Low-Density Lipoprotein Subfractions and the Long-Term Risk of Ischemic Heart Disease in Men

13-Year Follow-Up Data From the Québec Cardiovascular Study

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Objective—The objective of the present study was to investigate the association between large and small low-density lipoprotein (LDL) and long-term ischemic heart disease (IHD) risk in men of the Québec Cardiovascular Study.

Methods and Results—Cholesterol levels in the large and small LDL subfractions (termed LDL-$C_{\geq 260Å}$ and LDL-$C_{<255Å}$, respectively) were estimated from polyacrylamide gradient gel electrophoresis of whole plasma in the cohort of 2072 men of the population-based Québec Cardiovascular Study. All men were free of IHD at the baseline examination and followed-up for a period of 13 years, during which 262 first IHD events (coronary death, nonfatal myocardial infarction, and unstable angina pectoris) were recorded. Our study confirmed the strong and independent association between LDL-$C_{<255Å}$ levels as a proxy of the small dense LDL phenotype and the risk of IHD in men, particularly over the first 7 years of follow-up. However, elevated LDL-$C_{\geq 260Å}$ levels (third versus first tertile) were not associated with an increased risk of IHD over the 13-year follow-up (RR=0.76; P=0.07).

Conclusions—These results indicated that estimated cholesterol levels in the large LDL subfraction were not associated with an increased risk of IHD in men and that the cardiovascular risk attributable to variations in the LDL size phenotype was largely related to markers of a preferential accumulation of small dense LDL particles. (***Arterioscler Thromb Vasc Biol*. 2005;25:553-559.)**

Key Words: low-density lipoprotein particle size ■ low-density lipoprotein cholesterol ■ ischemic heart disease ■ risk

Although increased plasma low-density lipoprotein (LDL) cholesterol (LDL-C) concentration is considered one of the most important risk factors for ischemic heart disease (IHD), many individuals in whom IHD develops have LDL-C levels in the normal range.[1] This observation challenges the traditional approach of using LDL-C concentrations as the main lipid target in the management of IHD risk.[2]

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Small dense LDL particles have been associated with an increased IHD risk in several retrospective case-control studies[3,4] and in most prospective studies with relatively short periods of follow-up.[5,6] Using 5-year prospective data from the Québec Cardiovascular Study, we have previously shown that men with an elevated proportion of LDL with a diameter <255Å (termed LDL $\%_{<255Å}$) had a 6-fold increased risk of IHD compared with men with relatively normal LDL $\%_{<255Å}$ levels.[7] It must be stressed that the association between the small dense LDL phenotype and incident IHD has not been entirely consistent,[8–10] with studies suggesting that larger LDL particles may in fact be associated with an increased cardiovascular risk.[8,10] These conflicting observations have fueled the current debate as to which subclass of LDL (small or larger) is most predictive of an increased risk of IHD. The association between the small dense LDL phenotype and incident IHD over periods of follow-up >10 years also remains unknown. All previous prospective studies on this topic were either case-control in design or were conducted using a follow-up <5 years.[7]

The principal objective of the present study therefore was to investigate the long-term risk of IHD associated with large LDL and small LDL using extended 13-year follow-up data in men from the Québec Cardiovascular Study.

Methods

Study Population and Follow-Up

The study population and follow-up methods have been described previously.[7,11] Briefly, a random sample of 4635 French Canadian men (aged 35 to 64 years), representing 65.5% of the population screened from 7 towns in the Québec City metropolitan area, were

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selected in 1974 for the study of cardiovascular disease risk factors. Data collected in 1985 were used as the baseline characteristics for the present prospective analyses. Among the 2552 men re-evaluated in 1985, 102 volunteers and 265 patients with a history of IHD before 1985 were excluded from the present prospective analyses. Diagnosis of diabetes was considered in men who self-reported the disease. In 1990 to 1991 and in 1998, participants were contacted by mail and invited to complete a short questionnaire, which provided information history of cardiovascular diseases and type 2 diabetes. For those who reported such diseases and those who died, hospital charts were reviewed by cardiologists of the study. Among the 2185 subjects eligible for follow-up, only 8 subjects (0.4%) were lost to follow-up. Among the remaining 2177 men, plasma was no longer available in 59 subjects and LDL-C levels could not be calculated in 46 participants because triglyceride levels were >4.5 mmol/L. Thus, analyses were conducted in a cohort of 2072 men. Only 5.7% and 2.9% of men were using β-blockers and diuretics, respectively, at the 1985 baseline evaluation. The risk of IHD in men using either medication was similar. Medication used at baseline was therefore categorized as a single variable representing the use (versus nonuse) of β-blockers and/or diuretics. Only 1.9% of men were using hypolipidemic medication in 1985, mainly clofibrate and cholestyramine, and this variable was not associated with variation in IHD risk. It was therefore not included in the analyses. It must be stressed that multivariate modeling of IHD risk including all 3 medications as a single entity or as a combined variable produced similar results.

Definition of IHD Events

First IHD events, which included unstable angina (typical symptoms with new ischemic electrocardiogram changes), coronary death, and nonfatal myocardial infarction, were diagnosed as detailed previously.[11] A total of 262 first cases of IHD were recorded over the 13-year follow-up: 162 first cases of nonfatal myocardial infarction, 50 first cases of unstable angina pectoris, and 50 fatal coronary events.

Laboratory Analyses

Twelve-hour fasting blood samples were obtained at the 1985 baseline evaluation and immediately used for lipid and apolipoprotein measurements. Methods used to determine plasma lipid and apolipoprotein levels and insulin and C-reactive protein concentrations have been detailed in previous publications.[7,12,13] Nondenaturing 2% to 16% polyacrylamide gradient gel electrophoresis was used to characterize LDL particle size as described previously using plasma stored at −80°C.[7] In the present analyses, we have characterized LDL particles using different measures: (1) LDL peak particle size, which corresponded to the estimated diameter of the major peak in each individual; (2) the relative proportion of LDL particles having a diameter <255Å or ≥260Å (termed LDL%$_{<255Å}$ and LDL %$_{\geq 260Å}$, respectively), which was ascertained by computing the relative area of the densitometric scan on the gel using 2 arbitrary cut points (255Å or 260Å) as previously described;[7] and (3) the cholesterol in small and large LDL subfractions (arbitrarily termed LDL-C$_{<255Å}$ and LDL-C$_{\geq 260Å}$, respectively), which were estimated by multiplying the total plasma LDL cholesterol levels by the relative proportion of small (LDL %$_{<255Å}$) and large LDL (LDL %$_{\geq 260Å}$).[7]

Statistical Analyses

Mean baseline characteristics of incident IHD cases and of IHD-free men during follow-up were compared by Student *t* test for parametric

TABLE 1. Baseline Characteristics of the 1810 Men Without IHD and the 262 Men Who Had a First IHD Event During the 13-Year Follow-Up

	Ischemic Heart Disease			
	Without	With		
Variables	(N=1810)	(N=262)	% Difference	*P*
Age, y	56.2±6.9	58.8±7.3	4.6	<0.001
Body mass index, kg/m^2	26.0±3.7	26.9±4.3	3.5	0.002
Systolic blood pressure, mm Hg	129±17	138±20	7.0	<0.001
Type 2 diabetes mellitus, % (N)	4.1 (74)	10.7 (28)	6.6	<0.001
Smokers, % (N)	22.4 (406)	27.9 (73)	5.5	0.05
Total cholesterol, mmol/L	5.7±1.0	6.0±1.1	5.3	<0.001
LDL cholesterol, mmol/L	3.8±0.9	4.1±1.0	7.9	<0.001
HDL cholesterol, mmol/L	1.04±0.26	0.99±0.24	−4.8	0.002
Apolipoprotein B, mg/L	116±30	128±34	10.3	<0.001
Triglycerides, mmol/L	1.6±1.5	1.8±1.5	12.5	<0.001
LDL peak particle size, Å	257.0±5.8	256.5±5.8	−0.2	0.15
LDL %$_{<255Å}$, %	39.4±20.1	43.1±19.5	9.4	0.006
LDL %$_{255–260Å}$, %	23.6±9.8	23.2±10.3	−1.7	0.6
LDL %$_{\geq 260Å}$, %	37.0±18.2	33.7±17.1	−8.9	0.005
LDL-C$_{<255Å}$, mmol/L	1.5±0.9	1.8±1.0	20.0	<0.001
LDL-C$_{255–260Å}$, mmol/L	0.91±0.43	0.96±0.47	5.5	0.07
LDL-C$_{\geq 260Å}$, mmol/L	1.4±0.8	1.4±0.8	0.0	0.56

HDL indicates high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; LDL %$_{<255Å}$, proportion of total LDL with a diameter <255Å; LDL %$_{255–260Å}$, proportion of total LDL with a diameter between 255 and 260Å; LDL %$_{\geq 260Å}$, proportion of total LDL with a diameter ≥260Å; LDL-C$_{<255Å}$, estimated cholesterol concentration within the LDL subfraction <255Å; LDL-C$_{255–260Å}$, estimated cholesterol concentration within the LDL subfraction 255–260Å; LDL-C$_{\geq 260Å}$, estimated cholesterol concentration within the LDL subfraction ≥260Å.

Values are mean±SD.

Plasma triglyceride levels are presented as geometric mean.

Smokers are men who smoke ≥20 cigarettes/day.

variables and by the Wilcoxon test for nonparametric variables. Differences in frequency data were tested by χ^2 analysis. The nonlipid and lipid risk variables in men classified in different tertiles of LDL-$C_{\geq 260Å}$ or LDL-$C_{<255Å}$ levels were compared using a general linear model with the Tukey post-hoc test to locate subgroup differences. Duration of follow-up was calculated in person-years by using the follow-up of each participant from the 1985 baseline evaluation until death, onset of IHD, or the last contact. Kaplan–Meier survival probability (estimated probability of not having IHD during follow-up) was computed for tertiles of each variable. The log-rank test was used to compare parallelism of survival curves among the tertiles of LDL size variables. Cox proportional hazards models were used to estimate rates of IHD events. For all Cox models, the proportional hazards assumptions were formally tested. Age, body mass index, systolic blood pressure, type 2 diabetes (presence versus absence), smoking habits (smokers of >20 cigarettes/d versus others), medication use (versus nonuse) at baseline, plasma log-transformed triglyceride levels, and the high-density lipoprotein (HDL) cholesterol were included as potential confounders. Statistical analyses were performed on SAS (SAS Institute, Cary, NC).

Results

Participants in whom a first IHD event developed during the 13-year follow-up compared with IHD-free men were older and had a deteriorated risk profile (Table 1), including a greater proportion of small LDL particles and higher levels of LDL-$C_{<255Å}$. However, there were no statistically significant difference in LDL peak particle size and in LDL-$C_{\geq 260Å}$ levels between incident IHD cases and IHD-free individuals.

As shown in Table 2, individuals with increased LDL-$C_{\geq 260Å}$ levels (third tertile) had higher plasma HDL cholesterol levels (+16.8%; $P<0.001$), reduced plasma triglyceride (−26.3%; $P<0.001$), and fasting insulin levels (−12.9%; $P<0.001$), and a lower proportion of LDL with a diameter <255Å (−35.8%; $P<0.001$) compared with men with reduced LDL-$C_{\geq 260Å}$ concentrations (first tertile). However, subjects with elevated LDL-$C_{\geq 260Å}$ levels had significantly higher LDL cholesterol and apolipoprotein B concentrations (+22.9% and +7.0%, respectively; $P<0.001$) compared with individuals with low LDL-$C_{\geq 260Å}$ concentrations. Inversely and as expected, individuals with elevated LDL-$C_{<255Å}$ levels (third versus first tertile) had a higher body mass index, elevated plasma triglyceride, and insulin levels, lower HDL cholesterol concentrations, smaller LDL particles (Table 3).

Figure 1 depicts the Kaplan–Meier survival curves among each tertile of LDL-$C_{\geq 260Å}$ levels (top). The survival probabilities in men of the third tertile of LDL-$C_{\geq 260Å}$ versus tertiles 1 and 2 dissociated rapidly over the first 6 years of follow-up, after which they became virtually parallel until the end of follow-up. However, the apparently reduced 13-year risk of IHD in men with elevated LDL-$C_{\geq 260Å}$ levels (third versus other 2 tertiles) did not reach statistical significance ($P=0.09$). Men with elevated LDL-$C_{<255Å}$ levels were at increased risk for IHD (log-rank test $P<0.001$). However, the assumption of hazard proportionality for LDL-$C_{<255Å}$ levels was not respected, as evidenced by a significant interaction with follow-up duration in modulating the 13-year risk of IHD ($P<0.001$). Even though the interaction between LDL-$C_{\geq 260Å}$ levels and follow-up did not reach statistical significance ($P=0.17$), subsequent multivariate survival analyses were undertaken by arbitrarily stratifying the data using the

TABLE 2. Characteristics of the 2072 Men According to Tertiles of Cholesterol in Large LDL Particles (LDL-$C_{\geq 260Å}$)

	Tertile of LDL-$C_{\geq 260Å}$ (mmol/L)		
Variables	<1.03 (n=689)	1.03–1.68 (n=693)	≥1.68 (n=690)
Age, y	56.3±7.0	56.8±7.1	56.6±6.9
Body mass index, kg/m^2	26.7±3.9	26.1±3.8*	25.7±3.6*†
Systolic blood pressure, mm Hg	131±17	131±17	129±17
Total cholesterol, mmol/L	5.4±1.0	5.6±1.0*	6.1±0.9*†
LDL cholesterol, mmol/L	3.5±0.9	3.8±0.9*	4.3±0.9*†
HDL cholesterol, mmol/L	0.95±0.24	1.04±0.24*	1.11±0.27*†
Apolipoprotein B, mg/dL	114±31	116±31	122±29*†
Triglycerides, mmol/L	1.9±1.6	1.5±1.5*	1.4±1.4*†
C-reactive protein, mg/L	1.9±3.0	1.8±3.1	1.7±3.2
Insulin, pmol/L	72.9±1.6	68.4±1.5*	63.5±1.5*†
LDL peak particle size, Å	253.2±5.5	257.0±4.5*	260.7±4.8*†
LDL %$_{<255Å}$, %	57.7±16.3	40.1±12.7*	21.9±11.8*†
LDL %$_{255–260Å}$, %	23.7±13.8	23.6±7.0	23.3±7.5
LDL %$_{\geq 260Å}$, %	18.6±8.8	36.3±9.5*	54.8±12.6*†
LDL-$C_{<255Å}$, mmol/L	2.1±0.9	1.6±0.8*	1.0±0.7*†
LDL-$C_{255–260Å}$, mmol/L	0.83±0.54	0.90±0.32*	1.01±0.40*†
LDL-$C_{\geq 260Å}$, mmol/L	0.62±0.25	1.3±0.19*	2.29±0.50*†

Values are mean±SD.
Plasma triglyceride, C-reactive protein, and insulin levels are presented as geometric mean.
*Significantly different from tertile 1.
†Significantly different from tertile 2.

TABLE 3. Characteristics of the 2072 Men According to Tertiles of Cholesterol in Small LDL Particles (LDL-$C_{<255Å}$)

	Tertile of LDL-$C_{<255Å}$ (mmol/L)		
Variables	<1.07 (n=689)	1.07–1.86 (n=693)	≥1.86 (n=690)
Age, y	56.6±6.9	56.9±7.1	56.2±6.9
Body mass index, kg/m^2	25.7±3.9	26.1±3.8	26.6±3.6*†
Systolic blood pressure, mm Hg	129±17	131±17	132±17*
Total cholesterol, mmol/L	5.3±0.9	5.6±0.9*	6.3±0.9*†
LDL cholesterol, mmol/L	3.5±0.9	3.8±0.8*	4.4±0.9*†
HDL cholesterol, mmol/L	1.13±0.28	1.03±0.24*	0.94±0.22*†
Apolipoprotein B, mg/dL	101±27	113±25*	137±28*†
Triglycerides, mmol/L	1.3±1.5	1.6±1.5*	2.0±1.5*†
C-reactive protein, mg/L	1.6±3.2	1.8±3.1	2.0±3.0*†
Insulin, pmol/L	64.4±1.5	68.1±1.5*	72.3±1.5*†
LDL peak particle size, Å	260.9±5.0	257.6±4.5*	252.4±4.6*†
LDL $\%_{<255Å}$, %	18.9±10.6	40.9±10.6*	59.8±12.1*†
LDL $\%_{255–260Å}$, %	27.5±12.7	24.2±7.5*	18.9±6.1*†
LDL $\%_{≥260Å}$, %	53.6±15.8	34.8±10.8*	21.4±9.5*†
LDL-$C_{<255Å}$, mmol/L	0.62±0.28	1.47±0.23*	2.59±0.58*†
LDL-$C_{255–260Å}$, mmol/L	0.97±0.53	0.92±0.39*	0.84±0.36*†
LDL-$C_{≥260Å}$, mmol/L	1.9±0.8	1.4±0.6*	1.0±0.5*†

Values are mean±SD.
Plasma triglyceride, C-reactive protein, and insulin levels are presented as geometric mean.
*Significantly different from tertile 1.
†Significantly different from tertile 2.

median follow-up duration among incident IHD cases (7.3 years). This approach allowed us to test the temporal relationship between variations in plasma LDL-$C_{<255Å}$ levels and IHD risk and also allowed for a standardized comparison of the IHD risk associated with elevated LDL-$C_{<255Å}$ and LDL-$C_{≥260Å}$ levels. Arbitrarily stratifying the follow-up data at 5 or 9 years yielded essentially similar results (not shown).

As shown in Figure 2, elevated plasma LDL-$C_{≥260Å}$ levels (third versus first tertile) were associated with a significant 50% reduction in the risk of IHD when considering the first 7.3 years of follow-up, which remained significant after multivariate adjustment for nonlipid (RR=0.59; P=0.03), as well as for nonlipid and lipid risk factors (RR=0.52; P=0.009). Inversely, men with elevated LDL-$C_{<255Å}$ levels (third versus first tertile) were at greater risk of IHD over the first 7.3 years of follow-up. Adjustment for nonlipid (RR=3.4; P<0.001) and nonlipid and lipid risk variables (RR=2.5; P<0.001) did not materially modify this association. When considering a follow-up ≥7.3 years, the variation in the risk of IHD associated with elevated LDL-$C_{≥260Å}$ or LDL-$C_{<255Å}$ levels was no longer observed in both univariate or multivariate analyses.

Elevated plasma apoB levels (third versus first tertile) predicted an increased multivariate risk of IHD over the short-term as well as long-term follow-up period (RR=2.4; 95% CI, 1.5 to 3.8; and RR=1.6; 95% CI, 1.0 to 2.5, respectively, not shown). The correlation between plasma apoB concentrations and LDL-$C_{<255Å}$ and LDL-$C_{>260Å}$ levels were r=0.52 and r=0.11 (P<0.001), respectively. The combined impact of concomitant variations in LDL-$C_{<255Å}$ and of apoB levels on the short-term risk of future IHD events is shown in Figure 3. The short-term IHD risk in men with apoB <116 mg/dL (median of cohort) was significantly increased in men with elevated LDL-$C_{<255Å}$ concentrations (RR=2.1; P=0.02). High levels of apoB were also associated with an increased risk of IHD in men with relatively low levels of LDL-$C_{<255Å}$ (RR=1.9; P=0.04). However, the combination of small LDL and high apoB levels was associated with the highest short-term risk of IHD (RR=3.1; P<0.001). Data in Figure 3 (right) also suggest that increased levels of large LDL, by being associated with a globally more favorable risk profile, attenuated the increased risk attributable to elevated plasma apoB levels.

Discussion

These data first suggest that the increased short-term IHD risk attributable to a preferential accumulation of small dense LDL (LDL-$C_{<255Å}$) may be attenuated over a long-term period. These data also suggested no evidence of an increased risk of IHD attributed to elevated cholesterol concentrations in large LDL particles. In fact, men with elevated cholesterol levels within large LDL subfraction had a generally more favorable cardiovascular risk profile compared with individuals with low levels of LDL-$C_{≥260Å}$, and had a 50% lower IHD risk over the first 7 years of follow-up.

Small LDL and IHD Risk

Several prospective case-control or population-based studies conducted over relatively short periods of follow-up have indicated that small dense LDL particles were associated with

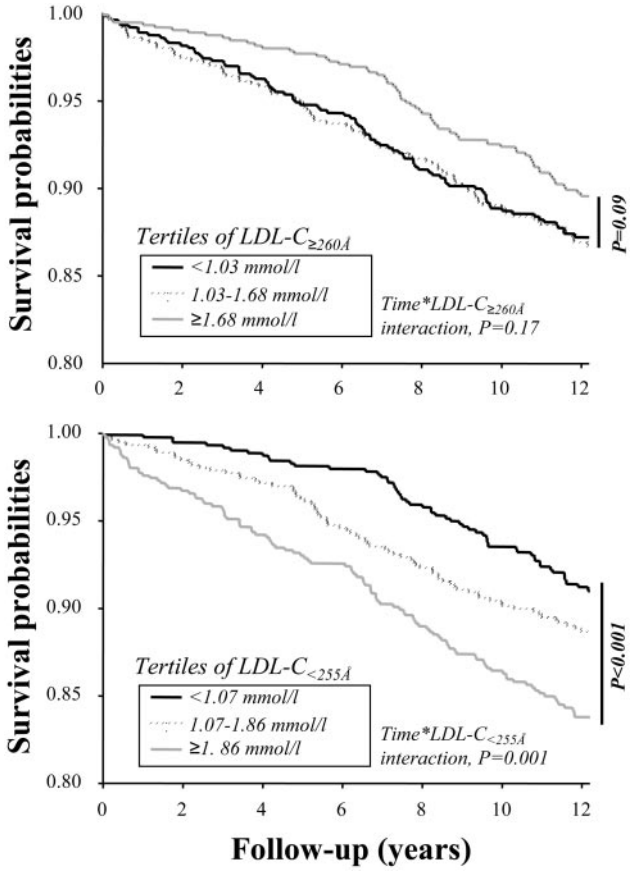

Figure 1. Estimated probability for not having ischemic heart disease (IHD) during the 13-year follow-up for each tertile of LDL-$C_{\geq 260Å}$ (top panel) and of LDL-$C_{<255Å}$ levels (lower panel). Interaction terms correspond to interactions between duration of follow-up and LDL-$C_{<255Å}$ or LDL-$C_{\geq 260Å}$ levels in modulating the 13-year IHD risk.

an increased risk of IHD.[6,14] In many of these studies, however, the association between various features of the small dense LDL phenotype and the risk of IHD was attenuated after multivariate adjustment for other lipid risk factors such as plasma triglyceride or HDL cholesterol levels.[14–16] Previous nested case-control studies, even those with a longer follow-up, could not by design investigate the impact of follow-up duration on the relationship between LDL particle size phenotype and IHD risk. This would have been possible only if incident IHD cases had been matched with controls for time to event, which was apparently not the case in those studies. The Québec Cardiovascular study also remains to date the only study in which LDL particle size phenotype was measured in its entire cohort and not only in a case-control subsample of participants. We did not have an a priori hypothesis that the association between small LDL and long-term IHD risk would be modulated by duration of follow-up. However, the significant interaction between LDL-$C_{<255Å}$ levels and follow-up duration ($P<0.001$) suggested that the impact of this risk factor on IHD risk was not consistent across the range of follow-up. Stratification of follow-up was therefore justified and used to overcome this statistical issue. Data revealed that the strong and independent short-term association between LDL-$C_{<255Å}$ levels and IHD

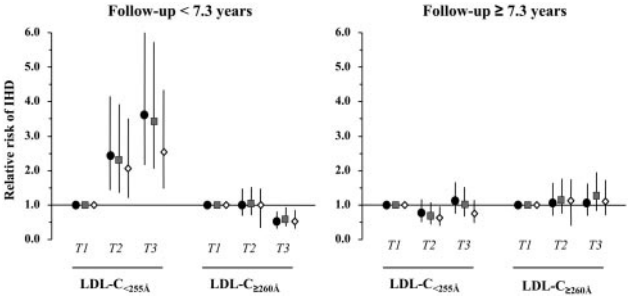

Figure 2. Comparison of the multivariate relative risk (RR) of ischemic heart disease associated with plasma LDL-$C_{\geq 260Å}$ or LDL-$C_{<255Å}$ levels in men after stratification for duration of follow-up. The RR of IHD in each tertile (T) of LDL-$C_{\geq 260Å}$ or LDL-$C_{<255Å}$ levels were computed using men in the first tertile as a reference group (RR=1.0). Black circles represent the unadjusted RR. Gray squares represent RR after adjustment for nonlipid risk factors: age, body mass index, systolic blood pressure, diabetes, smoking, and medication use at baseline. White diamonds represent RR after adjustment for nonlipid and lipid risk factors: HDL cholesterol, log-transformed triglycerides, and apolipoprotein B.

risk was attenuated over a longer-term follow-up (≥7.3 years).

We hypothesize that this time-dependent relationship between features of the LDL size phenotype and IHD risk may be attributed to a survival effect, according to which a majority of men with the atherogenic small dense LDL phenotype and the metabolic syndrome may have had an early IHD event, thus leaving participants with a more favorable LDL size phenotype but a nonetheless high risk profile to be susceptible to having IHD over a longer period of time. Concordant with this hypothesis, we found that patients in whom IHD events were recorded within the first 7 years of follow-up had features of the small dense LDL phenotype, whereas individuals who had IHD after the first 7 years of follow-up did not (data not shown). Mykkanen et al[17] have previously shown using a nested-case control study design that LDL size was not a predictor of coronary artery disease risk in elderly white subjects aged 65 to 74 years, an observation that is consistent with a potential survival bias

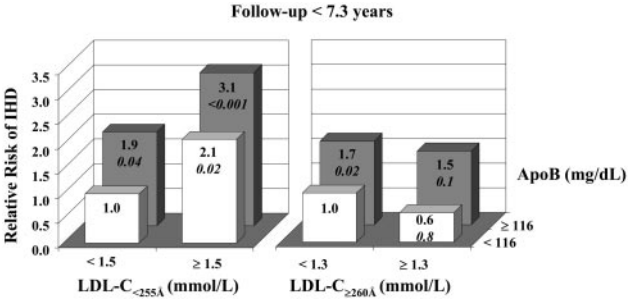

Figure 3. Short-term relative risk of IHD and *P* levels according to levels of LDL-$C_{<255Å}$ (above or below median of 1.5 mmol/L) or LDL-$C_{\geq 260Å}$ (median 1.3 mmol/L) further stratified according to apolipoprotein B (apo B) levels (median=116 mg/dL). The groups with low apoB and low LDL-$C_{<255Å}$ or low LDL-$C_{\geq 260Å}$ concentrations were used as reference (RR=1.0). Relative risks were adjusted for age, body mass index, systolic blood pressure, type 2 diabetes, medication use at baseline, and smoking habits.

effect modulating the risk attributed to the small dense LDL phenotype over different periods of time.

Large LDL and IHD Risk

Individuals with increased LDL-$C_{\geq 260Å}$ concentrations were characterized by lower plasma triglyceride and insulin levels, lower body mass index, and higher plasma HDL cholesterol concentrations compared with participants with lower LDL-$C_{\geq 260Å}$ levels. They were also at lower risk for IHD. However, men with elevated LDL-$C_{\geq 260Å}$ concentrations had concurrent elevations in plasma LDL cholesterol and apolipoprotein B levels, which may have contributed to modulate their long-term risk of IHD by promoting atherosclerosis over a prolonged period of time. One limitation of the present study is that LDL size phenotype and other components of the cardiovascular risk profile were measured only at baseline. Thus, we cannot exclude the possibility that the apparently favorable cardiovascular risk profile of individuals with elevated LDL-$C_{\geq 260Å}$ at baseline may have deteriorated over time, thereby contributing to the disappearance of the apparent short-term cardioprotection associated with having increased LDL-$C_{\geq 260Å}$ levels. The extent to which statin use may have confounded the assessment of risk in men with elevated LDL-$C_{\geq 260Å}$ levels remains unknown because data on statin therapy at time of last contact were not available.

These data are at variance with some previous reports on this topic. Campos et al have shown that LDL particle size distribution characterized by a predominance of the largest of 3 LDL subclasses (>268Å) was more prevalent among men and women with coronary artery disease (43%) than among control subjects (25%).[8] It must be stressed that this study was retrospective in design and excluded individuals with triglyceride levels >250 mg/dL, thus resulting in a very low prevalence of the small dense LDL phenotype. Campos et al[10] also recently reported that survivors of myocardial infarction with large LDL (mean 266Å) in the CARE study had a 4-fold increased risk of coronary artery disease compared with myocardial infarction survivors having a reduced LDL size (mean 245Å). Surprisingly, and consistent with our own data, patients in the highest quintiles of the LDL size distribution (with large LDL) had a less deteriorated risk profile as evidenced by lower body mass index, lower plasma triglyceride and total cholesterol levels, and higher HDL cholesterol concentrations compared with patients in the lower portion of the LDL size distribution.[10] These characteristics among men with large LDL particles are usually not associated with a higher risk of IHD. We suspect that the association between large LDL and IHD reported by the CARE investigators may be largely explained by the fact that this was a secondary prevention study with a potential survival effect bias distorting the relationship between LDL size phenotype and IHD risk. The fact that patients in CARE were purposefully selected on the basis of having low LDL-C levels may also have contributed to their discordant observations compared with a majority of studies that have emphasized the strong association between small dense LDL and the risk of IHD.

LDL Particle Number and IHD Risk

Our results further indicated that LDL particle number, as approximately estimated by total plasma apoB concentrations, is also an important correlate of IHD risk. Plasma apoB levels were an independent risk factor for IHD in multivariate analyses, even after taking into account variables related to the LDL size phenotype. Data also indicated that plasma apoB levels modified the short-term risk of IHD attributable to the small dense LDL phenotype, as characterized by elevated levels of LDL-$C_{<255Å}$. These results suggest that information on both LDL size and LDL particle number may be of important value in projecting a more accurate view of a patient's risk of IHD over a given period of time.

In conclusion, our results support the notion that the small dense LDL phenotype confers an increased risk of IHD, particularly over a short period of follow-up, and that levels of large LDL are not associated with an increased risk of IHD in men. This does not imply that large LDL particles enriched in cholesteryl esters are not atherogenic as they have in fact been shown to be.[18,19] We rather propose that the strong association between elevated LDL-$C_{\geq 260Å}$ levels and the presence of other cardioprotective aspects of the risk profile, rather than elevated LDL-$C_{\geq 260Å}$ per se, may contribute to explain this apparent reduction in the risk of IHD. Our data further support the concept that total LDL cholesterol levels is only a crude marker of the overall atherogenicity of LDL because different LDL subclasses show very different associations with the risk of IHD.

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