HDL, HDL₂, and HDL₃ Subfractions, and the Risk of Acute Myocardial Infarction
A Prospective Population Study in Eastern Finnish Men

Jukka T. Salonen, MD, PhD, MPH; Riitta Salonen, MD; Kari Seppänen, MPh; Rainer Rauramaa, MD, PhD; and Jaakko Tuomilehto, MD, PhD

Background. We investigated the association of cholesterol concentrations in serum high density lipoprotein (HDL) and its subfractions HDL₂ and HDL₃, with the risk of acute myocardial infarction in 1,799 randomly selected men 42, 48, 54, or 60 years old.

Methods and Results. Baseline examinations in the Kuopio Ischaemic Heart Disease Risk Factor Study were done during 1984–1987. In Cox multivariate survival models adjusted for age and examination year, serum HDL cholesterol of less than 1.09 mmol/l (42 mg/dl) was associated with a 3.3-fold risk of acute myocardial infarction (95% confidence intervals [CI], 1.7–6.4), serum HDL₂ cholesterol of less than 0.65 mmol/l (25 mg/dl) was associated with a 4.0-fold risk of acute myocardial infarction (95% CI, 1.9–8.3), and serum HDL₃ cholesterol of less than 0.40 mmol/l (15 mg/dl) was associated with a 2.0-fold (95% CI, 1.1–4.0) risk of acute myocardial infarction. Adjustments for obesity, ischemic heart disease, other cardiovascular disease, maximal oxygen uptake, systolic blood pressure, antihypertensive medication, serum low density lipoprotein cholesterol, and triglyceride concentrations reduced the excess risks associated with serum HDL, HDL₂, and HDL₃ cholesterol in the lowest quartiles by 52%, 48%, and 41%, respectively. Additional adjustments for alcohol consumption, cigarettes smoked daily, smoking years, and leisure time energy expenditure reduced these excess risks associated with low HDL, HDL₂, and HDL₃ cholesterol levels by another 26%, 24% and 21%, respectively.

Conclusions. Our data confirm that both total HDL and HDL₂ levels have inverse associations with the risk of acute myocardial infarction and may thus be protective factors in ischemic heart disease, whereas the role of HDL₃ remains equivocal. (Circulation 1991;84:129–139)

Several prospective population studies have reported an inverse association between circulating high density lipoprotein (HDL) cholesterol concentration and risk of ischemic heart disease (IHD)¹⁻¹¹; in two studies, the inverse relation did not achieve statistical significance,¹²,¹³ and in two other studies, no association was found.¹⁴,¹⁵ Most studies have reported decreased levels of both HDL₂ and HDL₃ cholesterol in patients with acute myocardial infarction (AMI) and in those with severe coronary artery disease.¹⁶ The only prospective study of IHD from which data on HDL₂ and HDL₃ subfractions have been reported¹ observed a stronger inverse association for HDL₃ than for HDL₁ cholesterol with the risk of IHD. In none of the previous studies was the effect of the random variability in individual HDL cholesterol values taken into account. Nor have the potential confounders of the association between HDL cholesterol and AMI risk been comprehensively controlled. Because previous findings were controversial and inconclusive, we investigated the associations of serum HDL, HDL₂, and HDL₃ cholesterol levels with the risk of AMI.

Methods

Subjects

The Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) is a population study with the purpose of investigating previously unestablished risk factors for AMI and carotid atherosclerosis¹⁷ in Eastern Finnish men, the population with the highest recorded incidence of IHD and mortality from IHD.¹⁸ The present analysis is based on those who participated in KIHD between March 1984 and December

From the Department of Community Health and General Practice (J.T.S.), University of Kuopio, Kuopio, the Research Institute of Public Health (R.S.), University of Kuopio, Kuopio, the Kuopio Research Institute of Exercise Medicine (K.S., R.R.), Kuopio, and the Department of Epidemiology, National Public Health Institute of Finland (J.T.), Helsinki, Finland.

Supported by grants from the Finnish Academy and the Ministry of Education of Finland.

Address for correspondence: Professor Jukka T. Salonen, University of Kuopio, P.O. Box 6, 70211 Kuopio, Finland.

Received June 27, 1990; revision accepted February 19, 1991.
were a batch of 65 men. Serum HDL cholesterol values were calculated for 1,799 men. No exclusions were made for the present analysis.

**Lipid Analysis**

The examination protocol and measurements have been previously described in detail. Subjects gave blood specimens for lipoprotein separation between 8:00 and 10:00 AM on Tuesday, Wednesday, or Thursday after having abstained from ingesting alcohol for 3 days, from smoking for 12 hours, and from eating for 12 hours. After the subject had rested in the supine position for 30 minutes, blood was drawn with Terumo Venject VT-100PZ vacuum tubes (Terumo Corp., Tokyo). No tourniquet was used.

For lipoprotein analysis, serum was separated by centrifugation at 20°C for 10 minutes at 2,000 g after coagulation at room temperature for 1 hour. Samples were stored at 4°C for no more than 2 days before separation of lipoproteins. All ultracentrifugations were done at 10°C with a Kontron TGA-65 ultracentrifuge (Kontron, Zürich). The main fractions (very low density lipoprotein [VLDL], low density lipoprotein [LDL], and HDL) were separated as described by Carlson. Serum was centrifuged for 16 hours at 115,000 g. VLDL was recovered as the top fraction and HDL was recovered as the supernatant after precipitation of the bottom fraction with dextrose sulfate and magnesium chloride. The cholesterol concentration in LDL was calculated as the difference between the bottom and HDL fractions.

The HDL2 and HDL3 subfractions were separated during a second ultracentrifugal spin at 108,000 g for 62 hours against a density of 1.25 g/cm³ as described by Kirsten and Carlson. The top and bottom fractions were separated by the tube slicing technique. In this procedure, the HDL1 subfraction, a negligible component of total HDL, is included in the HDL2 subfraction. Thus, the HDL subfraction, calculated as the difference between total HDL and HDL3, consists mainly of HDL2.

The cholesterol contents of all lipoprotein fractions were measured enzymatically (CHOD-PAP method, Boehringer Mannheim, Mannheim, FRG) on the day after the last spin. All tests were run in duplicate. A Seronorm Lipid (Nycemed, Oslo, Norway) control serum sample was included in each daily batch of cholesterol determinations. The between-batch coefficient of variation during 1984–1987 was 2.2% for total HDL, 5.2% for LDL, 9.2% for HDL, 10.8% for HDL2, and 14.2% for HDL3 cholesterol (210 batches). There was no linear trend in the quarterly control mean values over time. Tracking of serum HDL cholesterol values over time was studied annually by taking repeat serum HDL cholesterol measurements using the same method in random subsamples from the study cohort of approximately 65 men. Repeat measurements of serum HDL cho-

Other Risk Factor Measurements

The current number of cigarettes, cigars, and pipefuls of tobacco smoked daily as well as the duration of regular smoking (years); history of myocardial infarction, angina pectoris, and other IHD; and presence of hypertension and use of antihypertensive medication were recorded using a self-administered questionnaire, which was checked by an interviewer. The participants were reinterviewed regarding medical history by a physician. A subject was considered a current smoker if he had ever smoked on a regular basis or had smoked cigarettes, cigars, or a pipe within the past 30 days. Subjects who had not smoked within the past 30 days were defined as nonsmokers and given a score of 0 for number of cigarettes, cigars, and pipefuls of tobacco smoked daily. Smoking years were considered the sum of years of smoking regardless of when smoking started and stopped and whether it had occurred continuously or during several periods. Alcohol consumption was assessed by instructed and interview-checked 4-day food recording. Total leisure time energy expenditure was measured with a modified Taylor questionnaire concerning the activities within the previous 12 months.

Resting blood pressure was measured between 8:00 and 10:00 AM on the first examination day by one nurse with a random-zero mercury sphygmomanometer. The measuring protocol included, after a supine rest of 5 minutes, three measurements in the supine position, one after 1 minute of standing, and two in the sitting position with 5-minute intervals. The mean of all six systolic blood pressure values was used in the present analysis. In the present study, hypertension was defined as supine systolic blood pressure (mean of two last measurements) of 160 mm Hg or more, sitting systolic blood pressure (mean of two measurements) of 165 mm Hg or more, preexercise sitting systolic blood pressure of 170 mm Hg or more, supine diastolic blood pressure (mean of two last measurements) of 95 mm Hg or more, sitting diastolic blood pressure (mean of two measurements) of 100 mm Hg or more, preexercise sitting diastolic blood pressure of 105 mm Hg or more, or self-reported history of hypertension or use of current antihypertensive medication.

The respiratory gas exchange was measured on a breath-by-breath basis with the MGC 2001 system (Medical Graphics Corp., Minneapolis, Minn.) during a symptom-limited exercise test. The testing protocol consisted of a linear increase of work load by 20 W/min. Highest oxygen uptake (average of 8 seconds) during the test was defined as VO2 max. Men were determined to have IHD if they reported a history of myocardial infarction, angina pectoris, or other IHD; reported use of medication for angina
pectoris; or had ischemia during the maximal exercise tolerance test. Exercise electrocardiograms were coded manually by one cardiologist. The criteria for ischemia were 1) ischemic electrocardiogram, defined as horizontal or down-sloping ST depression of 0.5 mm or more or upsloping ST depression of 1.0 mm or more; 2) typical angina pectoris pain leading to discontinuation of exercise; or 3) maximal heart rate during exercise of 130 beats/min or less. Diabetes was defined as previous diagnosis of diabetes or fasting blood glucose of 8.0 mmol/l or more.

**Determination of Follow-up Events**

A prospective registry for AMI was established in the province of Kuopio as a part of the World Health Organization MONICA Project in 1982. This registry collected detailed diagnostic information on all suspected fatal and nonfatal AMI events among the population, which includes the present cohort. The events are then classified according to explicitly defined, uniform diagnostic criteria into the categories of definite AMI, possible AMI, no AMI, and insufficient data. This classification was based on autopsy, serial electrocardiographic findings, cardiac enzymes, symptoms, and history of IHD. About half of the fatal cases were autopsied. Serial electrocardiographic changes were classified according to the Minnesota Code into five categories. Cardiac enzymes were routinely determined each day since hospitalization of the patient. Aspartate aminotransferase, lactate dehydrogenase (LD), LD2, creatinine kinase, and creatinine kinase–MB levels were used, and the determination of enzymes was standardized among several hospitals participating in the AMI Registry Study in Finland. Enzymes were coded as definite if the highest value of any of the enzymes was twice the upper limit of the normal range and no other cause for the elevation was apparent. Hospitalized patients were interviewed shortly after hospital admission. In fatal cases, data on symptoms were obtained from medicolegal reports and, when necessary, by interviewing relatives. Symptoms were coded as typical if the chest pain lasted for at least 20 minutes in the absence of noncardiac, nonatherosclerotic causes.

In the present study, fatal and nonfatal events with the diagnostic category of definite or possible AMI were regarded as end points. An event was regarded as a definite AMI if at least one of the following conditions was met: definite electrocardiographic changes; typical, atypical, inadequately described symptoms combined with probable electrocardiogram and abnormal enzymes; typical symptoms and abnormal enzymes; or naked-eye appearance of fresh AMI and/or recent coronary occlusion found at necropsy regardless of other findings. Possible nonfatal AMI was confirmed if there were typical symptoms combined with probable electrocardiogram or equivocal enzymes or atypical or inadequately described symptoms combined with probable electrocardiogram and equivocal enzymes. Possible AMI among fatal cases required an exclusion of any other good evidence for another cause of death and either typical, atypical, or inadequately described symptoms; evidence of chronic coronary occlusion, stenosis, or old myocardial scarring at necropsy without typical, atypical, or inadequately described symptoms; or a good history of chronic IHD.

The coverage of the registry is checked against the computerized national hospital discharge and death certificate registers. We obtained the diagnostic information and dates of all heart attacks in our study cohort by record linkage based on the uniform Finnish personal identification code (social security number). No personal identification codes were missing in either our study cohort or the heart attack registry data. There were no losses to follow-up.

Between March 1984 and December 1988, a “definite” or “possible” fatal or nonfatal AMI was registered in 97 subjects of the present study. In the case of multiple events, the first one for each subject was considered to be the endpoint in our present analysis. The follow-up periods for individual subjects varied between 1 and 4½ years.

**Statistical Methods**

Three dummy (0, 1) variables were constructed that compared the three lowest (1st, 2nd, and 3rd) quartiles of serum HDL, HDL2, and HDL3 cholesterol with the highest (4th) quartile: 1st versus 4th, 2nd versus 4th, and 3rd versus 4th. These were entered simultaneously into BMDP Cox proportional hazards models, separate models for serum HDL, HDL2, and HDL3 cholesterol dummies, and a joint model for both HDL2 and HDL3 dummies. Three different sets of covariates were entered: first, age and examination year (as dummy variables, 1985 versus other, 1986 versus other, and 1987 versus other); second, age, examination year (as above), body mass index (kg/m2), history of or prevalent IHD (yes versus no), other cardiovascular disease (yes versus no), maximal oxygen uptake (ml/kg/min), systolic blood pressure (mean of six measurements), antihypertensive medication (yes versus no), serum LDL cholesterol concentration (mmol/l), and serum triglyceride concentration (mmol/l); and third, all variables listed above plus alcohol consumption (g/day), cigarette pack-years, and total leisure time energy expenditure (kcal/12 mo).

The goodness of fit of the proportional hazards models was examined by analyzing changes in the proportionality of hazards with time and with risk factor levels. The results indicated that the application of the models was appropriate. In addition, the simplest models (including three dummy variables for HDL, age, and examination year) were fitted using events in only the first 2½ or the last 2½ years of follow-up time. According to the deviance test for homogeneity of coefficients, there were no significant differences in the coefficients for HDL dummies between these two models.
Risk factor–adjusted relative hazards were estimated as antilogarithms of coefficients for binary (0, 1) independent variables. Their confidence intervals were estimated based on the assumption of the asymptomatic normality of estimates. Risk factor–adjusted population attributable fractions for multica
terical risk factors were computed according to Walter26 using the estimates of relative hazards derived from the Cox models.

The attenuation of the relative hazard estimates due to random variability over time was corrected by replacing the baseline quartile–specific baseline serum HDL cholesterol mean values with mean values in the same groups in repeat measurements of serum HDL cholesterol during the follow-up period. Product–moment correlations between baseline and repeat values were computed. This procedure corresponds to the nonparametric method to correct for the regression dilution bias in relative hazard estimates suggested by MacMahon and coworkers.27 Second, the “usual” serum HDL cholesterol concentrations were approximated using the simple imputation method. The linear regression equation, based on all 322 repeat measurements, was 0.63 times baseline HDL cholesterol + 0.39. All tests of significance were two sided.

**Results**

The mean values and ranges of serum HDL, HDL₁, and HDL₃ cholesterol concentrations were 1.29, 0.85, and 0.44 mmol/l and 0.52–3.05, 0.07–2.77, and 0.13–0.83 mmol/l, respectively. The unadjusted correlation between serum HDL₁ and HDL₃ cholesterol concentrations was 0.138. The strongest correlates of serum HDL, HDL₁, and HDL₃ cholesterol are presented in Tables 1 and 2. Men receiving antihypertensive medication had 12.0% lower serum HDL cholesterol and 15.9% lower serum HDL₁ cholesterol levels than those not receiving antihypertensive medication (Table 1). Fasting plasma insulin level, antihypertensive medication, alcohol consumption, maximal oxygen uptake, body mass index, and age were the strongest determinants of serum HDL and HDL₁ cholesterol levels in multivariate regression models (Table 2). Men with diabetes, IHD, or other cardiovascular disease also had decreased serum HDL, HDL₁, and HDL₃ cholesterol levels (Table 1), but these conditions had no independent association with serum HDL or its subfractions in the multivariate regression analysis. Alcohol consumption, plasma insulin concentration, antihypertensive medication, maximal oxygen uptake, and years of smoking were most strongly partially associated with serum HDL₁ cholesterol (Table 2).

The strongest predictors of AMI other than HDL cholesterol in the present study cohort were current cigarettes smoked daily, history of IHD, maximal oxygen uptake (inverse), age, antihypertensive medication, systolic blood pressure, and, if the current systolic blood pressure was not entered into the model, years of hypertension. Neither serum LDL cholesterol, serum total triglycerides, body mass index, fasting blood glucose, fasting plasma insulin concentration (mIU/l), nor diabetes (yes versus no) had a statistically significant association with the risk of AMI in the present analysis.

Cox models adjusted for age and examination years compared the three lowest quartiles of HDL cholesterol and its subfractions (as three dummy variables) with the highest quartiles. In these models, HDL cholesterol of less than 1.09 mmol/l (42 mg/dl)
TABLE 2. Main Behavioral and Biological Correlates of Serum HDL, HDL_{2}, and HDL_{3} Cholesterol Concentrations

<table>
<thead>
<tr>
<th></th>
<th>Serum HDL cholesterol</th>
<th>Serum HDL_{2} cholesterol</th>
<th>Serum HDL_{3} cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted r</td>
<td>Partial β*</td>
<td>Unadjusted r</td>
</tr>
<tr>
<td>Fasting plasma insulin (mIU/l)</td>
<td>-0.22</td>
<td>-0.10</td>
<td>-0.20</td>
</tr>
<tr>
<td>Antihypertensive medication (yes versus no)</td>
<td>-0.22</td>
<td>-0.14</td>
<td>-0.20</td>
</tr>
<tr>
<td>Alcohol consumption (g/day)</td>
<td>0.13</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td>Maximal oxygen uptake (ml/kg/min)</td>
<td>0.22</td>
<td>0.11</td>
<td>0.20</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>-0.22</td>
<td>-0.12</td>
<td>-0.22</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>-0.02</td>
<td>0.10</td>
<td>-0.01</td>
</tr>
<tr>
<td>Smoking years</td>
<td>-0.06</td>
<td>-0.07</td>
<td>-0.05</td>
</tr>
<tr>
<td>Cigarettes, cigars, and pipes smoked daily</td>
<td>-0.12</td>
<td>-0.04</td>
<td>-0.12</td>
</tr>
<tr>
<td>Plasma fibrinogen (g/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple r²</td>
<td>0.130</td>
<td>0.113</td>
<td>0.040</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein.
*Standardized partial coefficient from step-up least-squares regressions models including all independent variables shown in the order of entry to the model for serum HDL cholesterol. Correlation and regression coefficients with absolute value of at least 0.06 depart statistically significantly from 0.
†Not entered in the step-up procedure (β is too low to enter).

was associated with a 3.32-fold risk of AMI, serum HDL_{3} cholesterol of less than 0.65 mmol/l (25 mg/dl) was associated with a 4.00-fold risk of AMI, and serum HDL_{3} cholesterol of less than 0.40 mmol/l (15 mg/dl) was associated with a 2.04-fold risk of AMI (Table 3). Adjustments for body mass index, diabetes (yes versus no), and serum triglyceride and LDL cholesterol concentrations did not influence these relative hazards. When additional adjustments were made for body mass index, history of or prevalent IHD or other cardiovascular disease, maximal oxygen uptake, systolic blood pressure, antihypertensive

TABLE 3. Excess Risk of Acute Myocardial Infarction in Three Lowest Quartiles of Serum HDL, HDL_{2}, and HDL_{3} Cholesterol Concentrations Compared With Highest Quartile

<table>
<thead>
<tr>
<th>Serum cholesterol concentration</th>
<th>Adjusted for age and examination year</th>
<th>Adjusted for potential confounders and behavioral determinants of HDL*</th>
<th>Adjusted for potential confounders, behavioral determinants of HDL* and the other HDL subtraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol/l</td>
<td>Relative hazard</td>
<td>95% confidence intervals</td>
<td>Relative hazard</td>
</tr>
<tr>
<td>mg/dl</td>
<td>Relative hazard</td>
<td>95% confidence intervals</td>
<td>Relative hazard</td>
</tr>
<tr>
<td>HDL</td>
<td>1.07 – 1.24</td>
<td>41 – 47</td>
<td>3.22</td>
</tr>
<tr>
<td>&lt;0.65</td>
<td>0.81 – 1.01</td>
<td>31 – 39</td>
<td>1.97</td>
</tr>
<tr>
<td>&lt;1.01</td>
<td>&gt;39</td>
<td>&gt;57</td>
<td>1.00</td>
</tr>
<tr>
<td>HDL_{2}</td>
<td>0.65 – 0.80</td>
<td>25 – 30</td>
<td>2.56</td>
</tr>
<tr>
<td>&lt;0.81</td>
<td>0.45 – 0.50</td>
<td>17 – 19</td>
<td>1.44</td>
</tr>
<tr>
<td>&lt;0.50</td>
<td>&gt;19</td>
<td>&gt;39</td>
<td>1.00</td>
</tr>
<tr>
<td>HDL_{3}</td>
<td>0.40 – 0.44</td>
<td>15 – 16</td>
<td>1.61</td>
</tr>
<tr>
<td>&lt;0.50</td>
<td>&gt;19</td>
<td>&gt;39</td>
<td>1.00</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; NA, not applicable.
*Age (yr) (NS), examination years (three dummy variables) (NS, NS, p<0.01), body mass index (kg/m²) (NS), history of or prevalent ischemic heart disease (yes versus no) (p<0.01), other cardiovascular disease (yes versus no) (NS), maximal oxygen uptake (ml/kg/min) (p<0.05), systolic blood pressure (mean of six measurements, mm Hg) (NS), antihypertensive medication (yes versus no), serum low density lipoprotein cholesterol (mmol/l) (NS), serum triglyceride concentration (mmol/l) (NS), alcohol consumption (g/day) (NS), cigarettes smoked daily (p<0.001), smoking years (NS), and total leisure time energy expenditure in the previous 12 months (kcal).
TABLE 4. Partial Excess Risks of Acute Myocardial Infarction Associated With Blood Lipids, Smoking, and Hypertension

<table>
<thead>
<tr>
<th>Serum HDL cholesterol (mM and mg/dl)</th>
<th>Adjusted for age and examination year*</th>
<th>Adjusted for potential confounders and behavioral determinants of HDL†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative hazard</td>
<td>95% confidence intervals</td>
</tr>
<tr>
<td>&lt;1.07 (&lt;41)</td>
<td>2.80</td>
<td>1.44–5.45</td>
</tr>
<tr>
<td>1.07–1.24 (41–47)</td>
<td>1.74</td>
<td>0.87–3.50</td>
</tr>
<tr>
<td>1.25–1.47 (48–57)</td>
<td>1.39</td>
<td>0.67–2.87</td>
</tr>
<tr>
<td>&gt;1.47 (&gt;57)</td>
<td>1.00</td>
<td>...</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mM and mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥4.77 (≥184)</td>
<td>1.09</td>
<td>0.60–1.98</td>
</tr>
<tr>
<td>4.00–4.76 (154–183)</td>
<td>1.16</td>
<td>0.63–2.11</td>
</tr>
<tr>
<td>3.38–3.99 (130–153)</td>
<td>0.94</td>
<td>0.49–1.81</td>
</tr>
<tr>
<td>&lt;3.38 (&lt;130)</td>
<td>1.00</td>
<td>...</td>
</tr>
<tr>
<td>Current smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes‡</td>
<td>1.96</td>
<td>1.30–2.94</td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>...</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.85</td>
<td>1.20–2.85</td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>...</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; LDL, low density lipoprotein.

*From Cox models including age, examination year (three dummy variables), and eight terms for risk factors shown in the table.
†Age, examination year (three dummy variables), body mass index (kg/m²), history of prevalent ischemic heart disease (yes versus no), other cardiovascular disease (yes versus no), maximal oxygen uptake (ml/kg/min), serum triglyceride concentration (mmol/l), alcohol consumption (g/day), total leisure time energy expenditure in the previous 12 months (kcal), and eight terms for risk factors shown in the table.
‡Ever smoked on a regular basis or smoked cigarettes, cigars, or a pipe within 30 days before baseline examination.

Medication (yes versus no), and serum LDL cholesterol or serum triglyceride concentration, the excess risks (relative hazard minus 1) associated with serum HDL, HDL₂, and HDL₃ cholesterol in the lowest quartile decreased by 52%, 48%, and 41%, respectively. The decrease in excess risk was mainly a result of history of IHD and VO₂max. Inclusion of diabetes (yes versus no) in the model did not change the relative hazards. Additional adjustments for alcohol consumption (g/day), current cigarette, cigars, and pipefuls smoked daily, smoking years, and leisure time energy expenditure (kcal/yr) reduced these excess risks an additional 26%, 24%, and 21%, respectively (Table 3).

In a Cox model including in addition to age and examination years both serum HDL₂ and HDL₃ cholesterol dummies, the three lowest quartiles of HDL₂ cholesterol were associated with 3.74-fold (95% CI, 1.80–7.78), 2.48-fold (95% CI, 1.16–5.33), and 1.97-fold (95% CI, 0.89–4.33) risks of AMI. When HDL₂ was simultaneously included in the model, the impact of HDL₃ cholesterol was reduced. The respective relative hazards for the three lowest quartiles of HDL₃ cholesterol were 1.66 (95% CI, 0.84–3.27), 1.40 (95% CI, 0.70–2.78), and 1.38 (95% CI, 0.68–2.80). In a Cox model adjusted for potential confounders (second model in Table 3), the relative hazards (with 95% CIs) for the three lowest quartiles of HDL₂ and HDL₃ cholesterol, when entered simultaneously, were 2.44 (95% CI, 1.12–5.28), 1.60 (95% CI, 0.72–3.54), and 1.62 (95% CI, 0.73–3.59) for HDL₂ and 1.46 (95% CI, 0.72–2.95), 1.34 (95% CI, 0.66–2.71), and 1.31 (95% CI, 0.64–2.70) for HDL₃, respectively. The respective relative hazards adjusted for all potential confounders and behavioral determinants of HDL are shown in Table 3.

In a Cox model that includes age, examination year, serum HDL and LDL cholesterol levels (three dummy variables each), smoking (within 30 days), and hypertension, the excess risk in men in the lowest quartile of serum HDL cholesterol was greater (relative hazard, 2.80) than that for smoking (relative hazard, 1.96) and hypertension (relative hazard, 1.85) (Table 4). When history of or prevalent cardiovascular disease and maximal oxygen uptake were added into the model, these excess risks were appreciably reduced (by 18% for smoking and 53% for hypertension). The inclusion of terms for interactions between HDL dummies and smoking or between HDL dummies and hypertension did not affect the relative hazard estimates for HDL quartiles to a significant degree. Also, the addition of interaction terms did not improve the fit of the model, as judged based on the change in the deviance for the entire model.
The percentages of AMIs attributable to low HDL cholesterol were 67% when adjusted for age and examination years, 35% when adjusted for the potential confounding factors listed in the first footnote of Table 3, and 24% when also adjusted for behavioral determinants of HDL listed in the footnote of Table 3. The respective population attributable percentages for smoking and hypertension were 23% and 30% with adjustments for age and examination year and 19% and 17% with additional adjustments for the potential confounders defined in Table 4.

In the multivariate Cox models adjusted for age and examination year, both serum HDL ($z=-3.61, p<0.001$) and serum HDL$_2$ ($z=-3.43, p<0.001$) cholesterol concentrations had significant independent associations with the risk of AMI. The association of serum HDL$_3$ cholesterol with AMI did not reach statistical significance in a two-sided test ($z=-1.92, p=0.07$). An increase of 1.0 mmol/l in HDL, HDL$_2$, and HDL$_3$ cholesterol reduced the risk of AMI by 79%, 79%, and 94%, respectively. When adjusted for age, examination year, maximal oxygen uptake, prevalent IHD, other cardiovascular disease, body mass index, baseline serum LDL cholesterol and triglyceride concentrations, systolic blood pressure, and anti-hypertensive medication, an increase of 1.0 mmol/l in baseline HDL cholesterol was associated with a 61% decline of AMI risk. The respective decline associated with an increase (mmol/l) in the usual HDL cholesterol was 76%.

The unadjusted correlations between baseline measurement and repeat measurements 1–3 years after baseline measurement were 0.72 ($n=130$) for serum HDL cholesterol and 0.57 ($n=365$) for serum LDL cholesterol concentrations. The respective correlation coefficients for baseline versus 3–5-year postbaseline measurements were 0.67 ($n=192$) and 0.52 ($n=481$). The baseline quartile-specific mean serum HDL cholesterol concentrations for measurements at baseline and 1–3 and 3–5 years after baseline are presented in Table 5. The differences in serum HDL cholesterol mean values between the lowest and the highest baseline quartiles were 0.74 mmol/l at baseline and 0.44 mmol/l (41% less) an average of 1–5 years after baseline (during the follow-up period of the present study). The average difference between quartile specific mean values was 0.25 mmol/l for baseline measurements and 0.15 mmol/l (41% less) for measurements 1–5 years after baseline.

The relative hazards in the quartiles of baseline serum HDL cholesterol concentration with adjustment for the potential confounding factors listed in the footnote of Table 3 are presented in Figure 1. The relative hazards are plotted against quartile-specific mean values of both baseline HDL cholesterol and HDL cholesterol during the follow-up. The latter curve represents the association between serum HDL cholesterol and AMI risk when corrected for the regression dilution bias.

When adjusted for age, examination year, IHD, other cardiovascular disease, systolic blood pressure, antihypertensive medication, and serum LDL cholesterol, an increment of 1 mg/dl in serum HDL cholesterol was associated with a 2.4% (4.1% with correction for regression dilution bias) reduction in AMI risk, and an increment of 10% in HDL cholesterol was associated with a 10% (corrected, 17%) reduction in AMI risk. An additional adjustment for cigarette pack-years decreased the respective (uncorrected) decrements of AMI risk to 2.2% and 9%.

**Discussion**

Earlier, we reported preliminary findings concerning the roles of HDL$_2$ and HDL$_3$ subfractions with regard to the risk of AMI. The present data confirm that HDL$_2$ subfraction has an inverse association with the risk of AMI, whereas the role of HDL$_3$ remains equivocal. The association was stronger for HDL$_2$ than HDL$_3$, and the association of HDL$_3$ with AMI lost its statistical significance when adjustment was made for HDL$_2$. The impact of neither total HDL nor its subfractions was weakened by adjustments for age, serum LDL cholesterol or triglyceride concentrations, obesity, or diabetes. Decreased serum HDL$_2$ cholesterol was found in men who received antihypertensive medication, had high body mass index or high fasting plasma insulin, or currently smoked. Elevated serum HDL$_3$ cholesterol was associated with high alcohol consumption and high maximal oxygen uptake. Serum
HDL cholesterol correlated appreciably with alcohol consumption and weakly with plasma insulin, antihypertensive medication, maximal oxygen uptake, and duration of smoking.

In the present analysis, a very conservative approach was taken, and the major behavioral determinants of HDL (smoking, alcohol consumption, and physical activity) were controlled for. Because HDL may be a major mediating biological mechanism in the effect of smoking, alcohol, and exercise on IHD risk, the analytical approach may also be considered to represent an overadjustment.

The strong age-adjusted inverse association of total serum HDL cholesterol with the risk of AMI is in accord with the observations of earlier prospective population studies. The relative hazard, an estimator of relative risk, was 3.3 when the lowest quartile (less than 42 mg/dl) and the highest quartile (57 mg/dl or more) were compared. In the Framingham study, serum HDL cholesterol in the bottom quintile (less than 35 mg/dl) was associated with a 4.1-fold increased risk factor-adjusted 12-year mortality from IHD compared with HDL cholesterol of 55 mg/dl or more in 1,007 men free of IHD who were 50–79 years old. In the same men, both the age-adjusted and the risk factor–adjusted relative hazards of myocardial infarction in the lowest quintile (36 mg/dl or less) was 1.7 (95% CI, 1.0–2.9) compared with the highest quintile (53 mg/dl or more).

Low serum HDL cholesterol was also significantly associated with increased mortality from IHD in the Livermore Study, Honolulu Heart Study, Framingham Study, Tromsø Heart Study, Oslo Heart Study, Israeli Ischaemic Heart Disease Study, Multiple Risk Factor Intervention Trial, Coronary Primary Prevention Trial, Lipid Research Clinics Prevalence Mortality Follow-up Study, Helsinki Heart Study, and British Regional Heart Study. Statistically nonsignificant inverse trends were observed in the Minnesota Businessmen Study and the Finnish cohort of the Seven Countries Study. In the first analysis of the British Regional Heart Study, a significant inverse association was observed between nonfasting serum HDL cholesterol and incidence of IHD, but this association was substantially weakened and lost its statistical significance by adjusting for the standard coronary risk factors, including non-HDL cholesterol. In a later analysis based on a longer follow-up, the inverse association continued to be significant after risk factor adjustment. Men in the lowest quintile (less than 0.93 mmol/l, or 36 mg/dl) had a 2.0-fold risk compared with those in the highest quintile (at least 1.33 mmol/l, or 51 mg/dl). In two studies, there was no association between HDL and IHD risk. Of these two studies, the study from Gothenburg was based on only 18 case–control pairs, including several men on lipid-altering medication.

Previous studies of HDL cholesterol and IHD risk have not taken into account the attenuation of the relation resulting from random measurement variability and the regression of HDL cholesterol values toward the mean over time. If no correction for this regression dilution bias is applied, the present data agree with the estimates from four prospective US studies that a 1-mg/dl (0.026-mmol/l) increment in HDL cholesterol level was associated with a 2–3%
decrement in IHD risk.9 With the correction, the respective decrement of AMI risk in our data was approximately 4%. According to our data, the potential impact of HDL cholesterol in the prevention of IHD is impressive. If the association between HDL and IHD was causal, the elevation of all HDL values of less than 1.5 mmol/l (58 mg/dl) to above this value would theoretically prevent between 35% and 67% of AMIs, depending on which other factors are assumed to change with HDL cholesterol. In the present study cohort with a short average follow-up, a low serum HDL cholesterol concentration appears to be a stronger risk factor for AMI than smoking, hypertension, or serum LDL cholesterol concentration. In addition to the shortness of follow-up, the weakness of the association between serum LDL cholesterol and AMI risk could have been a result of the weaker tracking (greater random variability) of LDL than HDL cholesterol over time.

Quantitative estimates of the association between HDL and IHD must be interpreted cautiously because differences can be caused by a number of factors. In prospective studies, the characteristics of the subjects, length of follow-up, choice and determination of follow-up events, blood sampling, treatment of serum or plasma samples, and method of HDL measurement may be relevant. In the present study, blood samples were drawn from resting subjects in the supine position after a 12-hour fast and abstinence from smoking in the morning, and lipoprotein fractions were separated from unfrozen serum samples, and analyzed within 2 days. Serum HDL was separated with a combination of ultracentrifugation and precipitation, and HDL3 was separated with a second ultracentrifugal spin at the site of blood sampling. Despite the time-consuming analytical procedure, the measurement variabilities of HDL, HDL2, and HDL3 cholesterol measurements were considerably large. Because random variability in the risk factor measurements attenuates associations with disease outcomes, the greater measurement variability for HDL3 than for HDL2 cholesterol may account for a part of or the entire difference in the strength of their association with AMI risk.

The early Livermore Study is the only prospective population study that has investigated the association of HDL subfractions with the risk of IHD.1 In that study, both HDL2 and HDL3 fractions were significantly inversely associated with the risk of an IHD event, even though the association was stronger for HDL2 than for HDL3 fraction. In most case–control studies comparing subjects with and without angiographically assessed coronary artery disease, lower levels of HDL2 and HDL3 cholesterol have been observed in cases than in controls.16 In most studies, there has been a greater proportionate difference in HDL2, although there have been some exceptions.33 In addition, in the National Heart, Lung, and Blood Institute Type II Intervention Study,34 the extent of coronary atherosclerosis associated with both HDL subfractions but significantly with only HDL3. The question, however, of whether only HDL2 or HDL3 subfraction is protective of IHD may be irrelevant because both fractions participate in the reverse cholesterol transport.35

Theoretically, serum HDL cholesterol could be an indicator of another factor rather than a true protective factor per se. First, it reflects the levels of cigarette smoking, physical activity, and alcohol consumption and is associated with obesity, diabetes, and hypertension. The inverse association of serum HDL and HDL2 cholesterol with the risk of AMI persisted in our data, however, even after adjustments for all of these factors in a multivariate analysis. Also, in the Lipid Research Clinics Follow-up Study, the association of cigarette smoking with cardiovascular disease mortality was independent of HDL cholesterol, and the inverse relation between alcohol consumption and cardiovascular disease mortality was only partially mediated through HDL cholesterol.31 It is also possible that decreased serum HDL is an indicator of another metabolic disorder that could be the true etiologic factor. Reduced HDL could reflect inefficient catabolism of triglyceride-rich lipoproteins and be associated with chylomicron remnants. Low HDL cholesterol is associated with decreased lipoprotein lipase activity and increased hepatic lipase activity. Low serum HDL cholesterol concentration can be a consequence of either a decreased number of HDL particles in the circulation or a reduced size of HDL particles resulting from cholesterol depletion. These two conditions may not have the same etiologic relevance. The absence of accelerated atherogenesis in patients with low or no HDL35–37 speaks against a direct causal role of HDL in atherogenesis. In addition, coronary artery disease has been reported in persons with familial hyper-HDL2–cholesterolemia.38

In our study cohort with a very high incidence of AMI and a short average follow-up, serum HDL cholesterol concentration was one of the strongest predictors of AMI. The lack of the predictive power of serum LDL cholesterol may be a result of the short follow-up because LDL conceivably increases the risk of AMI through promotion of the progression of coronary atherosclerosis as well as a result of large random variability over time. Serum LDL cholesterol was the strongest determinant of the progression of carotid atherosclerosis in a 2-year follow-up study of a subsample of the present subjects with B-mode ultrasonography, whereas serum HDL cholesterol had no association with atherosclerosis progression in this small cohort.39 An identical finding was observed in a 2-year coronary atherosclerosis progression study with repeat angiograms.40 On the other hand, in two other angiography studies,34,41 HDL cholesterol had an inverse relation with atherosclerosis progression. These observations provide some support for the hypothesis of differential pathogenetic mechanisms for the effects of serum LDL and HDL cholesterol in IHD.

Because the protective impact of serum HDL cholesterol concentration becomes detectable in such
a short follow-up, the effect of HDL may be-mediated through pathways other than atherogenesis; the reverse cholesterol transport from arteries to the liver may not be the only antiatherogenic mechanism of HDL. There are other possible alternative or parallel mechanisms for the protective effect of HDL. First, HDL may act as an antioxidant; that may explain why it is associated in cell culture with increased prostacyclin production and reduced platelet aggregability. As an antioxidant, HDL inhibits the oxidation of cholesterol and the formation of lipid peroxides, which are cytotoxic for the endothelium and inhibit prostaglandin synthesis at certain levels. Also, apo-
lipoprotein A-I has been observed to stabilize secreted prostacyclin. Second, HDL has been found to promote fibrinolysis. Third, high levels of HDL may reduce the uptake of LDL by endothelial cells by competing for the LDL receptor. Fourth, HDL may prevent the formation of LDL aggregates, which appear to facilitate the influx of LDL to the endothelial cells. Finally, HDL counteracts in vitro the activation of platelets by LDL. Apolipoprotein A-I has been found to increase the solubility of cholesterol in bile.

The role of HDL as an antioxidant and its impact on platelet function and thrombogenesis deserve thorough studies. The effect of HDL and its subfra-
tions on atherosclerosis progression must be investigated in large population cohorts with repetitive assessments of atherosclerosis. In conclusion, the present data provide confirmation of the inverse association between serum HDL and HDL2 cholesterol and the risk of IHD, whereas the role of HDL3 remains only suggestive. Our findings are consistent with the concept that high HDL blood levels or associated metabolic factors are protective for IHD.

Acknowledgments

We are grateful to Professor Kalevi Pyörälä and Dr. Pertti Palomäki, Kuopio University Hospital, for providing access to data of the AMI Registry in the province of Kuopio, to Dr. Esko Taskinen and Dr. Juha Venäläinen for supervising the maximal exercise tests, and to Dr. Jaakko Eränen for coding the exercise electrocardiograms.

References

8. Gordon DJ, Knoke J, Probstfield JL, Superko R, Tyroler HA, for the Lipid Research Clinics Program: High density lipoprotein cholesterol and coronary heart disease in hypercholes-
KEY WORDS: clinical trials • high density lipoprotein • acute myocardial infarction
HDL, HDL2, and HDL3 subfractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men.
J T Salonen, R Salonen, K Seppänen, R Rauramaa and J Tuomilehto

Circulation. 1991;84:129-139
doi: 10.1161/01.CIR.84.1.129

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/84/1/129

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/