Low Density Lipoprotein Particle Size and Risk of Early-Onset Myocardial Infarction in Women

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Previous studies of middle-aged men have shown a univariate association between low density lipoprotein (LDL) particle diameter (size) and coronary heart disease, but this association has yet to be examined in younger women. Using a subsample from a population-based case-control study of women living in western Washington State, the authors examined the association between LDL particle size and risk of early-onset myocardial infarction (MI) in 1992–1995. Gradient gel electrophoresis was used to characterize LDL subclasses in nonfasting blood samples from 72 MI cases and 159 controls aged 20–44 years. Mean LDL particle size in cases was significantly smaller compared with controls (26.4 vs. 26.9 nm, \( p < 0.001 \)), with an odds ratio of 2.3 (\( p < 0.0001 \)) for a 1-nm smaller LDL particle size. These results were independent of age, menopausal status, smoking, diabetes, hypertension, and LDL cholesterol (odds ratios = 1.9–2.3 for a 1-nm smaller LDL particle size, all \( p < 0.02 \)) but were not independent of body mass index, high density lipoprotein cholesterol, or triglyceride (odds ratios = 1.4, 1.4, and 1.1, respectively; all \( p > 0.05 \)). Therefore, in age-adjusted analyses, smaller LDL particle size was associated with MI in young women, but the risk was attenuated after adjustments for metabolic factors related to both LDL particle size and MI. Am J Epidemiol 2001;153:939–45.

The causal relation between increased levels of low density lipoprotein (LDL) cholesterol and risk of coronary heart disease is well established (1–6). However, LDL particles are heterogeneous in size, density, and composition, and this variation can be used to define specific LDL subclasses in individual subjects (7).

A number of case-control and cross-sectional studies have examined the relation between LDL heterogeneity and risk of coronary heart disease. An early study by Fisher (8) found that “polydisperse” LDL was more prevalent in persons with atherosclerosis than in controls. In a study by Crouse et al. (9), male coronary artery disease cases were found to have significantly lower LDL molecular weights than controls (\( p < 0.001 \)). In the first population-based case-control study on this topic, gradient gel electrophoresis was used to characterize individual LDL subclass phenotypes for 109 cases and 121 controls (10). After adjustment for age, sex, and relative weight, the study found that a predominance of small, dense LDL particles (LDL subclass phenotype B) was associated with a threefold increase in risk of myocardial infarction. In a study among a group of young males, Tornvall et al. subsequently reported that for myocardial infarction survivors, the LDL particle distribution fell into the more dense range compared with the distribution for healthy controls (11).

In the largest case-control study to date, Campos et al. found that cases had a larger LDL particle score (indicating a smaller LDL particle size) compared with controls, even after cases taking beta-blocker medication were excluded (12). Coresh et al. examined the associations of LDL particle size, density, and composition with premature coronary artery disease in a sample of male and female cases and controls (13); they showed that cases tended to have smaller LDL particles of lower molecular weights. Finally, the most recent case-control study compared men with and without coronary artery disease by angiography and found that men with coronary artery disease had significantly higher concentrations of small, dense LDL (LDL-III by density), with an odds ratio of 4.5 (\( p < 0.01 \)) (14). An even higher odds ratio was found when men who had survived a prior myocardial infarction were compared with healthy controls (odds ratio (OR) = 6.9, \( p < 0.001 \)) in the same study. Taken together, these case-control studies consistently suggest an association between small, dense LDL and atherosclerosis. However, most studies also demonstrated that the magni-

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MATERIALS AND METHODS

Women’s Cardiovascular Health Study

This analysis was based on a subsample of women from the Women’s Cardiovascular Health Study, a population-based study of myocardial infarction and stroke in young women (18–23). Briefly, this study was based on women 18–44 years of age residing in western Washington State (King, Pierce, or Snohomish County). Cases were women who had their first diagnosis of fatal or nonfatal myocardial infarction between July 1, 1991, and February 28, 1995, with no prior history of heart disease or stroke. Potential cases were identified through monthly review and abstraction of discharge diagnoses of acute ischemic heart disease provided by all hospitals within the study region, incident reports from emergency medical service systems, and death certificates listing out-of-hospital deaths from cardiovascular disease and related conditions (18). Criteria developed for the Cardiovascular Health Study were used to determine whether ascertained events represented myocardial infarction (24). A total of 208 women with probable or definite myocardial infarction were identified. Of these, 161 were living at the time of recruitment. Of these 161 women, 4 were not approached for recruitment at the request of their physicians, 40 declined to participate, and 10 could not be located. Thus, a total of 107 nonfatal myocardial infarction patients were recruited into the study between January 1992 and July 1995.

Controls were women 18–44 years of age who also resided in King, Pierce, or Snohomish County in Washington State during the case diagnosis period. Potential controls were identified through random digit dialing (25). Briefly, a computer algorithm was used to generate telephone numbers, and these numbers were used to contact potential controls. To identify women who fulfilled the eligibility criteria, a household census was conducted as contacts were made. After eligible controls were identified, 691 randomly chosen, eligible controls were approached for recruitment. These women were frequency matched to the combined age distribution of all stroke and myocardial infarction cases recruited into the study by using the following categories: 18–24, 25–29, 30–34, 35–39, and 40–44 years. Of the 691 controls, 6 were excluded because of a prior history of heart disease and stroke, and 1 woman was excluded because of her inability to communicate in English. Interviews were conducted with 526 (77 percent) of these 684 women; the remaining women declined to participate.

A structured, in-person interview was used to obtain information on a variety of cardiovascular disease risk factors (menopausal status, smoking status, history of diagnosis of diabetes, history of diagnosis of hypertension, history of myocardial infarction in first-degree relatives, height and weight, and demographic characteristics). Self-reported height and weight, and, for a few women, height and weight abstracted from medical records, were used to calculate body mass index (BMI) as kilograms divided by meters squared (kg/m²). Trained interviewers gathered information from each case based on the time period prior to the myocardial infarction. When a control was interviewed, a date randomly chosen (“reference date”) from all possible diagnosis dates for the cases was selected as the reference date for that control.

A nonfasting blood sample was collected from 79 living cases and from 361 controls. The remaining cases and controls declined to provide a blood specimen. A 30-ml venous blood sample was collected into vacuum tubes treated with ethylenediaminetetraacetic acid (EDTA), usually at the time of the interview. Aliquots of plasma were frozen at –70°C. Blood samples from cases were collected at least 3 months after myocardial infarction occurred. Blood samples from cases and controls were assayed for LDL cholesterol, HDL cholesterol, plasma triglyceride, and total cholesterol at the Northwest Lipid Research Laboratory in Seattle, Washington (26, 27).

LDL subclass analyses

At the time of this study, a previously unthawed plasma aliquot was available for LDL subclass analyses for 72 of the 79 cases. Controls (n = 159) were randomly selected for this study from among the 361 women who provided blood samples, after frequency matching to the age distribution of the cases.

Gradient gel electrophoresis was used to determine LDL subclass phenotypes and LDL particle size. The procedures used have been described elsewhere (28). Briefly, 2–14 percent polyacrylamide gels were produced, and electrophoresis procedures were applied by using plasma samples. A set of high-molecular-weight standards were also run on each
gel and were used to construct a quadratic calibration curve for estimating the diameter of LDL subclasses. In addition, two quality control samples with well-characterized LDL subclass phenotypes were run on each gel. Two gels were run on each sample by using whole plasma, and investigators were blinded to the case-control status of the study subject. The scan data for each sample lane on the gel were used to classify the LDL subclass phenotype (10) on the basis of blinded evaluations by three reviewers and to estimate the diameter of the major LDL subclass (LDL particle size) (28).

More specifically, LDL subclass phenotype A is characterized by a predominance of large, buoyant LDL particles with a peak diameter of generally more than 25.5 nm (10). In contrast, phenotype B is characterized by a predominance of small, dense LDL particles with a peak diameter of less than or equal to 25.5 nm (10). LDL particle size was defined as the estimated diameter of the major LDL subclass from the gels and was used as a continuous variable in the analyses. Associations between a 1-nm smaller LDL particle diameter and increased risk of myocardial infarction were evaluated.

**Statistical analysis**

Analyses were performed with LDL subclass phenotypes and LDL particle size as the exposures of interest. Age-adjusted t tests for independent samples were used to compare geometric mean lipid values and geometric mean LDL particle sizes. Odds ratios were used to estimate the relative risks of myocardial infarction associated with a smaller LDL particle size. Odd ratios and 95 percent confidence intervals were estimated by using unconditional logistic regression (29).

In our analyses of LDL particle size, we also examined whether a quadratic relation fit the data better than a simple linear relation by centering the LDL particle size values and conducting a likelihood ratio test for the addition of a quadratic term. Multivariate analyses were initially performed with adjustment for age, menopausal status, and smoking. Additional adjustments were also made for diagnosis of hypertension, diagnosis of diabetes, BMI, triglyceride, HDL cholesterol, LDL cholesterol, and total cholesterol. The latter risk factors were selected on the basis of their known associations with LDL particle size, triglyceride, or HDL cholesterol and thus their potential to act as confounders.

**RESULTS**

Characteristics of cases and controls are summarized in table 1. The average age at the diagnosis reference date was similar for cases and controls (means, 39.9 and 39.3 years, respectively). The majority of women (88 percent of cases, 91 percent of controls) were White, not of Hispanic origin. Cases and controls differed on smoking status, with a much greater proportion of cases reporting that they were current smokers. A higher proportion of cases than controls was postmenopausal, and average BMI was higher for cases than controls. Compared with controls, cases were also more likely to have a prior diagnosis of diabetes or hypertension.

Table 2 shows the mean lipid values for cases and controls. Cases had significantly higher plasma levels of total cholesterol, LDL cholesterol, and triglyceride and lower HDL cholesterol.
HDL cholesterol levels (all p < 0.0001). The proportion of cases with LDL subclass phenotype B was significantly larger than the proportion of controls (36 vs. 14 percent, p < 0.001, table 3), resulting in an age-adjusted odds ratio of 3.5 for the association between LDL subclass phenotype B and myocardial infarction.

The frequency distribution of LDL particle size for cases and controls (figure 1) showed that the LDL particles in myocardial infarction cases tended to be smaller when compared with controls. Mean LDL particle size was significantly smaller for cases than for controls (26.4 vs. 26.9 nm, p < 0.001). The risk of myocardial infarction increased twofold for every 1-nm smaller LDL particle size, with adjustment for age only (OR = 2.3, p < 0.0001) (table 3).

A 1-nm smaller LDL particle size was associated with myocardial infarction, after adjustment for age, menopausal status, and smoking (OR = 2.1, p = 0.001, table 4, model 1). The association between a 1-nm smaller LDL particle size and risk of myocardial infarction was also independent of hypertension and diabetes (OR = 2.3 and OR = 2.2, respectively; both p = 0.001; models 2 and 3) but was not independent of BMI (OR = 1.4, p = 0.183, model 4).

In addition, the LDL particle size association with myocardial infarction was independent of LDL cholesterol (OR = 1.9, p = 0.011, model 5) but was not independent of HDL cholesterol or triglyceride (OR = 1.4 and OR = 1.1, respectively; models 6 and 7). There was no significant improvement in the fit of the unconditional logistic models when a quadratic term for LDL particle size was added.

### DISCUSSION

In this sample of young women, a predominance of small LDL particles was associated with a more than threefold increased risk of myocardial infarction (OR = 3.5, p < 0.001). Similarly, a 1-nm smaller LDL particle size was associated with a risk of myocardial infarction (OR = 2.3, p < 0.001). This relation was independent of age, menopause, smoking, diabetes, hypertension, and LDL cholesterol levels, but it was not independent of BMI, HDL cholesterol, or triglyceride.

The results of this study are consistent with previous reports. For example, a meta-analysis of three available prospective studies (15–17), with a total sample size of 960 men, estimated an odds ratio of 1.6 (p < 0.05) for a 1-nm smaller LDL particle size (30). This result was independent of triglyceride, HDL cholesterol, and other covariates, although the odds ratio was decreased to 1.3 (p < 0.05) after adjustment. Similarly, in a prospective, nested case-control study of older Japanese-American men participating in the Honolulu Heart Program, smaller LDL particle size was associated with subsequent coronary heart disease (relative risk = 1.3 for a 1-nm smaller LDL particle size, p < 0.05) (31). However, this relation was not independent of HDL cholesterol or triglyceride levels.

It is important to note that nonfasting blood samples were used in this study. However, other studies, including the Physicians’ Health Study (15) and the Stanford Five City Project (16), also used nonfasting blood samples and reported results similar to ours. In the present study, the timing of blood draws during the day was distributed similarly

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### Table 2: Lipid values of cases and controls, Women’s Cardiovascular Health Study, western Washington State, 1992–1995

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Cases (n = 72)</th>
<th>Controls (n = 159)</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>231.2 (41.4)</td>
<td>193.4 (34.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL† cholesterol</td>
<td>139.1 (39.3)</td>
<td>107.2 (30.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL† cholesterol</td>
<td>43.4 (12.1)</td>
<td>55.0 (15.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>209.6 (128.7)</td>
<td>113.7 (68.8)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* SD, standard deviation; LDL, low density lipoprotein; HDL, high density lipoprotein.
† Based on age-adjusted t tests of differences in geometric means.

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### Table 3: Association between low density lipoprotein subclasses and myocardial infarction, Women’s Cardiovascular Health Study, western Washington State, 1992–1995

|   | Cases | Controls | Age-adjusted OR† | 95% CI† | p value  
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LDL subclass phenotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenotype B</td>
<td>26</td>
<td>36.1</td>
<td>22</td>
<td>13.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Phenotype A</td>
<td>46</td>
<td>63.9</td>
<td>137</td>
<td>86.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>100.0</td>
<td>159</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

|   | Cases | Controls | Age-adjusted OR‡ | 95% CI | p value  
<table>
<thead>
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</thead>
<tbody>
<tr>
<td>LDL size</td>
<td>72</td>
<td>26.4 (0.8)</td>
<td>159</td>
<td>26.9 (0.7)</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* OR, odds ratio; CI, confidence interval; LDL, low density lipoprotein.
† Value expressed as mean (standard deviation).
‡ For a 1-nm smaller size.
for cases and controls (data not shown). Two other limitations of this study were that, for cases, blood samples were obtained after the occurrence of myocardial infarction, and response rates were low (75 percent among cases and 69 percent among controls). Finally, data on use of hormone replacement therapy and other medications that may affect LDL subclasses were not available at the time of the blood draw. It would have been valuable to examine hormone replacement data and their possible role as a confounding variable in the association between LDL particle size and myocardial infarction.

In this study, the association between smaller LDL particle size and occurrence of myocardial infarction was not independent of triglyceride or HDL cholesterol. It is possi-


<table>
<thead>
<tr>
<th>Adjustment for nonlipid covariates*</th>
<th>OR†</th>
<th>95% CI†</th>
<th>p value</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL† particle size (1 nm smaller)</td>
<td>2.1</td>
<td>1.4, 3.3</td>
<td>0.001</td>
<td>2.3</td>
<td>1.4, 3.7</td>
<td>0.001</td>
<td>2.2</td>
<td>1.4, 3.5</td>
<td>0.001</td>
<td>1.4</td>
<td>0.9, 2.3</td>
<td>0.183</td>
</tr>
</tbody>
</table>

* Adjustments: model 1—age, menopause, and smoking; model 2—age, menopause, smoking, and hypertension; model 3—age, menopause, smoking, and diabetes; model 4—age, menopause, smoking, and body mass index.
† OR, odds ratio; CI, confidence interval; LDL, low density lipoprotein.
‡ Adjustments: model 5—age, menopause, smoking, and LDL cholesterol; model 6—age, menopause, smoking, and high density lipoprotein cholesterol; model 7—age, menopause, smoking, and triglyceride.
ble that the small sample size (n = 231) did not provide sufficient power to detect an effect of smaller LDL particle size independent of these highly related lipid covariates. Another possible explanation for the lack of statistically significant findings in the models that controlled for triglyceride and HDL cholesterol is the close association between LDL subclasses and other lipoprotein and apolipoprotein risk factors for atherosclerosis. The interrelations between triglyceride, HDL cholesterol, and LDL particle size may reflect the “atherogenic lipoprotein phenotype” (32), in which small, dense LDL is associated with a variety of other lipoprotein risk factors, including increased plasma triglyceride, increased apolipoprotein B, lower HDL cholesterol, and lower levels of apolipoprotein A-I. Furthermore, it has been hypothesized that LDL particle size is a marker for the insulin resistance syndrome (33, 34) and its association with atherosclerosis (35). Recent quantitative analyses also indicate significant genetic influences on combinations of these risk factors (36–38), raising the possibility of underlying genetic susceptibility to cardiovascular disease in this sample of young women.

Several mechanisms have been proposed to explain the association between smaller LDL particle size and risk of coronary heart disease. Because of their smaller diameter, it is possible that small LDL particles are more easily deposited in atheromas, contributing to accelerated atherosclerosis. It has also been shown that small, dense LDL particles are more susceptible to oxidation (39), illustrating another possible atherogenic mechanism (40). Small, dense LDL also has reduced affinity to the LDL-receptor binding site and alters the apolipoprotein B conformation (41). As a result, small LDL particles may bind with less affinity to the LDL receptor, increasing the amount of time they remain in the bloodstream. Finally, it is possible that more than one of these mechanisms are operating together to increase the risk of coronary heart disease.

In conclusion, this study of young women provides evidence for an association, in age-adjusted analyses, between small, dense LDL particles and increased risk of myocardial infarction. However, this association was not independent of triglyceride, HDL cholesterol, or BMI. Further studies are needed to determine the clinical utility of LDL particle size by examining whether increasing LDL particle size through behavioral or pharmacologic interventions can decrease the risk of myocardial infarction.

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REFERENCES


