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Associations of HDL_2 and HDL_3 Subfractions With Ischemic Heart Disease in Men

Prospective Results From the Québec Cardiovascular Study

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Abstract

Abstract Individuals with elevated plasma concentrations of HDL cholesterol are at lower risk for ischemic heart disease (IHD). Whether the cardioprotective effects of HDL can be attributed to one or both HDL subfractions (HDL_2 and HDL_3) remains, however, controversial. The relationship of HDL subfractions to the incidence of IHD was investigated in a sample of 1169 French-Canadian men younger than 60 years and living in the Quebec City suburbs. Between 1980 to 1981 and 1990, 83 of the 944 men with complete follow-up in 1990 (80.8%) had a first IHD. Men who developed IHD had lower HDL, HDL_2, and HDL_3 cholesterol concentrations at baseline than men who remained

Top
Abstract
Introduction
Methods
Results
Discussion
References

free from IHD. Adjusted relative risk (RR) of IHD was calculated among quartiles of HDL cholesterol and HDL subfractions with the use of Cox survival models. Men in the fourth quartile of HDL_2 (RR=0.21; 95% confidence interval [CI], 0.08 to 0.56) and HDL_3 cholesterol distributions (RR=0.37; 95% CI, 0.15 to 0.94) were at lower risk for IHD than men in the first quartile. Despite the fact that the respective contributions of HDL_2 and HDL_3 to IHD risk were of the same magnitude in a multivariate model that included both subfractions, the contribution of the HDL_2 subfraction was statistically significant (standardized RR=0.84; 95% CI, 0.74 to 0.95), whereas it did not reach significance for HDL_3 (standardized RR=0.87; 95% CI, 0.69 to 1.11). Neither the linear combination of HDL_2 and HDL_3 nor their ratio provided further information on the risk of IHD compared with HDL cholesterol alone or with the ratio of total to HDL cholesterol. >From a statistical standpoint, the present data suggest that the HDL_2 subfraction may be more closely related to the development of IHD than the HDL_3 subfraction. However, the qualitative difference in the relative predictive value of each subfraction was trivial, since it only corresponded to a modest quantitative difference. Thus, the possibility that a significant proportion of the cardioprotective effect of elevated HDL cholesterol levels may be mediated by the HDL_3 subfraction still cannot be excluded. Finally, from a clinical point of view and within the limits of resolution provided by these data, the measurement of HDL subfractions does not appear to provide any additional information on the risk of IHD than HDL cholesterol alone or the ratio of total to HDL cholesterol.

Key Words: HDL subfractions • ischemic heart disease

Introduction

Top
Abstract
Introduction
Methods
Results
Discussion
References

The protective role of HDL against the development of IHD is well accepted; several prospective studies[1] [2] [3] [4] [5] [6] have confirmed the early observations of Barr et al,[7] who first suggested more than 40 years ago that individuals with elevated plasma concentrations of HDL cholesterol were at lower risk for IHD. However, the mechanisms whereby elevated HDL cholesterol levels may prevent the development of premature atherosclerosis are not fully understood.[8] Among other factors, it has been suggested that any benefit underlying elevated HDL cholesterol levels could be mainly attributed to the HDL_2 subfraction,[9] and most cross-sectional analyses tend to support this concept.[8] [10] To date, only four prospective studies[9] [11] [12] [13] have compared the relative importance of HDL subfractions on IHD risk in middle-aged men, and results reported have been rather inconsistent. All four prospective studies have reported that both HDL_2 and HDL_3 subfractions were reduced among men who developed IHD.[9] [11] [12] [13] Only three have compared the discriminating potential of HDL_2 and HDL_3 subfractions on IHD risk using a multivariate approach.[11] [12] [13] In the Physicians' Health Study[11] and the Caerphilly and Speedwell Collaborative Heart Disease Studies,[12] HDL_3 cholesterol was the strongest predictor of IHD. Inversely, results from the Kuopio Ischemic Heart Disease Risk Factor Study[13] have suggested that the cardioprotective effect of elevated HDL cholesterol levels may be attributed to the HDL_2 subfraction. Whether our ability to predict IHD is improved by the measurement of cholesterol in HDL subfractions also requires further evaluation from prospective studies. In this report, we have investigated the respective contribution of HDL cholesterol and its subfractions as well as of other established risk factors to the development of IHD in a cohort of 944 French-Canadian men living in the Québec metropolitan area and followed over a period of approximately 10 years.

Methods

Top
Abstract
Introduction
Methods
Results
Discussion
References

Study Cohort and Follow-up

The study cohort has been previously described.[14] Briefly, a random sample of 4637 men (aged 35 to 64 years), 99% of French-Canadian descent, was recruited in 1973 for a study on cardiovascular disease risk factors that included the measurement of nonfasting cholesterol levels.[14] Of this sample, 266 men had clinically diagnosed IHD or stroke and were excluded from further follow-up. In 1980 to 1981, fasting blood lipid and lipoprotein measurements were performed, and their cross-sectional relationships to other risk factors for IHD such as age, body weight, and alcohol consumption in men younger than 60 years were investigated.[15] For this purpose, a subgroup of 2077 men free of IHD

were randomly selected to participate in this substudy. Seventy-four subjects had died, and 60 could not be located. The remaining 1943 men were invited to participate in the clinical evaluation: 427 (22.0%) were not interested in participating, 158 (8.1%) were excluded because they had pathologies or were under pharmacological treatment known to directly interfere with lipid metabolism, 36 (1.9%) did not appear at the clinic, and 153 (7.9%) had left the Québec City area. In 1990 to 1991, participants were contacted by mail and invited to answer a short standardized questionnaire inquiring about medication, status of cardiovascular diseases, and diabetes mellitus.[14] For those who reported such diseases and those who died, hospital charts were reviewed. Telephone calls were made to participants who did not answer a second letter or, if unsuccessful, to a close family member. HDL subfractions were measured in 1169 men in 1980 to 1981,[15] and of this subsample, 80.8% (n=944) had a complete follow-up by 1990.

Evaluation of Risk Factors

In 1980 to 1981, each of the participants completed a standardized questionnaire administered by trained nurses and further reviewed by a physician before the participants left the clinic. Demographic, medical, and lifestyle histories were recorded, and blood pressure, body weight, and body height were measured. Body mass index was computed as a function of weight (in kilograms) divided by height (in meters) squared. Blood pressure was measured after the subjects had rested for 5 minutes in a sitting position. Measurements were done with a calibrated mercury sphygmomanometer, with phases I and V of Korotkoff sounds used for systolic and diastolic blood pressures, respectively. The mean of two measures taken 5 minutes apart was used in the analyses. Alcohol intake was computed from the type of beverage (beer, wine, and spirits) consumed in ounces per week and then standardized as absolute quantity, with 1 oz of absolute alcohol equivalent to 22.5 g of alcohol.[16] Smoking habits were categorized as follows: 1, subjects who had never smoked; 2, ex-smokers (subjects who stopped smoking at least 1 year before the 1981 baseline visit); and 3, current smokers.

Definition of End Points

The diagnosis of a first IHD event included typical effort angina, coronary insufficiency, nonfatal MI, and coronary death according to the criteria proposed by Gillum et al.[17] The diagnosis of effort angina was based on typical retrosternal squeezing or pressure-type discomfort occurring on exertion and relieved by rest and/or nitroglycerin. The diagnosis of coronary insufficiency was considered if typical retrosternal chest pain, of at least 15 minutes' duration, was associated with transient ischemic changes on ECG (Minnesota codes 5-1 or 5-2) without significant elevation of the levels of creatine phosphokinase. The diagnosis of MI was based on evolutive ECG changes suggestive of myocardial necrosis (Minnesota code 1-1) or the presence of at least two of the following criteria: ECG evidence of myocardial necrosis according to Minnesota codes 1-2-1 to 1-2-5 and 1-2-7 or changes in repolarization (codes 9-2 and 5-1 or 5-2); abnormal enzymes defined by a value of total creatine phosphokinase at least twice the upper limit of normal; and typical retrosternal chest pain of at least 20 minutes' duration not relieved by rest and/or nitroglycerin. All ECGs were read by the same cardiologist, who was unaware of the participants' risk profile. Criteria for the diagnoses of coronary deaths were confirmed through death certificates or autopsy reports confirming the presence of coronary disease. MI was considered fatal if criteria for MI were met and death occurred within 4 weeks of MI or if acute MI was diagnosed at autopsy. Circumstances and time elapsed between symptoms or death were verified from close relatives and hospital files. Informed consent was obtained to review relevant hospital files. Autopsies were performed in approximately one third of deaths.

Laboratory Analyses

Blood samples were obtained after a 12-hour fasting period. Venipuncture was done while the participants were in a sitting position. A tourniquet was used but released before collection of blood samples. Venous blood was withdrawn in evacuated tubes (Becton-Dickinson) containing EDTA, and all measurements were performed on fresh plasma (within 3 hours after venipuncture). After separation of plasma from blood cells by centrifugation, total plasma cholesterol and triglyceride levels were determined on an Auto Analyzer II (Technicon Instruments Corp) as previously described.[18] Total plasma HDL cholesterol levels were measured in the supernatant fraction after precipitation of apoprotein B–containing lipoproteins with heparin/manganese chloride.[19] HDL_2 was then precipitated from the HDL fraction[20] with a 4% solution of low-molecular-weight dextran sulfate (15 to 20 kD) obtained from SOCHIBU. The cholesterol content of the supernatant fraction (HDL_3) was determined, and HDL_2 cholesterol levels were derived by subtracting HDL_3 from total HDL cholesterol concentrations. The measurements of HDL_2 and HDL_3 cholesterol levels yielded coefficients of variation of 9.8% and 6.3%, respectively. One subject with triglyceride levels above 10 mmol/L was excluded from the analyses because the possibility that such elevations in plasma triglyceride levels may be due to familial hyperchylomicronemia could not be excluded.[21]

Statistical Methods

Baseline means and frequency data between men who developed IHD (IHD+) and those who remained free of IHD during follow-up (IHD-) were compared with one-way ANOVA and the χ^2 test, respectively. Interrelationships among metabolic variables were assessed by correlational analyses with the Spearman coefficient, which takes into account the nonparametric distribution of variables. Log

transformation and the Spearman's statistic essentially yielded similar correlation coefficients. We calculated duration of follow-up in person-years using the follow-up of each participant from the 1980 to 1981 evaluation until the 1990 last contact, death, or onset of IHD. Cox proportional hazards models were used to assess the risk of IHD among quartiles of HDL, HDL_2, and HDL_3 cholesterol, triglycerides, and the HDL_2/HDL_3 and total/HDL cholesterol ratios. RRs were computed as the estimated relative rate of events using the quartile with the lowest concentration as reference, which by definition was assigned a risk of 1.0. Age, systolic blood pressure, smoking, and family history of IHD were included in all analyses as potential confounders. The Kaplan-Meier survival probability (estimated probability of not having IHD during follow-up) was computed for each quartile of HDL_2 and HDL_3 cholesterol. The log-rank test was used to compare parallelism of survival curves among quartiles. HDL cholesterol concentrations and its subfractions were also treated as continuous variables in further analyses. Results are presented as standardized RRs of IHD (also adjusted for confounders), which represent the change in risk of IHD associated with an increase of 20% in the cholesterol concentration. To compare the combined contribution of HDL subfractions to the contribution of HDL cholesterol alone in the risk assessment of IHD, the log-likelihood statistic, or deviance, is presented. The smaller the deviance, the better is the fit of the model in describing the response variable. The difference in deviance between two models essentially follows a χ^2 distribution. It is thus possible, with the use of this statistic, to compare the degree of fit of two models. Stepwise multiple survival analyses were also performed to identify the best predictors of IHD risk in this cohort of men. All statistics were performed with SAS software (SAS Institute).

Results

Top
Abstract
Introduction
Methods
Results
Discussion
References

Mean follow-up of the 944 men evaluated in 1980 to 1981 was 8.2 years and generated 7773 person-years. Among the 944 men without IHD at entry, 83 developed IHD during follow-up for an incidence rate of 107/10 000 person-years. First IHD events included 39 cases of MI, 32 cases of angina pectoris, and 12 coronary deaths. Men who developed IHD during this period [IHD(+)] were older and had a higher systolic blood pressure than men who remained free from IHD [IHD(-)] during the same period (Table 1). Body mass index and diastolic blood pressure were, however, comparable in both groups. Baseline smoking habits were also similar between the two groups. Approximately 45% of subjects were current smokers, whereas 16% had never smoked and 38% were ex-smokers. Among men who were current smokers, the number of cigarettes smoked per day was also similar between IHD(+) and IHD(-) men (29 versus 27 cigarettes per day, respectively). The frequency of heavy smokers (men smoking >25 cigarettes per day) was also identical in IHD(+) and IHD(-) men (26% in both groups). Finally, IHD(+) men consumed less alcohol than IHD(-) individuals (4.0 versus 5.5 oz of alcohol per week; P=.08) at entry. Mean plasma concentrations of HDL cholesterol and its subfractions as well as the ratio of HDL_2 to HDL_3 cholesterol measured at baseline are presented in Fig 1. Plasma levels of HDL cholesterol (0.91±0.20 versus 0.99±0.24 mmol/L) and of HDL_2 (0.29±0.12 versus 0.34±0.16 mmol/L) and HDL_3 cholesterol (0.62±0.13 versus 0.56±0.13 mmol/L) were all significantly decreased in IHD(+) men compared with IHD(-) men, with the HDL_2 cholesterol subfraction showing the largest difference (14.7%) between the two groups. The ratio of HDL_2 to HDL_3 cholesterol was also reduced in IHD(+) men compared with IHD(-) men (P=.005).

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Table 1. Baseline Risk Factors in IHD(-) and IHD(+) Men

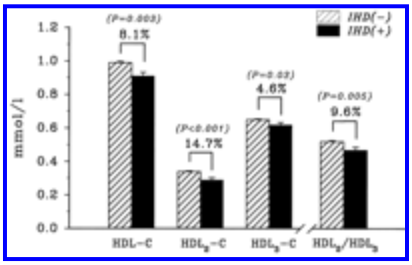

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Figure 1. Comparison of mean plasma concentrations of HDL cholesterol (C) and of its subfractions at baseline in IHD(+) and IHD(-) men. Values are mean±SEM. The relative difference (percent) in the cholesterol content of HDL, HDL_2, and HDL_3 as well as in the ratio of HDL_2 to HDL_3 in IHD(+) men compared with IHD(-) men is also presented.

The Kaplan-Meier estimated survival probability for each quartile of HDL_2 and HDL_3 is presented in Fig 2. The log-rank test for equality across HDL_2 quartiles was significant (*P*=.01), suggesting that men with lower HDL_2 cholesterol concentrations showed an increased probability of having IHD during follow-up compared with men in the highest quartile of HDL_2 cholesterol distribution. There was also an obvious trend for an increased probability of developing IHD in men with reduced HDL_3 cholesterol levels. However, the log-rank test for equality across HDL_3 quartiles did not reach statistical significance (*P*=.06).

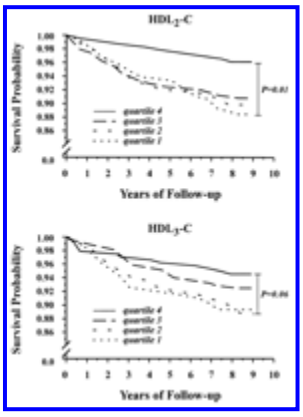

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Figure 2. Kaplan-Meier survival curves for quartiles of HDL_2 (top) and HDL_3 (bottom) cholesterol (C) concentrations expressed as the estimated probability of not having IHD during the 10-year follow-up. The log-rank test showed that the difference in estimated probability was different over the four HDL_2 cholesterol quartiles (*P*=.01). The test did not reach statistical significance for HDL_3 cholesterol quartiles (*P*=.06).

Table 2 presents the RRs and 95% CIs for each quartile of HDL cholesterol and its subfractions as well as for triglyceride levels and the ratio of total to HDL cholesterol. Also presented are probability values of trends for change in IHD risk with increasing concentrations or ratios when considered as continuous variables. Men in the highest quartile of the HDL_2 cholesterol distribution were characterized by a 4.8-fold decrease in the risk of IHD compared with men in the first quartile (RR=0.21; 95% CI, 0.08 to 0.56). The reduction in risk for men in the fourth quartile of HDL (RR=0.42; 95% CI, 0.22 to 0.79) and HDL_3 cholesterol (RR=0.37; 95% CI, 0.15 to 0.94) was also important but of lower magnitude than for HDL_2 cholesterol. Finally, an elevated ratio of total to HDL cholesterol was associated with a marked increase in the risk of IHD (RR=5.0 for men in the fourth quartile; 95% CI, 2.19 to 11.38).

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Table 2. Adjusted RR of IHD During the 10-Year Follow-up Across Quartiles of HDL Cholesterol, HDL Subfractions, Triglycerides, and Ratio of Total to HDL Cholesterol

Table 3 presents the standardized RRs of developing IHD for HDL cholesterol and its subfractions as well as for the combination of HDL_2 and HDL_3 cholesterol levels as continuous variables. The degree of fit of models (Δdeviance), which corresponds to the difference in deviance between each model and the model that included confounders only, is also presented. The standardized RR for increasing HDL cholesterol was 0.69 (95% CI, 0.50 to 0.83), suggesting that an increase of 0.20 mmol/L (or 20%) in HDL cholesterol levels was associated with a 31% decrease in the risk of developing IHD. The addition of HDL, HDL_2, or HDL_3 cholesterol levels to a model that included only the confounders reduced the deviance by 11.7 ($P<.001$), 10.9 ($P<.001$), and 5.0 ($P<.05$), suggesting that HDL and its subfractions significantly improved the prediction of risk over the use of nonmetabolic risk factors. Combination of HDL_2 and HDL_3 cholesterol also decreased the deviance significantly (Δdeviance=12.3; $P<.01$). Despite the fact that the contributions of both subfractions in this model were of the same magnitude, the contribution of the HDL_2 subfraction was significant (standardized RR=0.84; 95% CI, 0.74 to 0.95), whereas that of the HDL_3 subfraction was not (standardized RR=0.87; 95% CI, 0.69 to 1.11). Although the model that included both HDL_2 and HDL_3 subfractions yielded a better prediction of IHD risk (Δdeviance=12.3) than the model with HDL_2 cholesterol (Δdeviance=11.7), the difference between these two models was negligible and of no statistical significance. The ratio of HDL_2 to HDL_3 cholesterol was also a significant predictor of risk (not shown). The reduction in deviance for the model with the ratio of HDL_2 to HDL_3 cholesterol was, however, of the same magnitude as the reduction in deviance associated with HDL cholesterol only.

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Table 3. Respective Contribution of HDL and Its Subfractions to the Risk of IHD: Standardized RRs of Developing IHD

Additional analyses showed that HDL_2 remained significantly associated with the risk of IHD after control for triglyceride (standardized RR=0.83; 95% CI, 0.74 to 0.95) and LDL cholesterol levels (standardized RR= 0.89; 95% CI, 0.73 to 0.93) but not after adjustment for the ratio of total to HDL cholesterol (standardized RR=0.89; 95% CI, 0.76 to 1.03). HDL_3 cholesterol remained a significant predictor of IHD development after control for LDL cholesterol levels (standardized RR=0.74; 95% CI, 0.58 to 0.94). Statistical adjustment for triglycerides (standardized RR=0.82; 95% CI, 0.64 to 1.04) and for the ratio of total to HDL cholesterol (standardized RR=0.95; 95% CI, 0.72 to 1.25) attenuated the relationship between the HDL_3 subfraction and the risk of IHD to a point of insignificance. Among all lipoprotein/lipid variables, including HDL subfractions, the ratio of total to HDL cholesterol showed the strongest association with the risk of IHD in multivariate stepwise survival analyses (Wald's $\chi^2=15.9$; $P<.001$). Other risk factors included in the final model were age ($\chi^2=15.0$; $P<.001$) and systolic blood pressure ($\chi^2=3.6$; $P=.06$). Neither total HDL cholesterol nor its subfractions contributed significantly to the prediction of IHD after these three variables were included in the stepwise model.

In this French-Canadian cohort, concentrations of HDL_2 and HDL_3 cholesterol accounted for 35% and 65%, respectively, of total HDL cholesterol levels. The correlation coefficients between HDL subfractions and some of their potential correlates are presented in Table 4. Both HDL_2 and HDL_3 cholesterol levels were strongly correlated with total HDL cholesterol levels ($r=.8$; $P<.001$). However, the two subfractions were not as closely related to each other ($r=.38$). Weekly alcohol intake was related to HDL_3 ($r=.24$) but not to HDL_2 cholesterol levels ($r=.07$). Neither systolic blood pressure nor cigarette smoking showed any relationship with HDL_2 and HDL_3 cholesterol concentrations. The relationship of body mass index to both HDL subfractions was of similar magnitude ($r=.24$ for HDL_2 and $r=.20$ for HDL_3; $P<.001$).

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Table 4. Correlation Coefficients Between HDL and Its Subfractions and Some of Their Potential Correlates in the Sample of 944 Men

Discussion

Top
Abstract
Introduction
Methods
Results
Discussion
References

Although both HDL subfractions measured at baseline were reduced among IHD(+) men compared with IHD(-) men, the reduction in HDL_2 cholesterol levels was more substantial than for HDL_3 cholesterol. Men in the highest quartile of the HDL_2 cholesterol distribution were characterized by a 4.8-fold decrease in the risk of IHD compared with men in the lowest quartile. The relationship between IHD and levels of HDL_2 cholesterol, when examined as a continuous variable, was also highly significant. Elevated HDL_3 cholesterol levels were also associated with a significant reduction in IHD risk (2.7-fold decrease in the risk of IHD for men in the fourth quartile), but this association was of lower magnitude than the relationship between HDL_2 and IHD. When simultaneously studied in a multivariate Cox survival model, the reduction in risk associated with increasing HDL_2 cholesterol levels remained statistically significant after control for HDL_3 cholesterol levels, whereas adjustment for HDL_2 cholesterol attenuated the association between HDL_3 and IHD to a point of insignificance. From a statistical standpoint, the present data suggest that the HDL_2 subfraction may be more closely related to the development of IHD than the HDL_3 subfraction. However, the qualitative difference in the relative predictive value of each subfraction was trivial, since it corresponded to only a modest quantitative difference. Based on these observations, one cannot exclude the possibility that a significant proportion of the cardioprotective effect of elevated HDL cholesterol levels may be mediated by the HDL_3 subfraction.

Results from cross-sectional studies have supported, in most cases, the hypothesis that any beneficial effects underlying elevated HDL cholesterol levels could be attributed to a greater extent to the HDL_2 cholesterol subfraction.[8] [10] On the other hand, results from the four prospective studies available have produced equivocal results. The early observations of Gofman and colleagues[9] first suggested that both HDL subfractions were reduced in IHD(+) men. Multivariate analyses comparing the relative contribution of each subfraction were not performed in this original study. In the more recent prospective case-control Physicians' Health Study,[11] both HDL_2 and HDL_3 subfractions were reduced in men with MI. The HDL_3 subfraction was, however, more closely associated with MI than the HDL_2 subfraction. In the Kuopio Ischemic Heart Disease Risk Factor Study,[13] both HDL cholesterol and the HDL_2 cholesterol subfraction were negatively associated with acute MI. The HDL_3 subfraction was also inversely associated with IHD, but this association did not persist after adjustment for HDL_2 cholesterol levels. In British men from the Caerphilly and Speedwell Collaborative Heart Disease Studies, the association with incidence of IHD appeared to be stronger for HDL_3 than for HDL_2 cholesterol.[12]

A number of factors may account, at least partly, for the inconsistencies among previous prospective studies. It has been suggested that a greater proportion of HDL defined as HDL_2 may favor this subfraction in terms of its relative importance over HDL_3 in the assessment of IHD risk.[12] Results from the Kuopio study,[13] in which subjects had a relatively important proportion of their HDL cholesterol levels as HDL_2 (65%), have suggested that the HDL_2 subfraction provided more information on IHD risk than HDL_3. In contrast, the Physicians' Health Study[11] and the Speedwell Study,[12] with 10% and 18%, respectively, of total HDL cholesterol as HDL_2, concluded that HDL_3 was more strongly associated with IHD. Thus, a systematic underestimation of the HDL_2 cholesterol pool favoring the overestimation of HDL_3 may explain why the latter subfraction was, in some studies, more strongly associated with IHD than HDL_2.[12] In the present study the proportion of the total HDL cholesterol pool as HDL_2 (35%) is in agreement with the average distribution of cholesterol in HDL subfractions in men[20] and may help to explain why HDL_2 was a better correlate of IHD than HDL_3 in the present report.

Two of these recent prospective studies have isolated the HDL subfractions using sequential ultracentrifugation,[12] [13] whereas standardized precipitation methods with dextran sulfate were used in the Physicians' Health Study[11] as well as in the present report. It has been shown that the chemical composition of HDL_2 and HDL_3 isolated by precipitation methods agreed well with those of ultracentrifugally isolated HDL subfractions.[20] HDL_2 values obtained by these two methods also strongly correlated with each other.[22] Previous work has shown that a relatively large amount of alcohol consumed weekly and important variation in body mass index (5 kg/m^2) were associated with only minute changes in HDL_2 cholesterol levels.[23] It is therefore unlikely that lifestyle factors may be totally responsible for the heterogeneity in the relative proportion of HDL subfractions observed among prospective studies. Such variations in the cholesterol content of the HDL subfractions are thus more likely to be the result of laboratory manipulations. HDL_2 and HDL_3 cholesterol concentrations are largely determined by the simultaneous action of four enzymes present in the plasma, namely lecithin-cholesterol acyltransferase, cholesteryl ester transfer protein, hepatic triglyceride lipase, and lipoprotein lipase.[8] Whether these enzymes may have been under different genetic influences in the various populations studied is not known, and examining this possibility is far beyond the scope of the present prospective study.

Other factors such as coefficients of variations related to the measurement of HDL subfractions may also help to explain some of the discordance among the prospective analyses.[12] [13] The coefficients of variation for HDL_2 and HDL_3 measurements in studies previously reviewed varied from 7% to 15%[11] [12] and even reached 36% to 45% in one study.[13] In the present report coefficients of variation for HDL_2 and HDL_3 cholesterol were 9.8% and 6.3%, respectively. Since random variability in measurement of risk factors may attenuate the association with clinical outcomes, a greater variability in the measurement of a given HDL subfraction may partially explain differences among studies. All four prospective studies used MI and coronary death as end points for the study of the association between HDL subfractions and IHD. We performed additional analyses using only first or second cases of MI and coronary death as end points. Although the number of events was too small to accurately assess the risk of IHD, similar results were obtained, since a larger proportion of the reduction in risk associated with elevated HDL cholesterol levels could be attributed to the HDL_2 rather than HDL_3 subfractions.

Concentrations of HDL cholesterol subfractions may be altered by many factors, such as obesity (particularly abdominal obesity),[24] exercise,[25] diet,[26] and alcohol consumption.[8] [27] Alcohol intake appears to increase HDL_3 cholesterol without changing the cholesterol content of HDL_2,[28] thereby altering HDL composition. Results of the present study support this notion since alcohol intake was moderately associated with increases in HDL_3 cholesterol levels but not with HDL_2 cholesterol concentrations. Moderate drinkers have been shown to have less IHD than nondrinkers,[29] and it has been suggested that the inverse relationship between moderate alcohol consumption and cardiovascular disease mortality was only partially mediated by the concomitant increase in HDL cholesterol levels.[30] [31] The fact that alcohol increases HDL_3 but not HDL_2 and that HDL_3 did not appear to be as closely related to IHD tends to support this notion. Other studies have reported that the beneficial effects of moderate alcohol consumption on the risk of IHD could be mediated through its effects on both subfractions.[29] [32] [33] Adding alcohol consumption to the Cox proportional hazards model yielded results that were essentially similar to those obtained when alcohol intake was not considered. HDL may reduce IHD risk by mechanisms other than reverse cholesterol transport per se. Indeed, HDL may act as an antioxidant[34] and has also been shown to promote fibrinolysis.[35] High levels of HDL may also reduce LDL uptake by endothelial cells by competing for the LDL receptor.[36] Whether HDL_2 and HDL_3 subfractions play different roles in these processes will require further investigation.

Results presented in this report support the notion that the measurement of HDL subfractions in addition to HDL cholesterol levels does not improve the prediction of IHD. A number of cross-sectional studies[8] [37] and recent prospective reports[11] [12] [13] have also suggested that HDL subfractions were not superior to total HDL cholesterol alone in predicting IHD. It has been argued that this conclusion may be incorrect, since proportionally larger errors in measurements of the individual HDL subfractions, compared with errors associated with the HDL cholesterol assay, may result in a greater decrease in the goodness of fit of the models.[13] Phillips and Davey Smith[38] have described cases in which an important bias in RR estimate may be introduced when the independent variables are characterized by considerable measurement imprecision. This problem is particularly relevant when two independent variables strongly related to each other are included in one multivariate model to predict a response. Thus, the additive effect of measurement imprecision of two variables, in our case HDL_2 and HDL_3, may have reduced the goodness of fit of the model to a greater extent than when only one independent variable (HDL cholesterol) is used. In the present report, however, coefficients of variation for the assessment of HDL subfractions were relatively small, and the correlation between HDL_2 and HDL_3 cholesterol levels was weak (r=.38, 14% of shared variance). For these reasons, the potential

introduction of bias in the Cox proportional hazards model is not likely to be an important factor in the present analyses. A method that would adequately estimate the effect of imprecision on the overall fit of a model remains to be developed.[38]

Summary

A purely quantitative analysis of the data presented herein supports the hypothesis that HDL_2 may be more strongly associated with the development of IHD than HDL_3. The qualitative difference in the relative predictive value of each subfraction was, however, trivial and possibly had few clinical implications. The possibility that a significant proportion of the cardioprotective effect of elevated HDL cholesterol concentrations may be mediated by the HDL_3 subfraction cannot be excluded at this point on the basis of our results. Although the assessment of HDL subfractions may provide valuable information on the mechanisms potentially involved in the etiology of reduced HDL cholesterol levels,[8] [10] the inherent difficulty underlying their measurement and the sample size of most studies, including the present one, preclude definitive statements regarding the relative value of each subfraction. Finally, measurements of HDL_2 and HDL_3 cholesterol do not appear to provide additional information on the risk of IHD, particularly compared with the information provided by the ratio of total to HDL cholesterol, which was the best lipoprotein/lipid correlate of IHD risk in this cohort of men. The determination of HDL subfractions as a routine screening test for evaluation of IHD risk is not justified and therefore cannot be recommended at the present time.

Selected Abbreviations and Acronyms

CI	=	confidence interval
ECG	=	electrocardiogram, electrocardiographic
IHD	=	ischemic heart disease
IHD(+)	=	subjects who developed IHD during follow-up
IHD(-)	=	subjects who remained free of IHD during follow-up
MI	=	myocardial infarction
RR	=	relative risk

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Footnotes

†Dr Moorjani died October 1, 1995.

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References

- Top
- Abstract
- Introduction
- Methods
- Results
- Discussion
- References

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