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Lipids and Lipoproteins

Lipoprotein(a)-Cholesterol and Coronary Heart Disease in the Framingham Heart Study

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Background: **Increased plasma lipoprotein(a) [Lp(a)] concentrations have been reported to be an independent risk factor for coronary heart disease (CHD) in some prospective studies, but not in others. These inconsistencies may relate to a lack of standardization and the failure of some immunoassays to measure all apolipoprotein(a) isoforms equally.**

Methods: **We measured plasma Lp(a)-cholesterol [Lp(a)-C] in a Caucasian population of offspring and spouses of the Framingham Heart Study participants, using a lectin-based assay (Lipopro™). We compared the prevalence of increased Lp(a)-C to the presence of sinking pre-β-lipoprotein (SPB). We also related Lp(a)-C concentrations to the prevalence of CHD risk in the entire population.**

Results: **The mean (± SD) Lp(a)-C concentration in the Framingham population (n = 3121) was 0.186 ± 0.160 mmol/L, with no significant gender or age differences. The mean Lp(a)-C concentrations in the absence or presence of SPB were 0.158 ± 0.132 mmol/L and 0.453 ± 0.220 mmol/L, respectively (P <0.0001). The mean Lp(a)-C concentration in men with CHD (n = 156) was 0.241 ± 0.204 mmol/L, which was significantly (P <0.001) higher, by 34%, than in controls. The odds ratio for CHD risk in men with Lp(a)-C ≥0.259 mmol/L (≥10 mg/dL), after adjusting for age, HDL-cholesterol, LDL-cholesterol, smoking, diabetes, blood pressure, and body mass index, was 2.293 (confidence interval, 1.55–3.94; P <0.0005). Lp(a)-C values correlated highly with a Lp(a)-mass immunoassay [Apotek™ Lp(a); r = 0.832; P <0.0001; n = 1000].**

Conclusions: **An increased Lp(a)-C value ≥0.259 mmol/L (≥10 mg/dL) is an independent CHD risk factor in men with a relative risk of more than 2, but was inconclusive in women. Lp(a)-C measurements offer an alternative to Lp(a)-mass immunoassays and can be performed on automated analyzers.**

In 1963, Kåre Berg *(1)* first described lipoprotein(a) [Lp(a)][5] as a lipoprotein antigen that was more prevalent in the plasma of myocardial infarction survivors than in an age-matched control group of Scandinavian men.

Apolipoprotein (a) [apo(a)] is known to be a very heterogeneous glycoprotein that shares at least 75% homology with plasminogen, including domains of plasminogen referred to as kringle 4, kringle 5, and the protease domain. There is no unique domain of the apo(a) gene that does not share homology with plasminogen *(2)*. apo(a) is highly glycosylated *(3, 4)*, with numerous O-glycosidic linkages in the regions between the kringle domains *(5)*. At least 34 phenotypes are expressed, ranging from 12 to 50 kringles *(6, 7)*, and there are potentially more phenotypes and genotypes that may exist.

Increased Lp(a) concentrations have been described in numerous case-control studies as correlating with coronary heart disease (CHD) *(8–15)*. There have been several prospective studies that have identified Lp(a) as a CHD risk factor *(16–25)*, although not all have been positive

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[5] Nonstandard abbreviations: Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); CHD, coronary heart disease; SPB, sinking pre-β-lipoprotein; Lp(a)-C, lipoprotein(a)-cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; HRT, hormone replacement therapy; and CI, confidence interval.

(26–29). The measurement of Lp(a) mass has been the "standard" since the formulation of the first radioimmunoassay by Albers and Hazzard *(30)* in 1974. Because of variability in apo(a) isoform size, we do not believe that Lp(a) should be standardized on the basis of mass concentrations. Immunoassays may not provide an accurate measure of Lp(a) concentration if the antibodies in the assay bind to epitopes that may be present in apo(a) in multiple copies or if a single epitope within apo(a) is altered by conformational changes because of large variations in apo(a) size. Marcovina et al. *(31)* have reported that an isoform size bias, which misrepresents the true Lp(a) concentration, can occur with some assays, although this is less of a problem with other assays *(31, 32)*.

Three prospective studies that measure Lp(a) as sinking pre-β-lipoprotein (SPB) *(19–21)* strongly support Lp(a) as an important CHD risk factor. The method used in those studies may be reliable, in part, because of its lack of reliance on immunodetection, its dependence on lipid detection, and its measurement of fresh plasma samples, rather than frozen, archived samples. However, SPB analysis is only semiquantitative.

A novel assay has been designed to avoid the difficulties that may arise with immunodetection of various isoforms of apo(a) by nonspecifically trapping Lp(a) with a lectin, and then measuring the cholesterol in the lectin-bound fraction using standard enzymatic cholesterol assays, standardized to a lower range of detection [0–1.297 mmol/L (0–50 mg/dL)]. This method has been described, characterized, and shown to correlate highly with an immunoassay (Macra™; Wampole Laboratories) *(33)*. To further evaluate Lp(a)-cholesterol [Lp(a)-C] as a CHD risk factor, we measured Lp(a)-C in plasma samples collected from participants in the Framingham Offspring Study between 1990 and 1994 and compared them to SPB results generated on the same subjects between 1970 and 1974 *(19, 20)*. It is well documented that there are no significant changes in Lp(a) concentrations over time *(34)*, although cross-sectional data suggest some small effect *(35)*. In addition, we compared the results of Lp(a)-C analysis in a subset of 1000 subjects with Lp(a)-mass values generated with the Apotek™ Lp(a) Assay (Sigma).

Materials and Methods

Plasma samples from offspring and their spouses among the Framingham Heart Study participants were routinely collected, as described previously, after an overnight fast *(36)*. Samples collected from 3332 participants at exam 5, between 1991 and 1995, were stored at −80 °C for 2–6 years without being subjected to thawing and were then analyzed with the Lipopro™ Lp(a)-cholesterol kit (Genzyme Diagnostics), according to directions contained in the package insert. In brief, 200 μL of Lipopro Lp(a) reagent was added to the top filter chamber of a microtube. Plasma (200 μL) was then added and mixed by vortex-mixing *(33)*. The mixture was allowed to stand for 5 min at ambient room temperature before being centrifuged for 1 min at 12 000*g* in a microcentrifuge. Wash buffer (200 μL) was then added to the top filter chamber of each tube, vortex-mixed, and incubated for 5 min before the centrifugation step was repeated. After the second centrifugation, the lower chamber, containing unretained plasma and wash, was replaced with a new tube. Elution buffer (200 μL) was then added to the upper chamber, allowed to incubate for 5 min, and centrifuged for 3 min at 12 000*g*. This eluate that contained the Lp(a) was then subjected to a standard enzymatic cholesterol assay, calibrated to the range 0–1.3 mmol/L (0–50 mg/dL). Our laboratory participates in the CDC/National Heart, Lung, and Blood Institutes Cholesterol Standardization Program.

SPBs were obtained using methods described previously *(19, 20)*. In brief, fresh plasma samples were collected from subjects, ultracentrifuged to remove VLDL (d <1.006 kg/L), and immediately blotted onto cellulose acetate strips, which were then applied to an electrophoresis apparatus containing barbital buffer. Samples were then subjected to 1.5 V/cm for 16 h. The cellulose acetate strips were then stained with oil red O. Samples were then all read at the same time, using the same reader (WPC), and classified as "positive", "trace", or "not present".

To compare Lp(a)-C results directly to Lp(a)-mass concentrations, plasma samples from a subset of 1000 Framingham Offspring Study participants were also subjected to Lp(a)-mass measurement with the Apotek Lp(a)-mass assay, an immunoassay that traps Lp(a) with a polyclonal antibody to apo(a) and detects with polyclonal antibodies to apolipoprotein B, making the assay less likely to be affected by changes in isoform size. The assay was performed according to the package insert instructions, and the results have been published elsewhere *(32)*.

Other plasma lipoproteins were determined as described previously *(36)*. Total cholesterol was measured on an Abbott Spectrum CCX, using the cholesterol esterase/cholesterol oxidase method (Sigma), according to the package insert. Triglycerides were also measured using this automated method, measuring glycerol after hydrolysis, according to package insert specifications (Sigma). HDL-cholesterol (HDL-C) was determined using Mg^{2+}/dextran precipitation of lipoproteins containing apolipoprotein B, followed by cholesterol measurement of the unprecipitated HDL fraction, using a standard cholesterol esterase/cholesterol oxidase assay. LDL-cholesterol (LDL-C) was calculated using the formula developed by Friedewald et al. *(37)* for subjects with triglycerides <4.0 g/L (<400 mg/dL).

All prevalent CHD cases were identified at or before exam 5 (through 1995). A panel of three physicians examined the records of all potential CHD cases and confirmed the presence of CHD according to the criteria established for the analysis of the Framingham Heart Study, as described elsewhere *(38)*. CHD includes angina pectoris, myocardial infarction, coronary insufficiency,

and coronary death. No cases were prospectively identified.

The statistics were analyzed using the SAS program (SAS Institute). Subjects with prevalent CHD were compared with those with no evidence of CHD on a variety of known risk factors, including hypertension, diabetes, smoking history, LDL-C, HDL-C, and triglycerides as well as Lp(a)-C, SPB, and Lp(a) mass. A corrected LDL-C was calculated by directly subtracting Lp(a)-C from LDL-C. The Student *t*-test was used to compare the mean values of continuous measures. A χ^2 test was calculated for categorical factors. We log-transformed highly skewed continuous measures to normalize the distribution before assessing them in a comparison between subjects with and without CHD. Although the untransformed means and standard deviations were reported, *P* values for the analyses of the transformed variables were reported. To evaluate the relationship between Lp(a)-C and SPB, different percentiles of Lp(a)-C were compared with the presence of SPB (both borderline present and present). The approximate 75th percentile for Lp(a)-C (0.259 mmol/L) was chosen as a cutoff point because this is the approach taken by the National Cholesterol Education Program for LDL-C. The sensitivity and specificity for Lp(a) were calculated using SPB as the reference; thus the sensitivity was the proportion of SPB-positive subjects who had Lp(a) ≥0.259 mmol/L, and the specificity was the proportion of SPB-negative subjects who had Lp(a) <0.259 mmol/L. To adjust for known risk factors for CHD, we used logistic regression analysis for the presence or absence of prevalent CHD at exam 5. Lp(a)-C was assessed both as a continuous variable and by group analysis (less than or equal to, or greater than 0.259 mmol/L).

Results

Lp(a)-C values were obtained on a total of 3332 subjects. There were 149 subjects with duplicate identification numbers whose identities could not be verified and who were therefore removed from the analysis. Complete information on the CHD risk factors of an additional 62 subjects was not available. The final analysis of Lp(a)-C and CHD risk factors was performed on a total of 3121 subjects: 1488 men and 1633 women. This population was nearly all Caucasian.

The characteristics of the Framingham Offspring Subjects are shown in Table 1 and display the gender differences in this population. These data obtained from the Framingham Heart Study provide normal ranges for Lp(a)-C. There were no significant gender or age differences in Lp(a)-C concentrations. The mean Lp(a)-C values were 0.186 ± 0.160 mmol/L (7.21 ± 6.15 mg/dL) in men and 0.182 ± 0.166 mmol/L (7.06 ± 6.42 mg/dL) in women, with an overall mean of 0.185 ± 0.163 mmol/L (7.13 ± 6.29 mg/dL). As noted in the percentile distribution data in Table 2, the Lp(a)-C values were highly skewed, with a skewness of 1.76 (1.65 in men and 1.86 in women) and a kurtosis of 3.80 (3.17 in men and 4.30 in women). The distribution patterns were consistent with the distribution of Lp(a) mass seen in the Framingham population *(35)* and other Caucasian populations, measured with immunoassays *(39)*.

Lp(a)-mass results obtained with the Apotek Lp(a) assay for a subset of 1000 Framingham Heart Study

Table 1. Gender differences in the Framingham offspring (mean ± SD).

Characteristic	Units	Men	Women	*P*
n		1488	1633	
Age	years	55.5 ± 10.0	55.0 ± 9.9	NS[a]
Systolic BP	mmHg	129.3 ± 17.8	124.8 ± 20.2	<0.0001
Diastolic BP	mmHg	76.7 ± 10.1	72.8 ± 10.3	<0.0001
Glucose	mmol/L	5.75 ± 1.77	5.38 ± 1.52	<0.0001
	(mg/dL)	(105.5 ± 32.4)	(98.5 ± 27.8)	
Total cholesterol	mmol/L	5.20 ± 0.91	5.39 ± 0.98	<0.0001
	(mg/dL)	(201 ± 35)	(208 ± 38)	
Triglycerides	mmol/L	1.84 ± 1.36	1.54 ± 1.01	<0.0001
	(mg/dL)	(163 ± 121)	(136 ± 89)	
LDL-C	mmol/L	3.31 ± 0.81	3.25 ± 0.88	0.02
	(mg/dL)	(128 ± 31)	(125 ± 34)	
LDL-C (corr)[b]	mmol/L	3.13 ± 0.80	3.08 ± 0.85	0.03
	(mg/dL)	(121 ± 31)	(119 ± 33)	
HDL-C	mmol/L	1.10 ± 0.29	1.44 ± 0.40	<0.0001
	(mg/dL)	(42.7 ± 11.0)	(55.6 ± 15.3)	
Lp(a)-C	mmol/L	0.186 ± 0.160	0.182 ± 0.166	NS
	(mg/dL)	(7.21 ± 6.15)	(7.06 ± 6.42)	
Current smoking	%	19.1	19.4	NS
Prevalent CHD	%	10.4	3.5	<0.0001

[a] NS, not significant; BP, blood pressure.
[b] LDL-C (corr) = LDL-C − Lp(a)-C.

Table 2. Percentiles for Lp(a)-C distribution [mmol/L (mg/dL)].

			Percentile						
Gender	n	Mean ± SD	5	10	25	50	75	90	95
Male	1488	0.186 ± 0.160 (7.21 ± 6.15)	0.035 (1.35)	0.045 (1.73)	0.070 (2.70)	0.130 (5.04)	0.263 (10.16)	0.409 (15.82)	0.508 (19.62)
Female	1633	0.182 ± 0.166 (7.06 ± 6.42)	0.032 (1.24)	0.041 (1.58)	0.065 (2.50)	0.123 (4.74)	0.246 (9.51)	0.408 (15.75)	0.519 (20.06)
All	3121	0.185 ± 0.163 (7.13 ± 6.29)	0.034 (1.31)	0.043 (1.66)	0.067 (2.58)	0.127 (4.90)	0.252 (9.73)	0.409 (15.78)	0.514 (19.83)

participants were similarly skewed, with a skewness of 2.34 and a kurtosis of 9.42, a mean Lp(a) mass of 0.260 ± 0.295 g/L (25.97 ± 29.49 mg/dL), and no significant gender or age differences. Lp(a)-C was highly correlated with Lp(a) mass in both men and women ($P < 0.0001$), with Pearson correlation coefficients for the log-transformed Lp(a)-C of 0.829 and 0.835, respectively. Despite the skewed distribution, differences in measurements (cholesterol vs mass), and the wider total population variance of the Lp(a)-mass values (SD, 113% of mean) when compared with Lp(a)-C (SD, 88% of mean), comparisons of all subjects at the 75th percentile [Lp(a) mass of 0.344 g/L and Lp(a)-C of 0.259 mmol/L] were similar, with 93% specificity and 82% sensitivity in men and 93% specificity and 85% sensitivity in women.

In men, Lp(a)-C had a curvilinear relationship with age, grouped by decade (Table 3), but failed to attain statistical significance. In women, there was a linear relationship between Lp(a)-C and age delineated by decade, with the largest difference in the fifth decade, when most women reach menopause. Among the women, there also were differences in Lp(a)-C values that were dependent on menopausal status (Table 4). When pre- and postmenopausal women were compared, only 16.27% of premenopausal women had Lp(a)-C ≥0.259 mmol/L, compared with 26.73% of postmenopausal women ($P < 0.001$). Because menopause is an age-related phenomenon, it is difficult to separate the effect of age from menopause. When compared with men, women (Table 3) had a much larger gradation of Lp(a)-C values across increasing decades, which was sustained after the fifth decade, whereas in men, the gradation decreased dramatically after the sixth decade. Furthermore, an estrogen effect was supported by the 17.6% lower mean Lp(a)-C concentrations ($P < 0.01$) in women without CHD and receiving hormone replacement therapy [HRT; Lp(a)-C, 0.155 ± 0.150 mmol/L (5.98 ± 5.79 mg/dL); n = 281] compared with women the same mean age (55 ± 10 years) without CHD and not receiving HRT [Lp(a)-C, 0.188 ± 0.170 mmol/L (7.26 ± 6.57 mg/dL); n = 1284].

SBP measurements of Lp(a) were performed in this population and have been reported elsewhere to predict the risk of CHD in men <55 years *(20)* and in women >55 years *(19)*. Numerous studies, reviewed by Bostom et al. *(20)*, have demonstrated that the presence of an SPB band corresponds to Lp(a) and is highly specific for Lp(a)-mass concentrations of 0.3 g/L (30 mg/dL) or more. SPBs were reported as being "present", "borderline present", or "not present". A comparison of the mean Lp(a)-C concentrations for these three categories is shown in Table 5 and clearly shows a gradation from the absence of SPB [0.157 ± 0.131 mmol/L (6.08 ± 5.06 mg/dL)] to borderline present [0.359 ± 0.209 mmol/L (13.89 ± 8.06 mg/dL)] to present [0.453 ± 0.0.221 mmol/L (17.49 ± 8.54 mg/dL)], with very little overlap between the categories "absent" and "present". Subjects with Lp(a)-C concentrations ≥0.259 mmol/L (≥10 mg/dL) are 11.6 (odds ratio) times more likely to have a SPB band (present or borderline) than subjects with Lp(a)-C concentrations <0.259 mmol/L ($P < 0.0002$). The sensitivity and specificity with SPB as the reference are 72.1% and 81.8%, respectively.

The mean Lp(a)-C concentrations in men and women

Table 3. Differences in Lp(a)-C [mmol/L (mg/dL)] concentrations with age.

	Men		Women	
Age, years	n	Mean ± SD	n	Mean ± SD
30–39	81	0.173 ± 0.152 (6.70 ± 5.88)	80	0.148 ± 0.122 (5.73 ± 4.73)
40–49	363	0.182 ± 0.147 (7.04 ± 5.67)	434	0.156 ± 0.136 (6.02 ± 5.26)
50–59	477	0.193 ± 0.174 (7.46 ± 6.71)	524	0.188 ± 0.174 (7.25 ± 6.74)
60–69	446	0.192 ± 0.159 (7.41 ± 6.16)	470	0.205 ± 0.181 (7.93 ± 7.01)
70–79	116	0.165 ± 0.138 (6.38 ± 5.33)	118	0.195 ± 0.179 (7.54 ± 6.91)

Table 4. Comparison of pre- and postmenopausal women from Framingham offspring (mean ± SD).

Characteristic	Units	Premenopausal women	Postmenopausal women	P
n		677	1321	
Age	years	45.2 ± 5.4	59.7 ± 7.8	<0.0001
BMI[a]	kg/m^2	25.3 ± 5.5	26.8 ± 5.5	<0.0001
Systolic BP	mmHg	115.3 ± 17.5	128.9 ± 20.1	<0.0001
Diastolic BP	mmHg	71.6 ± 10.5	73.5 ± 10.1	<0.0003
Glucose	mmol/L	5.05 ± 0.86	5.53 ± 1.70	<0.0001
	(mg/dL)	(92.5 ± 15.7)	(101.2 ± 31.3)	
Total cholesterol	mmol/L	4.95 ± 0.87	5.61 ± 0.97	<0.0001
	(mg/dL)	(191 ± 34)	(217 ± 38)	
LDL-C	mmol/L	2.93 ± 0.78	3.40 ± 0.90	<0.0001
	(mg/dL)	(113 ± 30)	(131 ± 35)	
LDL-C (corr)[b]	mmol/L	2.79 ± 0.75	3.29 ± 0.85	<0.0001
	(mg/dL)	(108 ± 29)	(127 ± 33)	
HDL-C	mmol/L	1.45 ± 0.39	1.45 ± 0.41	NS
	(mg/dL)	(56.0 ± 14.9)	(55.9 ± 15.8)	
Triglycerides	mmol/L	1.25 ± 0.94	1.71 ± 1.27	<0.0001
	(mg/dL)	(111 ± 83)	(151 ± 112)	
Lp(a)-C	mmol/L	0.152 ± 0.130	0.197 ± 0.179	<0.0001
	(mg/dL)	(5.88 ± 5.02)	(7.63 ± 6.93)	

[a] BMI, body mass index; BP, blood pressure; NS, not significant.
[b] LDL-C (corr) = LDL-C − Lp(a)-C.

with and without CHD are compared in Table 6. There was a statistically significant difference between men with CHD [0.241 ± 0.166 mmol/L (9.32 ± 7.87 mg/dL)] and men without CHD [0.180 ± 0.152 mmol/L (6.96 ± 5.87 mg/dL); $P < 0.001$]. On the other hand, there was no significant difference between women with CHD [0.199 ± 0.158 mmol/L (7.71 ± 6.10 mg/dL)] and women without CHD [0.182 ± 0.166 mmol/L (7.03 ± 6.43)]. However, the prevalence of CHD in women was only 3.5% (68 cases out of 1633 subjects), compared with 10.4% (156 cases out of 1488 subjects) in men (see Table 1) in this cohort, which was relatively young (55 ± 10 years) for assessing CHD in women.

Logistics regression analysis (Table 7) for the association of Lp(a)-C with CHD was performed, using Lp(a)-C as a continuous variable and grouping Lp(a) ≥0.259 mmol/L (≥10 mg/dL), to compare the indicator that best correlated with the presence (present and borderline present) of SPB. The relative odds of CHD attributable to increased Lp(a)-C were adjusted for age, LDL-C, HDL-C, smoking, diabetes, blood pressure, and body mass index.

Table 5. Relationship between Lp(a)-C and SBP.[a]

Band status	n	Population, %	Lp(a)-C, mean ± SD
None	2971	88.4	0.157 ± 0.131 mmol/L
			(6.08 ± 5.06 (mg/dL)
Borderline	248	7.4	0.359 ± 0.209 mmol/L
			(13.89 ± 8.06 mg/dL)
Present	143	4.2	0.453 ± 0.221 mmol/L
			(17.49 ± 8.54 mg/dL)

[a] Sensitivity, 72.1%; specificity, 81.8%; odds ratio, 11.6 for SBP if Lp(a)-C ≥0.259 mmol/L (10 mg/dL) vs <0.259 mmol/L (10 mg/dL); P <0.0002.

The Lp(a)-C values were log-transformed to better normalize the distribution of this highly skewed indicator. As a continuous variable, Lp(a)-C in men had an odds ratio of 1.483 [95% confidence interval (CI), 1.186–1.854; P <0.0005] for each unit increase on a log scale, whereas the odds ratio of 1.102 was not significant (95% CI, 0.824–1.437) in women. To better isolate Lp(a)-C and LDL-C [which contains LDL and intermediate-density lipoprotein as well as Lp(a)-C], the analysis was repeated with a corrected LDL-C (LDL-C minus Lp(a)-C). Pearson correlation coefficients showed a significant correlation between LDL-C and Lp(a)-C in men (r =0.146; P = 0.0001), which disappeared when analysis was repeated with the LDL-C corrected for Lp(a)-C (r = −0.028; P = 0.2935). The logistics regression analysis with corrected LDL-C values was slightly lower than with the uncorrected LDL-C

Table 6. Relationship between Lp(a)-C and CHD.

Subjects	n	Mean ± SD
Men without CHD	1332	0.180 ± 0.152 mmol/L
		(6.96 ± 5.87 mg/dL)
Men with CHD	156	0.241 ± 0.204 mmol/L
		(9.32 ± 7.87 mg/dL)
Women without CHD	1565	0.182 ± 0.166 mmol/L[a]
		(7.03 ± 6.43 mg/dL)
Women with CHD	68	0.199 ± 0.158 mmol/L
		(7.71 ± 6.10 mg/dL)

[a] The mean value for women without CHD not receiving HRT was 0.188 ± 0.170 mmol/L (7.26 ± 6.57 mg/dL; n = 1204); for women with CHD not receiving HRT, the mean value was 0.200 ± 0.160 mmol/L (7.74 ± 6.17 mg/dL; n = 62). The mean value for women receiving HRT hormone was 0.155 mmol/L (5.98 mg/dL; n = 281).

Table 7. Logistic regression analysis for association of Lp(a)-C with CHD.[a]

	Men	Men[b]	Women
Lp(a)-C			
Odds ratio[c]	1.483	1.396	1.102
95% CI	(1.186–1.854)	(1.122–1.736)	(0.824–1.473)
P	<0.0005	0.0027	NS[d]
Lp(a)-C ≥0.259 mmol/L			
Odds ratio	2.293	2.074	0.760
95% CI	(1.549–3.94)	(1.407–3.057)	(0.390–1.481)
P	<0.0001	0.0002	NS

[a] Adjusted for age, LDL-C, HDL-C, smoking, diabetes, blood pressure, and body mass index.

[b] Calculated with LDL corrected for Lp(a)-C [LDL-C − Lp(a)-C, in mg/dL].

[c] Odds ratio per one-unit increase in log scale.

[d] NS, not significant.

values, but with tighter confidence intervals (Table 7). To compare the Lp(a)-C indicators that match SPB, the same adjusted analysis for Lp(a)-C ≥0.259 mmol/L (≥10 mg/dL) in men yielded an odds ratio of 2.293 (95% CI, 1.549–3.94; P <0.0001). Again, a repeat analysis with corrected LDL-C values yielded a slightly lower odds ratio, but with tighter confidence limits (Table 7). However, the odds ratio was not significant in women, with a value of 0.760 (95% CI, 0.390–1.481). The use Lp(a)-C values as a surrogate for SPB, a measurement of Lp(a) that confers attributable risk of CHD, yields similar results as previously published SPB prevalence data in men.

Discussion

These analyses from the Framingham Heart Study provide normal ranges for the Lipopro Lp(a)-cholesterol assay, as well as prevalence data to generate odds ratios for CHD with various concentrations of Lp(a)-C. This is the first report of Lp(a)-C measurements within a population large enough to determine these indicators, with distribution patterns being very similar to the Lp(a)-mass values measured for this same population, using the commercially available Apotek assay. Although Lp(a)-C measurements have not been used traditionally, they provide an easy comparison to the other cholesterol-containing lipoprotein measurements, as well as a true method for correcting LDL-C concentrations for the contribution of Lp(a)-C.

The direct comparison of Lp(a)-C to SPB values provides indicators that are believed to predict the relative risk of CHD in both men and women. The odds ratio of 11.6 for Lp(a)-C ≥0.259 mmol/L (10 mg/dL) to be found in subjects with the presence of SPB, with a sensitivity of 72.1% and a specificity of 81.8%, supports the similarities of these two measurements of Lp(a). Both of these methods are dependent on the detection of the lipid content of Lp(a), rather than an immunodetection method. Prevalence data support the use of Lp(a)-C ≥0.259 mmol/L (≥10 mg/dL) in predicting CHD risk in men, whereas the data are less clear for women. However, the prevalence of CHD in this population of women was only 3.5% (68 cases), whereas the prevalence of CHD in men was 10.4% (152 cases). Lp(a)-C will need to be assessed prospectively in this population to determine the true relative risk of CHD in both men and women.

The results in women may be further confounded by the effect of estrogen status on Lp(a)-C concentrations. Although Jenner et al. *(35)* demonstrated that Lp(a)-mass values corrected for age were not different in pre- and postmenopausal women, menopause is an age-related phenomenon that cannot be clearly separated from this indicator. To support this further, our study provides evidence that when women receiving HRT are compared with women of the same age not receiving estrogen, there is a 17.6% difference in the mean Lp(a)-C between these two groups. Furthermore, a comparison of Lp(a)-C mean concentrations by decade demonstrates a continuous upward gradation that is sustained in women beyond the fifth decade, but falls in men beyond the sixth decade. The effect of estrogen status on Lp(a)-C is further supported by the work of Kim et al. *(40)*, who reported a 23% reduction in Lp(a) mass in postmenopausal women placed on HRT (conjugated equine estrogens, 0.625 mg/day, and medroxy progesterone acetate, 2.5 mg/day); when HRT was discontinued, the concentration of Lp(a) mass returned to normal *(41)*.

In summary, this analysis in the offspring and spouses of the Framingham Heart Study establishes normal ranges of Lp(a)-C for men and women. Our analysis confirms a similarly skewed distribution pattern of Lp(a)-C values when compared with those we reported in another publication *(35)*, using another commercially available immunoassay (Macra). The Lp(a)-C values correlated highly with Apotek Lp(a) mass and with the presence or absence of SPB. However, in contrast to SPB, the Lp(a)-C assay provides a quantitative measure. Our data indicate that a Lp(a)-C concentration ≥0.259 mmol/L (≥10 mg/dL) is a useful tool in predicting the risk of CHD in men. Regarding CHD risk, the relationship between Lp(a) and LDL-C, which was first reported by Armstrong et al. *(42)*, is likely because of the presence of Lp(a)-C in the LDL-C value; this relationship can be directly removed from the analysis by subtracting Lp(a)-C from LDL-C when using the Lipopro Lp(a)-cholesterol assay. Lp(a)-C measurements provide useful information for CHD risk assessment. In assessing the risk of Lp(a)-C, especially in analyses that compare it with LDL-C, it may be appropriate to use a corrected LDL-C to isolate Lp(a)-C from the LDL-C value. It would be inappropriate to always correct LDL-C, because most LDL-C measurements include cholesterol from LDL, intermediate-density lipoprotein, and Lp(a). Moreover, the Lp(a)-C method may be easier to standardize, both nationally and internationally, than immunoassay methods.

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