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Apolipoprotein A-1 predicts coronary heart disease only at low concentrations of high-density lipoprotein cholesterol: an epidemiological study of Japanese-Americans

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Abstract Conventional epidemiological and clinical studies of apolipoprotein A-1 and high-density lipoprotein-cholesterol have demonstrated, when examined jointly, that high-density lipoprotein is a better predictor of coronary heart disease. This strategy does not take into account known lipid metabolic relationships. A statistical approach that takes into account apoliprotein A-1 being a constituent of the high-density lipoprotein particle is more appropriate. Among 1,177 Japanese-American men of the Honolulu Heart Program cohort free of disease at baseline (1980–1982), 182 new coronary heart disease cases developed over a 12-year follow-up period. After removing the linear relationship with high-density lipoprotein-cholesterol, a relative measure of apoliprotein A-1 concentration was derived. Based on joint conditions of "low" and "high" relative apoliprotein A-1 concentration and ≤40 and >40 mg/dl for the high-density lipoprotein-cholesterol distribution, four groupings were created. Among relative joint groupings of high/≤40, low/≤40, high/>40, and low/>40, respectively, the 12-year coronary heart disease incidence varied from 28.6, 18.2, 8.3, to 11.7 cases per 1,000 person-years. A test of statistical interaction was significant (P=0.028). Additional analyses revealed coronary heart disease cases were more likely among men with triglycerides >190 mg/dl. Observed patterns of relationships among relative apoliprotein A-1 level, high-density lipoprotein cholesterol, and triglycerides with incident coronary heart disease are consistent with patterns noted in clinical, laboratory, and transgenic animal research more capable of elucidating mechanisms of disease causation. This epidemiological study suggests similar mechanisms may be operating at a population level, and may contribute to the public health burden of coronary heart disease.

Key words Apolipoprotein A-1 · High-density lipoprotein-cholesterol · Triglycerides · Coronary arteriosclerosis · Epidemiology

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Introduction

Apolipoprotein A-1 (Apo-A1) is the major protein of the high-density lipoprotein (HDL) particle [1]. At early stages in the uptake and reverse transport of cholesterol from cells, it is the only protein associated with the HDL particle [2–4].

When examined jointly, epidemiological and clinical studies demonstrate HDL-cholesterol to be a better predictor of coronary heart disease (CHD) than Apo-A1 [5–17]. Population-based studies measuring Apo-A1 and HDL-cholesterol demonstrate very high correlations between each – of the order of 0.6–0.8 [10, 11, 13, 17, 18]. Such high correlations are expected because Apo-A1 is the structural backbone of the HDL particle. With this degree of co-linearity, the use of statistical models that adjust for linear relationships with HDL cholesterol significantly attenuate relationships of Apo-A1 with measures of CHD. In some studies no relationship remains; in others, the residual relation-

ship is so marginal in magnitude and in statistical significance as to cast doubt on whether the measurement of Apo-A1 adds anything of practical value [6, 7, 10, 14, 17].

Early studies attempted to account for this co-linear relationship by constructing ratios of HDL-cholesterol to Apo-A1. These studies generally demonstrated that cases have lower ratios than controls – implying that the amount of cholesterol associated with Apo-A1 was depressed in people developing clinical CHD [19]. Although a relative measure of Apo-A1 concentration may be an appropriate measure in a causal association with CHD, there are difficulties with the use of ratios. Further characterization and assessment of these are presented later in this paper.

This epidemiological study examines a random subset of middle-aged Japanese-American men of the Honolulu Heart Program cohort free of cardiovascular disease at the time of baseline measurement in 1980–1982, and followed 12 years for incident CHD. It focuses on the joint use of a relative measure of Apo-A1 concentration and absolute level of HDL-cholesterol as predictors of CHD. Addition of another level of joint stratification for triglyceride levels examines inter-relations among three metabolically linked lipids as predictors of CHD. The validity of the analysis rests on a premise that relationships between incident CHD and variation in these joint measures of lipid metabolism are causal.

Materials and methods

Study sample

The Honolulu Heart Program cohort was established at Kuakini Medical Center in Honolulu, Hawaii, in 1965-1968 in order to study the determinants and correlates of cardiovascular disease. The Program attempted to recruit all Japanese-American men then living on the island of Oahu, Hawaii and born between 1900 and 1919. Previous publications document the demographic characteristics and sampling strategies used to identify and recruit the 8,006 men of the cohort [20, 21]. The Kuakini Medical Center Research Institutional Review Board approved all examinations and informed consent protocols. All procedures were in accordance with institutional guidelines.

In 1970–1972, as part of the Cooperative Lipoprotein Phenotyping Study, a sub-sample of the cohort was recruited in order to study relationships of blood lipids and lipoproteins with incident cardiovascular disease [22–24]. Two additional examinations of this sub-sample were carried out, the last being in 1980–1982. It is the random sub-sample (n=1,379) of the original cohort from this third examination that constitutes the sampling frame of this report. At the time of the original examination in 1980–1982, plasma samples obtained after an overnight fast were sent to the Northwest Lipid Research Clinic in Seattle, Washington. The protocols and laboratory standards developed in collaboration with the Lipid Research Clinic Program were used to measure total cholesterol, HDL-cholesterol, and triglycerides [25–28]. HDL was measured in the supernatant fraction of plasma after heparin-manganese chloride precipitation, with a coefficient of variation of 6.8% [25, 29].

Plasma samples obtained at that examination were also frozen and maintained at –70°C. These were sent in 1996 to the Department of Medicine, Northwest Lipid Research Laboratories, University of Washington, Seattle, Wash., USA to measure Apo-A1, glucose, insulin, C-peptide, and lipoprotein (a) [Lp(a)] [30].

For Apo-A1 in particular, measurements were performed on a Behring Nephelometer Analyzer calibrated with WHO-IFCC International Reference Material. The method is used as a comparison method for the standardization of Apo-A1 and Apo-B; thus, rigorous criteria are followed for acceptance of an analytical run [30]. Quality control specimens consisted of three fresh-frozen serum pools with low, medium, and high concentrations of Apo-A1. The within- and between-assay coefficients of variability were consistently <2% and <2.5%, respectively.

Prevalent cases of cardiovascular disease as of 1980–1982, either cerebrovascular or coronary disease, were excluded from data analyses (n=202). Incident CHD cases (n=182) subsequent to the 1980–1982 examination were identified as part of ongoing surveillance activities. These include monitoring and categorizing events identified from hospital surveillance and from ongoing examination of the cohort. An incident case was identified as having: (1) definite clinical indications of an event [electrocardiogram (ECG) evidence of an acute myocardial infarction, unequivocal cardiac enzyme elevations, silent myocardial infarction evidence based on serial changes in the ECG, and clinical diagnosis of coronary insufficiency or angina pectoris leading to surgical intervention] or (2) suspected indications (equivocal ECG and cardiac enzyme changes, clinical symptoms of angina pectoris medically treated but not leading to surgical intervention). Upon demonstration of similar patterns of relationship with predictors regardless of the level of evidence for a clinical event compared with men with no evidence for CHD (data not shown), definite and suspected cases were combined.

Statistical modelling approach

Previous analysis of observational data reflect viewpoints of whether Apo-A1 as a main effect is, or is not, a "better" independent predictor of CHD than HDL-cholesterol. These viewpoints focus on finding a predictive equation of public healthy utility. The strategy does not take into account known metabolic relationships.

If the objective is to assess whether inter-relations reflecting mechanisms of causation are associated with the development of CHD in the population, then it is proposed that an "effect modification" statistical approach is more appropriate. This viewpoint focuses on statistical detection of joint inter-relations among metabolically linked lipids in the prediction of CHD. Statistical models are created to reflect known biological relationships between these moieties and the development of CHD. A specific detail takes into account Apo-A1 being a constituent of the HDL particle. For these reasons, the primary statistical analysis for this study was developed in two stages.

First, a relative measure of Apo-A1 was created by removing the linear relationship of HDL-cholesterol from variation in Apo-A1 using regression methods. It has units of Apo-A1 concentration, but varies from negative to positive values. For each observation, the relative measure of Apo-A1 is simply the observed Apo-A1 value minus the value predicted by the simple linear regression from the HDL concentration (Fig. 1, *vertical arrows*). This specific formulation is often called the residual Apo-A1 distribution; i.e., it

refers to the residual distribution of the Apo-A1 after the linear relationship with HDL-cholesterol has been removed.

This relative measure is not based on the conventional use of the HDL- cholesterol/Apo-A1 ratio. A ratio imposes a mathematical structure presuming a linear relation between the two variables with an intercept of zero (i.e., when HDL-cholesterol is zero, Apo-A1 concentration is zero). This is not correct for HDL-cholesterol and Apo-A1.

Secondly, statistical models are developed taking into account whether the relative Apo-A1 measure is observed at a high or low value of HDL cholesterol. Thus, the approach loses information about absolute levels of Apo-A1, but retains information about absolute levels of HDL-cholesterol. The approach as a whole is perhaps non-standard in that HDL-cholesterol and relative Apo-A1 are mathematically linked. However, this linkage violates no principle of statistics, and is analogous to part-correlation methods used in the social sciences [31].

Statistical methods

Initial analysis

In order to confirm previously established findings related to Apo-A1 concentration, the Apo-A1 distribution was divided into four groups based on the intervals ≤125, >125–140, >140–155, and >155 mg/dl. Sample sizes, unadjusted means, and standard deviations were calculated within these groups for the variables listed in Table 1. Logarithm transformation was performed on variables with skewed distributions, although geometric means and standard deviations are reported. Initial examination of relationships across groups revealed monotonic trends confirming previously established associations for most variables. Thus, *P* values for statistical tests of linear trend are reported based upon orthogonal polynomial contrasts across the four Apo-A1 groups.

After demonstrating no impact in altering magnitude of estimates within groups (Table 1), no adjustment for age was carried out. There was no adjustment for HDL-cholesterol because of the high co-linearity of Apo-A1 and HDL-cholesterol. Such adjustment would produce extrapolations to a "common" HDL value that would not exist in a general population.

Number of CHD cases, proportion of incident cases, person-years of follow-up, incidence density, and relative rates were assessed among the Apo-A1 groupings. Proportional hazards modelling was used to calculate relative rates using as reference "Apo-A1 ≤125 mg/dl". Ninety-five percent confidence intervals (95% CI) are reported for incidence densities and measures of association. Statistical tests of homogeneity among groups for logistic and proportional hazards models used a χ^2 test of equal

Table 1 Means and standard deviations (SD) of selected continuous variables among quartile groupings of apolipoprotein A-1 (Apo-A1) [*BMI* body mass index, *BP* blood pressure, *HDL* high-density lipoprotein, *Lp(a)* lipoprotein (a)]

		Apo-A1 (mg/dl)					Test for trend
Variable		≤125	>125 to ≤140	>140 to ≤155	>155	Total	*P* value
Patients *(n)*		270	317	277	313	1,177	
Age (years)	Mean	68.3	68.4	68.1	67.8	68.2	0.123
	SD	5.06	5.02	4.94	4.83	4.96	
BMI (kg/m^2)	Mean	24.3	24.0	23.5	22.5	23.6	<0.001
	SD	2.76	2.96	2.93	2.85	2.88	
Back skinfold (mm)	Mean[a]	15.9	15.2	14.5	12.5	14.4	<0.001
	SD[a]	1.38	1.38	1.42	1.42	1.40	
Arm skinfold (mm)	Mean[a]	10.8	10.5	10.4	8.9	10.1	<0.001
	SD[a]	1.39	1.41	1.43	1.49	1.43	
Systolic BP (mmHg)	Mean	140.3	138.2	138.5	138.5	138.8	0.304
	SD	17.6	18.2	18.5	17.1	17.8	
Diastolic BP (mmHg)	Mean	81.5	81.1	81.8	80.4	81.2	0.380
	SD	8.7	9.2	10.1	9.4	9.4	
Cholesterol (mg/dl)	Mean	197	209	215	219	210	<0.001
	SD	34.9	31.5	37.2	45.7	37.8	
HDL-cholesterol (mg/dl)	Mean	35.4	42.2	49.2	62.5	47.7	<0.001
	SD	5.5	6.0	7.4	12.8	8.6	
Triglycerides (mg/dl)	Mean[a]	157.3	146.9	133.5	122.0	138.9	<0.001
	SD[a]	1.67	1.75	1.86	1.85	1.78	
Lp(a) (nmol/l)	Mean[a]	16.5	16.4	16.5	18.2	16.9	0.350
	SD[a]	3.17	3.18	3.51	3.58	3.36	
Glucose (mg/dl)	Mean[a]	114.2	112.6	110.3	108.3	111.3	<0.001
	SD[a]	1.24	1.23	1.19	1.19	1.21	
Insulin (μU/ml)	Mean[a]	13.2	12.7	11.7	9.1	11.5	<0.001
	SD[a]	1.73	1.69	1.81	1.82	1.76	
C-Peptide (pg/ml)	Mean[a]	1.65	1.57	1.46	1.25	1.47	<0.001
	SD[a]	1.58	1.57	1.61	1.66	1.61	

[a] Geometric mean and SD (geometric SD is exponentiation of SD on natural log scale)

parameter estimates [32]. A χ^2 trend test among groups was used for proportion of cases and incidence density [33].

Primary analysis

The first set of analyses focused on the joint relationship of a relative measure of Apo-A1 and absolute level of HDL-cholesterol as predictors of CHD. Addition of another level of joint stratification for triglyceride levels examines inter-relations among three metabolically linked lipids as predictors of CHD.

Analyses reported in this paper do not adjust for associations that may exist for indirect markers or exogenous risk factors, such as age, disease status, alcohol or tobacco use, dietary habits, etc. No adjustment for age is justified based on the observation that doing so has no impact on altering magnitudes of associations. Controlling for any-and-all co-variates risks over-control. For the other co-variates, justification is based on beliefs that: (1) biochemistry tests reflecting endogenous lipid and carbohydrate metabolism are more proximate to the mechanism of causation of CHD than exogenous variables and (2) indirect markers of factors related to the development of CHD are mediated by more directly measured physiological and biochemical variables. These beliefs rest on a premise from evidence arising outside of epidemiology that joint variation in HDL and relative Apo-A1, and triglycerides, are causally linked to development of CHD.

A cut-off point was identified at ≥80th percentile of the relative Apo-A1 distribution (+9.2 mg/dl), forming two groups. The HDL-cholesterol distribution was divided into two groups based on the cut-off point ≤40mg/dl. Cut-off points at ≥90th percentile and ≤45 mg/dl, respectively, produced similar patterns of results. Relationships and descriptions of co-variates and incident CHD among joint combinations of the two groups of these two variables were assessed using analysis of variance methods for continuous variables, and proportions, incidence density, and relative rates for CHD incident events. Relative rates are referenced to the joint category "HDL>40 mg/dl and relative Apo-A1≥80th percentile".

The pattern of results of categorical analyses were verified by constructing a proportional hazards model in which HDL-cholesterol and relative Apo-A1 were specified as continuous variables. The best combination of predictors for all combinations of these two variables up to fourth-order interactions was assessed in the prediction of incident CHD. The formula resulting from this modelling is heuristic, designed to find the best predictive equation without implying a mathematical framework reflected in biology.

Statistical tests of multiplicative interaction between the joint HDL and relative Apo-A1 groupings are reported for estimates of proportions, incidence densities, and relative rates. For differences in incidence density between the two relative Apo-A1 groups, comparisons of these differences are done by contrasting the two HDL groups using a χ^2 test of directly pooled estimates for uniform incidence density differences [34].

An additional analysis examined incidence density stratified by joint combinations of the HDL-cholesterol and relative Apo-A1 groups plus a stratification of the triglyceride distribution at a cut-off point of ≤190 mg/dl (approximately the highest quartile). Using proportional hazards modelling, a statistical model examining all two-way and the three-way interactions was used in order to detect effect modification.

Results

Apo-A1 concentration and HDL-cholesterol level are highly correlated with each other in this sampling frame

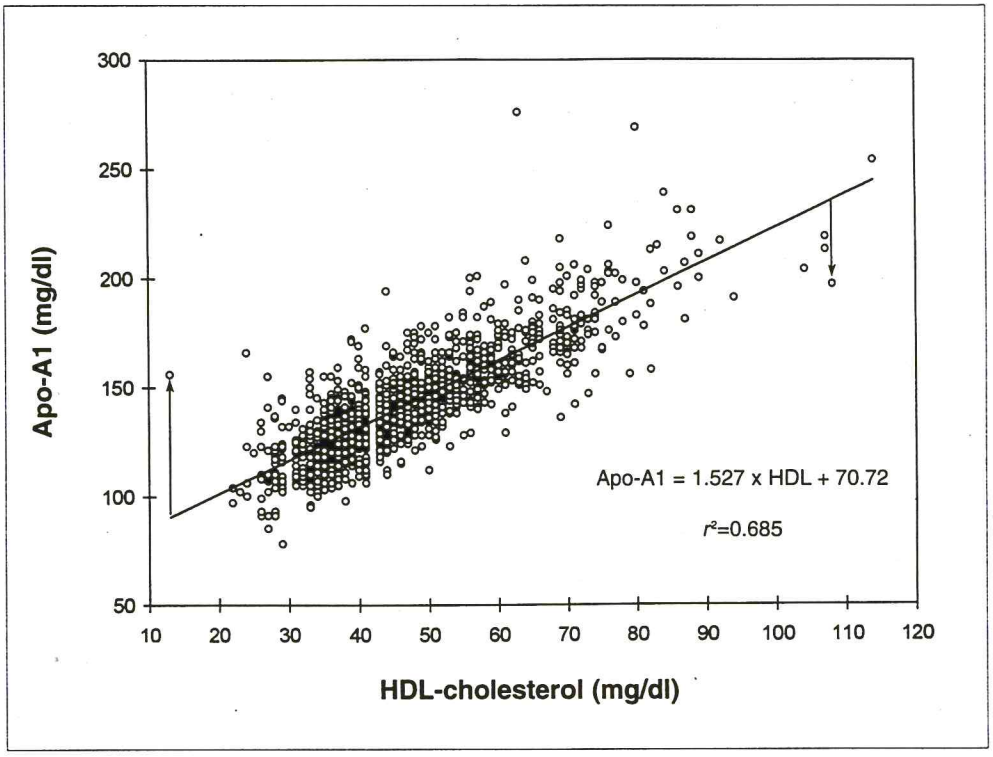

Fig. 1 Linear relationship between apolipoprotein A-1 *(Apo-A1)* and high-density lipoprotein *(HDL)*-cholesterol. *Vertical arrows* denote residual values for two points; i.e., observed Apo-A1 value minus value predicted by he linear relationship with HDL-cholesterol

(r^2=0.685, Fig. 1). Among quartile groups of Apo-A1, significant linear trends are noted for body mass index, subscapular (back) and triceps (arm) skinfold thickness, total cholesterol, HDL-cholesterol, triglycerides, blood glucose, insulin, and C-peptide (Table 1). However, no linear trends are noted for age, systolic and diastolic blood pressure, or Lp (a). The proportion of incident cases and the incidence density (number of cases per 1,000 person-years) notably and monotonically decreased with increasing level of Apo-A1 (Table 2).

Structuring the statistical analysis framework to reflect relationships among joint categories of HDL-cholesterol (≤40, >40 mg/dl) and relative Apo-A1 (≥80 percentile, <80 percentile, cut-off point at +9.2 mg/dl) produced statistically significant differences in mean values of co-variates among these four levels (Table 3).

The CHD incidence density was highest, 28.6 events per 1,000 person-years, in the joint stratum of HDL ≤40 mg/dl and Apo-A1 residual >80th percentile (Table 4). Conversely, the lowest incidence density, 8.3 events per 1,000 person-years, was noted in the joint stratum of HDL >40 mg/dl and relative Apo-A1 >80th percentile. Referenced to this latter joint stratum, a relative rate of 3.50 was noted among men with HDL ≤40 mg/dl and Apo-A1 residual >80th percentile.

CHD incidence density differences, and relative rates (Table 4), comparing relative Apo-A1 categories were heterogenous across levels of HDL, producing heterogeneity statistics of χ^2=4.11 (*df*=1, *P*=0.043) and χ^2=4.77 (*df*=1, *P*=0.028), respectively.

Using HDL-cholesterol and relative Apo-A1 as continuous measures and examining the heuristic predictive characteristics of the various linear, quadratic, cubic, and quartic terms produced the same pattern of relationship as noted for the categorical analysis (Fig. 2). This modelling procedure produced statistically significant (*P* values varying from 0.062 to 0.001) parameter estimates for third-order and fourth-order terms in HDL-cholesterol and relative Apo-A1 variables and their interaction.

The incidence density for CHD events among joint strata of HDL-cholesterol, relative Apo-A1, and triglycerides produced one cell (HDL≤40 mg/dl, triglycerides≤190 mg/dl, relative Apo-A1>80th percentile) with no CHD events and only 75.5 person-years of observation (Table 5). Heuristic statistical modelling of all main effects and all two-way and the three-way interactions retained the main effects of triglycerides (χ^2=10.25, *P*=0.001) and relative Apo-A1 (χ^2=6.62, *P*=0.010), and the three-way interaction (χ^2=10.00, *P*=0.002). Heterogeneity statistics of incidence density differences and relative rates comparing Apo-A1 categories across joint strata of HDL and triglycerides produced significant results (χ^2=16.67, *df*=3, *P*<0.001 and χ^2=7.25, *df*=3, *P*=0.064, respectively).

The difference in CHD incidence density between relative Apo-A1 strata is positive for all strata except HDL ≤40 mg/dl and triglycerides >190 mg/dl. In this stratum the difference reverses to a value of –9.6 events per 1,000 person-years, reflecting the highest incidence density of 31.6 events per 1,000 person-years among all joint strata in men with relative Apo-A1 >80th percentile. It is the contrast of this negative difference with the combination of all other positive differences that produces the statistically significant test of heterogeneity. The median triglyceride level was highest, 353 mg/dl, in the group with the highest CHD incidence density of 31.6 events per 1,000 person-years.

Table 2 Cumulative incidences and relative rates [95% confidence intervals (CI)] of coronary heart disease events among strata of Apo-A1 (*ID* incidence density)

	Apo-A1 groupings (mg/dl)					Homogeneity*
Parameter	≤125	>125–≤140	>140–≤155	>155	Total	*P* value
Cases	54	59	43	26	182	–
n	270	317	277	313	1,177	–
% Cases	20.0	18.6	15.5	8.3	15.5	<0.001
Person-years	2,907.9	3,389.8	3,075.0	3,512.9	12,885.6	–
ID, per 1,000 person-years	18.6	17.4	14.0	7.4	14.1	<0.001
(95% CI)	(14.0, 24.2)	(13.2, 22.4)	(10.1, 18.8)	(4.8, 10.8)	(12.2, 16.3)	–
Relative rate[a]	1	0.941	0.751	0.396	–	<0.001
(95% CI)	–	(0.65, 1.36)	(0.50, 1.12)	(0.25, 0.63)	–	–

* Homogeneity of estimates. A Wald χ^2 test that parameter estimates equal each other is used for logistic and proportional hazards models. χ^2 trend tests are used for unadjusted estimates of percentage cases and ID

[a] Proportional hazards model, referenced to lowest Apo-A1 concentration stratum

Table 3 Means and SD of selected continuous variables among joint strata of HDL-cholesterol and percentiles of residual Apo-A1 after accounting for linear relationship with HDL

		HDL≤40 mg/dl		HDL>40 mg/dl		*F* value*
Residual Apo-A1		>80%[a]	≤80%	>80%	≤ 80%	(*P* value)
Patients (*n*)		76	334	160	607	
Age (years)	Mean	67.1	68.3	67.3	68.4	3.50
	SD	4.36	4.98	4.87	5.01	(0.015)
BMI (kg/m^2)	Mean	24.8	24.4	23.2	23.0	22.9
	SD	3.17	275	268	295	(<0.001)
Back skinfold (mm)	Mean[b]	16.2	16.2	14.0	13.4	26.2
	SD[b]	1.40	1.37	1.37	1.43	(<0.001)
Arm skinfold (mm)	Mean[b]	10.7	10.9	9.5	9.7	9.73
	SD[b]	1.42	1.39	1.41	1.47	(<0.001)
Systolic BP (mmHg)	Mean	141.1	140.5	140.0	137.3	3.16
	SD	16.2	18.0	15.9	18.3	(0.024)
Diastolic BP (mmHg)	Mean	84.2	81.6	81.7	80.4	4.47
	SD	10.50	8.83	8.36	9.66	(<0.004)
Cholesterol (mg/dl)	Mean	227.5	202.5	225.0	208.5	18.6
	SD	73.9	34.3	38.4	32.4	(<0.001)
Apo-A1 (mg/dl)	Mean	143.4	119.8	175.2	148.3	–
	SD	10.7	10.9	227	18.5	–
HDL-cholesterol (mg/dl)	Mean	34.3	35.2	55.5	54.2	–
	SD	5.12	4.08	11.46	11.21	–
Triglycerides (mg/dl)	Mean[b]	389.1	178.3	172.1	100.5	291.3
	SD[b]	1.87	1.56	1.64	1.49	(<0.001)
Lp(a) (nmol/l)	Mean[b]	7.47	16.33	15.18	19.65	15.7
	SD[b]	3.95	3.27	3.85	3.07	(<0.001)
Glucose (mg/dl)	Mean[b]	122.6	114.6	111.2	108.1	14.3
	SD[b]	1.33	1.23	1.17	1.19	(<0.001)
Insulin (μU/ml)	Mean[b]	17.2	14.4	10.9	9.8	49.0
	SD[b]	1.74	1.61	1.71	1.81	(<0.001)
C-peptide (pg/ml)	Mean[b]	1.94	1.77	1.49	1.28	46.2
	SD[b]	1.52	1.51	1.60	1.62	(<0.001)

* F test of global association based on three degrees of freedom

[a] 80th percentile of Apo-A1 residual distribution is + 9.2 mg/dl

[b] Geometric mean and SD (geometric SD is exponentiation of SD on log-transformed variable). Statistical testing done on log-transformed variables

Table 4 Cumulative incidence, ID, and relative rates of coronary heart disease events among joint strata of HDL-cholesterol and of percentiles of residual Apo-A1 after accounting for linear relationship with HDL

		HDL≤40 mg/dl		HDL>40 mg/dl			
Residual Apo-A1		>80%[a]	≤80%	>80%	≤80%	Total	*P* value*
Cases		23	66	15	78	182	–
n		76	334	160	607	1,177	–
% Cases		30.3	19.8	9.4	12.9	15.5	0.025
Person-years		803.8	3,627.1	1,812.3	6,642.4	12,885.6	–
ID per 1,000 person-years		28.6	18.2	8.3	11.7	14.1	0.028
	(95% CI)	(18.1, 42.9)	(14.1, 23.2)	(4.6, 13.6)	(9.3, 14.7)	(12.2, 16.3)	–
ID difference between Apo-A1 residual groups		−10.4	–	3.5	–	–	0.043**
	(95% CI)	(−22.9, 2.1)	–	(−1.5, 8.4)	–	–	–
Relative rate[b]		3.50	2.22	1	1.44	–	0.028
	(95% CI)	(1.83, 6.71)	(1.27, 3.89)	–	(0.83, 2.49)	–	–

* Statistical test of multiplicative interaction betwen HDL and Apo-A1 residual groupings, excepting ID difference between Apo-A1 residual groups; ** Statistical test of directly pooled estimate of uniform ID differences

[a] 80th percentile of Apo-A1 residual distribution is +9.2 mg/dl

[b] Proportional hazards model, referenced to HDL>40 mg/dl and Apo-A1 residual >80th percentile (+9.2 mg/dl)

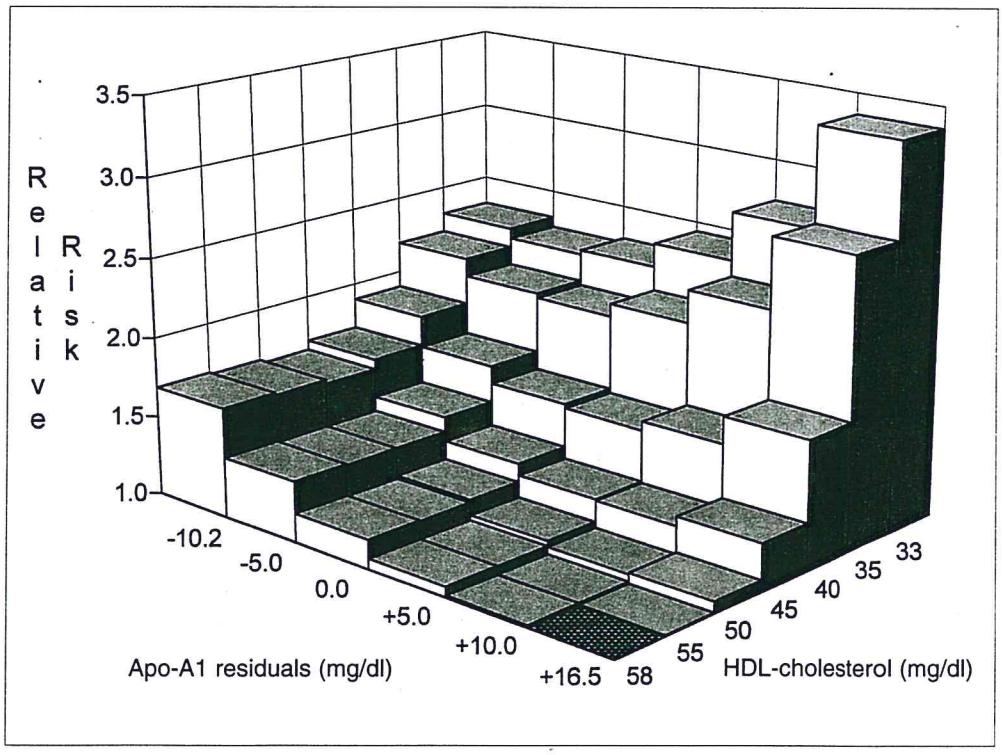

Fig. 2 Relative rate of coronary heart disease from proportional hazards model of continuous measures of HDL-cholesterol and the residual of Apo-A1 after accounting for the linear relationship with HDL-cholesterol. Relative rates are referenced to the joint conditions of the 80th percentile of HDL-cholesterol (58 mg/dl) and the 90th percentile of residual Apo-A1 (+16.5 mg/dl)

Table 5 ID, ID differences, and ID rate ratios of coronary heart disease events among joint strata of triglycerides, HDL-cholesterol, and percentiles of residual Apo-A1 after accounting for linear relationship with HDL

	HDL>40 mg/dl				HDL≤ 40 mg/dl				
	TG≤190 mg/dl		TG>190 mg/dl		TG≤190 mg/dl		TG>190 mg/dl		
Residual Apo-A1	>80%[a]	≤80%	>80%	≤80%	>80%	≤80%	>80%	≤80%	Total
Cases	9	70	6	8	0	33	23	33	182
n	96	575	64	31	6	190	70	144	1,176
Person-years	1095.0	6325.1	717.2	305.1	75.5	2126.4	728.3	1500.7	12,873.3
ID per 1,000 person-years	8.2	11.1	8.4	26.2	0.0	15.5	31.6	22.0	14.1
(95% CI)	(3.8, 15.6)	(8.6, 14.0)	(3.1, 18.2)	(11.3, 51.7)	(0.0, 48.9)	(10.7, 21.8)	(20.0, 47.4)	(15.1, 30.9)	(12.2, 16.4)
ID difference between Apo-A1 residual groups	2.8	–	17.9	–	15.5	–	–9.6	–	*
(95% CI)	(–3.1, 8.8)	–	(–1.5, 37.2)	–	(10.2, 20.8)	–	(–24.5, 5.3)	–	–
ID rate ratio between Apo-A1 residual groups	1.35	–	3.13	–	∞	–	0.70	–	**
(95% CI)	(0.67, 2.70)	–	(1.09, 9.03)	–	(0.31, ∞)	–	(0.41, 1.19)	–	–
Median TG (mg/dl)	135	96	249.5	220	148.5	140	353	254.5	132

* Statistical test of heterogeneity of ID differences: χ^2=16.67, *df*=3, *P*<0.001
** Statistical test of heterogeneity of ID risk ratios: χ^2=7.25, *df*=3, *P*<0.064
[a] 80th percentile of Apo-A1 residual distribution is +9.2 mg/dl

Discussion

Conceptualizing relative Apo-A1 is mentally challenging. While analogous to calculating a ratio of HDL to Apo-A1, it is more accurate to describe it as the excess (or deficit) Apo-A1 above and beyond (or below and beneath) what would be predicted from the linear relationship of Apo-A1 with HDL concentration (Fig. 1). One could consider HDL-cholesterol as the load, and Apo-A1 as trucks. These data illustrate that when there is not a lot of load but there are trucks in excess of that expected for the load, then the risk of developing cardiovascular disease is notably increased. Moreover, it would appear that by some linkage of lipid metabolism, if there is a lot of triglyceride load (carried by other trucks) in conjunction with the low HDL-cholesterol and relative excess of Apo-A1, then the risk of developing cardiovascular disease is even higher. What is the evidence that such associations, rather than due to some effect of an unknown confounding variable, are really being driven by variations in lipid metabolism?

Early work by Fielding [34], confirmed by others [35], suggests that a class of HDL-cholesterol particle (pre-β HDL) is preferentially elevated and total HDL-cholesterol is reduced in some hyperlipemic states, including triglyceridemias. Pre-β HDL consists of further subcategories, reflecting a progression to becoming an α-HDL particle in the metabolism and transport of cholesterol. Compared with α-HDL particles that contain higher levels of lipids and cholesterol ester, pre-β_1 HDL is high in Apo-A1 content [35]. The pre-β_1 subparticle appears to accept cholesterol from cells in the first steps of reverse cholesterol transport [36, 37]. Between 17 and 63% of all Apo-A1 may be associated with pre-β HDL in these hyperlipidemic conditions compared with 4±2% in normolipidemic people [38].

Elevations in pre-β HDL associated with CHD may be due either: (1) to impaired metabolism of pre-β HDL to α-HDL by lecithin-cholesterol acyltransferase (LCAT) that converts cholesterol to cholesteryl ester [35], or (2) to an enhanced production or recycling of pre-β HDL in CHD – possibly by an in situ promoting effect associated with cholesterol-laden macrophages present in atheromatous lesions [39]. Other studies support the importance of an interaction between LCAT activity and the presence of peripheral cells, as well as other lipid particles, in the regulation of pre-β HDL levels [40, 41]. How such phenomena may relate to results presented in this paper cannot be discerned, but raise potential avenues of inquiry for understanding mechanisms of causation for a lipid derangement leading to clinical CHD in a non-trivial portion of the population.

It cannot be discerned from this study whether associations among HDL-cholesterol, Apo-A1, and triglycerides are driven by metabolic mechanisms related to reverse cholesterol transport, or by a direct effect of triglyceride-rich lipoproteins on the development of atherosclerosis. Thus, it can only be generally concluded that these results indirectly support a hypothesis that some men with hypertriglyceridemia, depressed HDL cholesterol levels, and who go on to develop CHD may have a preferential redistribution in HDL particle sub-populations from the α-migrating to the pre-β form, resulting in a high relative Apo-A1 concentration. If there is an elevation in a pre-β HDL subfraction, it is associated with a much-larger overall depression in α-HDL levels, the predominant form of HDL-cholesterol in blood [39].

This explanation is consistent with observations in transgenic mice models having various combined genetic modifications. These modifications enhance the expression of dietary-induced atherosclerotic lesions concomitant with alterations in plasma triglycerides, pre-β and α-HDL cholesterol, and Apo-A1 [42]. Mice homozygous for a low-density lipoprotein (LDL) receptor defect manifest elevated levels of atherogenic LDL and intermediate-density lipoproteins (IDL). A synergy is produced upon addition of the human Apo-C3 gene into the model, producing (1) dietary-induced aortic lesion areas two to three times that of the LDL receptor defect background, (2) a tenfold increase in very low-density lipoprotein and IDL-LDL cholesterol levels along with elevated triglycerides, (3) a marked lowering of absolute Apo-A1 concentration, (4) a minor diminution in pre-HDL concentration, and (5) a doubling in the proportion of Apo-A1 carried by the pre-β particle from 16% to 33%. Addition of the human gene for cholesteryl ester transfer protein (1) lowers α-HDL-cholesterol levels as well as absolute Apo-A1 concentration, (2) depresses pre-β HDL-cholesterol concentration by 15%, (3) doubles again the proportion of Apo-A1 carried by the pre-β particle from 33% to 60%, and (4) further elevates plasma triglycerides.

The findings in a transgenic mouse model intended to produce features of familial combined hyperlipidemia in humans are characteristic of the features observed in this population-based study. This animal model suggests that the genetic mechanisms producing these variations in lipid metabolism lead to the decrease in HDL-cholesterol and concomitant increase in triglycerides associated with development of atherosclerosis-mediated CHD, and that an additional feature is the relative increase in Apo-A1 compared with the absolute level of HDL-cholesterol.

Future population-based studies may benefit by thoughtful structuring of statistical analyses to reflect understanding of disease mechanisms, and by the use of more-targeted and specific markers of metabolic function. Such an approach emphasizes better understanding of potential mechanisms causing morbidity and mortality, rather than development of equations to identify predictors of CHD for purposes of screening and assessment of public health impact.

A specific mechanism by which observational studies have demonstrated significant relationships between the joint conditions of hypertriglyceridemia and depressed HDL-cholesterol levels may partially, but not completely, be via derangements resulting in "enrichment" of HDL subparticles containing relatively higher levels of Apo-A1. Clinical and laboratory studies have provided detailed understanding of how such mechanisms may operate in selected groups with well-characterized defects in lipid metabolism. Transgenic studies in mice confirm the possibility of these causal mechanisms operating in humans. This epidemiological study suggests such mechanisms may be operating at a population level, and may be related to a non-trivial contribution to the public health burden of CHD.

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