Fifty-three year follow-up of coronary heart disease versus HDL2 and other lipoproteins in Gofman’s Livermore Cohort

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Abstract To assess the relationships of lipoprotein mass concentrations to all-cause and coronary heart disease (CHD) mortality, we analyzed the prospective 53-year follow-up of 1,905 men measured for lipoprotein mass concentrations by analytic ultracentrifugation between 1954 and 1957. Cause of death was determined from medical records and death certificates before 1979 and from National Death Index death diagnoses thereafter. Of the 1,329 men (69.8%) who died through 2008, CHD was listed as a contributing cause of death for 409 men, including 113 deaths from premature CHD (age < 65 years). When adjusted for age, the risk associated with the lowest HDL2 quartile increased 22% for all-cause (P = 0.001), 63% for total CHD (P < 10^-5), and 117% for premature CHD mortality (P = 0.0001). When adjusted for standard risk factors (age, total cholesterol, blood pressure, BMI, smoking) and the lowest HDL3 quartile, the corresponding risk increases were 14% (P = 0.05), 38% (P = 0.004), and 62% (P = 0.02), respectively. Men with HDL3 < 25th percentile had 28% greater total CHD risk (P = 0.03) and 71% greater premature CHD risk (P = 0.01). Higher LDL-mass concentrations increased total CHD risk by 3.8% (P < 10^-5) and premature CHD risk by 6.1% (P < 10^-5) per 10 mg/dl increase in concentration. Thus, low HDL2 is associated with increased CHD risk.—Williams, P. T. Fifty-three year follow-up of coronary heart disease versus HDL2 and other lipoproteins in Gofman’s Livermore Cohort. J. Lipid Res. 2012, 53: 266–272.

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• prevention

In 1954, John Gofman and colleagues reported that analytic ultracentrifuge measurements of high-density lipoproteins (HDL) in blood revealed two particle subclasses, HDL2 and HDL3, and that the concentrations of the more buoyant particles (HDL2) were 50% higher in women than men (1, 2). In 1966, they reported that 38 men who developed coronary heart disease (CHD) had HDL mass concentrations that were 32% lower for HDL2 and 8% lower for HDL3 (the less buoyant particles) compared with those who did not develop CHD (3).

Gofman’s initial observation gave rise to studies by others on the potential clinical utility of HDL2 and HDL3 measurements. Rather than employing Gofman’s original methodology, they measured HDL2- and HDL3-cholesterol by precipitation and HDL size classes by nuclear magnetic resonance (NMR) or, more rarely, ultracentrifugation. Many studies showed that high HDL2 was associated with lower CHD risk (4–10); however, the independent effects of HDL2 and HDL3 on CHD risk remained inconclusive (5, 7–10). Currently, the National Cholesterol Education Program Adult Treatment Panel (ATP-III) guidelines (11) state that “although small studies suggest greater predictive power of one or another HDL component, their superiority over HDL-cholesterol has not been demonstrated in large, prospective studies. Consequently, ATP III does not recommend the routine measurement of HDL subspecies in CHD risk assessment.” However, the clinical utility of HDL subclasses may be underappreciated in part due to the limitations of these alternative methods to adequately characterize HDL heterogeneity (12–16).

Between 1954 and 1956, John Gofman created a cohort of 1,905 men in whom lipoproteins were measured by analytic ultracentrifugation (3). Our rediscovery of Gofman’s original data enabled us to assess the relationships of lipoprotein mass concentrations to CHD during 29-year follow-up (17). Those analyses showed that (i) the lowest quartile of HDL2-mass increased the men’s risks of fatal plus nonfatal CHD by 38% and their risks of premature CHD (age < 65 years) by 61% when adjusted for traditional risk factors, (ii) the HDL2-CHD risk relationship remained significant when adjusted for HDL3, and (iii) the risk reduction per mg/dl of HDL-mass was significantly greater than

Abbreviations: BMI, body mass index; CHD, coronary heart disease; HR, hazard ratio.

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for HDL2 than HDL3 (17). The current report extends the mortality surveillance of Gofman’s cohort from 29 to 53 years of follow-up, assesses the dose-response relationships between lipoproteins and disease risk, and tests whether HDL2 affects CHD risk and mortality independent of total HDL levels.

MATERIALS AND METHODS

The cohort consisted of male employees of the Livermore Radiation Laboratory (currently Lawrence Livermore National Laboratory) who volunteered to have blood drawn between 1954 and 1956 and to be followed prospectively thereafter (3). The men are limited to the 1,905 subjects of Gofman’s original report who were free of prior ischemic heart disease at baseline. The criteria for entry into the study presumably corresponded to the criteria adopted by the Cooperative Study, which evaluated the predictive value of serum low-density lipoprotein fractions and total cholesterol on clinical complications of atherosclerosis, in which Gofman participated (18). Namely, potential subjects were disqualified on the basis of more than trace amounts of urine protein or sugar; diabetes mellitus; nephritis (except past history of pyelonephritis, nephrolithiasis, or loss of kidney); treatment with ACTH, cortisone, or related hormones; history of rheumatic heart disease; known congenital heart disease; or syphilis or Buerger’s disease. Height, weight, blood pressure, and casual (potentially nonfasting) blood draws were obtained during the employee’s annual medical physical examination. Cigarette consumption was determined by self-report. Serum total cholesterol was assayed by a modification of the Abell method (19). Corrected mass concentrations of low-density lipoprotein and total cholesterol were determined on clinical laboratories, in which Gofman participated (18). The current report extends the analysis of HDL2 to a total of 53 years of follow-up.

Survival analyses (Cox proportional hazard analyses) and logistic regression analyses were performed using JMP 5.1 (SAS Institute, Cary, NC). Hazard ratios (HR) are presented with their 95% confidence intervals (95% CI). Although there were no substantial deviations from the proportional hazards assumption, the robustness of our findings was insured by the requirement that they also achieved statistical significance by logistic regression analyses. The test for a linear versus a threshold HDL effect involved the simultaneous inclusion of both HDL mg/dl concentrations and an indicator function for the lowest HDL quartile in the model, with a significance of the indicator variable and nonsignificance of the per mg/dl concentration as evidence of a threshold effect.

RESULTS

Table 1 presents the characteristics of the 1,905 men in the cohort. At baseline, 31.1% were under age 30, 61.9% were between 30 and 49.9 years, 6.8% were between 50 and 64.9 years, and less than one-third of one percent were 65 years or older. Most were of healthy weight as measured by body mass index (BMI < 25 kg/m²; 64.4%), some were moderately overweight (25 kg/m² ≤ BMI < 30 kg/m²; 31.5%), and few were obese (BMI ≥ 30 kg/m²; 4.1%). Only 7.2% of the men were hypertensive (systolic blood pressure ≥ 140 or diastolic blood pressure ≥ 90 mm Hg), and 33.5% had serum total cholesterol > 240 mg/dl.

Table 1 presents the age-adjusted hazard ratios for HDL2 and HDL3 and the age-adjusted hazard ratios for total mortality and CHD mortality. The current analyses demonstrate a threshold for HDL2 with a significant association with total mortality and CHD mortality.

Table 1 presents the age-adjusted hazard ratios (95% confidence interval) for all-cause and CHD mortality versus individual risk factors during 53-year follow-up.

| Age (years) | 35.24 ± 8.82 |
|------------|--------------|
| Cigarettes | 8.89 ± 10.69 |
| BMI (kg/m²) | 24.15 ± 3.19 |
| Systolic BP | 120.35 ± 12.36 |
| Diastolic BP | 70.77 ± 9.06 |
| Total cholesterol | 223.96 ± 45.43 |
| HDL2 | 36.87 ± 28.24 |
| HDL3 | 229.29 ± 43.39 |
| LDL | 352.36 ± 86.56 |
| IDL | 49.12 ± 23.37 |
| Small VLDL | 90.24 ± 54.47 |
| Large VLDL | 50.36 ± 66.35 |

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all-cause and CHD mortality. As expected, greater cigarette consumption, BMI, and blood pressure increased the men’s risk for all-cause and CHD mortality during the 53 years of follow-up. Greater serum total cholesterol concentrations also increased their risk for CHD, but not for all-cause, mortality. The effects of BMI on all-cause mortality appeared to be attributable to CHD, whereas the effects of blood pressure and cigarette use appeared to include significant increases in non-CHD deaths. Specifically, the age-adjusted risks for non-CHD deaths increased with cigarette packs/day (HR = 1.48, 95% CI: 1.31 to 1.67, P = 10^{-5}), diastolic blood pressure (HR = 1.08 per 10 mm Hg, 95% CI: 1.00 to 1.17, P = 0.06), and systolic blood pressure (HR = 1.08 per 10 mm Hg, 95% CI: 1.02 to 1.13, P = 0.009). Table 2 shows that cigarette packs smoked per day, systolic blood pressure, and total cholesterol independently increased the risks for total and premature CHD when included in multiple linear regression models.

Higher serum HDL2 concentrations were significantly related to decreased total CHD, premature CHD, and all-cause mortality (Table 1). In addition, the lowest HDL2 quartile (≤17 mg/dl) had a 63% increased age-adjusted CHD risk (95% CI: 1.32 to 1.99) vis-à-vis its second through fourth quartiles (P < 10^{-5}, not shown). This increased risk for fatal CHD was only slightly diminished by adjustment for total HDL (i.e., HDL2 + HDL3, HR = 1.51; 95% CI: 1.19 to 1.92, P = 0.0008) or HDL3-mass (HR = 1.57; 95% CI: 1.26 to 1.95, P = 0.0001). The lowest HDL2 quartile had an even greater affect on the risk for premature fatal CHD (HR = 2.17; 95% CI: 1.48 to 3.14, P = 0.0001), which persisted when adjusted for total HDL (HR = 1.76; 95% CI: 1.13 to 2.73, P = 0.01) or HDL3 (HR = 1.95; 95% CI: 1.31 to 2.89, P = 0.001) in addition to age. The lowest quartile’s association with all-cause mortality was weaker (HR = 1.22; 95% CI: 1.08 to 1.38, P = 0.001) but it too

| TABLE 2. Age-adjusted hazard ratios (95% confidence interval) for reported total and premature CHD versus traditional risk factors and lipoprotein mass concentrations |
|----------------------------------|----------------------------------|----------------------------------|
|                                   | Premature CHD (age ≤ 65 years)   | Total CHD                         |
|                                   | Model 1 Subfractions included    | Model 1 Subfractions included    |
| Cigarettes (packs/day)            | 1.45 (1.04, 1.98)                | 1.15 (0.96, 1.69)                |
|                                  | P = 0.03                         | P = 0.10                         |
|                                  | 1.36 (0.97, 1.88)                | 1.27 (0.97, 1.67)                |
|                                  | P = 0.07                         | P = 0.04                         |
|                                  | 1.30 (0.92, 1.81)                | 1.19 (0.91, 1.58)                |
|                                  | P = 0.13                         | P = 0.11                         |
| BMI (kg/m²)                       | 1.05 (0.99, 1.11)                | 1.01 (0.99, 1.04)                |
|                                  | P = 0.09                         | P = 0.14                         |
|                                  | 1.03 (0.97, 1.09)                | 1.01 (0.98, 1.05)                |
|                                  | P = 0.37                         | P = 0.50                         |
|                                  | 1.04 (0.98, 1.10)                | 1.01 (0.98, 1.05)                |
| Systolic BP (per 10 mm Hg)        | 1.16 (1.02, 1.30)                | 1.22 (1.12, 1.30)                |
|                                  | P = 0.03                         | P = 0.04                         |
|                                  | 1.15 (1.01, 1.29)                | 1.21 (1.14, 1.31)                |
|                                  | P = 0.03                         | P = 0.04                         |
|                                  | 1.19 (1.03, 1.34)                | 1.23 (1.14, 1.31)                |
|                                  | P = 0.02                         | P = 0.04                         |
| Diastolic BP (per 10 mm Hg)       | 1.15 (0.89, 1.48)                | 1.02 (0.89, 1.17)                |
|                                  | P = 0.29                         | P = 0.79                         |
|                                  | 1.18 (0.91, 1.52)                | 1.03 (0.90, 1.18)                |
|                                  | P = 0.36                         | P = 0.66                         |
|                                  | 1.13 (0.87, 1.45)                | 1.01 (0.88, 1.15)                |
|                                  | P = 0.93                         |                                 |
| Total cholesterol (per 10 mg/dl)  | 1.07 (1.03, 1.12)                | 1.05 (1.02, 1.07)                |
|                                  | P = 0.0005                       | P = 0.0001                       |
|                                  | 1.07 (1.03, 1.12)                | 1.05 (1.02, 1.07)                |
|                                  | P = 0.0006                       | P = 0.0010                       |
|                                  | 0.96 (0.89, 1.04)                | 0.96 (0.92, 1.00)                |
|                                  | H < 10 mg/dl                     |                                 |
| HDL2 (lowest quartile)           | 1.62 (1.08, 2.41)                | 1.38 (1.11, 1.71)                |
|                                  | P = 0.02                         | P = 0.04                         |
|                                  | 1.62 (1.08, 2.41)                |                                 |
|                                  | P = 0.02                         |                                 |
|                                  | 1.71 (1.14, 2.53)                | 1.28 (1.02, 1.59)                |
|                                  | P = 0.03                         |                                 |
|                                  | 1.71 (1.14, 2.53)                |                                 |
|                                  | P = 0.03                         |                                 |
| LDL (per 10 mg/dl)               | 1.09 (1.05, 1.13)                | 1.05 (1.03, 1.07)                |
|                                  | P = 0.0001                       | P < 10^{-5}                      |
|                                  | 1.09 (1.05, 1.13)                |                                 |
|                                  | P = 0.0001                       |                                 |
| IDL (per 10 mg/dl)               | 0.91 (0.80, 1.03)                | 0.97 (0.91, 1.04)                |
|                                  | P = 0.15                         | P = 0.40                         |
|                                  | 0.91 (0.80, 1.03)                |                                 |
|                                  | P = 0.15                         |                                 |
| Small VLDL (per 10 mg/dl)        | 1.05 (0.99, 1.12)                | 1.05 (1.01, 1.08)                |
|                                  | P = 0.12                         | P = 0.009                         |
|                                  | 1.05 (0.99, 1.12)                |                                 |
|                                  | P = 0.12                         |                                 |
| Large VLDL (per 10 mg/dl)        | 1.00 (0.96, 1.05)                | 1.00 (0.97, 1.02)                |
|                                  | P = 0.84                         | P = 0.84                         |
|                                  | 1.00 (0.96, 1.05)                |                                 |
|                                  | P = 0.84                         |                                 |

The hazard ratios from the multivariate models are adjusted for the model’s other variables in addition to age. There were 409 total CHDs in 1,905 men, and 113 premature CHD events in 1,902 men (3 men were excluded for being 65 years or older at baseline). BP, blood pressure.
retained its significance when adjusted for total HDL (HR = 1.20; 95% CI: 1.08 to 1.39, \( P = 0.01 \)) or HDL3 (HR = 1.22; 95% CI: 1.08 to 1.39, \( P = 0.002 \)) in addition to age.

**Figure 1** shows that the majority of the risk reduction occurred between the first and second HDL2 quartiles. It also illustrates the negligible effects of HDL3 and total VLDL adjustment on the HDL2-CHD relationship. Table 2 shows that the total CHD risk associated with the lowest HDL2 quartile remained significant when adjusted for both standard risk factors and the lowest HDL3 quartile (\( P = 0.02 \)) and that, when adjusted, the risk reduction was nearly 2-fold greater for premature than total CHD deaths. Additional analyses (supplementary Table II) showed that when both the linear effect of HDL2-mass (i.e., per 10 mg/dl) and the lowest HDL2 quartile were included in the same analyses, the linear term was not displayed). Logistic regression analyses confirmed the decreasing odds of age-adjusted fatal CHD for increasing concentrations of HDL3-mass (3.2\% odds reduction per 10 mg/dl, \( P = 0.01 \)) and the increased odds for the lowest HDL3 quartile (32\% odds increase, \( P = 0.03 \)) and their continued significance when adjusted for standard risk factors (\( P = 0.004 \) and \( P = 0.02 \), respectively), including HDL2 (\( P = 0.02 \) and \( P = 0.05 \), respectively).

**LDL-, IDL-, and VLDL-mass concentrations**

Higher LDL-mass concentrations significantly increased the risk for total and premature CHD mortality when adjusted for age (Table 1). Its small but significant effect on total mortality is attributable to CHD mortality, as LDL had no significant effect on non-CHD mortality (\( P = 0.20 \), not displayed). **Figure 2** shows that CHD risk increased linearly with LDL-mass quartiles and that adjustment for total cholesterol concentrations had little effect on the LDL-CHD

![Adjusted for standard risk factors](image)

![Adjusted for age phc](image)

![Adjusted for age and total cholesterol](image)

**Fig. 1.** Risk ratio for fatal CHD from proportional hazard analyses of quartiles of HDL2-mass concentrations adjusted for age and other covariates as indicated. Fifty-three year follow-up of 1,905 men, of whom 409 had CHD listed as an underlying or contributing cause of death. *\( P \leq 0.05 \); †\( P < 0.01 \); ‡\( P \leq 0.005 \); §\( P \leq 0.001 \).

**Fig. 2.** Risk ratio for fatal CHD from proportional hazard analyses of quartiles of LDL-mass concentrations adjusted for age and other covariates as indicated. Fifty-three year follow-up of 1,905 men, of whom 409 had CHD listed as an underlying or contributing cause of death. *\( P \leq 0.05 \); †\( P < 0.01 \); ‡\( P \leq 0.001 \); §\( P \leq 0.0001 \).
risk relationship. Adjustment for LDL-mass concentrations eliminated the significant effect of total cholesterol on CHD risk in an analysis that adjusted for only age (from \(P < 10^{-5}\) to \(P = 0.97\), not displayed) or when adjusted for standard risk factors, IDL, and VLDL (Table 2). Higher concentrations of IDL-mass, small VLDL-mass, and large VLDL-mass also increased CHD risk when adjusted for age (Table 1), but IDL and large VLDL were not significant when adjusted for LDL and standard risk factors. Logistic regression analyses confirmed the increased odds for age-adjusted CHD mortality with increasing mass concentrations of LDL (\(P < 10^{-7}\)), IDL (\(P < 0.0001\)), small VLDL (\(P < 0.0001\)), and large VLDL (\(P = 0.006\)); the significance of LDL when included simultaneously with IDL, VLDL, and standard risk factors (\(P = 10^{-5}\)); and the elimination of the significance of total cholesterol when adjusted for LDL-mass concentrations (from \(P = 10^{-5}\) to \(P = 0.71\)).

**CHD as an underlying cause of death versus its inclusion as a contributing cause**

CHD was cited as the underlying cause of death in 286 (70%) of the 409 men who had CHD listed as a contributing cause (i.e., the entity-axis conditions of the National Death Index report). Restricting the analyses specifically to CHD as an underlying cause yielded results consistent with the preceding analyses, albeit with reduced statistical significance. Specifically, the lowest HDL2 quartile was significantly associated with total CHD risk when adjusted for age (HR = 1.55; 95% CI: 1.21 to 1.98, \(P = 0.0007\)), and when adjusted for standard risk factors plus the lowest HDL3 quartile (HR = 1.32; 95% CI: 1.01 to 1.71, \(P = 0.04\)). There was also significant evidence for a threshold risk increase for the lowest HDL2 quartile versus a linear decrease in CHD risk per mg/dl decrease in HDL2 (\(P = 0.01\)). CHD risk also increased significantly per 10 mg/dl increase in LDL-mass concentrations when adjusted for age alone (HR = 1.04; 95% CI: 1.03 to 1.06, \(P < 10^{-5}\)), and when adjusted for the standard risk factors (HR = 1.03; 95% CI: 1.01 to 1.06, \(P = 0.005\)). Age-adjusted CHD risk was not significantly related to HDL3-mass concentrations or the lowest HDL3 quartile (both \(P = 0.20\)).

**DISCUSSION**

The 53-year follow-up of Gofman’s Livermore Cohort is the longest epidemiological study of CHD to directly measure lipoproteins and HDL subclasses. The results demonstrate the importance of HDL2 as a CHD risk factor independent of traditional CHD risk factors (age, smoking, blood pressure, BMI, total cholesterol), VLDL-mass concentrations, HDL3, and total HDL concentrations. Moreover, they show that the majority of the risk falls within the lowest HDL2 quartile. These results are particularly significant because they differ sharply from findings based on other methodologies. Studies reporting HDL2- and HDL3-cholesterol have failed to consistently demonstrate the importance of one subfraction over another or of their significance over total HDL-cholesterol (11). Moreover, they report that the relationship of HDL-cholesterol to CHD risk is linear (11), in contrast to the HDL2-mass concentrations shown in Fig. 1. There is remarkable consistency between the risk estimates for fatal and nonfatal CHD during the first 29-year follow-up of this cohort and the risk estimates for the clinically much more important endpoints of fatal CHD during 53 years of follow-up. The 29-year follow-up included 179 CHD deaths among the 363 total fatal and nonfatal CHD events studied, which is less than one-half of the 409 fatal CHD analyzed in the current report.

The current ATP-III guidelines do not endorse routine clinical measurement of HDL subfractions (11). It may have been premature to discount the importance of HDL subclasses from epidemiological studies based on other methodologies. In part, the failure to establish the independent effects of HDL2 or HDL3 may relate to their reliance on HDL2- and HDL3-cholesterol. Unpublished analyses of the baseline data from 337 middle-aged men who participated in studies at our laboratory showed strong concordance between HDL-cholesterol and total HDL-mass (\(r = 0.85\) (21, 22)). Specifically HDL-cholesterol and HDL2-cholesterol [measured by precipitation (23)] show strong associations with HDL2-mass concentrations (\(r = 0.87\) and \(r = 0.85\), respectively) in these 337 men; however, this mostly reflects concordance among the higher concentrations. In contrast, 35% of men in the lowest quartile of HDL2-mass were not assigned to the lowest quartile of HDL-cholesterol or HDL2-cholesterol.

The current analyses show that the risk for premature fatal CHD (i.e., age \(\leq 65\) years) was over 2-fold greater, and for total fatal CHD, 63% greater for men who fell in the lowest HDL quartile vis-à-vis higher values. Although the Prospective Cardiovascular Munster Study reported a 3-fold greater coronary artery disease (CAD) risk for subjects with HDL-cholesterol levels \(< 35\) mg/dl vis-à-vis higher HDL-cholesterol (24), this result appears to be an exception. The pooled analyses of the Framingham Heart Study, the Lipid Research Clinics Primary Prevention Trial, the LRC Prevalence Mortality Follow-up Study, and the Multiple Risk Factor Intervention Trial suggest a linear relationship, in which each mg/dl increment in HDL-cholesterol reduced CHD risk by 2% in men and 3% in women (25). As cited above, HDL-cholesterol and HDL2-cholesterol frequently reclassifies the lowest quartile of HDL2-mass above the 25th HDL percentile, which could explain the difference. It is somewhat less obvious why the linear relationship reported for total HDL-cholesterol by Gordon et al. (25) was not reflected in the higher percentiles of the HDL2-mass distribution in Fig. 1, given their strong correlation (\(r = 0.85\)), albeit the correlation does leave 28% of the variance unexplained.

The biological variability in the analytic ultracentrifuge measurements was comparable to the more traditional measurements of lipids and lipoprotein cholesterol, albeit somewhat weaker. Specifically, unpublished analyses showed that the Pearson’s correlations for repeated measurements in 119 male and female control subjects (21, 22) measured one year apart were \(r = 0.70\) for HDL2-mass versus \(r = 0.80\).
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for HDL2-cholesterol; \( r = 0.62 \) for HDL3-mass versus \( r = 0.71 \) for HDL3-cholesterol; \( r = 0.77 \) for LDL-mass and \( r = 0.68 \) for IDL-mass versus \( r = 0.78 \) for LDL-cholesterol; and \( r = 0.70 \) for small VLDL-mass and \( r = 0.55 \) for large VLDL-mass versus \( r = 0.70 \) for VLDL-cholesterol. The somewhat weaker association for the analytic ultracentrifuge measurements is expected to attenuate the associations between lipoprotein subfractions and mortality; i.e., the results presented here underestimate the true risk ratios.

Table 2 addresses two fundamental questions proposed 60 years ago by Gofman and colleagues: i) whether analytic ultracentrifuge measurements of particular lipoproteins were specifically related to CHD and ii) whether particular lipoproteins accounted for serum cholesterol’s relationship to CHD. In 1950, the multicenter Cooperative Study (Cleveland Clinic, University of Pittsburgh, Harvard School of Public Health, and Donner Laboratory) was established to test prospectively in 5,000 middle-aged men whether the analytic ultracentrifuge’s lipoprotein measurements were a better predictor of cardiac events than total serum cholesterol levels (18). The study, plagued with difficulties in recruitment and in standardizing measurements across clinics, also had the problem of dealing with Gofman’s evolving methodology during the study. This included a labor-intensive correction in an adjustment for sedimentation rates at Donner Laboratory that the other laboratories were unable to implement due to time or equipment. The study’s majority conclusion was that CHD was related to small VLDL and total cholesterol but not IDL, and that cholesterol was more strongly predictive of CHD than were lipoproteins. The subfractions’ lack of improvement over total cholesterol dampened interest in their measurement. Dissatisfied with the Cooperative Study collaboration, between 1955 and 1957 Gofman established a new prospective study of Livermore Radiation Laboratory employees (3). In 1966, he reported that, when compared with mean serum concentrations of the total sample, the 38 men who developed clinical ischemic heart disease during 10 years of follow-up had 32% lower HDL2 (\( P < 0.01 \)), 8% lower HDL3 (\( P = 0.02 \)), 13% higher LDL (\( P < 0.001 \)), 25% higher IDL (\( P < 0.001 \)), and 21% higher small VLDL (\( P < 0.01 \)) (3). Table 2 shows that after 53 years of follow-up, low HDL2, low HDL3, and increasing levels of LDL and small VLDL predicted increased CHD risk and that adjustment for LDL-mass concentrations eliminated the significance of total cholesterol as a CHD risk factor in this study. The current work, together with our earlier analyses (17), offers persuasive evidence for a potent, independent effect of low HDL2-mass by analytical ultracentrifugation (compared with HDL-cholesterol subfractions) in predicting CHD incidence and mortality.

There are important limitations to these analyses. There is little information on the study protocol in Gofman’s 1966 report on the study. In addition, the lipoprotein measurements were nonfasting, which may be either a strength or weakness (26, 27). Although the participants were almost exclusively younger white males, the predictive value of most conventional risk factors for CHD, including the Framingham risk assessment, appears to be similar for other racial groups when recalibrated for different risk factor levels and disease rates (11). Although the analytic ultracentrifuge is not currently used for routine measurements of lipoprotein subfractions, the purpose of these analyses is to highlight the significance of the lipoprotein concentrations themselves rather than a particular methodology for their measurements.

In conclusion, these analyses provide further evidence for the clinical significance of low HDL2 in predicting fatal CHD. The association between HDL2 and CHD may not be simple. The Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE) trial produced higher all-cause mortality in patients whose HDL-cholesterol levels were raised by an average of 72% via CETP inhibition (28). The drug torcetrapib preferentially generated large CE-rich HDL2 particles in these patients (29). The discrepancy between clinical trial results and epidemiological findings suggest a manifest need to identify more precisely the specific components within HDL2 or the physiological measures metabolically linked to low HDL2 that are responsible for its association with CHD risk.

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