A Prospective Investigation of Elevated Lipoprotein (a) Detected by Electrophoresis and Cardiovascular Disease in Women

The Framingham Heart Study

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Background Sinking prebeta lipoprotein is a putative marker for elevated levels of lipoprotein (a). Although prospective data suggest that increased plasma lipoprotein (a) is an independent risk factor for coronary heart disease in men, no prospective studies are available in women.

Methods and Results From 1968 through 1975, sinking prebeta lipoprotein was determined by paper electrophoresis in 3103 women Framingham Heart Study participants who were free of prevalent cardiovascular disease. A sinking prebeta lipoprotein band was detectable in 434 of the women (14%) studied. The median follow-up interval was approximately 12 years. Incident cardiovascular disease was associated with band presence using a proportional hazards model that included age, smoking, body mass index, systolic blood pressure, glucose intolerance, low- and high-density lipoprotein cholesterol, and ECG left ventricular hypertrophy. Multivariable-adjusted relative risk estimates (with 95% confidence intervals) for outcomes in the band present versus absent groups were as follows: myocardial infarction (82 events), 2.37 (1.48 to 3.81); intermittent claudication (62 events), 1.94 (1.07 to 3.50); cerebrovascular disease (83 events), 1.88 (1.12 to 3.15); total coronary heart disease (174 events), 1.61 (1.13 to 2.29); and total cardiovascular disease (305 events), 1.44 (1.09 to 1.91). A subset analysis indicated that band presence was 50.9% sensitive and 95.4% specific for detecting plasma lipoprotein (a) levels of >30 mg/dL, the threshold value linked to increased cardiovascular disease risk in men.

Conclusions Sinking prebeta lipoprotein was a valid surrogate for elevated lipoprotein (a) levels in Framingham Heart Study women. Band presence and, equivalently, elevated plasma lipoprotein (a), was a strong, independent predictor of myocardial infarction, intermittent claudication, and cerebrovascular disease. Confirmation of these findings in other longitudinal studies of women is needed. (Circulation. 1994;90:1688-1695.)

Key Words: lipoprotein (a) • women • risk factors • myocardial infarction • coronary heart disease

In 1963, Berg1 discovered an antigenic determinant present in the low-density lipoprotein (LDL) fraction of some individuals and named it lipoprotein (a) [Lp(a)]. By 1974, several independent groups had reported the presence of an electrophoretic band in the density >1.006 g/mL fraction with prebeta mobility (hence the term “sinking prebeta”) on paper,2 cellulose acetate,3 or agarose.4 Early comparative studies5-7 using antisera provided by Berg strongly suggested that these sinking prebeta bands represented Lp(a); definitive confirmation was recently provided by Kawakami et al8 using the capillary transferable blotting method, followed by an enzyme-linked immunosorbent staining assay.

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Data derived from studies using crude rabbit antiserum immunosays alone or in combination with the presence or absence of an extra or sinking prebeta band indicated that Lp(a) was a qualitative, autosomal dominant genetic marker present in as many as 35% of Caucasian populations.2,3,9,10 The advent of more refined immunosays6-11 made it apparent that Lp(a) was a quantitative, not a qualitative trait, and that sinking prebeta positivity on electrophoresis generally corresponded to quantitative Lp(a) levels of ≥20 to 30 mg/dL.6,12,13 Although similar to LDL in core lipid composition and having B-100 as a surface apolipoprotein (apo), Lp(a) became recognized as a separate lipoprotein class [i.e., Lp(a)] that also contained the unique glycoprotein apo (a), probably disulfide-linked to apo B-100.14,15

Investigations from the 1970s suggested that the presence of an “additional”15 (equivalently, “sinking”16,17) prebeta band on cellulose acetate or agarose gels was linked to coronary heart disease (CHD) in men. Case-control studies using quantitative immunosays confirmed these initial observations, demonstrating that men with Lp(a) levels >30 mg/dL were at significantly increased risk for CHD.18,19 Discovery of the striking structural homology between apo (a) and human plasminogen in the mid 1980s20,21 intensified the
investigation of Lp(a) as a possible "link" between atherosclerosis and thrombosis. Subsequently, numerous retrospective case-control studies in which the participants were exclusively (eg, References 25, 26, 30, and 38) or predominantly (eg, References 27 to 29, 31 to 37, 39, and 40) men, have associated elevated levels of Lp(a) with CHD (References 25 to 32 and 40), cerebrovascular disease (References 33 to 36), and peripheral vascular disease (PVD) (References 37 to 39). Retrospective studies of Lp(a) and atherosclerotic cardiovascular disease (CVD) that have included small numbers of women (ie, References 27 to 29, 31 to 37, 39, and 40) are available. Despite limited power, analyses of subgroups from these studies suggest that elevated Lp(a) levels confer a modest (References 27, 31, and 33 to 36) to significantly increased (References 37 and 39) risk for clinical atherosclerosis in women.

In seven recent prospective studies of Lp(a) or apo(a) levels and subsequent CHD in men, five reported a significant association, and two did not. Six of the studies used long-term (≥6 years) frozen samples in their analyses. Variations in storage conditions and assay methodologies may have accounted, in part, for these discordant results. For example, Craig et al have shown that serum Lp(a) concentrations may decline considerably over time when subjected to long-term freezing at −20°C, as in the Helsinki Heart Study. They stressed the importance of carefully matching samples for storage temperature and length of time in prospective case-control studies.

No prospective studies evaluating the relation between Lp(a) levels, or the presence of sinking prebeta lipoprotein on electrophoresis, and atherosclerotic cardiovascular disease outcomes in women have been reported. During examination 11 (1968 through 1971) of the original Framingham Heart Study Cohort and examination 1 (1971 through 1975) of the Framingham Offspring, paper electrophoresis was performed on fresh plasma samples for the identification of sinking prebeta lipoprotein in 3103 women free of clinical cardiovascular disease. Longitudinal follow-up of these individuals provided a unique opportunity to examine whether sinking prebeta lipoprotein band presence was a significant, independent risk factor for the development of CVD in women.

**Methods**

**Study Population**

Details of the design and methods for The Framingham Heart Study have been published previously. In brief, two groups of men and women are followed. The original population-based sample (The Cohort) from Framingham, Mass., were recruited in 1948 and are examined every 2 years. The children of the original cohort and their spouses (The Offspring) were enrolled in the early 1970s and are examined every 3 to 4 years. Data from medical examinations at each cycle and information on morbidity and mortality from hospitals and other sources are routinely recorded.

The group studied in this investigation was composed of women participants in Cohort examination 11 (1968 through 1971) and Offspring examination 1 (1971 through 1975). The protocol included an updated (Cohort) or complete (Offspring) medical history, a physical examination, a 12-lead ECG, and a fasting lipid profile. Presence of a sinking prebeta lipoprotein band (as absent, definite, or trace) was also determined. A total of 3103 women who were free of prevalent congestive heart failure, CHD (ie, angina pectoris, myocardial infarction, or coronary insufficiency), stroke (atherothrombotic or hemorrhagic brain infarction) or transient ischemic attack, and intermittent claudication were included in the present analyses.

**CVD Outcomes**

Follow-up outcome data were available through Cohort examination 20 (1988 through 1989) and Offspring examination 3 (1983 through 1987). The outcomes that were analyzed included total CHD (ie, myocardial infarction, angina pectoris, coronary insufficiency, and CHD death), total cerebrovascular disease (ie, atherothrombotic brain infarction, transient ischemic attack, or stroke death), and intermittent claudication. Detailed operational definitions of these outcomes are provided elsewhere.

**Lipoprotein Analyses**

After an overnight fast of 12 to 14 hours, blood specimens were collected in an aqueous solution of disodium EDTA, providing 1.275 mg/mL of blood, and the plasma was extracted. Plasma lipoproteins were isolated according to a protocol developed by the Laboratory for Molecular Disease of the National Heart, Lung, and Blood Institute. High-density lipoprotein (HDL) cholesterol was determined after the precipitation of apo B-containing lipoproteins with heparin–manganese chloride using a modification of the technique described by Burstein and Samaille. Plasma was subjected to ultracentrifugation (39 000 rpm, 4°C, density of 1.06 g/mL, 15 hours), the infranate was collected, and its cholesterol content was determined. LDL cholesterol was calculated by subtracting the HDL cholesterol from the bottom fraction (density >1.006 g/mL) cholesterol. Very low-density lipoprotein (VLDL) cholesterol was measured by subtracting the bottom fraction cholesterol from the total cholesterol. Cholesterol concentrations were determined by the Abell-Kendall method, and triglycerides were determined by a modification of the Kessler-Lederer method.

Presence of a sinking prebeta lipoprotein band was determined as follows. A fresh 20-μL aliquot of the 1.006 g/mL infranate was subjected to electrophoresis in albumin containing barbital buffer at pH 8.6 on Whatman #1 filter paper strips. Electrophoresis was performed for 16 hours at 120 V. The strips were then dried, stained with Oil Red O dissolved in 60% ethanol, dried again, and examined visually. Bands were scored as “no” (absent), “yes” (definite), or “trace” by the same examiner for all determinations.

Quantitative assessment of Lp(a) levels was performed on a subset of women (n=1340) from the Offspring during examination 3 (1983 through 1987) as described previously by Jennner et al. In brief, plasma Lp(a) was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (Terumo Medical Corporation). Standards and controls provided by the manufacturer were calibrated to a reference preparation of human plasma obtained from Dr John Albers (Northwest Lipid Research Clinic). A double-antibody radioimmunoassay calibrated with purified Lp(a) was used to derive the value of the reference preparation. Mean interassay and intraassay coefficients of variation for the ELISA were 4.3% and 2.8%, respectively. Plasma concentrations of Lp(a) are reported as total Lp(a) mass (mg/dL).

Fibrinogen levels were also determined between 1966 and 1971 in a smaller subset of women (n=375) included in the present analyses as described by Kannel et al.

**Other Covariate Measures**

**Body Mass Index**

Height and weight for each participant were measured with the individual dressed in an examining gown wearing no shoes. Body mass index was expressed as weight in kilograms divided by height in meters raised to the second power (kg/m²).
Pressure

ECG Left Ventricular Hypertrophy

Smoking

Blood Pressure

Physician-determined blood pressure was measured in the left arm after the individual had been seated for at least 5 minutes.

Smoking

Cigarette use was based on smoking habits in the year prior to examination and included assessment of the number smoked per day.

ECG Left Ventricular Hypertrophy

Standard 12-lead ECGs were obtained on all participants. ECG diagnosis of left ventricular hypertrophy (LVH) was made using previously published criteria.49

Glucose Intolerance

Individuals were considered to have glucose intolerance if they met one of the following criteria: casual glucose ≥120 mg/dL (original cohort) or fasting glucose ≥110 mg/dL (offspring cohort) at the current examination, use of oral hypoglycemic agents or insulin, or glycosuria.

Statistical Analysis

Differences for continuous variables were assessed by t tests and for discrete variables by χ² or Fisher's exact tests, according to smoking prebeta lipoprotein band status (ie, definite or trace pooled versus absent). Crude incidence densities (per 1000 person-years of follow-up) and relative risk estimates (hazards ratios) for the outcomes of myocardial infarction, intermittent claudication, total cerebrovascular disease, total CHD, and total CVD were determined by smoking prebeta lipoprotein status. Multivariable adjusted relative risk estimates were then calculated using a proportional hazards model that included age, body mass index, cigarettes smoked per day, systolic blood pressure, LDL cholesterol, HDL cholesterol, glucose intolerance, and ECG LVH. Evaluation of effect modification was accomplished by analysis of stratified data and formal testing with interaction terms in the multivariable proportional hazards models. All analyses, including checking the assumption of constant proportional hazards, were performed using SAS.57

Results

Quantitative Lp(a) data were available from a large subset of the study population (n=1340). The distributions (see Fig 1) and mean plasma Lp(a) levels (±SEM) in the pooled definite or trace (n=169) group (39.9±1.6 mg/dL) were significantly different from those in the absent (n=1171) group (11.9±0.4; P<.0001 by t test). Lp(a) concentrations of >38 mg/dL are above the 90th percentile for the Framingham Offspring women.55 Band presence (at Offspring examination 1) was 50.9% sensitive and 95.4% specific (see Table 1) for plasma Lp(a) >30 mg/dL (at Offspring examination 3), a threshold value previously linked to increased CVD risk in men.18,55 Using the same cutoff point (ie, 30 mg/dL), the sensitivity was lower (50.9% versus 63.5%) and the specificity was essentially equal (95.4% versus 97.8%) to the values reported by Bruckert et al53 based on simultaneous quantitative determination of Lp(a) by immunonephelometry and sinking prebeta lipoprotein band presence on agarose electrophoresis in 843 mostly hyperlipidemic patients.

A sinking prebeta lipoprotein band was detectable (trace or definite, pooled) in 434 of the 3103 women (14.0%) studied. Results of covariate measures according to sinking prebeta lipoprotein band status (ie, definite and trace pooled versus absent) are presented in Table 2. Band presence versus absence was associated with older age and greater body mass index, systolic blood pressure, and LDL cholesterol. HDL cholesterol, cigarettes smoked per day, glucose intolerance, and ECG LVH did not differ significantly by band status. Subtraction of Lp(a) cholesterol [ie, 0.328 × total Lp(a)]53 from LDL cholesterol in the subset of women with both measures available (n=1339) eliminated the apparent difference in LDL cholesterol levels.

Table 1. Sensitivity and Specificity of Sinking Prebeta Lipoprotein Band Presence

<table>
<thead>
<tr>
<th>Band</th>
<th>Lp(a) &gt;30 mg/dL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>169</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1171</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity, 118/232 (50.9%); specificity, 1057/1108 (95.4%).

Table 2. Characteristics According to Sinking Prebeta Lipoprotein Band Status

<table>
<thead>
<tr>
<th>Band</th>
<th>Present (n=434)</th>
<th>Absent (n=2679)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>45.1±14.1†</td>
<td>41.5±13.7</td>
<td>.0001†</td>
</tr>
<tr>
<td>BMI</td>
<td>25.1±4.6</td>
<td>24.3±4.6</td>
<td>.0010</td>
</tr>
<tr>
<td>SBP</td>
<td>125.2±20.4</td>
<td>122.8±19.8</td>
<td>.0217</td>
</tr>
<tr>
<td>LDL-C</td>
<td>183.8±39.4</td>
<td>124.3±37.2</td>
<td>.0001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>57.9±14.0</td>
<td>56.9±15.1</td>
<td>.1817</td>
</tr>
<tr>
<td>Cigarettes/d</td>
<td>8.5±12.8†</td>
<td>8.7±12.3</td>
<td>.8357</td>
</tr>
<tr>
<td>Glucose intolerance</td>
<td>18±4.2</td>
<td>11±4.2</td>
<td>.9740§</td>
</tr>
<tr>
<td>ECG LVH</td>
<td>1±0.23</td>
<td>10±0.37</td>
<td>0.999</td>
</tr>
</tbody>
</table>

‡ Number (percentage) with glucose intolerance or left ventricular hypertrophy on ECG (ECG-LVH).

† t test of differences in mean values between band "definite" or "trace" pooled vs "absent."

§χ² or Fisher's exact test of differences in percentages with glucose intolerance or ECG-LVH between band "definite" or "trace" pooled vs "absent."

Fig 1. Bar graph of frequency distributions of lipoprotein (a) [Lp(a)] plasma levels according to sinking prebeta lipoprotein band status in a subset (n=1340) of Framingham Heart Study women.
between the band present and absent groups (116.5 versus 113.5, \( P = .2747 \)).

Consistent with the recent findings of Jenner et al\textsuperscript{5} based on quantitative Lp(a) data from the Framingham Offspring, we observed no significant difference in the age-adjusted prevalence of sinking prebeta lipoprotein among premenopausal and postmenopausal women in the current pooled Cohort/Offspring study group (premenopausal, 11.3%; postmenopausal, 15.9%; \( P = .085 \) by Mantel-Haenzel \( \chi^2 \)). Furthermore, age-adjusted band prevalence did not differ significantly between users (n=559) and nonusers (n=2530) of estrogen-containing compounds (users, 12.7%; nonusers, 14.3%; \( P = .114 \) by Mantel-Haenzel \( \chi^2 \)). A limited subset of our subjects (n=375) had both band status and fibrinogen levels assessed between 1966 and 1971. Fibrinogen did not differ significantly (\( P = .2766 \), by \( t \) test) between the band absent (n=295; mean fibrinogen, 300.2 mg/dL) and present groups (n=81; mean fibrinogen, 292.8 mg/dL).

During 39,156 person-years of follow-up through 1989, 305 incident CVD events were documented. Table 3 shows the absolute number of events, the unadjusted incidence densities per 1000 person-years of follow-up, and crude relative risk estimates (ie, hazard ratios, band present divided by band absent) for myocardial infarction, intermittent claudication, total cerebrovascular disease, total CHD, and total CVD. An age-adjusted Kaplan-Meier plot with myocardial infarction as the outcome is illustrated in Fig. 2. The risk ratios (with 95% confidence intervals) derived from a proportional hazards model adjusted for age, body mass index, cigarettes smoked per day, HDL cholesterol, LDL cholesterol, glucose intolerance, and ECG LVH are also displayed in Table 3. Multivariable adjusted relative risk estimates were as follows: myocardial infarction, 2.37 (1.48 to 3.81); intermittent claudication, 1.94 (1.07 to 3.50); total cerebrovascular disease, 1.88 (1.12 to 3.15); total CHD, 1.61 (1.13 to 2.29); and total CVD, 1.44 (1.09 to 1.91). Comparison of these results with the crude relative risk estimates revealed minimal confounding by the major established CVD risk factors.

With myocardial infarction as the outcome, only the HDL cholesterol–band present interaction term (\( P = .0410 \)) revealed any evidence of effect modification between sinking prebeta lipoprotein and the other independent risk factor covariates modeled (ie, age, body mass index, diabetes, cigarettes smoked per day, ECG LVH, systolic blood pressure, and LDL cholesterol). The relative risk estimate for myocardial infarction with a band present versus absent increased steadily above an HDL of 30 mg/dL, perhaps reflecting that at markedly low HDL levels, "hyperalphalipoproteinemia" overwhelmed the effect of an elevated plasma Lp(a). Additional analyses indicated that the menopausal status–band present and hormone use–band present interaction terms were also nonsignificant with myocardial infarction as the outcome. Finally, formal testing revealed that the assumption of constant proportional hazards was not violated in our analyses.

The Adult Treatment Panel (II) recommended nonfasting determination of both total and HDL cholesterol for screening, and defined "high-risk" cutoff points as a total cholesterol of \( > 240 \) mg/dL and an HDL cholesterol of \(< 35 \) mg/dL.\textsuperscript{59} To assess the attributable risk of sinking prebeta lipoprotein band presence in comparison to these traditional lipid/lipoprotein risk factors, total and HDL cholesterol were dichotomized as high total (\( > 240 \) mg/dL or \( \leq 240 \) mg/dL) and low HDL (\(< 35 \) mg/dL or \( \geq 35 \) mg/dL), respectively. Relative risk estimates were then calculated from a proportional hazards model in which these discrete variables for total cholesterol and HDL cholesterol replaced the continuous variables for LDL and HDL cholesterol, with the remaining covariates

### Table 3. Crude and Multivariable Adjusted Outcomes by Sinking Prebeta Lipoprotein Band Status

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. of Events</th>
<th>Band Present*</th>
<th>Band Absent*</th>
<th>Crude RR†</th>
<th>MARR‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial infarction</td>
<td>82</td>
<td>5.0</td>
<td>1.7</td>
<td>2.77 (1.74-4.41)</td>
<td>2.37 (1.48-3.81)</td>
</tr>
<tr>
<td>Intermittent claudication</td>
<td>62</td>
<td>3.0</td>
<td>1.4</td>
<td>2.21 (1.25-3.90)</td>
<td>1.94 (1.07-3.50)</td>
</tr>
<tr>
<td>Total cerebrovascular disease</td>
<td>83</td>
<td>3.9</td>
<td>2.0</td>
<td>1.91 (1.15-3.17)</td>
<td>1.88 (1.12-3.15)</td>
</tr>
<tr>
<td>Total coronary heart disease</td>
<td>174</td>
<td>8.2</td>
<td>4.1</td>
<td>1.94 (1.37-2.75)</td>
<td>1.61 (1.13-2.29)</td>
</tr>
<tr>
<td>Total cardiovascular disease</td>
<td>305</td>
<td>12.9</td>
<td>7.7</td>
<td>1.66 (1.26-2.19)</td>
<td>1.44 (1.09-1.91)</td>
</tr>
</tbody>
</table>

*Unadjusted incidence densities (per 1000 person-years of follow-up) and \( \text{t crude and } \text{t multivariate adjusted relative risk estimates (with 95% confidence intervals) for atherosclerotic cardiovascular disease outcomes in subjects with "definite" and "trace" bands pooled vs those with "absent" bands derived from separate proportional hazards models.}

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**Fig. 2.** Plot of age-adjusted Kaplan-Meier comparison of sinking prebeta lipoprotein band groups. MI indicates myocardial infarction.
left intact. Given that HDL of <35 mg/dL is perhaps too stringent a definition for low HDL in women, we also substituted HDL <45 mg/dL for low HDL in an additional model. The relative risk estimates (with 95% confidence intervals) for myocardial infarction in each model are depicted in Table 4. With myocardial infarction as the outcome, the population attributable risk percentage (PAR%) for each of these dichotomous lipid/lipoprotein variables was then calculated according to the formula: \( \text{PAR}\% = \frac{P_{d}(\text{RR}-1)}{P_{d}(\text{RR}-1)+1} \times 100 \), where \( P_{d} \) is equal to the prevalence of the dichotomous lipid/lipoprotein risk factor, and RR is equal to the relative risk estimate for myocardial infarction from the multivariable model. The results displayed in Table 4 reveal that for myocardial infarction, sinking prebeta lipoprotein band presence was associated with a population attributable risk percentage (17.6%) essentially equivalent to elevated total cholesterol (18.7%) and a low HDL cholesterol defined as <45 mg/dL (18.8%).

**Discussion**

Our data confirm previous reports\(^8\,\,^{8,12,13}\) that the detection of a prebeta migrating band during electrophoresis of density >1.006 g/mL plasma is a valid surrogate for substantially increased Lp(a) levels. The analyses are unique in providing the first prospective evidence that elevated plasma Lp(a) detected by electrophoresis is an independent risk factor for CHD in women. In addition, we report the first prospective association between elevated Lp(a) and non-CHD atherothrombotic sequelae, i.e., intermittent claudication and cerebrovascular disease.

We validated sinking prebeta lipoprotein band presence as a surrogate for elevated plasma Lp(a) by comparing quantitative Lp(a) levels determined during Offspring examination 3 (1984 through 1987) with band presence or absence at Offspring examination 1 (1971 through 1975). Despite the difference in sampling dates, Lp(a) concentrations, in contrast to LDL and apo B, appear to remain constant over time and are largely independent of age.\(^5\,\,^{55,60,61}\) Rifai et al.\(^60\) for example, demonstrated that neonatal concentrations of Lp(a) plateaued at stable adult levels by 2 years of age. In addition, cross-sectional data from approximately 9000 women in the Atherosclerosis Risk in Communities\(^6\) and Framingham Offspring\(^6\) studies revealed only a minimal increase in plasma Lp(a) between 40 and 70 years of age. Finally, Jauhiainen et al.\(^6\) found that individual Lp(a) concentrations remained relatively constant over 8.5 years in serial samples drawn from 33 male subjects aged 40 to 55 years old whose plasma Lp(a) was 20 mg/dL.

Only one sizable study\(^8\) has evaluated associations between sinking prebeta lipoprotein band presence and multiple established atherothrombotic risk factors in women. Kawakami et al.\(^8\) noted that the frequency of band positivity was not influenced by age in a hospital-based sample of 529 women ranging in age from 20 to 84 years old. The presence or absence of hypertension, diabetes mellitus, hypercholesterolemia, and smoking was not associated with band status in a subsample of 115 women with proven atherosclerotic disease.\(^8\) We observed modest increases in baseline mean age, systolic blood pressure, body mass index, and LDL cholesterol in a comparison of the band present with the band absent groups. Minor differences in the crude compared with the multivariable adjusted relative risk estimates for all outcomes are evidence of minimal confounding of the effect of band status by these risk factors.

Although negative findings from investigations of limited size have been reported,\(^26\,\,^{28,29,32}\) other retrospective case-control\(^27\,\,^{31,33-36}\) and large cross-sectional studies\(^40\,\,^{52,63}\) demonstrating a positive relation between Lp(a) and clinical atherothrombotic disease in women are consistent with our prospective study. For example, two case-control studies have linked elevated Lp(a) with PVD in women,\(^27\,\,^{39}\) with the data of Norrgard et al.\(^37\) suggesting that Lp(a) may be a better predictor of PVD in women than in men. Dahlen et al.\(^40\) were the first to report a significant association between Lp(a) levels and the extent of coronary artery disease on angiography in a moderately sized (n=86) series of women. More recently, Solymoss et al.\(^42\) demonstrated that Lp(a) level was the best predictor of both the presence and severity of coronary atherosclerosis, after control for nonlipoprotein risk factors, in a consecutive series of 300 young women (<60 years old) undergoing elective arteriography for ischemic heart disease. Finally, data from the largest angiographic series where Lp(a) levels were determined in both women (n=323) and men (n=731) have been reported by Labeur et al.\(^53\) Comparing the highest with lowest quintile groups for Lp(a) concentration, they observed a significant multivariable adjusted odds ratio for angiographic CHD presence in women but not men.

Lp(a) may promote arteriosclerosis through atherogenic and thrombogenic pathomechanisms.\(^25\,\,^{24,64,78}\) Re-combinant apo (a)\(^64\) native Lp(a),\(^64\) and oxidatively modified Lp(a)\(^65\) can all be taken up by macrophages in vitro, suggesting a role for Lp(a) in foam cell formation. In vivo, Lp(a)/apo (a) accumulated in atherosclerotic plaques excised from either killed nonhuman primates\(^66\)
or transgenic mice expressing human apo (a)67 that were fed atherogenic diets. Earlier, Rath et al68 demonstrated that Lp(a) accumulated in arterial wall biopsies taken from the ascending aorta in patients undergoing aortocoronary bypass surgery. A significant correlation between serum and aortic tissue levels was demonstrable for apo (a) but not apo B.68 Potential antifibrinolytic effects of Lp(a) have also been described in vitro.69-74 High concentrations of Lp(a) reduce plasminogen binding to endothelial cells, mononuclear cells, and platelets, possibly interfering with plasminogen activation on the endothelial surface and the lysis of platelet-rich thrombi.69,70 Lp(a) may further interfere with plasminogen activation on the thrombus surface by competitively inhibiting plasminogen and tissue-type plasminogen activator (TPA) binding to fibrin.71,72 Reversible binding of TPA to surface-bound Lp(a) has been shown in vitro73; in vivo, a similar interaction may occur. Lp(a) binding to the cell surface glycosaminoglycans, which normally inhibit thrombin formation, ie, heparin and heparan sulfate, has also been demonstrated.74 Evidence that Lp(a) inhibits fibrinolysis and promotes occlusive thrombosis in vivo was provided by two recent studies.75,76 Williams et al75 experimentally injured a common carotid artery segment in 18 cynomolgus monkeys with low (n=6), medium (n=6), or high (n=6) plasma Lp(a) concentrations. All six monkeys with high (>35 mg/dL) Lp(a) concentrations developed occlusive thrombosis with permanent cessation of flow versus only one of the six low-Lp(a) monkeys. Moliterno et al76 studied 105 myocardial infarction survivors who had not received thrombolytic therapy. Patients were divided into patent (n=52) or occluded (n=53) infarct-related artery groups. Clinical features and lipoprotein or heparinostatic plasma constituents did not differ between the groups except for a mean Lp(a) of 49.1 mg/dL in the patients with occluded infarct-related arteries versus 18.5 mg/dL in those with patent infarct-related arteries.

Potential noncausal explanations for our findings include misclassification of either exposures or outcomes and confounding. By design, baseline assessment of exposure status (ie, various risk factors, including band status) and prospective outcome determination were performed independently, precluding serious differential misclassification. Nondifferential misclassification of the main independent variable, band status, was quite possible given the modest sensitivity (~51%) of this measure but would probably have biased our results toward lower relative risk estimates for the various outcomes.77 Comparison of the crude and multivariable adjusted relative risk estimates for all the atherothrombotic outcomes studied indicates that there was little confounding of the effect of band status by the major Framingham risk factors. Weak to modest correlations between Lp(a) and fibrinogen levels have been noted by some78,79 but not other investigators.80 A subset analysis suggested that our results were not likely to have been confounded by an association between elevated fibrinogen concentrations and band status. Important unmeasured confounders could have influenced our results. Distinct from plasma Lp(a) concentration, apo (a) isoform size81 and percent lysine binding capacity82 may confer independent antifibrinolytic properties. Apo (a) isoform size distribution and lysine binding affinity were not determined in the present study, so we are unable to exclude the possibility that our results were confounded by these qualitative characteristics of Lp(a). Future studies of the Framingham population will be incorporating such qualitative Lp(a) assays.

Lp(a) has proven refractory to standard lipid-lowering pharmacotherapy with resins,83 fibrates,84 HMG-CoA reductase inhibitors,85,86 and probucol.87 Reductions in plasma Lp(a) have been reported with seemingly contradictory hormonal regimens consisting of either androgenic88 or estrogenic compounds.89 The clinical efficacy of estrogen replacement therapy for Lp(a) lowering in women needs to be established in controlled trials. Consistent reductions in plasma Lp(a) have been reported, however, with nicotinic acid.90,91 Until further confirmation of our prospective findings is reported, primary or secondary CVD prevention trials of Lp(a) reduction in women (eg, with nicotinic acid) would not appear to be warranted.

In summary, detection of a sinking prebeta lipoprotein band on electrophoresis was a valid marker for elevated Lp(a) levels in Framingham Heart Study women who were free of prevalent CVD. Band presence and, equivalently, elevated plasma Lp(a) predicted the development of atherothrombotic outcomes, especially myocardial infarction, in these women. Confirmation of these findings in other longitudinal studies of women is needed.

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