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## Lipoprotein-Associated Phospholipase A<sub>2</sub> Activity Improves Risk Discrimination of Incident Coronary Heart Disease Among Women

Ida J Hatoum, ScD, Nancy R Cook, ScD, Jeanenne J Nelson, PhD, Kathryn M Rexrode, MD, and Eric B Rimm, ScD

Departments of Nutrition and Epidemiology (IH, ER), Harvard School of Public Health, Boston; Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston (ER); Donald W. Reynolds Center for Cardiovascular Research and the Center for Cardiovascular Disease Prevention, Division of Preventive Medicine (NC, KR), Brigham and Women's Hospital, Boston; GlaxoSmithKline, Research Triangle, North Carolina (JN)

### Abstract

**Background**—This study sought to determine the relation between and discriminative capability of Lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) and coronary heart disease (CHD) in a large population of disease-free women.

**Methods**—Among participants of the Nurses' Health Study who provided a blood sample, there were 421 cases of incident myocardial infarction (MI) during 14 years of follow-up. Controls were matched to cases 2:1 using risk-set sampling based on age, smoking, and blood draw date.

**Results**—Conditioning on the matching factors, Lp-PLA<sub>2</sub> activity was significantly associated with MI (RR=2.86 for extreme quartiles; 95% Confidence Interval (CI): 1.98, 4.12). After adjustment for lipid, inflammatory, and clinical risk factors, the relative risk remained statistically significant was assessed by (RR=1.75; 95% CI: 1.09, 2.84). The discriminative capability of Lp-PLA<sub>2</sub> comparing area under the receiver operating characteristic curves (AUROC) for models with and without Lp-PLA<sub>2</sub>, and by calculating the net reclassification improvement index (NRI). Adding Lp-PLA<sub>2</sub> activity to a multivariable-adjusted model increased the AUROC from 0.720 to 0.733, and significantly improved the NRI (p = 0.004).

**Conclusions**—Levels of Lp-PLA<sub>2</sub> activity were significantly associated with incident CHD among women. In addition, Lp-PLA<sub>2</sub> activity added significantly to CHD risk discrimination.

### INTRODUCTION

Heart disease is the leading cause of death for both men and women in industrialized countries.<sup>1</sup> Although a number of risk factors for heart disease have been identified, coronary heart disease (CHD) still occurs in people who lack any traditional risk factors.<sup>2</sup> The recognition that CHD is an inflammatory disease<sup>3</sup> has led to the search for potential biomarkers that play a role in the etiology of CHD or serve as predictors of CHD risk. Such

Correspondence: Ida Hatoum, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115; [ihatoum@hsph.harvard.edu](mailto:ihatoum@hsph.harvard.edu); 617-432-1843; Fax: 617-432-2435.

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studies have examined a number of these general inflammatory biomarkers, including C-reactive protein,<sup>4</sup> interleukin 6,<sup>5</sup> and tumor necrosis factor  $\alpha$ .<sup>6</sup>

One recently identified inflammatory biomarker, lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>), is a calcium-independent member of the superfamily of phospholipase A<sub>2</sub> enzymes. This 45.4 kDa protein is secreted by monocytes, macrophages, T lymphocytes, and mast cells, and binds to the carboxy terminus of apolipoprotein B-100 to circulate with LDL. Eighty percent of Lp-PLA<sub>2</sub> circulates bound to LDL, with apparent binding preference for small, dense and electronegative LDL.<sup>7</sup> Lp-PLA<sub>2</sub> hydrolyzes the *sn*2 ester bond of oxidized phospholipids to produce lysophosphatidacholine and non-esterified fatty acids,<sup>8</sup> two inflammatory compounds that act within the intima of atherosclerotic lesions to perpetuate inflammation.<sup>8</sup>

Epidemiologic studies of circulating Lp-PLA<sub>2</sub> and cardiovascular disease have consistently demonstrated a positive association for both Lp-PLA<sub>2</sub> concentration<sup>9–11</sup> and Lp-PLA<sub>2</sub> activity<sup>11–14</sup>, in both healthy populations<sup>10, 12, 13</sup> and clinical populations.<sup>9, 11, 14</sup> However, few of these studies of Lp-PLA<sub>2</sub> activity were in large healthy populations. Furthermore, few studies have characterized the predictive capability and potential clinical utility of Lp-PLA<sub>2</sub> activity.<sup>15, 16</sup>

Therefore, the aim of this study is to assess the association between Lp-PLA<sub>2</sub> activity and incident CHD in a large population of disease-free women. In addition, we seek to examine the discriminatory ability of Lp-PLA<sub>2</sub> by analyzing change in AUC as well as the net reclassification after adding Lp-PLA<sub>2</sub> activity to a multivariable model using a novel method that accounts for the sampling structure in this case-control study. In addition to providing information about risk prediction, markers such as Lp-PLA<sub>2</sub> that can add to prediction also can uncover important etiologic pathways for cardiovascular disease.

## METHODS

### Study population

The Nurses' Health Study (NHS) is a prospective cohort study of 121,700 U.S. female nurses who were 30–55 years old at baseline in 1976. Between 1989 and 1990, 32,826 women provided a blood sample. Participants are followed through biennial mailed questionnaires that collect information on lifestyle factors and health behaviors. Participants were followed until their first cardiovascular event, incidence of cancer, or death. Through June 30, 2004, we documented an incident myocardial infarction (MI) among 421 women who were free of cardiovascular disease and cancer at the time of blood draw. These included 352 cases of nonfatal MI and 69 cases of fatal CHD. Mean follow-up time among cases was 7.5 years (SD = 3.9; range 1 month – 14 years). Through risk set sampling methods, two controls free of cardiovascular disease and cancer up to the date of diagnosis of the paired case were randomly selected and matched on age (in two-year categories), smoking (never, past, current: 1–14 cigarettes/day, 15+ cigarettes/day), month of blood draw, fasting status and reported problems during blood draw. This study was approved by the institutional review board of Brigham and Women's Hospital and the Human Subjects Committee Review Board of Harvard School of Public Health. This study was supported by research grant HL34594 from the National Heart Lung and Blood Institute. Additional support was provided by GlaxoSmithKline. GlaxoSmithKline had no access to the data and the academic institution had full and final right to publish.

### Endpoint

Nonfatal MI was confirmed according to the World Health Organization criteria of symptoms plus either typical electrocardiographic changes or elevated cardiac enzyme

levels by physicians blinded to exposure status. MIs that required hospital admission and for which confirmatory information was obtained by interview or letter, but for which no medical records were available (n=9), were designated as probable and included in the analysis. Participant deaths were reported by next of kin, the postal system, and through the records of the National Death Index; follow-up for deaths is estimated to be greater than 98% using these three sources combined.<sup>17</sup> Fatal CHD was confirmed by reviewing medical records or autopsy reports with permission from next of kin. Fatal CHD was defined as ICD-9 codes 410 to 412 if confirmed by hospital records or autopsy, or if CHD was the most likely cause and was listed as the cause of death on the death certificate, along with evidence of prior CHD. We designated as probable fatal CHD those cases in which CHD was the underlying cause on the death certificate but for which no medical records concerning the death were available (n=16), and included these cases in the analyses. Potential cases for whom CHD could not be confirmed were excluded, as were their matched controls. Physicians who reviewed records were blinded to risk factor status.

### Blood collection and laboratory measurements

Participants were sent a blood collection kit that included instructions and supplies. Blood samples were collected in heparin blood tubes, chilled, and sent back by prepaid overnight courier. Upon arrival, samples were centrifuged and stored in cryotubes as plasma, buffy coat, and red blood cells. Cryotubes were then stored in liquid nitrogen freezers at  $-130^{\circ}\text{C}$  or lower.

Lp-PLA<sub>2</sub> activity was measured by CAM-colorimetric activity test automated assay (GlaxoSmithKline, Research Triangle Park, NC) performed on a clinical chemistry analyzer using a colorimetric substrate for Lp-PLA<sub>2</sub>, which is similar to platelet activating factor with the addition of a nitro-phenol label at the sn2 position. Hydrolysis of the colorimetric substrate is monitored by changes in visible absorbance over time (nmols/min/mL) using a standard curve for nitrophenol absorbance. Coefficients of variation (CVs) were 4.3%.

Concentrations of total cholesterol, triglycerides, and HDL cholesterol were measured simultaneously on the Hitachi 911 analyzer using reagents and calibrators from Roche Diagnostics (Indianapolis, IN); CVs were <1.8%. LDL cholesterol concentration was measured by a homogenous direct method from Genzyme (Cambridge, MA); CVs were <3.1%. HbA<sub>1c</sub> concentrations were based on turbidimetric immunoinhibition with hemolyzed whole blood or packed red cells; CVs were <3.0%. ICAM-1 was measured by a commercial enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, Minn); CVs were <3.6%. CRP was measured with the US CRP ELISA kit (Diagnostic Systems Laboratories, Inc, Webster, TX); CV was <5.1%.

### Assessment of lifestyle exposures

On biennial questionnaires participants provided information about their age, weight, smoking status, aspirin use, cholesterol-lowering medication use, hormone therapy use, and physical activity. We calculated BMI as the ratio of weight (in kilograms) over height (in meters) squared. Physical activity was calculated as metabolic equivalents per week using the duration of moderate or vigorous forms of exercise multiplied by the intensity of the activity. History of hypertension, diabetes, and family history of MI were determined from self-reports before blood collection. Alcohol use was estimated with a dietary food frequency questionnaire. Lifestyle exposures used for these analyses were those assessed in 1990.

## Statistical analysis

Differences in baseline characteristics between cases and controls were assessed using Student's t-test for variables expressed as means, by Wilcoxon's rank-sum test for variables expressed as medians, and by the chi-square test for variables expressed as percentages. Age-adjusted Spearman correlations were computed to assess the association between Lp-PLA<sub>2</sub> activity and cardiovascular risk biomarkers. Lp-PLA<sub>2</sub> activity was divided into quartiles based on the distribution among the controls. Odds ratios and 95% confidence intervals were calculated using conditional logistic regression, conditioning on the matching factors.

Because risk set sampling was used, odds ratios directly estimate the incidence rate ratio. Multivariable models additionally adjusted for BMI (kg/m<sup>2</sup>), physical activity (MET hours/week), family history of MI, history of hypertension, history of diabetes, alcohol intake (grams/day), aspirin use (none, 1–2/week, 3–6/week, 7–14/week, 15+/week), cholesterol-lowering medication use, HDL, LDL, HbA<sub>1c</sub>, log CRP, and hormone replacement therapy (never, past, current). Because 55% and 67% of participants were missing ICAM and fasting triglycerides, respectively, these two variables were not included in multivariable models. Tests for linear trends were conducted by treating the median value for each quintile of Lp-PLA<sub>2</sub> activity as a continuous variable. We modeled Lp-PLA<sub>2</sub> activity continuously and tested for deviations from linearity using restricted cubic splines; using this method we found no deviations from linearity. We stratified by time (0 – 6 years of follow-up, > 6 years of follow-up) and assessed the relation between Lp-PLA<sub>2</sub> and CHD using conditional logistic regression based on the overall Lp-PLA<sub>2</sub> distribution among controls. We assessed potential effect modification by HRT use, LDL, CRP, and HDL using -2 Log Likelihood tests comparing an unconditional logistic regression model with the matching factors and main effects only and a model that included the matching factors, main effects, and an additional cross-classification term.

Because this sample is a matched case-control study, any measures that rely on specificity calculations or absolute risk (e.g., the area under the receiver operating characteristic (AUROC) curve) are not valid, as the distribution among controls does not reflect the population distribution.<sup>18</sup> We therefore calculated weights that represent the distribution of age and smoking strata in the total cohort, and used these in all prediction analyses to re-weight the case-control sample to reflect the population distributions of age and smoking among cases and controls. To examine the discriminative capability of Lp-PLA<sub>2</sub> we compared the AUROC for a multivariable unconditional logistic regression model with and without adjustment for Lp-PLA<sub>2</sub>. We also calculated the net reclassification improvement (NRI) of adding Lp-PLA<sub>2</sub> in the model by forming weighted reclassification tables separately for cases and controls.<sup>19</sup> Participants were first categorized into quartiles based on the rank of their predicted risk derived from a multivariable unconditional logistic regression model that included all measured risk factors but that did not include Lp-PLA<sub>2</sub> activity. We then calculated the percentage women classified into higher or lower relative risk categories after inclusion of Lp-PLA<sub>2</sub> into the model. All analyses were performed using SAS software, version 9.1 (SAS Institute Inc, Cary, NC).

## RESULTS

Lp-PLA<sub>2</sub> activity was higher in cases than in controls (Table I). As expected, traditional CHD risk factors, including LDL, HDL, triglycerides, BMI, history of hypertension, history of high cholesterol, history of diabetes, and HbA<sub>1c</sub> were significantly different in cases and controls.

Among controls, Lp-PLA<sub>2</sub> activity was modestly positively correlated with total and LDL cholesterol, apolipoprotein B, fasting triglycerides, ICAM, BMI, and age. Lp-PLA<sub>2</sub> was negatively correlated with HDL cholesterol. No correlation between CRP or HbA<sub>1c</sub> and Lp-PLA<sub>2</sub> activity was observed (Table II).

Conditioning on matching factors, the relative risk of CHD was 2.86 (95%CI: 1.98, 4.12; *P* for trend < 0.001), comparing persons in the highest quartile of Lp-PLA<sub>2</sub> activity to the lowest quartile. After multivariable adjustment for traditional non-lipid risk factors, this relationship was not affected. The multivariate risk of CHD comparing extreme quartiles was 2.60 (95%CI: 1.72, 3.92; *P* for trend < 0.001). Additional adjustment for lipid-related variables, including LDL, HDL, history of high cholesterol, and cholesterol-lowering medication use, only modestly affected the association (RR=1.75; 95%CI: 1.09, 2.84) (Table III). The relation between Lp-PLA<sub>2</sub> activity and CHD is primarily affected by adjustment for HDL in women. There were no significant interactions between Lp-PLA<sub>2</sub> and HDL, LDL, CRP, or hormone therapy use ( $-2 \log$  likelihood *P*-values > 0.05 in all models; data not shown).

Lp-PLA<sub>2</sub> circulates bound to LDL and therefore may contribute to LDL's effect on cardiovascular disease. In the multivariable-adjusted model without Lp-PLA<sub>2</sub>, LDL quartile was a significant predictor of CHD (RR = 1.59 for extreme quartiles; 95%CI: 1.03, 2.45). After further adjustment for Lp-PLA<sub>2</sub> activity, this relationship was attenuated (RR=1.28; 95% CI: 0.81, 2.06).

We examined the relation between Lp-PLA<sub>2</sub> activity and CHD within early (the first six) and later (the last eight) years of follow-up. After multivariable adjustment including LDL, the relation between Lp-PLA<sub>2</sub> and CHD was qualitatively stronger during the first six years of follow-up (Table IV), although an interaction by length of follow-up was not significant (*P*=0.53). The relative risk comparing extreme quartiles in a model conditioning on the matching factors was 3.50 (95%CI: 2.12, 5.77; *P* for trend < 0.001) during the first six years of follow-up and was 2.20 (95%CI: 1.27, 3.80; *P* for trend < 0.001) during the following eight years of follow-up. After multivariable adjustment, this relationship remained statistically significant for the first six years (*p*=0.04), but not for the last eight years (*p*=0.13).

To determine whether Lp-PLA<sub>2</sub> activity adds predictive information to models of CHD risk, we calculated the AUROC for multivariable-adjusted models with and without Lp-PLA<sub>2</sub> activity, weighted according to the population distribution of age and smoking. Adding Lp-PLA<sub>2</sub> activity to a multivariable model increased the AUROC from 0.720 to 0.734. We also looked at improvement in classification (Table V). Adding Lp-PLA<sub>2</sub> activity to the multivariable-adjusted model led to 23.3% reclassification among cases and 32.3% reclassification among controls. Among the 421 cases, inclusion of Lp-PLA<sub>2</sub> activity reclassified 13.3% of women as higher but 9.9% of women as lower, indicating a reclassification improvement of 3.4% among cases. Among the 800 controls, inclusion of Lp-PLA<sub>2</sub> activity reclassified 19.0% of women as lower, but 13.6% of women as higher, indicating a reclassification improvement of 5.4% among controls. Adding Lp-PLA<sub>2</sub> activity to the model led to a statistically significant improvement in reclassification (*P* = 0.004), with an overall net reclassification improvement of 8.8%.

## DISCUSSION

In this nested case-control study we found that Lp-PLA<sub>2</sub> activity was significantly associated with CHD among women. The relationship between Lp-PLA<sub>2</sub> activity and CHD among was not explained by traditional lifestyle, clinical, lipid, or inflammatory risk factors.

In addition, Lp-PLA<sub>2</sub> activity added significantly to risk prediction, as assessed by both the area under the receiver operating curve and the net reclassification improvement index.

In the present study, the relation between Lp-PLA<sub>2</sub> activity and CHD appeared stronger during the first six years of follow-up. However, the present study may be underpowered to detect an effect during later follow-up, and future studies should confirm this observation. Although there is no clear pattern in the literature of Lp-PLA<sub>2</sub> activity and CHD by length of follow-up, in a previous analysis among diabetics, we found a similar pattern, with the effect of Lp-PLA<sub>2</sub> activity more pronounced for earlier CHD events.<sup>20</sup> This is consistent with observations that high levels of Lp-PLA<sub>2</sub> have been found in vulnerable and ruptured plaques, implicating Lp-PLA<sub>2</sub> in promotion of plaque instability.<sup>21</sup> Epidemiological evidence has been consistent with this finding, as studies that have looked at cardiovascular endpoints earlier in disease progression, such as coronary calcification<sup>22</sup> and extracoronary atherosclerosis,<sup>23</sup> have found no significant association between Lp-PLA<sub>2</sub> activity and these outcomes after adjusting for traditional risk factors.

To address whether Lp-PLA<sub>2</sub> activity provides predictive information beyond traditional risk factors, studies have looked at the added prognostic value of including Lp-PLA<sub>2</sub> in risk prediction models. In a recent investigation in the population-based ARIC study, the authors found that of 19 novel biomarkers, Lp-PLA<sub>2</sub> concentration was the only biomarker to add significantly to the area under the receiver operating characteristic curves.<sup>16</sup> However, analyses of the receiver operating curves are insensitive when examining change in the AUC from adding new predictors to the model.<sup>19, 24</sup> The net reclassification index is a more sensitive method for assessing the predictive ability of new markers that quantifies correct reclassification – up in risk for cases, down in risk for controls – relative to the amount of incorrect reclassification – down in risk for cases, up in risk for controls. In a recent study of novel predictors of CHD, Lp-PLA<sub>2</sub> activity did not significantly improve net reclassification.<sup>15</sup> In the present study, however, addition of Lp-PLA<sub>2</sub> activity to a traditional risk factors model led to a statistically significant increase in prediction ability, as measured by both the AUROC and by the net reclassification index among women. After accounting for the sampling structure employed during matching, Lp-PLA<sub>2</sub> activity led to a net of 7.9% improvement in prediction, with a 3.5% gain in predictive ability among cases, and a 4.4% gain among female controls.

This study has several limitations. First, biomarkers including Lp-PLA<sub>2</sub> have only been measured at baseline, which may lead to modest misclassification over time when estimating long-term average Lp-PLA<sub>2</sub> activity. Although a pilot study among men from the Health Professionals' Follow-up Study demonstrated that Lp-PLA<sub>2</sub> activity is highly reproducible when comparing two samples measured one year apart (Intraclass  $r = 0.87$ ;  $n=15$ ; unpublished observation), we have no information about consistency of Lp-PLA<sub>2</sub> activity over longer periods of time. Second, these analyses were performed on a cohort of overwhelmingly Caucasian (>95% in each cohort) women, which may lead to lack of generalizability to other races. Other studies have documented differences in levels of Lp-PLA<sub>2</sub> by race, but no study has documented effect modification of the relation between Lp-PLA<sub>2</sub> and CHD. Third, this study analyzed the relation between CHD and Lp-PLA<sub>2</sub> activity in women only, and these results may not be extrapolated to men. Finally, we have measured only Lp-PLA<sub>2</sub> activity and have no measurements on Lp-PLA<sub>2</sub> concentration. Reported correlations between Lp-PLA<sub>2</sub> concentration and activity have ranged from 0.36 in a population with prevalent CHD<sup>14</sup> to 0.86 in a general population.<sup>25</sup> It is unclear which parameterization of Lp-PLA<sub>2</sub> will ultimately be most relevant for prediction and etiology, although a recent pooled analysis indicated similar and consistent across both measurements.<sup>26</sup> Future studies with both measurements should tease out these distinct pathways.

In summary, we found that Lp-PLA<sub>2</sub> activity was associated with increased risk of incident CHD in women. In addition, we found that Lp-PLA<sub>2</sub> activity significantly added to prediction of CHD above and beyond traditional risk factors and significantly improved risk classification. Differences by length of follow-up indicate it may be beneficial for future studies to account for this when assessing the relation between Lp-PLA<sub>2</sub> activity and CHD. Future studies may reproduce these results and further clarify whether Lp-PLA<sub>2</sub> is useful as a clinical marker of cardiovascular risk.

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**Table I**

Baseline characteristics of 1221 women from the Nurses' Health Study in 1990

	Cases (n=421)	Controls (n=800)	P-value
Lp-PLA <sub>2</sub> ng/mm/mL Mean (range)	169.2 (50.3 – 292.0)	153.7 (45.9 – 371.4)	<0.001
Age, (mean years)	59.9	59.9	--
Current smoking, %	27	27	--
BMI, (mean kg/m <sup>2</sup> )	26.7	25.1	<0.001
Postmenopausal Hormone use, %	35	38	0.27
Family history of MI, %	21	12	<0.001
History of hypertension, %	50	27	<0.001
History of hypercholesterolemia, %	53	41	<0.001
History of diabetes, %	14	6	<0.001
Cholesterol medication use %	4	3	0.08
LDL (mean mg/dl)	144.1	136.1	<0.001
HDL (mean mg/dl)	52.3	60.0	<0.001
Triglycerides (median mg/dl)*	126.0	99.0	<0.001
HbA1c (%)	5.9	5.5	<0.001
CRP (median mg/L)	2.6	2.0	0.01
ICAM (median ng/mL)†	293.0	279.0	0.08
Physical activity (mean MET-hr/wk)	18.5	19.2	0.60
Alcohol (mean g/day)	4.8	6.2	0.02

Abbreviations: n, Number of Participants

\* 293 cases and 534 controls have fasting triglyceride measurements

† 253 cases and 422 controls have ICAM measurements

**Table II**

Age-adjusted Spearman correlation coefficients (r) between plasma Lp-PLA<sub>2</sub> activity and selected cardiovascular risk factors among 800 controls from the Nurses' Health Study in 1990

Characteristic	r	
Age, years	0.10	<i>P</i> -value
Cholesterol (mg/dl)	<0.001	
Total	0.30	<0.001
LDL	0.48	<0.001
HDL	-0.41	<0.001
Triglycerides (mg/dl)*	0.15	<0.001
Apolipoprotein B (mg/dl)	0.41	<0.001
CRP (mg/dl)	-0.09	<0.001
HbA1c (%)	0.02	0.01
BMI (kg/m <sup>2</sup> )	0.09	0.49
ICAM <sup>†</sup>	0.16	0.01

Abbreviations: r, Correlation Coefficient

\* N = 539 for fasting triglycerides

<sup>†</sup>N = 422

Relative risks and 95% confidence intervals for CHD from the Nurses' Health Study (1990 – 2004) according to baseline Lp-PLA<sub>2</sub> activity levels

**Table III**

Women	Quartiles of Lp-PLA <sub>2</sub>				p-trend
	Q1	Q2	Q3	Q4	
Cases	60	83	116	162	
Controls	200	198	202	200	
Mean Lp-PLA <sub>2</sub> (range); cases*	107.5 (50.3 – 126.6)	142.2 (128.9 – 151.9)	163.9 (152.2 – 175.5)	209.8 (175.8 – 292.0)	
Mean (range); controls*	110.1 (45.9 – 128.0)	139.5 (128.4 – 152.1)	163.0 (152.2 – 175.7)	205.4 (175.8 – 371.4)	
Matching factors adjusted model <sup>†</sup> RR	1.0	1.46	1.93	2.86	<0.001
95% CI		0.98, 2.17	1.33, 2.80	1.98, 4.12	
Multivariable + lipid adjusted model <sup>‡</sup> RR	1.0	1.32	1.59	1.75	0.03
95% CI		0.85, 2.05	1.03, 2.45	1.09, 2.84	

Abbreviations: CHD, Coronary Heart Disease; Q, Quartile; RR, relative risk; CI, Confidence Interval

\* Quartiles based on the distribution of the controls

<sup>†</sup> RR estimated by conditional logistic regression after conditioning on matching factors (smoking, age, fasting status, month of blood draw)

<sup>‡</sup> Multivariable model adjusted for BMI, history of high cholesterol, history of diabetes, history of hypertension, alcohol use, aspirin use, cholesterol-lowering medication use, physical activity, CRP, HDL, postmenopausal hormone use, HDL, LDL, history of high cholesterol, and cholesterol-lowering medication use

Table IV

Relative risks and 95% confidence intervals for CHD from the Nurses' Health Study (1990 – 2004) according to baseline Lp-PLA<sub>2</sub> activity levels stratified by early vs. late events

		Q1	Q2	Q3	Q4	P-trend
<b>Quartiles of Lp-PLA<sub>2</sub></b>						
<b>EARLY (≤ 6 years of follow-up)</b>						
<b>Women</b>						
Cases		30	50	69	94	
Matching factors adjusted model <sup>*</sup> RR		1.0	1.93	2.33	3.50	<0.001
95% CI			1.13, 3.31	1.42, 3.83	2.12, 5.77	
Multivariable + lipid adjusted model <sup>†</sup> RR		1.0	1.67	1.90	1.94	0.04
95% CI			0.91, 3.07	1.07, 3.40	1.04, 3.74	
<b>LATE (&gt; 6 years of follow-up)</b>						
<b>Women</b>						
Cases		30	33	47	68	
Matching factors adjusted model <sup>*</sup> RR		1.0	1.0	1.51	2.20	<0.001
95% CI			0.55, 1.83	0.86, 2.64	1.27, 3.80	
Multivariable + lipid adjusted model <sup>†</sup> RR		1.0	1.02	1.35	1.58	0.13
95% CI			0.53, 1.97	0.68, 2.68	0.74, 3.37	

Abbreviations: CHD, Coronary Heart Disease; Q, Quartile; RR, relative risk; CI, Confidence Interval

<sup>\*</sup> RR estimated by conditional logistic regression after conditioning on matching factors (smoking, age, fasting status, month of blood draw)

<sup>†</sup> Multivariable model adjusted for BMI, history of high cholesterol, history of diabetes, history of hypertension, alcohol use, aspirin use, cholesterol-lowering medication use, physical activity, CRP, postmenopausal hormone use, HDL, LDL, history of high cholesterol, and cholesterol-lowering medication use

**Table V**

CHD risk reclassification\* comparing multivariable-adjusted<sup>†</sup> logistic regression models with and without Lp-PLA<sub>2</sub> activity among 1221 women from the Nurses' Health Study (1990 – 2004), weighted by age and smoking categories<sup>‡</sup>

		Multivariable Model* (Lp-PLA <sub>2</sub> )			
		Quartiles of Lp-PLA <sub>2</sub> activity <sup>§</sup>			
		Q1	Q2	Q3	Q4
Cases	Number of Cases	100	100	100	100
	Number of Controls	100	100	100	100
	Number of Controls	100	100	100	100
	Number of Controls	100	100	100	100
Controls	Number of Controls	100	100	100	100
	Number of Controls	100	100	100	100
	Number of Controls	100	100	100	100
	Number of Controls	100	100	100	100
Total		200	200	200	200
NRI		0.04	0.04	0.04	0.04
95% CI		0.01	0.07	0.01	0.07
P-value		0.001	0.001	0.001	0.001

Abbreviations: Q, Quartile; NRI, Net Reclassification Improvement Index

\* Light gray shading indicates correct reclassification with the addition of Lp-PLA<sub>2</sub> activity in the multivariable model (higher predicted risk for cases, lower predicted risk for controls); dark grey indicates incorrect reclassification (lower predicted risk for cases, higher predicted risk for controls); unshaded indicates no reclassification.

<sup>†</sup> Multivariable model conditioned on matching factors (smoking, age, fasting status, month of blood draw) and adjusted for BMI, history of high cholesterol, history of diabetes, history of hypertension, alcohol use, aspirin use, cholesterol-lowering medication use, physical activity, CRP, HDL, and postmenopausal hormone use

<sup>‡</sup> Models are weighted according to the population (total cohort) distribution of age and sex categories; thus control cells reflect the weighted individuals in the case-control sample

<sup>§</sup> Quartiles based on the distribution of risk score from the multivariable model (without Lp-PLA<sub>2</sub> activity)