

# Low-density lipoprotein particle diameter and mortality: the Ludwigshafen Risk and Cardiovascular Health Study

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## Aims

The aim of the study was to examine whether differences in average diameter of low-density lipoprotein (LDL) particles were associated with total and cardiovascular mortality.

## Methods and results

We studied 1643 subjects referred to coronary angiography, who did not receive lipid-lowering drugs. During a median follow-up of 9.9 years, 398 patients died, of these 246 from cardiovascular causes. We calculated average particle diameters of LDL from the composition of LDL obtained by  $\beta$ -quantification. When LDL with intermediate average diameters (16.5–16.8 nm) were used as reference category, the hazard ratios (HRs) adjusted for cardiovascular risk factors for death from any cause were 1.71 (95% CI: 1.31–2.25) and 1.24 (95% CI: 0.95–1.63) in patients with large (>16.8 nm) or small LDL (<16.5 nm), respectively. Adjusted HRs for death from cardiovascular causes were 1.89 (95% CI: 1.32–2.70) and 1.54 (95% CI: 1.06–2.12) in patients with large or small LDL, respectively. Patients with large LDL had higher concentrations of the inflammatory markers interleukin (IL)-6 and C-reactive protein than patients with small or intermediate LDL. Equilibrium density gradient ultracentrifugation revealed characteristic and distinct profiles of LDL particles in persons with large (approximately even distribution of intermediate-density lipoproteins and LDL-1 through LDL-6) intermediate (peak concentration at LDL-4) or small (peak concentration at LDL-6) average LDL particle diameters.

## Conclusions

Calculated LDL particle diameters identify patients with different profiles of LDL subfractions. Both large and small LDL diameters are independently associated with increased risk mortality of all causes and, more so, due to cardiovascular causes compared with LDL of intermediate size.

## Keywords

Cholesterol • Triglycerides • Low-density lipoproteins • Subfractions • Mortality

## Introduction

Low-density lipoproteins (LDL) include a heterogeneous mixture of particles which can be separated by ultracentrifugation,<sup>1–3</sup> chromatography,<sup>4</sup> nuclear magnetic resonance spectroscopy,<sup>5</sup> or gradient gel electrophoresis.<sup>6</sup> Experimental and turnover studies have raised the possibility that dense LDL may be more atherogenic

than buoyant LDL. Potential mechanisms include easier transfer of dense LDL from the vessel lumen into the subintimal space,<sup>7</sup> decreased binding to LDL receptors and increased plasma residence time,<sup>8–11</sup> and higher affinity to proteoglycans compared with buoyant LDL.<sup>12,13</sup>

Dense LDL have been linked to surrogate vascular endpoints including endothelial dysfunction<sup>14</sup> or carotid intima media

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thickness.<sup>15,16</sup> Prospective epidemiological studies have mostly used gradient gel electrophoresis to investigate the relationship between LDL subfractions and coronary artery disease (CAD). However, results have not been consistent. Predominance of small dense LDL was indeed found associated with CAD, but overall the link was not independent from low high-density lipoproteins (HDL) cholesterol (HDL-C) and/or high triglycerides (TG).<sup>17–21</sup> Further studies have, in contrast, claimed that small dense LDL are associated with CAD independent of low-density lipoprotein cholesterol (LDL-C) and HDL-C, TG or lipoprotein(a).<sup>22,23</sup> Finally, others claim that buoyant LDL may be more atherogenic than dense ones.<sup>24,25</sup> In studies using nuclear magnetic resonance spectroscopy, small and large LDL were associated with atherosclerosis and CAD, but the LDL size was no longer predictive after adjustment for lipid parameters.<sup>26–28</sup> These controversial findings prompted us to examine whether mean LDL particle diameters are related to total and cardiovascular mortality and, if such a relationship would exist, whether it is independent from the major lipid parameters and established risk factors for CAD.

## Methods

### Ludwigshafen Risk and Cardiovascular Health Study

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study is an ongoing prospective cohort study of patients referred for coronary angiography. In total, 3316 subjects were recruited between June 1997 and May 2001.<sup>29</sup> Recruitment was done consecutively on regular working days and the number of subjects included per day was only limited by the capacity of the staff involved. There was no deliberate exclusion of subjects for reasons other than the exclusion criteria specified in the study protocol. Low-density lipoprotein composition was available in 3266 individuals with coronary angiograms; of these, 1623 with lipid-lowering drugs were excluded from the analysis. Among the remaining 1643 subjects, 1070 had angiographically proven CAD ( $\geq 20\%$  stenoses), and 573 had no CAD. Of the 1070 subjects with CAD, 743 presented with stable CAD, while the remaining patients were admitted with unstable angina pectoris ( $n = 243$ ), non-ST-elevation myocardial infarction (NSTEMI) (TroponinT  $> 0.1 \mu\text{g/L}$ ,  $n = 47$ ), or ST-elevation myocardial infarction (STEMI) (TroponinT  $> 0.1 \mu\text{g/L}$ ,  $n = 37$ ). During a median follow-up of 9.9 years, 398 patients died, of these 246 from cardiovascular causes according to death certificates obtained from local health authorities. No patient was lost to follow-up, death certificates were missing in 13 decedents who were included in the analysis for total mortality, but excluded from analysis of cardiovascular mortality. The study was approved by the ethics committee at the 'Ärztelkammer-Rheinland-Pfalz'. Informed written consent was obtained from all participants.

### Studies of low-density lipoprotein subfractions

To examine the relationship between LDL particle size and the distribution of LDL subfractions in detail, we studied two groups of patients not included in the LURIC study: (i) 114 patients with fasting plasma glucose between 1.1 and 2.0 g/L, established impaired glucose tolerance or type 2 diabetes mellitus not receiving oral antidiabetics, insulin or lipid-lowering agents (LDL-C  $> 1.30 \text{ g/L}$ , TG  $> 1.0 \text{ g/L}$ );<sup>30</sup> (ii) 84 patients with coronary heart disease (CHD) or CHD risk equivalent with LDL-C between 1.0 and 1.6 g/L.<sup>31</sup> Of these, 39 had vascular disease (34%) and 76 (90%) had diabetes mellitus. Low-density lipoprotein subfraction profiles used for the current evaluation were obtained while all patients were off lipid-lowering drugs.

## Laboratory procedures

Laboratory methods and lipoprotein analysis are described in detail in the Supplementary material online. In brief, lipoproteins were separated by a combined ultracentrifugation–precipitation method ( $n = 1643$ , LURIC study) ( $\beta$ -quantification; very-low-density lipoproteins (VLDL), LDL, HDL)<sup>32</sup> or by equilibrium density gradient centrifugation ( $n = 198$ , population outside the LURIC study) (VLDL, intermediate-density lipoproteins (IDL), LDL, six LDL subfractions, HDL).<sup>1</sup> The average radius of LDL was calculated from apoB and major lipids as described.<sup>1</sup>

## Statistical analysis

Participants of the LURIC study were classified into three groups according to the calculated mean LDL diameters (Supplementary material online, Figure S1): large ( $> 16.8 \text{ nm}$ ), intermediate ( $16.5–16.8 \text{ nm}$ ), and small LDL ( $< 16.5 \text{ nm}$ ). Clinical and anthropometric characteristics were compared between these strata by  $\chi^2$ -contingency table testing, ANOVA or logistic regression using covariables as indicated (Table 1). We used the Cox proportional hazards model to examine the association between average LDL size and mortality from all or cardiovascular causes. As indicated by log-minus-log diagnostic plots, the proportional hazards assumption was met. Multivariable adjustment was carried out for age, sex or in addition for CAD status (absence of angiographic CAD, stable CAD, unstable angina pectoris, NSTEMI, or STEMI), body mass index, diabetes mellitus, hypertension, smoking, estimated glomerular filtration rate, LDL-C, HDL-C, and TG (Figure 1, Table 2).

The assumption that residuals are normally distributed was examined using plots of observed vs. predicted values. In none of the analyses did we obtain an indication that this assumption was violated. We are reporting estimated marginal means of the dependent variables along with their 95% CI. The least significant difference *t*-test was used for *post hoc* comparisons. The statistical tests were two-sided;  $P < 0.05$  was considered significant. The SPSS 19.0 statistical package (SPSS Inc.) was used.

## Results

### Characteristics of study participants according to mean low-density lipoprotein diameters

The proportion of men significantly increased in parallel to decreasing LDL size (Table 1). Patients with small LDL were on average younger than those with large or intermediate LDL. Current or past smoking, diabetes mellitus and hypertension were more prevalent among patients with small LDL. There was no obvious association of LDL size with angiographic CAD. In parallel to decreasing LDL size, body mass index, TG, and systolic and diastolic blood pressure increased, while HDL-C decreased. Low-density lipoprotein cholesterol was highest among persons with intermediate LDL size, second highest among those with large LDL, and lowest among those with small LDL.

### Low-density lipoprotein size, total mortality, and mortality from cardiovascular causes

Mortality from all causes was lowest in patients with intermediate LDL ( $16.5–16.8 \text{ nm}$ ), while it was significantly higher in patients with larger and smaller LDL. This finding was robust against adjustment for age and sex and additional adjustment for other cardiovascular risk factors including LDL-C (Figure 1, Table 2, Model 3). The hazard ratios (HRs) of the fully adjusted model were 1.71 (95% CI: 1.31–2.25) and 1.24 (95%

**Table 1** Clinical characteristics of study participants according to low-density lipoprotein size

LDL size	Large	Intermediate	Small	P <sup>a</sup>	P <sup>b</sup>	P <sup>c</sup>
Particle diameter (nm) <sup>d</sup>	> 16.8	16.5–16.8	< 16.5			
N	469	470	704			
Sex						
Men	246 (53%)	289 (62%)	563 (80%)	<0.001 <sup>e</sup>	0.005 <sup>e</sup>	<0.001 <sup>e</sup>
Women	223 (47%)	181 (38%)	168 (20%)			
Age (years)	64 ± 11	63 ± 10	61 ± 12	<0.001 <sup>f</sup>	0.752 <sup>f</sup>	0.001 <sup>f</sup>
Smoking				<0.001 <sup>g</sup>		
Never	209 (45%)	210 (45%)	228 (32%)			
Previous	161 (34%)	170 (36%)	311 (44%)			
Current	99 (21%)	90 (19%)	165 (23%)			
Coronary artery disease				0.270 <sup>g</sup>		
None	185 (39%)	161 (34%)	227 (32%)			
Stable CAD	196 (42%)	210 (45%)	337 (48%)			
Unstable (TnT–)	63 (13%)	76 (16%)	104 (15%)			
NSTEMI (TnT+)	15 (3%)	15 (3%)	17 (2%)			
STEMI (TnT+)	61 (2%)	8 (2%)	19 (3%)			
Body mass index (kg/m <sup>2</sup> )	26.5 ± 4.0	27.3 ± 4.2	28.0 ± 4.2	<0.001 <sup>h</sup>	0.005 <sup>h</sup>	<0.001 <sup>h</sup>
Diabetes mellitus (%)	124 (26%)	129 (27%)	253 (36%)	<0.001 <sup>i</sup>	0.632 <sup>i</sup>	<0.001 <sup>i</sup>
LDL-C (g/L)	1.24 ± 0.33	1.31 ± 0.32	1.19 ± 0.30	<0.001 <sup>h</sup>	0.001 <sup>h</sup>	0.001 <sup>h</sup>
HDL-C (g/L)	0.44 ± 0.13	0.42 ± 0.11	0.37 ± 0.09	<0.001 <sup>h</sup>	0.030 <sup>h</sup>	0.001 <sup>h</sup>
Triglycerides (g/L) <sup>j</sup>	1.23 (1.18–1.29)	1.31(1.26–1.37)	1.73 (1.67–1.79)	<0.001 <sup>h</sup>	0.042 <sup>h</sup>	<0.001 <sup>h</sup>
Hypertension	325 (69%)	341 (73%)	531 (75%)	0.068 <sup>i</sup>	0.190 <sup>i</sup>	0.016 <sup>i</sup>
Systolic blood pressure (mmHg)	140 ± 23	142 ± 23	144 ± 23	0.001 <sup>k</sup>	0.228 <sup>k</sup>	0.001 <sup>k</sup>
Diastolic blood pressure (mmHg)	81 ± 12	82 ± 11	82 ± 12	0.101 <sup>k</sup>	0.133 <sup>k</sup>	0.608 <sup>k</sup>

<sup>a</sup>P for trend: large, intermediate, and small LDL.

<sup>b</sup>Post hoc P (least square difference): large vs. intermediate LDL.

<sup>c</sup>Post hoc P (least square difference): small vs. intermediate LDL.

<sup>d</sup>Calculated from apoB and major lipids determined by  $\beta$ -quantification.

<sup>e</sup>Logistic regression adjusted for age.

<sup>f</sup>ANOVA adjusted for sex.

<sup>g</sup> $\chi^2$ -test.

<sup>h</sup>ANOVA adjusted for age and sex.

<sup>i</sup>Logistic regression adjusted for age and sex.

<sup>j</sup>Triglycerides reported as medians and 25th and 75th percentile; triglycerides were log transformed before being used in ANOVA.

<sup>k</sup>ANOVA adjusted for age, sex, and for use of antihypertensive drugs.

CI: 0.95–1.63) for large and small LDL, respectively. Comparable results were obtained for the association with cardiovascular mortality (Figure 1, Table 2). Adjustment for apoB instead of LDL-C did not substantially change the association of LDL size with total and cardiovascular mortality (Supplementary material online, Table S1). There was no relationship between LDL-C and LDL-apoB with mortality, neither in the entire study population nor in any of the LDL size groups.

The association of small LDL with total mortality was stronger in women than in men ( $P$  for interaction = 0.023), while there was no significant interaction between sex and LDL diameter for the association with cardiovascular mortality ( $P$  for interaction = 0.759). The HRs for total and cardiovascular mortality were similar in patients with and without type 2 diabetes mellitus ( $P$  for interaction: 0.182 and 0.745, respectively).

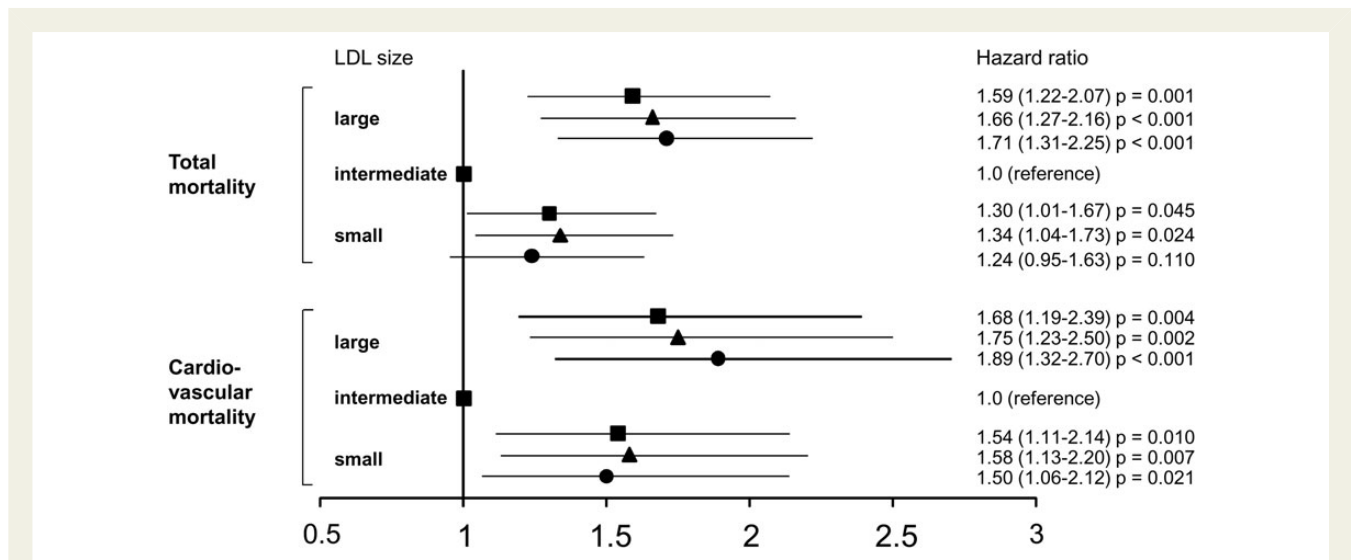
With the  $\beta$ -quantification method, lipoprotein(a) is in the LDL fraction. Thus, we calculated HRs in a subgroup with lipoprotein(a)

concentrations <0.30 g/L ( $n = 1183$ ). The resulting HRs were only slightly different compared with the entire study population indicating that lipoprotein(a) did not impact on our findings (Supplementary material online, Table S2).

The size and composition of LDL are related to the metabolism of TG-rich lipoproteins.<sup>33</sup> However, VLDL-apoB and VLDL-C were not significantly associated with total or cardiovascular mortality in the current study (Supplementary material online, Table S3).

### Low-density lipoprotein mean particle diameter and low-density lipoprotein composition

We investigated the composition of LDL in the strata of LDL size (Supplementary material online, Table S4). In patients with small LDL, the concentration of LDL-C was lower compared with those



**Figure 1** Hazard ratios and 95% confidence intervals for total and cardiovascular mortality by low-density lipoprotein size. The study participants were classified into three groups according to the calculated mean low-density lipoprotein diameters: large ( $> 16.8$  nm), intermediate (16.5–16.8 nm), and small low-density lipoprotein ( $< 16.5$  nm). Squares, unadjusted hazard ratios; triangles, adjusted for age and sex; circles, additionally adjusted for body mass index, diabetes mellitus, hypertension, smoking, clinical status, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and estimated glomerular filtration rate.

with intermediate-sized LDL. At the same time, the concentration of LDL-apoB was not decreased compared with intermediate LDL. Thus, at a given LDL-C the molar concentration of LDL particles was higher in individuals with small LDL. Consistently, small LDL contained less lipid molecules per particle than LDL in patients with intermediate-sized LDL (on average 1503 compared with 1675, respectively). In patients with large LDL, LDL-C was slightly lower than in patients with intermediate LDL, but LDL-apoB was much lower, indicating that these individuals have larger, lipid-rich LDL (on average 1851 molecules vs. 1675 per particle).

### Low-density lipoprotein mean particle diameter and markers of inflammation and oxidative stress

Oxidized LDL and Lp-PLA<sub>2</sub> were significantly higher in individuals with small LDL compared with the groups with large and intermediate LDL. Interestingly, the inflammatory markers C-reactive protein (CRP) and interleukin (IL)-6 were highest in the group with large LDL (Table 3). Adjustment for inflammatory markers slightly attenuated the association of large LDL with mortality, suggesting that inflammation is involved in the atherogenic effects of these particles (Table 2, Supplementary material online, Table S5).

### Low-density lipoprotein mean particle diameter and low-density lipoprotein subfractions

We examined the relationship between the three categories of mean LDL particle diameters and the distribution of LDL subfractions. Because LDL subfractions have not been determined by equilibrium density gradient ultracentrifugation in the LURIC study, we exploited baseline results obtained in participants of two independent,

previously completed, randomized clinical studies of lipid lowering. We calculated mean LDL diameters in these patients from the composition of the entire LDL fraction (1.0063 kg/L through 1.063 kg/L) and subsequently stratified the results according to the particle diameter thresholds used LURIC. As LDL contain one molecule apoB per particle, we considered the apoB contents of LDL subfractions to reflect the concentrations of particles in each of the density fractions LDL-1 to LDL-6. Low-density lipoprotein subfraction profiles were clearly distinct between the groups (Figure 2). In patients with large LDL, the LDL particle concentrations were almost equal across the density spectrum with LDL-1 assuming the highest concentration. In patients with LDL of intermediate mean size, LDL particle concentration reached a peak at LDL-4. Finally, in patients with the smallest calculated LDL diameters, the concentration of LDL particles was highest in LDL-6, followed by LDL-5. The compositions of LDL subfractions hardly differed across the strata with different average LDL sizes, with the exception of an enrichment of TG in the LDL subfractions in patients with large LDL (Supplementary material online, Tables S6 and S7). This overall indicates that changes in the calculated average size of LDL are mainly due to changes in the relative proportions of each of the subfractions rather than to compositional differences in individual subfractions.

## Discussion

This study demonstrates that the mean particle diameter of LDL is associated with long-term total and cardiovascular mortality in patients undergoing coronary angiography, essentially independent of the main acknowledged cardiovascular risk factors. Using the composition of LDL inferred from a  $\beta$ -quantification procedure, we stratified patients into those with large, intermediate, and small

**Table 2** Low-density lipoprotein size and mortality

LDL size	Model 1, HR (95% CI)	P	Model 2, HR (95% CI)	P	Model 3, HR (95% CI)	P	Model 4, HR (95% CI)	P
<b>Total mortality</b>								
Large (> 16.8 nm)	1.59 (1.22–2.07)	0.001	1.66 (1.27–2.16)	<0.001	1.71 (1.31–2.25)	<0.001	1.60 (1.21–2.11)	0.001
Intermediate (16.5–16.8 nm)	1.0 <sup>reference</sup>		1.0 <sup>reference</sup>		1.0 <sup>reference</sup>		1.0 <sup>reference</sup>	
Small (< 16.5 nm)	1.30 (1.01–1.67)	0.045	1.34 (1.04–1.73)	0.024	1.24 (0.95–1.63)	0.110	1.30 (1.00–1.74)	0.052
<b>Cardiovascular mortality</b>								
Large (> 16.8 nm)	1.68 (1.19–2.39)	0.004	1.75 (1.23–2.50)	0.002	1.89 (1.32–2.70)	<0.001	1.67 (1.16–2.41)	0.006
Intermediate (16.5–16.8 nm)	1.0 <sup>reference</sup>		1.0 <sup>reference</sup>		1.0 <sup>reference</sup>		1.0 <sup>reference</sup>	
Small (< 16.5 nm)	1.54 (1.11–2.14)	0.010	1.58 (1.13–2.20)	0.007	1.50 (1.06–2.12)	0.021	1.50 (1.05–2.14)	0.025

Model 1: unadjusted.

Model 2: adjusted for age and sex.

Model 3: in addition adjusted for body mass index, diabetes mellitus, hypertension, smoking, clinical status at presentation, LDL-C, HDL-C, triglycerides (log transformed), and estimated glomerular filtration rate.

Model 4: Model 3 additional adjusted for oxidized LDL, Lp-PLA<sub>2</sub> activity, CRP, and IL-6.

average calculated particle size. Intriguingly, the relationship between particle size and mortality was U-shaped, placing both patients with large and small LDL particles at a higher risk of fatal events.

It has been known for long that LDL include a mixture of particles differing regarding density<sup>1–3</sup> and size.<sup>4,6</sup> Experimental studies have suggested that small dense LDL are particularly atherogenic.<sup>7–9,11–13</sup> Epidemiological evidence based on clinical endpoints for a role of small dense LDL in the development or progression of vascular disease has, however, been surprisingly sparse. Some studies have relied on surrogate vascular endpoints only, such as endothelial dysfunction<sup>14</sup> or carotid intima media thickness.<sup>15,16</sup> In epidemiological studies using nuclear magnetic resonance spectroscopy to determine LDL size, either small LDL<sup>26</sup> were predictive for CAD or both small and large LDL were associated with subclinical atherosclerosis<sup>28</sup> or CAD,<sup>27,34</sup> but only in one study the risk estimates were independent of other lipid parameters.<sup>34</sup> Some prospective epidemiological studies using gradient gel electrophoresis have found small dense LDL associated with vascular disease after adjusting for either low HDL or high TG.<sup>22,23</sup> The majority of studies, however, did not show such an independent association.<sup>17–21</sup> This is evidently due to the fact that high concentrations of small dense LDL are part of the atherogenic lipid triad (in addition characterized by high TG) which in turn is a feature of the metabolic syndrome. It is, in this regard, worth to note that we were able to demonstrate that small LDL were essentially independently associated with mortality from all causes and from cardiovascular causes in the current study. Of interest, we also obtained evidence for adverse effects not only of predominantly small but also large LDL compared with LDL of intermediate size. This finding is consistent with studies claiming that buoyant rather than small LDL are associated with atherosclerosis<sup>25</sup> and an increased CAD risk.<sup>24,35</sup>

To validate our approach of using the average calculated diameter of LDL as a surrogate for the distribution of LDL subfractions, we examined the relationship between calculated LDL particle diameters and actual LDL profiles obtained by equilibrium density gradient ultracentrifugation. As expected, patients with low-LDL diameters exhibited distinct preponderance of dense LDL. In patients with LDL of intermediate size, the LDL particle concentration had a peak at LDL-4 (1.037–1.040 kg/L). At the largest average LDL size, the distribution of LDL particles was almost even, with LDL-1 (1.019–1.031 kg/L) reaching the highest concentration. The three groups of our mortality study thus had distinct patterns of LDL subfractions.

Low-density lipoprotein particles can be synthesized via two pathways: the lipolysis of TG-rich lipoproteins<sup>33</sup> and direct hepatic secretion.<sup>36</sup> In our study, the composition of LDL in patients with large, intermediate, and small LDL differed characteristically. In patients with large LDL, the LDL-TG concentration and the number of lipid molecules per particle were highest among the three groups; LDL were also relatively enriched in TG. It is thus reasonable to assume that incompletely catabolized remnants of TG-rich lipoproteins accumulate in these individuals. On the other hand, patients with smaller LDL had LDL poor in lipids but relatively enriched in cholesterol esters. Those small LDL are the product of hydrolysis of TG-rich precursors by hepatic lipase.<sup>33,36</sup> However, some of them may directly originate from the liver, because there is a substantial contribution of hepatic synthesis of small LDL to plasma LDL in hypertriglyceridemia.<sup>37</sup>



**Table 3** Estimated marginal means of biological markers according to low-density lipoprotein diameter<sup>a</sup>

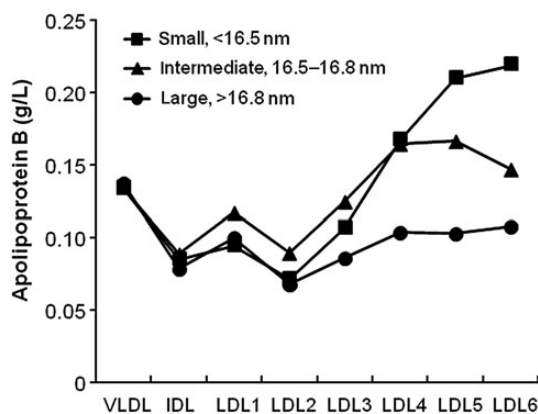
LDL size	Large (>16.8 nm)	Intermediate (16.5–16.8 nm)	Small (<16.5 nm)	P <sup>b</sup>	P <sup>c</sup>	P <sup>d</sup>
Oxidized LDL (U/L)	71 (68–73)	75 (72–78)	80 (78–83)	<0.001	0.018	0.004
Lp-PLA <sub>2</sub> activity (U/L)	487 (477–498)	492 (482–502)	519 (510–527)	0.009	0.497	<0.001
C-reactive protein (mg/L)	10.0 (8.7–11.4)	7.8 (6.4–9.11)	6.4 (5.3–7.6)	<0.001	0.020	0.142
IL-6 (ng/L)	5.80 (5.27–6.32)	4.84 (4.33–5.36)	4.59 (4.16–5.02)	0.002	0.011	0.460

<sup>a</sup>Estimated marginal means and 95% CI obtained in a general linear model (ANOVA) adjusted for age, sex, BMI, diabetes mellitus, hypertension, clinical status at presentation, estimated glomerular filtration rate, and use of lipid-lowering drugs.

<sup>b</sup>Overall P-values.

<sup>c</sup>Post hoc P (least square difference): large vs. intermediate LDL.

<sup>d</sup>Post hoc P (least square difference): small vs. intermediate LDL.



**Figure 2** Distribution of VLDL, IDL, and low-density lipoprotein subfractions obtained by equilibrium density gradient ultracentrifugation in 114 patients with fasting glucose between 1.1 and 2.0 g/L, established impaired glucose tolerance or type 2 diabetes mellitus<sup>30</sup> and 84 patients with coronary heart disease or coronary heart disease risk equivalent with low-density lipoprotein cholesterol between 1.0 and 1.6 g/L<sup>31</sup> stratified according to calculated average diameters of low-density lipoprotein (1.0063–1.063 kg/L). Circles, large low-density lipoprotein; triangles, intermediate low-density lipoprotein; squares, small low-density lipoprotein.

We sought to evaluate mechanisms underlying the association of buoyant and dense LDL with adverse vascular outcomes. We therefore compared markers of inflammation and oxidative stress between the strata of different average LDL sizes. We observed elevated concentrations of oxidized LDL in individuals with small LDL compared with the other groups. Further, Lp-PLA<sub>2</sub>, which by large promotes destabilization of atherosclerotic lesions, was also higher in this group. It is well conceivable that this finding is attributable to the preferential association of this enzyme with small LDL.<sup>38</sup> Small dense LDL appear to be cleared from the plasma at a decreased rate,<sup>11</sup> possibly by pathways different from the LDL receptor.<sup>39,40</sup> As a consequence of their prolonged time of residence in the circulation and their decreased content of core antioxidants, small dense LDL are more susceptible to oxidation,<sup>41–43</sup> which is in line with the current findings.

The concentrations of inflammatory markers such as CRP and IL-6 were highest in patients with buoyant LDL. The significance of this unexpected finding, however, remains elusive at this time. In particular, it is hard to decide whether systemic inflammation causes the accumulation of buoyant LDL or whether these lipoproteins are themselves responsible for the inflammatory reaction. Disorders of LDL metabolism and low-grade inflammation have so far been conceived to be atherogenic by completely distinct pathways.<sup>44</sup> However, the TG contents of LDL may be implicated in inflammation and atherogenesis. In humans, LDL-TG are mainly hydrolyzed by hepatic lipase, which is subject to modulation by inflammatory cytokines.<sup>45,46</sup> Downregulation of lipases is considered to explain, at least in part, the elevation of TG during the acute phase response.<sup>47</sup> Low-grade systemic inflammation could thus be the cause rather than the consequence of the accumulation of buoyant LDL. The observation, however, that LDL-TG were associated with both CAD and circulating adhesion molecules independent of CRP is in favor of the idea that TG-enrichment of LDL might itself be pro-inflammatory.<sup>48–50</sup>

Our study has three major limitations. First, it included patients scheduled for angiography. This hospital-based population may be not representative of a random population sample. Second, we excluded subjects receiving lipid-lowering drugs and thus may have selected individuals with low-LDL-C. Third, estimating the atherogenicity of LDL with the  $\beta$ -quantification method used here is still technically demanding and the clinical application of our findings may therefore not be straightforward. Future investigations will therefore have to address the question whether other methods to estimate mean LDL particle size would be equally informative.

In summary, this is the first study to show a significant association of both, large and small LDL particle size on total and cardiovascular mortality. Our results underline the impact of markers of the LDL metabolism on clinical endpoints beyond elevated LDL-C concentration.

## Supplementary Material

Supplementary material is available at *European Heart Journal* online.

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