High-Density Lipoprotein Cholesterol, High-Density Lipoprotein Particle Size, and Apolipoprotein A-I: Significance for Cardiovascular Risk

The IDEAL and EPIC-Norfolk Studies

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Objectives	This study was designed to assess the relationship of high-density-lipoprotein cholesterol (HDL-C), HDL particle size, and apolipoprotein A-I (apoA-I) with the occurrence of coronary artery disease (CAD), with a focus on the effect of very high values of these parameters.
Background	High plasma levels of HDL-C and apoA-I are inversely related to the risk of CAD. However, recent data suggest that this relationship does not hold true for very high HDL-C levels, particularly when a preponderance of large HDL particles is observed.
Methods	We conducted a post-hoc analysis of 2 prospective studies: the IDEAL (Incremental Decrease in End Points through Aggressive Lipid Lowering; n = 8,888) trial comparing the efficacy of high-dose to usual-dose statin treatment for the secondary prevention of cardiovascular events, and the EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk case-control study, including apparently healthy individuals who did (cases, n = 858) or did not (control patients, n = 1,491) develop CAD during follow-up. In IDEAL, only HDL-C and apoA-I were available; in EPIC-Norfolk, nuclear magnetic resonance spectroscopy-determined HDL particle sizes were also available.
Results	In the IDEAL study, higher HDL-C proved a significant major cardiac event risk factor following adjustment for age, gender, smoking, apoA-I, and apoB. A similar association was observed for HDL particle size in EPIC-Norfolk. Increased risk estimates were particularly present in the high ends of the distributions. In contrast, apoA-I remained negatively associated across the major part of its distribution in both studies.
Conclusions	When apoA-I and apoB are kept constant, HDL-C and HDL particle size may confer risk at very high values. This does not hold true for very high levels of apoA-I at fixed levels of HDL-C and apoB. These findings may have important consequences for assessment and treatment of CAD risk. (J Am Coll Cardiol 2008;51:634–42) © 2008 by the American College of Cardiology Foundation

Epidemiological data have convincingly demonstrated (1,2) an independent and inverse relationship between the concentration of cholesterol in high-density lipoproteins (HDL-C) and the risk of coronary artery disease (CAD). Studies focusing on the biological mechanisms responsible for this association suggest a major role for the main protein constituent of HDL, apolipoprotein A-I (apoA-I) (3). These observations have led to the development of novel therapies that raise plasma levels of HDL-C or apoA-I to decrease the risk of CAD that remains in patients treated with statins. Among these novel therapies, elevation of HDL-C by inhibition of the cholesteryl ester transfer protein (CETP) has attracted the most attention to date.

Recently, 2 clinical studies with the CETP inhibitor torcetrapib were published (4,5) evaluating the effect of this novel compound on atherosclerosis progression in humans. In both studies, considerable increases of plasma HDL-C were observed in patients receiving torcetrapib, with achievement of very high levels of this lipid fraction (72.1 and 81.5 mg/dl, respectively). Nevertheless, torcetrapib did not induce the expected regression of atherosclerosis. One possible explanation for this unexpected outcome pertains to the structural changes of the HDL particle induced by CETP inhibition. In fact, it has been hypothesised (6) that the very large HDL particles, which become predominant when HDL-C levels rise upon CETP inhibition, may be less effective in exerting anti-atherogenic functions. Although several in vitro experiments have addressed this specific issue (6–8), no robust epidemiological studies are available yet.

Therefore, the objective of the present study was to assess the relationships of plasma HDL-C and HDL particle size with CAD risk, with a particular emphasis on very high values of these parameters. Similarly, we evaluated the relationship for apoA-I. We used data from the large IDEAL (Incremental Decrease in End Points through Aggressive Lipid Lowering) trial (n = 8,888) (9), which compared the efficacy of high-dose to usual-dose statin treatment for the secondary prevention of cardiovascular events, and from a prospective case-control study (858 cases, 1,491 control patients) nested in the EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk cohort (10). The IDEAL dataset contained data on HDL-C and apoA-I, whereas the EPIC-Norfolk dataset additionally had values of HDL particle size as measured by nuclear magnetic resonance (NMR) spectroscopy. Here we present our results.

Methods

Study cohorts. The IDEAL study has been published previously (9). In summary, IDEAL is a prospective randomized trial comparing the efficacy of high- to usual-dose statin therapy for the secondary prevention of cardiovascular events. A total of 8,888 patients with a history of myocardial infarction (MI) were enrolled and randomized to either simvastatin 20 mg or atorvastatin 80 mg. Median follow-up was 4.8 years. Mean on-treatment levels of low-density lipoprotein cholesterol (LDL-C) were 104 mg/dl (2.7 mmol/l) in the simvastatin group and 81 mg/dl (2.1 mmol/l) in the atorvastatin group. The intensive treatment regime did not significantly reduce the occurrence of the primary outcome (major coronary event, MCE) but did reduce the risk of other composite secondary end points and nonfatal acute MI.

The EPIC-Norfolk study included 25,663 men and women ages 45 to 79 years resident in Norfolk, United Kingdom (10). Participants were recruited from general practices in Norfolk as part of the 10-country collaborative EPIC study, designed to investigate dietary and other determinants of cancer. Additional data were obtained in EPIC-Norfolk to enable the assessment of determinants of other diseases, including CAD. At the baseline survey between 1993 and 1997, participants completed a detailed health and lifestyle questionnaire. All individuals were flagged for death certification at the United Kingdom Office of National Statistics, with vital status ascertained for the entire cohort. In addition, participants admitted to hospital were identified using their unique National Health Service number by data linkage with the East Norfolk Health Authority (ENCORE) database, which identifies all hospital contacts throughout England and Wales for Norfolk residents. Participants were identified as having CAD during follow-up if they had a hospital admission and/or died with CAD as underlying cause. Coronary artery disease was defined as codes 410-414 according to the International Classification of Diseases-9th revision. The case-control dataset used for the present study included individuals who developed CAD during follow-up until November 2003 (mean follow-up 6 years). Control patients were study participants who remained free from CAD during this follow-up period. Cases and control patients were matched by age (within 5 years), gender, and time of enrollment (within 3 months) on a 1:2 basis. Individuals who reported a history of heart attack/stroke or use of lipid-lowering drugs at the baseline clinic visit were not included in the case-control study.

End point definition. The occurrence of an MCE was selected as outcome variable for this analysis. In the IDEAL study, this was the primary end point, defined as coronary death, nonfatal MI, or resuscitation after cardiac arrest. In

Abbreviations and Acronyms

ApoA-I = apolipoprotein A-I

ApoB = apolipoprotein B

CAD = coronary artery disease

CETP = cholesteryl ester transfer protein

HDL-C = high-density lipoprotein cholesterol

LDL-C = low-density lipoprotein cholesterol

MCE = major coronary event

MI = myocardial infarction

NMR = nuclear magnetic resonance

OR = odds ratio

RR = relative risk

EPIC-Norfolk, MCE included fatal or nonfatal CAD, defined as codes 410-414 according to the International Classification of Diseases-9th revision.

Selection of study participants. For the present analysis in the IDEAL study, lipid and apolipoprotein values were averages of the measurements performed at 3 and 6 months on treatment. Only patients with a complete set of lipid and apolipoprotein measurements at these time points were used, and all patients who developed MCE in this study before 6 months of treatment were excluded from the present analysis. Similarly, only patients with complete lipid, apolipoprotein, and HDL particle size measurements at the baseline survey in EPIC-Norfolk were used for the present analysis.

Laboratory measurements. Lipid and apolipoprotein measurements were performed on fasting blood samples in the IDEAL study and on nonfasting samples in EPIC-Norfolk. Levels of total cholesterol, HDL-C, and triglycerides were quantified using standard methodologies. In both studies, plasma concentration of apoA-I and apolipoprotein B (apoB) were determined by immunonepholometry (Behring Nephelometer BNII, Marburg, Germany), with calibration traceable to the International Federation of Clinical Chemistry primary standards (11).

In EPIC-Norfolk, mean HDL particle size was measured by NMR spectroscopy as previously described (12). Briefly, concentrations of HDL size subclasses were derived from the measured amplitudes of the distinct lipid methyl group NMR signals they emit. Weighted-average HDL particle sizes in nanometers (nm) were then calculated from the subclass levels, and the diameters were assigned to each subclass.

Statistical analyses. In the IDEAL dataset, the relationships of HDL-C and apoA-I with MCE were calculated by a Cox proportional hazards model, yielding values for relative risk (RR) for a 1-SD increase of HDL-C or apoA-I with 95% confidence intervals. The basic regression model included covariates for age, gender, and smoking status (current, former, never) recorded at baseline. Body mass index (BMI) was not taken into account because this parameter did not significantly contribute to the regression models (data not shown). Data on alcohol consumption were not available in the IDEAL database. In EPIC-Norfolk, the relationships of HDL-C, HDL particle size, and apoA-I with MCE were determined by conditional logistic regression analysis that took into account the matching for age, gender, and enrollment period and included the covariates smoking status (current, former, never), BMI, and alcohol consumption (number of units per week) (basic model). The MCE risk estimates were expressed as odds ratios (OR) for a 1-SD increase of HDL-C, HDL particle size, or apoA-I with 95% confidence intervals. The basic models for HDL-C (IDEAL and EPIC-Norfolk) and HDL particle size (EPIC-Norfolk only) were then adjusted for apoA-I, and those for apoA-I were adjusted for HDL-C (IDEAL and EPIC-Norfolk) or

HDL particle size (EPIC-Norfolk only). In addition, all models were adjusted for apoB to account for the pro-atherogenic lipoprotein fraction.

To assess the effect of very high values of HDL-C, HDL particle size, and apoA-I on the risk of MCE, patients were categorized into 6 subgroups for each of these parameters in the 2 studies. These subgroups were defined on the basis of the percentages of study participants that belonged to the following HDL-C subgroups in IDEAL: <40, 40 to 49, 50 to 59, 60 to 69, 70 to 79, and >80 mg/dl. The MCE risk estimates were than calculated for patients in each subgroup using the lowest category as reference. Adjustment procedures were identical as described earlier. A p value was calculated for each subgroup indicating whether the corresponding risk estimate significantly differed from the reference category. Also, statistical significance was assessed for a linear trend across the risk estimates connected to the 6 subgroups.

Results

Study populations. Of 8,888 IDEAL study participants, 324 patients (195 MCEs) were excluded because of missing values at 3 or 6 months or both or because they experienced an MCE before the 6-month visit. Thus, data were analyzed from 8,564 IDEAL study participants, among whom 679 experienced an MCE during follow-up. The EPIC-Norfolk case-control study contained 1,133 cases and 2,237 control patients. Of these, 239 cases and 324 control patients were excluded because of missing values at the baseline survey. Among the remaining study subjects, 36 cases were excluded because they had no matching control patients, as were 422 control patients because they were without matching cases. Consequently, the present analysis of EPIC-Norfolk used data from 858 cases and 1,491 control patients (225 cases with 1 matching control patient, 633 cases with 2 control patients).

Table 1 reports baseline demographic and clinical characteristics, as well as on-treatment (IDEAL) or baseline (EPIC-Norfolk) plasma levels of lipids and apolipoproteins per study. Patients in IDEAL were somewhat younger than those included in EPIC-Norfolk, and the IDEAL dataset contained a higher percentage of men. Levels of HDL-C and apoA-I were comparable. Levels of total cholesterol, LDL-C, and apoB were substantially lower in IDEAL than in EPIC-Norfolk, reflecting the fact that all IDEAL patients received statin therapy, whereas none of the EPIC-Norfolk participants used lipid-lowering medication.

Relationships among HDL-C, HDL particle size, or apoA-I and MCE. Table 2 displays the relationships among levels of HDL-C, HDL particle size (EPIC-Norfolk only) or apoA-I, and risk of MCE per study. Without adjustment for apoA-I and apoB, HDL-C was significantly and inversely related with MCE risk in both

Table 1	Demographic, Clinical, and Lipoprotein Characteristics of Study Subjects in IDEAL and EPIC-Norfolk	
	IDEAL (n = 8,564)	**EPIC (n = 2,349)**
Age (yrs)	61.7 ± 9.4	65.0 ± 7.9
Gender (% male)	80.9	62.7
Smoking (%)		
Current	20.4	11.2
Former	58.5	50.5
Never	21.1	38.3
BMI (kg/m^2)	27.3 ± 3.8	26.6 ± 3.6
Total cholesterol (mg/dl)	163.0 ± 33.7	243.9 ± 44.7
LDL-C (mg/dl)	91.7 ± 27.6	160.4 ± 39.8
HDL-C (mg/dl)	46.5 ± 11.9	50.9 ± 15.2
ApoB (g/l)	0.94 ± 0.28	1.32 ± 0.31
ApoA-I (g/l)	1.39 ± 0.22	1.60 ± 0.30

Values are presented as mean ± SD or as percentage. Demographic and clinical characteristics for IDEAL study subjects presented here are recorded at baseline. Lipid and apolipoprotein values in this study are the average from measurements at 3 and 6 months of treatment.

apoA-I = apolipoprotein A-I; apoB = apolipoprotein B; BMI = body mass index; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.

studies, as was HDL particle size in EPIC-Norfolk. In IDEAL, a 1-SD increase in HDL-C (+11.9 mg/dl) was associated with an RR of 0.92 (p = 0.04) and in EPIC-Norfolk (+15.2 mg/dl) with an OR of 0.78 (p < 0.0001). A similar increase of HDL particle size in EPIC-Norfolk (+0.48 nm) yielded an OR of 0.86 (p = 0.006). When regression models were adjusted for apoA-I, risk estimates for HDL-C and HDL particle size moved toward unity and lost significance. Upon additional adjustment for apoB, these risk estimates moved further upward, such that HDL-C (in IDEAL) and HDL particle size (in EPIC-Norfolk) became significantly positively related to the oc-

currence of MCE (RR 1.21, p = 0.04 in IDEAL; OR 1.23, p = 0.005 in EPIC-Norfolk, respectively).

In the basic regression models, apoA-I was negatively related to MCE occurrence in both studies (RR 0.90, p = 0.01 in IDEAL; OR 0.79, p < 0.0001 in EPIC-Norfolk). Upon adjustment for HDL-C, statistical significance of this relation was lost (RR 0.86, p = 0.11 in IDEAL; OR 0.87, p = 0.09 in EPIC-Norfolk). Adjustment for HDL particle size (EPIC-Norfolk only) had no effect. Upon additional adjustment for apoB, the relationship for apoA-I returned to a significant negative sign (RR 0.74, p = 0.002 in IDEAL; OR 0.74, p = 0.001 in EPIC-Norfolk, adjusted for HDL-C and apoB or OR 0.69, p < 0.0001, adjusted for HDL particle size and apoB).

Relationships among subgroups of HDL-C, HDL particle size or apoA-I, and MCE. The predefined HDL-C subgroups in IDEAL contained 30.2%, 34.4%, 24.0%, 7.6%, 2.5%, and 1.3% of the study participants. Tables 3 and 4 present risk estimates for subcategories of HDL-C levels (Table 3), HDL particle size (Table 3) (EPIC-Norfolk only), and apoA-I (Table 4) calculated by the basic models and following full adjustment for lipoproteins. In the IDEAL study, unadjusted analyses showed an inverse trend in MCE risk up to an HDL-C level of 70 mg/dl (RR 0.91, 95% CI 0.76 to 1.09; RR 0.77, 95% CI 0.62 to 0.95; RR 0.71, 95% CI 0.51 to 0.99 for the second, third, and fourth categories compared to the first, respectively) (Table 3). For the subcategories above 70 mg/dl, the relative risks lost statistical significance (RR 1.03, 95% CI 0.65 to 1.63; RR 0.96, 95% CI 0.51 to 1.82). This appeared to be due to wider 95% CIs but also to regression of the point estimates toward unity. No significant linear trend could be observed across

Table 2	Risk Estimates for a Major Coronary Event Per 1-SD Increase of HDL-C, HDL Particle Size, and ApoA-I in the IDEAL and EPIC-Norfolk Studies					
	IDEAL			**EPIC-Norfolk**		
	RR*	**95% CI**	**p Value**	**OR***	**95% CI**	**p Value**
HDL-C						
Basic model†	0.92	0.85–1.00	0.04	0.78	0.70–0.87	<0.0001
+ ApoA-I	1.05	0.96–1.15	0.59	0.87	0.74–1.03	0.10
+ ApoA-I + ApoB	1.21	1.01–1.46	0.04	1.07	0.89–1.28	0.49
HDL size‡						
Basic model	—	—	—	0.86	0.78–0.96	0.006
+ ApoA-I	—	—	—	0.98	0.87–1.11	0.74
+ ApoA-I + ApoB	—	—	—	1.23	1.07–1.42	0.005
ApoA-I						
Basic model	0.90	0.83–0.98	0.01	0.79	0.71–0.87	<0.0001
+ HDL-C	0.86	0.71–1.03	0.11	0.87	0.74–1.02	0.09
+ HDL-C + ApoB	0.74	0.61–0.90	0.002	0.74	0.62–0.88	0.001
+ HDL size	—	—	—	0.79	0.70–0.90	<0.0001
+ HDL size + ApoB	—	—	—	0.69	0.61–0.79	<0.0001

*Risk estimates for a 1-SD deviation increase of HDL-C (+11.9 mg/dl in IDEAL; +15.2 mg/dl in EPIC-Norfolk), HDL particle size (+0.48 nm in EPIC-Norfolk) and apoA-I (+0.22 g/l in IDEAL; +0.30 g/l in EPIC-Norfolk). †Basic model includes age, gender, and smoking for IDEAL and age, gender (matched variables), smoking, body mass index, and alcohol consumption (units/week) for EPIC-Norfolk. ‡Measured by nuclear magnetic resonance (NMR) spectroscopy.

apoA-I = apolipoprotein A-I; apoB = apolipoprotein B; CI = confidence interval; HDL = high-density-lipoprotein cholesterol; OR = odds ratio; RR = relative risk.

| Table 3 | Risk Estimates for a Major Coronary Event by Categories of HDL-C (IDEAL and EPIC-Norfolk) or HDL Particle Size (EPIC-Norfolk), Unadjusted or Adjusted for ApoA-I and B |

	Subgroup	MCE	Basic Model*			+ ApoA-I + ApoB		
			Risk Estimate	95% CI	p Value	Risk Estimate	95% CI	p Value
	HDL-C (mg/dl)							
IDEAL	<40	222	1.00	—		1.00	—	
	40–49	238	0.91	0.76–1.09	0.30	1.10	0.88–1.37	0.42
	50–59	145	0.77	0.62–0.95	0.02	1.12	0.81–1.55	0.50
	60–69	44	0.71	0.51–0.99	0.04	1.25	0.76–2.04	0.37
	70–79	20	1.03	0.65–1.63	0.91	2.19	1.12–4.28	0.02
	>80	10	0.96	0.51–1.82	0.90	2.49	1.04–5.95	0.04
	p trend		*0.06*			*0.08*		
			Basic Model†					
EPIC	<42.5	390	1.00	—		1.00	—	
	42.5–54.1	259	0.73	0.59–0.91	0.006	0.98	0.76–1.26	0.87
	54.1–69.6	141	0.60	0.46–0.78	<0.0001	1.00	0.70–1.43	0.99
	69.6–81.2	35	0.52	0.33–0.84	0.007	1.11	0.62–1.98	0.73
	81.2–96.7	26	0.60	0.35–1.03	0.07	1.47	0.75–2.89	0.26
	>96.7	7	0.41	0.16–1.02	0.05	1.41	0.50–3.97	0.52
	p trend		*<0.0001*			*0.40*		
	HDL Particle Size (nm)							
EPIC	<8.60	293	1.00	—		1.00	—	
	8.60–9.05	319	0.98	0.78–1.23	0.84	1.34	1.05–1.72	0.02
	9.05–9.53	165	0.64	0.49–0.84	0.001	1.20	0.87–1.64	0.27
	9.53–9.85	56	0.83	0.60–1.22	0.33	1.99	1.25–3.16	0.004
	9.85–10.07	16	0.78	0.41–1.47	0.43	2.32	1.12–4.77	0.02
	>10.07	9	1.01	0.44–2.34	0.98	3.49	1.37–8.89	0.009
	p trend		*0.03*			*0.003*		

Risk estimate indicates relative risk for IDEAL and odds ratio for EPIC-Norfolk. *Basic model includes age, gender, and smoking; †Basic model includes age and gender as matching factors and smoking, body mass index, and alcohol consumption (U/week).

MCE = number of major coronary events in each subcategory; other abbreviations as in Table 2.

the subgroups (p for linear trend 0.06). Upon adjustment for apoA-I and apoB, the lower HDL-C subcategories were no longer associated with MCE risk. However, patients in the categories above 70 mg/dl were at significantly increased risk (RR 2.19, 95% CI 1.12 to 4.28 and RR 2.49, 95% CI 1.04 to 5.95 in the fifth and sixth categories, respectively; Table 3). Again, no significant linear trend was observed (p for linear trend 0.08).

In EPIC-Norfolk, an inverse relationship for HDL-C was present in the basic regression model (p for linear trend <0.0001). In line with the analyses presented in Table 2, additional adjustment for apoA-I and apoB resulted in loss of significance for all subcategories (p for linear trend 0.40). A similar analysis for HDL particle size showed a significant downward trend for the unadjusted risk estimates (p for linear trend 0.03), although most subgroups were not statistically significantly related to MCE. Upon full adjustment for apoA-I and apoB, the risk estimates for the fourth, fifth, and sixth subcategories indicated a significantly increased MCE risk for people in those categories compared with those in the reference group (OR 1.99, 95% CI 1.25 to 3.16.; OR 2.32, 95% CI 1.12 to 4.77; OR 3.49, 95% CI 1.37 to 8.89, respectively; Table 3), with a statistically significant positive trend (p for linear trend 0.003).

Table 4 shows risk estimates for apoA-I, with additional adjustment for apoB and HDL-C, or apoB and HDL particle size (EPIC-Norfolk only). Across the apoA-I subcategories, unadjusted risk estimates were significantly inversely associated with MCE risk in both studies (p for linear trend 0.03 in IDEAL and <0.0001 in EPIC-Norfolk). Following full adjustment, apoA-I remained a significant protective MCE risk factor in some subgroups, whereas it lost statistical significance in others. Loss of statistical significance was particularly observed in the tails of the apoA-I distributions, probably through loss of statistical power. Importantly, none of the apoA-I subcategories demonstrated a switch toward increased MCE risk.

Collectively, upon adjustment for apoA-I and apoB, the plasma concentration of HDL-C was positively related to MCE risk in the IDEAL study, although this effect was limited to very high levels of this lipid parameter (>70 mg/dl) (Fig. 1A). In EPIC-Norfolk, a similar pattern was observed for NMR-measured HDL particle size, with increased risk estimates restricted to the highest categories of this parameter (Fig. 1B). In contrast, apoA-I remained a protective factor in both studies when full adjustment was performed. The risk estimates lost statistical significance in some subgroups, probably because of limited power, but

| Table 4 | Risk Estimates for a Major Coronary Event by Categories of ApoA-I in IDEAL and EPIC-Norfolk, Unadjusted or Adjusted for HDL Cholesterol Content and Apolipoprotein B |

	Subgroup	MCE	Basic Model*			+ HDL-C + ApoB		
			Risk Estimate	95%CI	p Value	Risk Estimate	95% CI	p Value
	ApoA-I (g/l)							
IDEAL	<1.25	239	1.00	—		1.00	—	
	1.25–1.45	247	0.85	0.71–1.02	0.07	0.79	0.63–0.98	0.03
	1.45–1.65	133	0.77	0.62–0.95	0.02	0.67	0.48–0.94	0.02
	1.65–1.80	37	0.75	0.53–1.07	0.12	0.63	0.38–1.05	0.08
	1.80–1.95	13	0.77	0.44–1.36	0.37	0.64	0.31–1.34	0.24
	>1.95	10	0.93	0.49–1.75	0.81	0.71	0.29–1.75	0.46
	p trend		*0.03*			*0.05*		
			Basic Model†					
EPIC	<1.43	314	1.00	—		1.00	—	
	1.43–1.67	280	0.65	0.52–0.82	<0.0001	0.68	0.53–0.88	0.003
	1.67–1.96	187	0.64	0.49–0.82	0.001	0.71	0.51–1.00	0.05
	1.96–2.21	57	0.68	0.46–1.00	0.052	0.78	0.47–1.31	0.35
	2.21–2.43	11	0.30	0.15–0.62	0.001	0.40	0.17–0.92	0.03
	>2.43	9	0.69	0.30–1.61	0.40	0.83	0.30–2.25	0.71
	p trend		*<0.0001*			*0.10*		
			+ HDL Particle Size + ApoB					
EPIC	<1.43	314	1.00	—		1.00	—	
	1.43–1.67	280	0.65	0.52–0.82	<0.0001	0.62	0.49–0.78	<0.0001
	1.67–1.96	187	0.64	0.49–0.82	0.001	0.56	0.42–0.76	<0.0001
	1.96–2.21	57	0.68	0.46–1.00	0.052	0.56	0.36–0.88	0.01
	2.21–2.43	11	0.30	0.15–0.62	0.001	0.26	0.12–0.55	<0.0001
	>2.43	9	0.69	0.30–1.61	0.40	0.50	0.20–1.27	0.15
	p trend		*<0.0001*			*<0.0001*		

Risk estimate indicates relative risk for IDEAL and odds ratio for EPIC-Norfolk. *Basic model includes age, gender, and smoking. †Basic model includes age and gender as matching factors, and smoking, body mass index, and alcohol consumption (U/week).
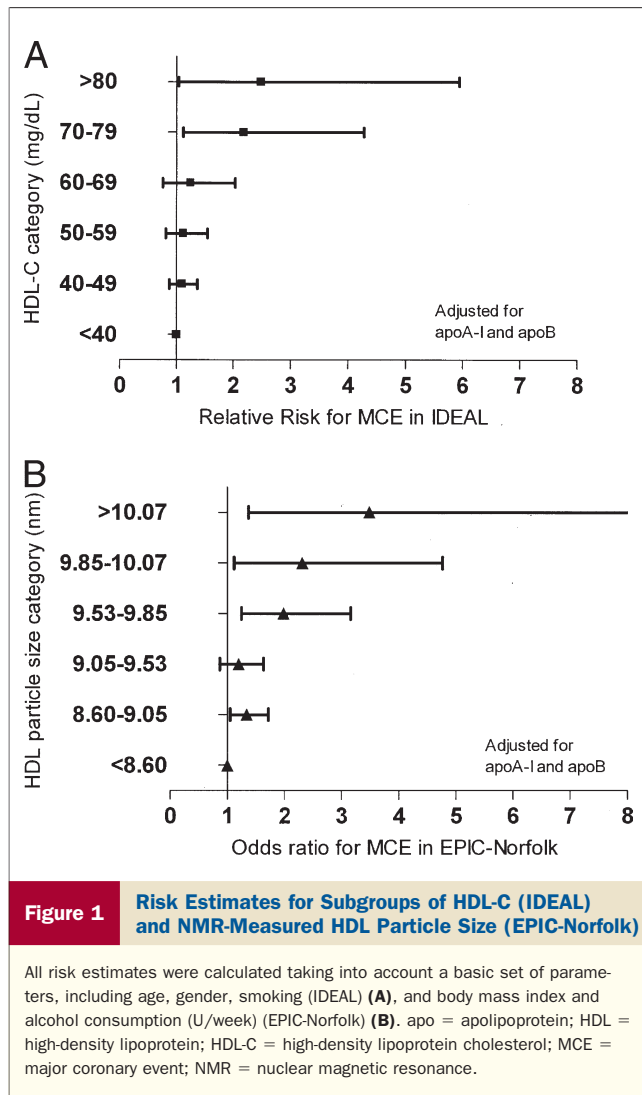Abbreviations as in Table 3.

apoA-I did not appear to turn into a significant risk factor at the highest plasma levels (Figs. 2A to 2C).

Discussion

In the present analysis of the IDEAL and EPIC-Norfolk studies, we assessed the relationships of HDL-C, HDL particle size, and apoA-I with risk of MCE. After adjustment for apoA-I and apoB, HDL-C (in IDEAL) and HDL particle size (in EPIC-Norfolk) became significantly positively related to MCE occurrence in the highest categories of the distribution. In contrast, apoA-I exhibited no positive relationship with MCE in any model, although statistical significance was lost in some subgroups, probably because of limited statistical power.

Comparison with other studies. Our observation that very high plasma HDL-C and very large HDL particles are associated with increased CAD risk is remarkable. Unfortunately, the number of studies reporting associations for HDL-C following adjustment for apoA-I and for the pro-atherogenic lipid fraction is rather modest. In fact, such data are available only from 2 post-hoc analyses, the first performed in the PRIME (Prospective Epidemiological Study of Myocardial Infarction) (13) and the second in the ARIC study (Atherosclerosis Risk in Communities) (14). The PRIME study reported loss of association for HDL-C

on adjustment for apoA-I, LDL-C, and triglycerides; the ARIC study showed that HDL-C remained negatively associated with CAD risk following adjustment for apoA-I and apoB. These results clearly differ from the observations in the present study. The numerous differences, however, among the PRIME, ARIC, and IDEAL studies in terms of study design, study size, patient eligibility, and laboratory and statistical methodology prevent any further insights or comparisons. Of note, the PRIME and ARIC studies did not include HDL-C subgroup analysis, and therefore any positive relationship with MCE at high levels of HDL-C may remain undetected. No large studies are available that prospectively investigated the relationship between NMR-measured HDL particle size and risk of CAD. The prevailing view is that an increased concentration of large HDL particles confers lower CAD risk (15), but this conclusion relies on the observation that larger HDL particles are virtually absent in subjects with overt CAD (16). Taken together, the current literature does not contain data as provided by the present analysis in the IDEAL and EPIC-Norfolk studies. Therefore, comparable analyses in other datasets with robust statistical power are urgently needed to validate these remarkable findings. Of note, simple quartile or even quintile analyses in our datasets did not detect the observed increased risk at the ends of the distributions for
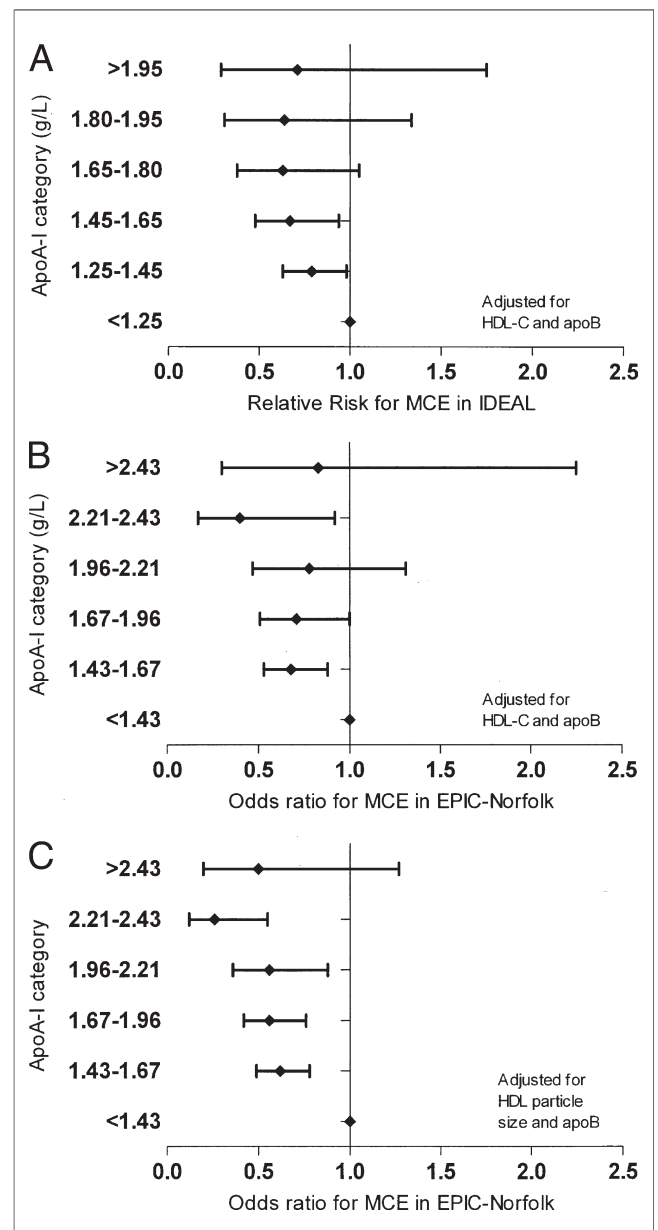
HDL contributes to its anti-atherogenic potency, several studies have demonstrated that HDL also can turn into a pro-inflammatory particle (21). Possibly, via these 2 latter mechanisms, a very high plasma concentration of large HDL particles might in fact induce a pro-atherogenic lipoprotein profile. However, whether either of these mechanisms has any physiological relevance in humans needs to be confirmed in further studies.

Although very high plasma HDL-C and very large HDL particles are associated with increased risk in the present

Figure 1   Risk Estimates for Subgroups of HDL-C (IDEAL) and NMR-Measured HDL Particle Size (EPIC-Norfolk)

All risk estimates were calculated taking into account a basic set of parameters, including age, gender, smoking (IDEAL) **(A)**, and body mass index and alcohol consumption (U/week) (EPIC-Norfolk) **(B)**. apo = apolipoprotein; HDL = high-density lipoprotein; HDL-C = high-density lipoprotein cholesterol; MCE = major coronary event; NMR = nuclear magnetic resonance.

Figure 2   Risk Estimates for Subgroups of apoA-I in IDEAL and EPIC-Norfolk

All risk estimates were calculated taking into account a basic set of parameters, including age, gender, smoking (IDEAL) **(A)**, and body mass index and alcohol consumption (U/week) (EPIC-Norfolk) **(B and C)**. Abbreviations as in Figure 1.

HDL-C and HDL particle size (data not shown), emphasizing the need to focus on extreme values for these parameters in future studies. Also, prospective epidemiological data are required to delineate the exact nature of the apparent nonlinear relationships for both parameters, as they could not be accurately deduced from our data.

Biological considerations. Our data suggest that very high values of plasma HDL-C and HDL particle size can confer increased risk of atherosclerotic disease. Although this epidemiological observation parallels the findings of some previous animal studies (17–19), there is no clear biological explanation of how HDL can become pro-atherogenic. This permits only speculation. First, some of the exchange of cholesterol esters between HDL and peripheral cells is known to be bidirectional, in part mediated by the scavenger receptor class B1 (SR-B1) (20). This observation gives rise to the hypothesis that very large HDLs, which are cholesterol enriched, may at some point become cholesterol donors instead of acceptors. Second, although it has widely been acknowledged that the anti-inflammatory capacity of

study, our data showed apoA-I to remain protective across the major part of its distribution. Most importantly, apoA-I did not exhibit a switch toward a positive relationship at higher levels. This may support apoA-I as an active component of HDL particles, possibly defining the atheroprotective capacity of this lipoprotein fraction. Indeed, several experimental studies have pointed to a crucial role for apoA-I in protection against atherosclerosis (22,23).

Clinical implications. The results from the present study may have several clinical implications. First, cardiovascular risk management may be more accurate if risk assessment relies on more precise measurements of HDL metabolism than HDL-C. Given that high plasma apoA-I more uniformly represents lower risk, this widely available parameter may be a valuable alternative marker. However, it might also be useful to develop novel biomarkers that actually provide information about HDL functionality. Second, the results of the present study may have important consequences for the development of pharmacological strategies that target the HDL pathway to decrease risk of atherosclerotic disease. On the basis of these data, interventions that primarily raise plasma HDL-C but do not or hardly change apoA-I levels may not be expected to have potent beneficial effects on atherosclerosis. More importantly, such strategies may even increase risk of atherosclerotic disease when achieving very high levels of HDL-C and hence HDL particle size. In contrast, given the present results, it can be hypothesized that strategies primarily raising plasma apoA-I levels with small molecular compounds, infusion of small lipid-poor apoA-I particles, or apoA-I gene therapy (24) will have a more pronounced effect on atherogenesis.

Study limitations. The present study has several limitations. First, the IDEAL and EPIC-Norfolk studies differ substantially in terms of baseline characteristics, study design, methods, and outcome validation. Importantly, all IDEAL participants used statin therapy during follow-up, whereas none of the EPIC-Norfolk participants did. However, statin treatment is known to have only limited effects on plasma HDL-C and apoA-I concentrations (25), and the relationship of HDL-C to CAD occurrence has been shown to be unaffected by statin therapy (26). Second, the IDEAL dataset did not contain information on alcohol consumption, which is widely known to affect plasma HDL-C, apoA-I, and risk of cardiovascular disease. However, exclusion of this parameter from the statistical model in EPIC-Norfolk hardly affected the results (data not shown). Third, fasting samples were used in IDEAL for laboratory measurements, whereas nonfasting measurements were performed in EPIC-Norfolk. This difference is not expected to induce significant confounding of our observations, because time of fasting hardly, if at all, affects the parameters used in the present study. Fourth, in contrast to the EPIC-Norfolk study, the use of lipoprotein parameters measured at 3 and 6 months in the IDEAL study resulted in the exclusion of some early events that occurred before these measurements. Finally, given the small number

of patients having very high HDL-C, HDL particle size, or apoA-I, the power to detect risk estimates at the far end of their distributions is limited. Despite these limitations, the fact that comparable findings were observed in substantially different study populations and different study designs might support the validity of our findings.

Conclusions

In summary, our data suggest that very high plasma HDL-C and very large HDL particles may represent increased CAD risk when levels of apoA-I and apoB remain unaffected. In contrast, apoA-I appears not to turn into a significant risk factor at high plasma concentrations. These observations may have important consequences for future CAD risk assessment and novel treatment strategies.

Author Disclosures

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