Association of Low HDL and HDL2 Cholesterol with Coronary Heart Disease in Noninsulin-Dependent Diabetics

Markku Laakso, Erkki Voutilainen, Kalevi Pyörälä, and Helena Sarlund

Lipids and lipoproteins were measured in 139 men and 145 women who were noninsulin-dependent diabetics (NIDDs) aged 45 to 64 years. Of these, 27 men and 16 women had had a previous definite myocardial infarction (MI). The NIDDs with MI (MI+) showed lower values of HDL and HDL2 cholesterol concentrations than NIDDS without previous MI (MI−) or NIDDS without any symptoms or electrocardiographic signs of coronary heart disease (CHD−). The inverse relationship between HDL, HDL2, and CHD was evident in both sexes, but it was particularly strong among male NIDDs. The difference in HDL and HDL2 cholesterol concentrations between the MI+ and MI− groups or between the MI+ and CHD− groups persisted after adjustment by analysis of covariance for the effect of physical activity, alcohol intake, obesity, duration of diabetes, and glycemic control. It is concluded that in a cross-sectional study, even among NIDDs with generally low HDL and HDL2 cholesterol concentrations, the presence of CHD is associated with a further depression of HDL and HDL2 cholesterol levels. Prospective studies are needed, however, to confirm that the association is predictive and not a consequence of CHD.

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Low concentration of serum high density lipoprotein (HDL) cholesterol has been shown to be strongly associated with an increased risk of coronary heart disease (CHD) in nondiabetic populations.1-6 Among diabetics, patients with noninsulin-dependent diabetes (NIDDS) have a lower level of HDL cholesterol than corresponding nondiabetic control subjects.7 At present, it is unclear whether the presence of CHD is associated with a further lowering of HDL cholesterol in this diabetic population which has a generally low level of HDL cholesterol. Therefore, our study compared lipid and lipoprotein levels in NIDDs with and without CHD.

Methods

Patients

All diabetic patients in Finland needing drug therapy are provided with it free of charge according to the Sickness Insurance Act. The Social Insurance Institution maintains a central register of diabetics who receive drug reimbursement. On the basis of this register, we identified 791 diabetic patients aged 45 to 64 years who had been diagnosed as diabetic after the age of 30 years and who lived near Kuopio University Central Hospital, East Finland. The main purpose was to obtain a population for a study that would compare the prevalence of CHD and its risk factors in middle-aged NIDDS living in East and West Finland.8

Among the total population of 791 diabetics, we selected 284 persons (139 men, 145 women) for the present study. The criteria were as follows:

1. Diet-treated diabetics. There were 104 eligible diabetics (57 men, 47 women) of whom 88 participated (48 men, 40 women; participation rate, 85%). These patients’ diabetes medication had been stopped at least 3 months before the study.

2. Diabetics treated with oral hypoglycemic drugs. A random sample of 173 diabetics (90 men, 83 women) was taken from a total of 448 diabetics treated with oral drugs. The number of diabetics in this random sample who participated in the study was 140 (73 men, 67 women; participation rate, 81%).

3. Diabetics treated with insulin. All 239 patients who were receiving insulin treatment were invited to
participate in an examination that included the determination of postglucagon plasma C-peptide response, and 170 patients participated (participation rate, 71%). Of these, 56 patients (18 men, 38 women) whose plasma C-peptide concentration was at least 0.60 nmol/liter 6 minutes after 1 mg of intravenous glucagon formed the final study group. A postglucagon C-peptide concentration of 0.60 nmol/liter was chosen for the cut-off level because diabetics having a C-peptide response higher than that probably have noninsulin-dependent diabetes.9

Figure 1 shows the subgroups of male and female diabetics. Altogether, 27 male diabetics and 16 female diabetics had had a previous myocardial infarction (MI) at least 1 year or more before the study. The diagnosis of MI was based either on previous hospital-treated MI that was diagnosed according to the criteria used in the WHO MONICA Project10 or by the presence of Q/QS-abnormalities on an electrocardiogram (ECG) (Minnesota codes 1.1 to 1.2). Among male and female diabetics without MI, those without coronary heart disease (CHD–) were identified. These patients had no cardiovascular symptoms (by the Rose cardiovascular questionnaire11) and their resting ECGs were normal. Altogether, 45 male diabetics and 42 female diabetics fulfilled these criteria.

![Figure 1](image_url)

Figure 1. Formation of study groups. NIDDM = noninsulin-dependent diabetes; MI+ = patients with myocardial infarction; MI− = patients without myocardial infarction; CHD− = patients free from coronary heart disease.

All the diabetics in the final study population fulfilled the WHO criteria for diabetes mellitus.12 All subjects were free from renal, liver, and thyroid diseases, and none were receiving hypolipidemic drug therapy.

**Methods**

Body mass index (BMI) was calculated according to the formula 

\[ \text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}^2} \]

Alcohol intake was determined according to a patient's estimate of the average number of drinks of beer or wine per week (transformed to absolute alcohol in grams/week). Exercise level was divided into two categories: 1) little or no physical exercise during leisure or work; 2) regular physical exercise during leisure time (e.g., walking, bicycling, jogging, or swimming for at least 30 minutes at least twice a week) and/or heavy physical activity at work (e.g., work in heavy industry, farming, or lumbering).

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 modifications13 of the method of Havel et al.14 All spinnings were done at 10°C with a Kontron TGA-65 ultracentrifuge. Serum samples were centrifuged at d = 1.006 (105,000 g, for 18 hours); VLDL (d < 1.006) was recovered as the top fraction. Total HDL was separated by spinning serum samples at d = 1.063 (105,000 g, for 18 hours). LDL (d = 1.006 – 1.063, including IDL) was calculated as the difference between the bottom fractions. For control of the ultracentrifugation procedure, HDL was also determined directly with dextran sulfate and magnesium chloride precipitation.13 The HDL3 and HDL2 subfractions were separated by running the total HDL fraction at d = 1.215 (105,000 g, 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). 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.15 C-peptide was measured by radioimmunoassay (antibody M 1230, Novo, Denmark). Glycosylated hemoglobin A\(_1\) (GHbA\(_1\)) was determined by commercial affinity chromatography (Quick-Sep Fast Haemoglobin Test System, Isolab Incorporated, Akron, Ohio) after incubation in 0.9% saline solution for 12 hours.

The results are expressed as means ± SEM. The differences between the groups were assessed by the \(\chi^2\) test, Student's two-tailed t test for independent samples, and analysis of variance. We compared the patients who had MI and those who were free from...
Table 1. Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MI +</td>
<td>MI -</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>27</td>
<td>112</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet alone</td>
<td>9</td>
<td>39</td>
</tr>
<tr>
<td>Oral drugs</td>
<td>13</td>
<td>60</td>
</tr>
<tr>
<td>Insulin</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>56.5 ±1.1</td>
<td>55.9 ±0.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.3 ±0.6</td>
<td>28.0 ±0.4</td>
</tr>
<tr>
<td>Alcohol intake (g/week)</td>
<td>26.6 ±6.2</td>
<td>63.9 ±8.5†</td>
</tr>
<tr>
<td>Physically active (%)</td>
<td>44.4</td>
<td>50.0</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>25.9</td>
<td>27.7</td>
</tr>
<tr>
<td>Hypertensives (%)</td>
<td>48.2</td>
<td>68.8</td>
</tr>
<tr>
<td>Duration of diabetes (yrs)</td>
<td>9.3 ±0.8</td>
<td>8.7 ±0.4</td>
</tr>
<tr>
<td>GHbA₁ (%)</td>
<td>9.2 ±0.3</td>
<td>9.5 ±0.2</td>
</tr>
</tbody>
</table>

Hypertensives were defined as those receiving drug therapy for hypertension and/or having a systolic/diastolic blood pressure ≥160/95 mm Hg. BMI = body mass index; GHbA₁ = glycosylated hemoglobin A₁; MI+ = patients with myocardial infarction; MI− = patients without myocardial infarction; CHD− = patients free from coronary heart disease.

*p < 0.05; †p < 0.01; ‡p < 0.001, vs the MI+ group.

No difference in the mode of treatment was observed in males between the MI+ and CHD− groups, but in females the groups differed significantly from each other due to insulin therapy, which was more often used in the MI+ group. In both sexes, no difference between the MI+ and CHD− groups was observed in BMI, alcohol intake, smoking, duration of diabetes, or GHbA₁; however, female NIDDs with no CHD were younger and more physically active than female diabetics with MI.

Table 2 shows the levels of serum total and lipoprotein cholesterol and triglycerides in the groups of diabetics by sex. No difference was observed between MI+ and MI− groups in total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, or HDL triglycerides. Both male and female diabetics without MI had significantly higher values of HDL and HDL₂ cholesterol than did diabetics with MI. Total triglyceride, LDL triglyceride, and VLDL triglyceride concentrations were higher in female diabetics with MI, but such a difference was not observed among males. The comparison of MI+ and CHD− groups with respect to serum total and lipoprotein cholesterol and triglycerides were similar to those reported above for MI+ and MI− groups.

To find the variables influencing HDL and HDL₂ cholesterol concentration, we carried out separate multiple regression analyses of the male and female diabetics with MI, those without MI, and those without CHD. Age, smoking, physical activity, alcohol intake, BMI, duration of diabetes, GHbA₁, and total triglyceride concentration (logarithmic transformation) were the independent variables, and HDL and HDL₂ cholesterol (separately) were the dependent variables. The total triglyceride concentration and BMI seemed to affect HDL and HDL₂ cholesterol.
terol concentration (p < 0.05) in diabetics without MI or in CHD-free diabetics (Table 3). In female diabetics with MI, only physical activity had a significant association with HDL cholesterol, but none of the variables had an association with HDL₂ cholesterol. Correspondingly, among male diabetics with MI, none of the variables had a significant association with HDL cholesterol, and only duration of diabetes had a significant association with HDL₂ cholesterol.

On the basis of multiple regression analyses, we selected physical activity, alcohol intake, BMI, duration of diabetes, GHbA₁, and total triglyceride concentration (logarithmic transformation) as covariates for the analysis of covariance comparing diabetics by sex and by study group (the MI + group vs the MI - group or the MI + group vs the CHD - group). In both sexes, the adjustment for physical activity, alcohol intake, BMI, duration of diabetes, and GHbA₁ did not abolish the difference in HDL and HDL₂ cholesterol between the MI + and MI - groups or between the MI + and CHD groups (Figure 2). If, in addition, an adjustment was made for the total triglyceride concentration, the difference in the HDL and HDL₂ cholesterol concentrations between the MI + and MI - groups or between the MI + and CHD groups disappeared in the women, but remained significant in men between the MI + and MI - groups in HDL and HDL₂ cholesterol concentrations (significance of F 0.038 for HDL and 0.040 for HDL₂ and between the MI + and CHD groups in the HDL₂ cholesterol concentration (significance of F 0.031).

### Discussion

Several epidemiological and clinical studies have associated low HDL and HDL₂ cholesterol levels with an increased risk of CHD in nondiabetic populations. Very little is known about the association of HDL cholesterol with CHD in diabetes. Gordon et al. showed in the Framingham Study that a low HDL cholesterol value appeared to raise the CHD incidence in female diabetics relative to that of male diabetics. Reckless et al. found that vascular disease in diabetes was related to low concentrations of HDL cholesterol, but that an even stronger association existed with a raised LDL cholesterol level. Bihari-Varga et al. reported a lowering of HDL cholesterol in NIDDs with ischemic heart disease, but the decrease was not statistically significant.

In the present study, NIDDs with MI showed lower HDL and HDL₂ cholesterol concentrations than NIDDs without MI or NIDDs without CHD. The inverse relationship between HDL, HDL₂, and CHD was evident in both sexes, but was particularly strong among male NIDDs. The difference in HDL and HDL₂ cholesterol concentrations between the MI + and MI - groups or between the MI + and CHD - groups did not depend on age, smoking, physical activity, alcohol consumption, obesity, duration of diabetes, or glycosylated hemoglobin A₁. The HDL cholesterol difference between the MI + and MI - groups disappeared in women, but not in men, after inclusion of total triglycerides in the analysis in addition to the variables mentioned. This supports

### Table 2. Values of Serum Total and Lipoprotein Cholesterol and Triglycerides

<table>
<thead>
<tr>
<th>Value</th>
<th>Men (mmol/liter)</th>
<th>Women (mmol/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MI+</td>
<td>MI-</td>
</tr>
<tr>
<td></td>
<td>MI+</td>
<td>MI-</td>
</tr>
<tr>
<td>Total C</td>
<td>6.63 ± 0.25</td>
<td>6.72 ± 0.13</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.95 ± 0.03</td>
<td>1.16 ± 0.03‡</td>
</tr>
<tr>
<td>HDL₂-C</td>
<td>0.55 ± 0.03</td>
<td>0.74 ± 0.03‡</td>
</tr>
<tr>
<td>LDL-C</td>
<td>4.10 ± 0.20</td>
<td>4.24 ± 0.09</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>1.58 ± 0.20</td>
<td>1.33 ± 0.10</td>
</tr>
<tr>
<td>Total TG</td>
<td>5.67 ± 0.46</td>
<td>6.26 ± 0.26</td>
</tr>
<tr>
<td>HDL-TG</td>
<td>0.15 ± 0.02</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>LDL-TG</td>
<td>0.42 ± 0.02</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>2.67 ± 0.42</td>
<td>2.15 ± 0.24</td>
</tr>
</tbody>
</table>

*p < 0.05; †p < 0.01; ‡p < 0.001, vs the MI + group.

For cholesterol values, 1 mmol/liter = 39 mg/dl. For triglyceride values, 1 mmol/liter = 86 mg/dl. C = cholesterol; TG = triglycerides; MI + = patients with myocardial infarction; MI - = patients without myocardial infarction; CHD - = patients free from coronary heart disease.

### Table 3. Variables Influencing the HDL and HDL₂ Cholesterol Concentrations by Multiple Regression Analysis (p < 0.05) by Sex in Diabetics with (MI +) and Without MI (MI -) and In Diabetics Free from CHD (CHD -)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Men</th>
<th>Women</th>
<th>HDL cholesterol</th>
<th>HDL₂ cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI +</td>
<td>None</td>
<td>Physical activity</td>
<td>Duration of diabetes</td>
<td>None</td>
</tr>
<tr>
<td>MI -</td>
<td>Triglycerides, GHbA₁</td>
<td>BMI, triglycerides</td>
<td>BMI, GHbA₁, triglycerides</td>
<td>BMI, triglycerides</td>
</tr>
<tr>
<td>CHD -</td>
<td>Alcohol intake, BMI, GHbA₁, triglycerides</td>
<td></td>
<td>Alcohol intake, duration of diabetes, triglycerides</td>
<td></td>
</tr>
</tbody>
</table>

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HDL CHOLESTEROL, MYOCARDIAL INFARCTION, AND DIABETES

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Figure 2. Comparison of HDL and HDL2 cholesterol concentrations between groups of patients with (MI +) and without (MI -) myocardial infarction and between groups of patients with MI (MI +) and those who were free from coronary heart disease (CHD -) by analysis of covariance (adjusted for physical activity, alcohol intake, body mass index, duration of diabetes, and glycosylated hemoglobin A1).

Female diabetics with MI had higher levels of total, LDL, and VLDL triglycerides than female diabetics without MI or without CHD; however, such a difference was not observed in males. High levels of LDL triglycerides in NIDDM with CHD may be due to abnormal metabolism of VLDL and/or to chylomicrons producing accumulation of remnant particles (IDL, chylomicron remnants) that are atherogenic.21 Total triglyceride concentration was shown to be an independent risk factor for CHD in nine population samples from the WHO Multinational Study,22 but in that study the concentration of HDL cholesterol was not measured.

The data presented in this study include only information on prior episodes of CHD. The finding that HDL and HDL2 cholesterol concentration is inversely related to MI does not prove that low HDL and HDL2 cholesterol levels predict MI in NIDDM. This question of preclusive association can only be answered by prospective studies of HDL and HDL2 cholesterol and MI incidence. In nondiabetics, some prospective studies have shown that low HDL cholesterol levels are followed by greater CHD incidence, and this may also be the case in NIDDM. Behavioral changes may occur after a MI in such things as the level of physical activity, smoking, the degree of obesity, and alcohol intake, and these factors may influence the HDL cholesterol level.7 In our study, these variables can, however, hardly explain our results concerning the association of low HDL and HDL2 cholesterol with MI, because no statistically significant difference was found between the MI+ and CHD- groups with respect to physical activity, smoking, obesity, and alcohol intake.

In conclusion, even among noninsulin-dependent diabetics with generally low HDL and HDL2 cholesterol concentrations, the presence of MI is associated with particularly low HDL and HDL2 cholesterol levels. Prospective studies are needed, however, to assess the predictive value of HDL and HDL2 cholesterol level with respect to CHD risk in noninsulin-dependent diabetics.

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Index Terms: HDL cholesterol • HDL2 cholesterol • coronary heart disease • noninsulin-dependent diabetes
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