD events (CHD death: 4.16±0.72 vs 2.72±0.22 mmol/L, P<.01; all CHD events: 3.69±0.52 vs 2.72±0.24 mmol/L, P<.01). LDL triglyceride (CHD death: 0.46±0.03 vs 0.41±0.01 mmol/L, P=NS; all CHD events: 0.45±0.03 vs 0.41±0.01 mmol/L, P=NS) and HDL triglyceride levels (CHD death: 0.16±0.01 vs 0.15±0.01 mmol/L, P=NS; all CHD events: 0.15±0.01 vs 0.15±0.01 mmol/L, P=NS) did not differ between NIDDM patients with and without CHD events. VLDL triglyceride levels were significantly higher in patients with CHD events than in those without CHD events (CHD death: 3.54±0.69 vs 2.15±0.21 mmol/L, P<.01; all CHD events: 3.09±0.50 vs 2.16±0.23 mmol/L, P<.01).

Table 2 gives the correlations of lipids and lipoproteins in the whole series of patients (N=313). Total cholesterol correlated with LDL and VLDL cholesterol fractions but also with total triglycerides and HDL, LDL, and VLDL triglycerides. HDL cholesterol had a stronger correlation with HDL₃ subfraction than with HDL₄ subfraction. In general, the correlations of HDL cholesterol and HDL₄ cholesterol with other lipids and lipoproteins were quite similar. VLDL cholesterol correlated strongly with total, HDL, LDL, and VLDL triglyceride levels.

Tables 3 and 4 summarize the association of lipid and nonlipid variables with CHD events on the basis of univariate logistic regression analysis. Of lipids and lipoproteins, VLDL cholesterol, total triglycerides, and VLDL triglycerides were positively associated and HDL and HDL₄ cholesterol negatively associated with CHD death and all CHD events in the whole study population (Table 3). When the patients with previous MI were excluded, the results remained essentially unchanged. In addition, LDL triglycerides were associated with CHD death and all CHD events. Of nonlipid variables, age and the duration of diabetes were related to CHD death.
death, and all CHD events and GHbA1 and fasting glucose were related to CHD death in the whole study population (Table 4). When the patients who had had an MI before the baseline examination were excluded, age and fasting glucose were associated with CHD death and age was associated with all CHD events.

Multivariate logistic analyses were performed, including variables that were significantly associated with CHD events in univariate logistic regression analyses

Table 2. Correlations of Lipids and Lipoproteins

<table>
<thead>
<tr>
<th></th>
<th>Total C</th>
<th>LDL C</th>
<th>HDL C</th>
<th>HDL2 C</th>
<th>HDL3 C</th>
<th>VLDL C</th>
<th>Total TG</th>
<th>HDL TG</th>
<th>LDL TG</th>
<th>VLDL TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total C</td>
<td>1.000</td>
<td>0.487†</td>
<td>0.047</td>
<td>0.026</td>
<td>0.063</td>
<td>0.640†</td>
<td>0.551†</td>
<td>0.428†</td>
<td>0.506†</td>
<td>0.506†</td>
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<tr>
<td>LDL C</td>
<td>1.000</td>
<td>0.318†</td>
<td>0.265†</td>
<td>0.163*</td>
<td>0.006</td>
<td>-0.077</td>
<td>-0.037</td>
<td>0.026</td>
<td>-0.093</td>
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<tr>
<td>HDL C</td>
<td>1.000</td>
<td>0.941†</td>
<td>0.196†</td>
<td>-0.430†</td>
<td>-0.502†</td>
<td>-0.057</td>
<td>-0.332†</td>
<td>-0.530†</td>
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<tr>
<td>HDL2 C</td>
<td>1.000</td>
<td></td>
<td>-0.147*</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>HDL3 C</td>
<td>1.000</td>
<td></td>
<td>-0.079</td>
<td>-0.101</td>
<td>0.013</td>
<td>-0.171*</td>
<td>-0.086</td>
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<tr>
<td>VLDL C</td>
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<td>0.827†</td>
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<tr>
<td>Total TG</td>
<td>1.000</td>
<td>0.507†</td>
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<tr>
<td>HDL TG</td>
<td>1.000</td>
<td>0.455†</td>
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<tr>
<td>LDL TG</td>
<td>1.000</td>
<td>0.687†</td>
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<tr>
<td>VLDL TG</td>
<td>1.000</td>
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<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

C indicates cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; and TG, triglycerides.

*P < .05, †P < .01.

Table 3. Lipids and Lipoproteins Associated With Coronary Heart Disease Death and All Coronary Heart Disease Events in Patients With Non–Insulin-Dependent Diabetes by Univariate Logistic Regression Analysis

Table 4. Nonlipid Risk Factors Associated With Coronary Heart Disease Death and All Coronary Heart Disease Events in Patients With Non–Insulin-Dependent Diabetes by Univariate Logistic Regression Analysis

MI indicates myocardial infarction; CHD, coronary heart disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; and VLDL, very-low-density lipoprotein. All CHD events include CHD death or nonfatal MI.

Unstandardized β-coefficients are shown. *P < .05, †P < .01, ‡P < .001.

(Table 5). Because of a high intercorrelation of VLDL cholesterol with VLDL triglycerides and of total triglycerides with VLDL triglycerides, only VLDL triglycerides were included in statistical analyses; similarly, HDL cholesterol and HDL2 cholesterol were not included simultaneously in the same model. Age and HDL cholesterol were associated with CHD events in all patients. After exclusion of patients with previous MI, age and HDL cholesterol were associated with CHD death, and age was associated with all CHD events. The associations were similar when HDL cholesterol was substituted by HDL2 cholesterol (data not shown).

To investigate in more detail the predictive role of VLDL triglycerides for future CHD events, we also...
TABLE 5. Risk Factors Associated With Coronary Heart Disease Death and All Coronary Heart Disease Events in Patients With Non–Insulin-Dependent Diabetes by Multivariate Logistic Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>CHD Death Events (56/313)</th>
<th>All CHD Events (61/313)</th>
<th>CHD Death (43/270)</th>
<th>All CHD Events (65/270)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.1011†</td>
<td>0.0681*</td>
<td>0.1562‡</td>
<td>0.0975f</td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>0.0693</td>
<td>0.0609</td>
<td>0.0154</td>
<td>0.0286</td>
<td></td>
</tr>
<tr>
<td>Glycated hemoglobin A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.1363</td>
<td>0.0232</td>
<td>0.1451</td>
<td>0.0294</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-1.6332*</td>
<td>-1.0747*</td>
<td>-1.5869*</td>
<td>-0.9482</td>
<td></td>
</tr>
<tr>
<td>LDL triglycerides</td>
<td>-1.2073</td>
<td>-0.9169</td>
<td>0.0925</td>
<td>0.3391</td>
<td></td>
</tr>
<tr>
<td>VLDL triglycerides</td>
<td>0.5408</td>
<td>0.4653</td>
<td>0.2840</td>
<td>0.2743</td>
<td></td>
</tr>
</tbody>
</table>

MI indicates myocardial infarction; CHD, coronary heart disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; and VLDL, very-low-density lipoprotein. All CHD events include CHD death and nonfatal MI. Unstandardized β-coefficients are shown. *P<.05, †P<.01, ‡P<.001.

performed multivariate logistic regression analyses in subgroups of diabetic patients (Table 6). Subgroups were formed on the basis of median LDL cholesterol (4.30 mmol/L) and HDL cholesterol (1.12 mmol/L) levels of all patients with or without a history of MI before the baseline examination. In patients with low LDL cholesterol level (≤4.30 mmol/L), VLDL triglycerides were significantly associated with CHD events, whereas HDL cholesterol was not significantly associated with CHD risk. In patients with high LDL cholesterol levels (>4.30 mmol/L), HDL cholesterol was associated with all CHD events but VLDL triglycerides failed to show any significant association with CHD risk.

Association of CHD events and lipoprotein levels was also investigated by calculating age-adjusted odds ratios (OR) (Table 7). Cutoff points for high total (>6.2 mmol/L) and LDL cholesterol (>4.1 mmol/L) and low HDL cholesterol (<0.9 mmol/L) and high total triglyceride levels (>2.3 mmol/L) were based on high-risk category of the National Cholesterol Education Program<sup>23</sup> and the modification of these recommendations for patients with NIDDM.<sup>24</sup> High total and LDL cholesterol levels were not associated with an increased risk of future CHD events. The risk of NIDDM patients having low HDL cholesterol (23% of patients), whatever their triglyceride level, to die from CHD was fourfold (P<.001) and for all CHD events, twofold (P=.002). High triglyceride level (45.4% of patients) was associated with a twofold risk for CHD death (P=.001) and all CHD events (P=.008). High non-HDL cholesterol (LDL cholesterol plus VLDL cholesterol) >5.3 mmol/L (83.7% of subjects) was not associated with increased risk of CHD events.

The simultaneous presence of low HDL cholesterol (<0.9 mmol/L) and high total triglyceride level (>2.3 mmol/L) (18.8% of subjects) did not increase the risk for CHD death (OR=3.7 [2.0 to 7.2], P<.001) or all CHD events (OR=2.0 [1.1 to 3.9], P=0.003) compared with that of low HDL cholesterol alone (Fig 3, upper panel). In contrast, the simultaneous presence of low HDL cholesterol (<0.9 mmol/L), high total triglycerides (>2.3 mmol/L), and high LDL cholesterol (>4.1 mmol/L) (7.0% of subjects) increased the risk of CHD death (OR=4.0 [1.7 to 9.5], P<.001) and all CHD events.

TABLE 6. Risk Factors Associated With All Coronary Heart Disease Events in All Patients With Non–Insulin-Dependent Diabetes by Multivariate Logistic Regression Analysis by Low-Density and High-Density Lipoprotein Cholesterol Levels

<table>
<thead>
<tr>
<th>LDL Cholesterol</th>
<th>HDL Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low, ≤4.30 mmol/L</td>
<td>High, &gt;4.30 mmol/L</td>
</tr>
<tr>
<td>(37/157)</td>
<td>(39/156)</td>
</tr>
<tr>
<td>Age</td>
<td>0.0618*</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>0.1304</td>
</tr>
<tr>
<td>Glycated hemoglobin A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>-0.0053</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.3089</td>
</tr>
<tr>
<td>LDL triglycerides</td>
<td>-0.2528</td>
</tr>
<tr>
<td>VLDL triglycerides</td>
<td>1.0266*</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; and VLDL, very-low-density lipoprotein. All CHD events include CHD death and nonfatal myocardial infarction. Unstandardized β-coefficients are shown. *P<.05, †P<.01.

TABLE 7. Age-Adjusted Odds Ratios and 95% Confidence Intervals for Predicting Coronary Heart Disease Death or All Coronary Heart Disease Events

<table>
<thead>
<tr>
<th></th>
<th>CHD Death</th>
<th>P</th>
<th>All CHD Events</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol &gt;6.2 mmol/L</td>
<td>1.4 (0.7-3.1)</td>
<td>NS</td>
<td>1.8 (0.9-3.2)</td>
<td>.008</td>
</tr>
<tr>
<td>LDL cholesterol &gt;4.1 mmol/L</td>
<td>1.0 (0.0-3.6)</td>
<td>NS</td>
<td>1.3 (0.6-2.3)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol &lt;0.9 mmol/L</td>
<td>3.9 (2.1-7.3)</td>
<td>&lt;.001</td>
<td>2.0 (1.1-3.6)</td>
<td>.002</td>
</tr>
<tr>
<td>Non-HDL cholesterol &gt;5.3 mmol/L</td>
<td>1.4 (0.7-2.9)</td>
<td>NS</td>
<td>1.4 (0.8-2.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Total triglycerides &gt;2.3 mmol/L</td>
<td>2.2 (1.2-4.0)</td>
<td>.001</td>
<td>1.6 (1.0-2.8)</td>
<td>.008</td>
</tr>
</tbody>
</table>

CHD indicates coronary heart disease; LDL, low-density lipoprotein; and HDL, high-density lipoprotein. All CHD events include CHD and nonfatal myocardial infarction. Confidence intervals are shown in parentheses.
Laakso et al.'s study on Coronary Heart Disease Risk and Lipoproteins in Diabetes

The risk also increased in patients with the simultaneous presence of low HDL cholesterol, high total triglycerides, and high total cholesterol (>6.2 mmol/L) (14.4% of subjects) (CHD death: OR=4.0 [2.0 to 7.9], P<.001; all CHD events: OR=2.9 [1.5 to 5.6], P<.001).

The risk for future CHD events remained essentially similar after the exclusion of patients with previous MI (Fig 3, lower panel). The simultaneous presence of low HDL cholesterol and high total triglycerides was associated with a fourfold risk of CHD death (OR=3.7 [1.7 to 7.9], P<.001) and a twofold risk of all CHD events (OR=2.0 [1.0 to 4.3], P=.007); the simultaneous presence of low HDL cholesterol, high total triglycerides, and high LDL cholesterol was associated with a threefold risk of CHD death (OR=3.1 [1.0 to 9.9], P=.006) and all CHD events (OR=3.0 [1.0 to 8.6], P=.005), and the simultaneous presence of low HDL cholesterol, high total triglycerides, and high total cholesterol was associated with a fourfold risk of CHD death (OR=4.2 [1.9 to 9.5], P<.001) and a threefold risk of all CHD events (OR=3.3 [1.5 to 7.1], P<.001).

**Discussion**

Although risk factors for CHD in NIDDM have been extensively studied, no previous population-based study so far has investigated the role of lipoprotein fractions in modifying the risk for atherosclerotic vascular disease.

FIG 3. Bar graphs: Risk of coronary heart disease (CHD) death or all serious CHD events (CHD death or nonfatal myocardial infarction) in non-insulin-dependent diabetic patients with respect to dyslipidemia during a 7-year follow-up in the whole study population (upper panel) and in patients without a history of myocardial infarction before the baseline examination (lower panel). Numbers 0 to 5 at left indicate odds ratio. HDL C indicates high-density lipoprotein cholesterol; TG, triglycerides; LDL C, low-density lipoprotein cholesterol; and total C, total cholesterol. **P<.01, ***P<.001.
in NIDDM patients. Indeed, previous studies have correlated only the levels of total cholesterol and total triglycerides with the risk of CHD. Both hypercholesterolemia and hypertriglyceridemia have been associated with an increased occurrence of CHD in both cross-sectional and prospective studies. In the WHO Multinational Study\textsuperscript{25} and in the Paris Prospective Study,\textsuperscript{26} the single risk factor that correlated most closely with the occurrence of CHD in diabetic subjects was plasma total triglyceride concentration. Unfortunately, these two studies assessed only total plasma triglyceride and cholesterol concentrations and did not evaluate various lipoprotein fractions. A cross-sectional study in patients with NIDDM provided the evidence that low HDL cholesterol and its HDL\textsubscript{2} subfraction\textsuperscript{27} contribute to enhanced atherosclerosis, but no follow-up studies have been previously published.

In our study population, low HDL cholesterol concentration was the most important single predictor of future CHD events. The risk for CHD death was fourfold and for all CHD events (CHD death or nonfatal MI), twofold among NIDDM patients with low HDL cholesterol level (<0.9 mmol/L). Furthermore, inverse relation with CHD was due mainly to HDL\textsubscript{2} cholesterol subfraction, as has been reported previously in studies including nondiabetic subjects.\textsuperscript{28,29} High triglyceride level or high triglyceride level combined with high total cholesterol or high LDL cholesterol levels, in addition to the presence of low HDL cholesterol concentration, did not substantially increase the risk of CHD events compared with that of low HDL cholesterol alone (Fig 3). However, low HDL cholesterol was a strong predictor of CHD events in the subgroup of patients with high LDL cholesterol (≥4.30 mmol/L) but not in the subgroup of low LDL cholesterol (<4.30 mmol/L). This indicates, in accordance with previous studies in nondiabetic subjects,\textsuperscript{30} that high LDL cholesterol and low HDL cholesterol have important joint effects on the CHD risk.

An inverse relation between HDL levels and the incidence of CHD has been documented in several epidemiological studies on nondiabetic subjects\textsuperscript{31-35} and also in an adult diabetic population.\textsuperscript{36} The hypothesis has been presented that HDL cholesterol has a central role in a system of reverse cholesterol transport; however, it remains unclear whether reverse cholesterol transport mediates the protective effect of HDL observed in epidemiological studies.\textsuperscript{37} An alternative hypothesis\textsuperscript{38} has been presented proposing that low HDL levels might be only a marker for the accumulation of chylomicron or VLDL remnants in plasma because of inefficient hepatic clearance of these particles. The remnants would enter the artery wall and be taken up by macrophages, giving rise to atheroma foam cells. Indeed, Patsch et al\textsuperscript{39} have shown that the elevation of postprandial triglyceride levels is strongly and inversely correlated with fasting HDL and HDL\textsubscript{2} cholesterol levels.

NIDDM patients with CHD had higher levels of total and LDL cholesterol than patients without CHD events. The risk for CHD events was twofold in NIDDM patients with high total triglyceride levels (>2.3 mmol/L). These results are in accordance with the findings reported previously on nondiabetic\textsuperscript{40} and diabetic subjects.\textsuperscript{25,26} The independent association of total triglycerides with CHD disappeared in multivariate logistic regression analyses when other lipid risk factors, particularly HDL cholesterol, was taken into account. However, in the subgroup of patients with low HDL cholesterol (≤1.12 mmol/L), high VLDL triglycerides were predictive of future CHD events, indicating that these lipoproteins have strong joint effects on the risk of CHD as reported also in previous studies in nondiabetic subjects.\textsuperscript{30,41,42}

Although controversy continues as to whether an increase in plasma triglyceride concentration is a primary risk factor for CHD, several possible mechanisms can be presented to support the notion that triglyceriderich lipoproteins, particularly VLDL lipoproteins, could be atherogenic. Besides direct atherogenicity, they could be indirectly atherogenic through inducing the decrease in HDL cholesterol and the increase in small, dense LDL particles. In the majority of hypertriglyceridemic subjects, the total number of small VLDL particles in circulation is increased, also reflecting abnormally high VLDL cholesterol. These particles contain apolipoprotein E and probably have enhanced activity for lipoprotein receptors on arterial wall cells, increasing their atherogenicity.\textsuperscript{43} Indeed, VLDL cholesterol was associated with the increased risk of future CHD events in our study population, supporting the hypothesis that at least a part of the risk relating to hypertriglyceridemia is related to high levels of VLDL cholesterol.

Extensive evidence from animal studies, clinical trials, and epidemiological studies has confirmed the causal role of LDL cholesterol in atherosclerosis.\textsuperscript{44} In our diabetic study population, serum total and LDL cholesterol levels did not, however, differ between the NIDDM patients with and without CHD events. Furthermore, the predictive value of high total and LDL cholesterol with respect to the risk of CHD was relatively weak (Tables 4 and 6). Several possibilities can be offered to explain this result. First, the mean duration of NIDDM at baseline was 9 years. Because of repeated dietary instructions, the patients may have changed their diets to contain less saturated fat and cholesterol, and, therefore, LDL concentrations at baseline may be lower and their range more narrow than they would be without dietary advice. It should be emphasized, however, that mean LDL levels in NIDDM patients were still markedly high (4.3 mmol/L). Second, we determined only the levels of LDL cholesterol (also including intermediate-density lipoprotein) and did not characterize in more detail qualitative changes in lipoprotein composition. Particularly small, dense LDL particles, which are increased in NIDDM,\textsuperscript{45} were not determined. These atherogenic lipoprotein particles\textsuperscript{46} remain unnoticed if only LDL cholesterol levels are measured. Moreover, LDL cholesterol in NIDDM can be modified, eg, oxidized or glycosylated,\textsuperscript{47} which all add the risk of atherosclerosis in these patients. Third, NIDDM patients may have increased LDL turnover (increased synthesis, increased catabolism) despite normal plasma LDL levels,\textsuperscript{48} which also can contribute to the increased risk of coronary atherosclerosis in these patients. Finally, LDL cholesterol is not a good predictor of CHD in older subjects.\textsuperscript{49}

We observed that high LDL triglyceride level was associated with the risk of CHD events in NIDDM
patients who had no history of MI before the baseline study (Table 4). High LDL triglyceride level, in addition to high VLDL cholesterol concentration, reflects an altered composition of VLDL and LDL particles. With respect to the risk of atherosclerosis, these abnormalities may be of great importance since VLDL remnants or intermediate-density lipoprotein arising during catabolism of VLDL to intermediate-density lipoprotein and LDL are atherogenic and associated with atherosclerosis in animal and human studies. Taking all of our findings together, it is interesting to note that lipoprotein abnormalities related to CHD risk in NIDDM patients (low HDL and HDL cholesterol, high total and VLDL triglycerides, and VLDL cholesterol) are associated with insulin resistance measured by the euglycemic clamp technique, not only in nondiabetic subjects but also in patients with NIDDM. Therefore, our results give evidence for the hypothesis that insulin resistance is associated with lipid and lipoprotein abnormalities that increase the risk for CHD in NIDDM.

With respect to nonlipid variables, only age was consistently associated with CHD death and all CHD events. This study showed, as did previous studies, that NIDDM abolishes the protective effect of female sex with respect to CHD risk. Indeed, almost a similar percentage of diabetic women (17.5%) as of diabetic men (18.3%) died of CHD during the follow-up. Also, the duration of NIDDM was associated with CHD events, but this relation lost its statistical significance when patients with previous MI were excluded. It is widely accepted that frank clinical NIDDM is preceded by a long prediabetic stage, and there is accumulating evidence that this prediabetic stage is characterized by a clustering of cardiovascular risk factors favoring atherosclerosis. For example, lipid and lipoprotein levels become abnormal in a precursor stage of NIDDM, in impaired glucose tolerance, and even in the relatives of diabetics. Thus, even long before the development of chronic hyperglycemia, there are quantitative and qualitative metabolic changes that predispose an individual to atherosclerosis. Interestingly, in this study, the various parameters of glycemic control did not relate to the risk of CHD. This can be partly due to relatively narrow range and high levels of HbA1 among NIDDM patients at baseline examination. Because the patients included in our study were originally identified from patients receiving drug reimbursement, they probably represent the more severe part of the distribution of the diabetic state.

In conclusion, our 7-year follow-up study gives the first evidence that low HDL and HDL cholesterol, high VLDL cholesterol, and high total and VLDL triglycerides are powerful risk indicators for CHD events in patients with NIDDM. Since lipoprotein changes were more closely associated with CHD risk than other conventional risk factors, these results challenge us to normalize these lipoprotein abnormalities in addition to reducing total and LDL cholesterol with diet, weight reduction, regular exercise, or drug therapy.

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