Cardiovascular Events With Increased Lipoprotein-Associated Phospholipase A\textsubscript{2} and Low High-Density Lipoprotein-Cholesterol
The Veterans Affairs HDL Intervention Trial


Objective—Lipoprotein-associated phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}), a proinflammatory enzyme that predominantly circulates with low-density lipoprotein (LDL), has been shown in general populations to predict cardiovascular (CV) events. We sought to determine whether increased Lp-PLA\textsubscript{2} would also predict CV events in the absence of high LDL-cholesterol (LDL-C), in a population with low high-density lipoprotein-cholesterol (HDL-C).

Methods and Results—Plasma Lp-PLA\textsubscript{2} activity was measured at baseline and after 6 months on-trial in 1451 men with low HDL-C (mean, 32 mg/dL) and low LDL-C (mean 110 mg/dL), randomized to either placebo or gemfibrozil therapy in the Veterans Affairs HDL Intervention Trial (VA-HIT). Over a quartile range of increasing Lp-PLA\textsubscript{2} there was a significant increase in LDL-C and decrease in HDL-C ($P<0.0001$), and an increased percentage of myocardial infarction (MI), stroke, or CHD death ($P=0.03$ for trend). In Cox models, adjusted for major CV risk factors, a 1-SD increase in Lp-PLA\textsubscript{2} was associated with a significant increase in CV events (hazard ratio [HR] 1.17 95% CI 1.04 to 1.32). Although gemfibrozil reduced Lp-PLA\textsubscript{2} only modestly (6.6%), at higher levels of Lp-PLA\textsubscript{2} gemfibrozil produced a significant reduction in CV events.

Conclusions—In VA-HIT, a population with low HDL-C and LDL-C, high Lp-PLA\textsubscript{2} independently predicted CV events that were reduced by gemfibrozil. (Arterioscler Thromb Vasc Biol. 2008;28:1172-1178)

Key Words: cardiovascular events • lipoprotein-associated phospholipase A\textsubscript{2} • inflammation • high-density lipoproteins • gemfibrozil

Lipoprotein-associated phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}) or platelet-activating factor acetylhydrolase (PAF-AH) is a proinflammatory enzyme that has been shown in a number of population studies to predict major cardiovascular (CV) events, independent of both traditional risk factors and other markers of inflammation.\textsuperscript{1-5} In plasma, Lp-PLA\textsubscript{2} is predominantly bound to low-density lipoprotein (LDL),\textsuperscript{6} and higher activity levels of this enzyme are especially associated with small-size LDL particles\textsuperscript{7} that are commonly viewed as more atherogenic than larger LDL particles. However, Lp-PLA\textsubscript{2} is also associated with high-density lipoproteins (HDL) and, indeed, there is evidence that this enzyme may migrate between LDL and HDL particles in the circulation.\textsuperscript{6,8} Furthermore, and in contrast to a positive association of Lp-PLA\textsubscript{2} with LDL, this particular phospholipase has been found to be negatively correlated with plasma HDL-cholesterol (HDL-C) concentrations,\textsuperscript{2,9,10} suggesting that higher levels of Lp-PLA\textsubscript{2} might especially be associated with proatherogenic processes that are predominantly associated with lower levels of HDL-C.

Phospholipids, and more specifically phosphatidylcholine molecules, may play a key role in the cardioprotective functions of HDL. In addition to the well-known capacity of HDL to promote cholesterol efflux from arterial macrophages, a process in great part mediated by HDL-phospholipids,\textsuperscript{11,12} HDL has been shown to suppress early inflammatory changes in the arterial wall during the development of atherosclerosis.\textsuperscript{13,14} In this process the phosphatidylcholines of HDL have been shown to be the principal active components of HDL that inhibit endothelial cell adhesion molecule formation\textsuperscript{15} that, in turn, leads to an influx of blood monocytes into the arterial intima that are transformed into macrophages and, ultimately, foam cells.

The present analysis was undertaken in the Veterans Affairs HDL Intervention Trial (VA-HIT),\textsuperscript{16} a secondary prevention study in men with uniformly low levels of both
HDL-C and LDL-cholesterol (LDL-C), to explore the possibilities that in this population increased Lp-PLA2 activity might be associated with an increase in CV events even in the absence of a high LDL-C, that an increased CV risk that accompanies a low HDL-C might be associated with high Lp-PLA2 activity independent of other CV risk factors, and that therapy with the fibrate gemfibrozil might lower the incidence of new CV events in conjunction with a reduction in Lp-PLA2 activity.

Methods

Study Population
VA-HIT was undertaken as a placebo-controlled, 5-year intervention trial with gemfibrozil, 1.2 g/d, to determine whether raising a low HDL-C (mean at baseline, 32 mg/dL) would reduce the major CV events of nonfatal myocardial infarction (MI) and CHD death. Twenty Department of Veterans Affairs Medical Centers took part in this trial. The entrance criteria, baseline characteristics, and major results of VA-HIT have been previously described.16,17 This study was approved by the Human Rights Committee of the Cooperative Studies Program Coordinating Center, by each of the 20 study-site’s institutional review boards, and by the Cooperative Studies Program Evaluation Committee. All subjects gave written informed consent. VA-HIT is registered as NCT00283335.

A group of 2531 men were recruited for VA-HIT and 2175 returned for a second follow-up visit after 6 to 7 months of therapy (Visit 3). From this group 1451 individuals (725 taking placebo, 726 taking gemfibrozil) representing 66.7% of those at this follow-up visit were randomly selected for the present analysis. This selection provided an overall 5-year rate of MI, CHD death, and stroke that approximated the rate for these events in the entire VA-HIT population.

Laboratory Procedures
Plasma, collected with EdTA as an anticoagulant, was obtained from fasting subjects at baseline and at specified intervals during follow-up. Lipids, apoproteins, and insulin were measured by previously cited methods18,19 and C-reactive protein (CRP) by an automated high-sensitivity, latex-enhanced nephelometric procedure (Wako CRP-UL, Wako Diagnostics), all at the VA-HIT Central Laboratory at the USDA Human Nutrition Research Center at the Tufts-New England Medical Center. Blood glucose and white cell counts were done at each local participating Veterans Administration Medical Center.

Measurement of Lp-PLA2 Activity
Plasma stored at −80°C was used for this analysis. Lp-PLA2 activity was measured by diaDexus Inc by a colorimetric activity method (CAM), as previously used by Koenig et al.7 For this assay, Lp-PLA2 activity is measured in a 96-well microplate with a colorimetric method (Wako CRP-UL, Wako Diagnostics) and then adjusted for the cardiac risk factors of age, hyperglycemia, smoking, diabetes, BMI, CRP, and lipids (LDL-C, HDL-C, and triglycerides). A test of interaction of gemfibrozil treatment with Lp-PLA2 activity was regularly performed, and in no analysis was this found to be significant. No adjustment needed to be made for the use of other lipid drugs at baseline because these were not permitted by protocol at entry into this trial. However, during the course of this trial other lipid drugs were used by 10.3% of placebo and 9.5% of gemfibrozil treatment groups and an adjustment was made for off-trial drug use where appropriate. For all Cox models the proportional hazards assumption was tested and confirmed. The SAS statistical package 9.1 was used for all analyses.

Results
Table 1 shows the major characteristics at baseline of this Lp-PLA2 study group of 1451 men who attended follow-up Visit 3, all men who attended Visit 3 (n=2175), and the entire VA-HIT population (n=2531). For none of the parameters shown were values significantly different between the group selected for study and all participants that attended Visit 3. Notably, the 5-year total CV event rate was very similar for the study group at Visit 3 and all those that attended Visit 3. At Visit 3 the CV event rate was, however, somewhat less than for the entire VA-HIT population at baseline, due perhaps in part to 28 CHD deaths (1.1% of the baseline population) that occurred before Visit 3.

Correlations of Lp-PLA2 activity with a variety of CV risk factors are shown in Table 2. At baseline, Lp-PLA2 was most strongly related to levels of LDL-C, apolipoprotein (apo) B, and HDL-C. Of note, Lp-PLA2 was only weakly related to body mass, hypertension, diabetes, triglycerides, glucose, and plasma insulin or those CV risk factors that are frequently associated with the metabolic syndrome or insulin resistance. Furthermore, in this population Lp-PLA2 was not related to more general measures of inflammation as assessed by the blood white cell count or levels of CRP.

Over a quartile range of increasing Lp-PLA2 activity (Table 3) levels of both LDL-C and apoB were significantly increased whereas HDL-C was decreased. There was no change in triglycerides. With increasing Lp-PLA2 there was a progressive increase in CV events with placebo, as shown by 5-year event rates, with a significant test for trends.

In multivariate analyses, adjusted for major CV risk factors, elevated baseline levels of Lp-PLA2 activity as well as elevated levels after 6 to 7 months in the study predicted a significant increase in the combined end point of MI, CHD death, and stroke over the 5-year period of trial (Table 4). As Table 4 also shows with an increase in Lp-PLA2 activity the single events of nonfatal MI as well as CHD death were also
significantly predicted by an increase in baseline levels of Lp-PLA₂, although the incidence of stroke was not. Moreover, an increase in Lp-PLA₂ also predicted an increase in CV events in fully adjusted models over a tertile range (1.85 HR, 95% CI 1.38 to 2.50, \( P < 0.0001 \)), comparing tertile 3 to 1, and also over a quartile range (1.70 HR, 95% CI 1.20 to 2.41, \( P < 0.002 \)), comparing quartile 4 to 1. For both the tertile and quartile analysis a test for trends was significant at \( P < 0.0001 \) and \( P < 0.0004 \), respectively.

As shown by Kaplan–Meier plots adjusted for major CV risk factors (Figure 1), the cumulative incidence of a CV event at baseline was greatest for groups with Lp-PLA₂ activity above the median value (168.5 nmol/min/mL), regardless of levels of LDL-C (shown as lower or higher than the median LDL-C value of 112 mg/dL) (Figure 1A) and regardless of levels of HDL-C (shown as lower or higher than the median HDL-C value of 31.5 mg/dL) (Figure 1B).

Furthermore, subjects with the highest levels of Lp-PLA₂ coupled either with LDL-C greater than the median (averaging 126 \( \pm \) 11 mg/dL) or with HDL-C less than the median (averaging 28 \( \pm \) 3 mg/dL) had the greatest risk of a CV event. The difference in cumulative incidence of a CV event comparing higher to lower Lp-PLA₂ was similar for subjects with low LDL-C (comparing curve 1 with curve 3, fully adjusted HR of 1.37 (95% CI 0.97 to 1.95, \( P = 0.08 \)) and subjects with high LDL-C (comparing curve 2 with curve 4, HR of 1.35 (95% CI 0.98 to 1.85, \( P = 0.06 \)). However, in contrast to results for LDL-C, for strata of HDL the cumula-

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Table 1. Major Characteristics at Baseline and Cardiovascular Event Rates for All VA-HIT Subjects and the Study Group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All at Baseline n=2531</th>
<th>All at Visit 3 n=2175</th>
<th>Study Group n=1451</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64.2 (7.2)</td>
<td>64.1 (7.1)</td>
<td>64.1 (7.2)</td>
<td>0.89</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.0 (4.8)</td>
<td>29.0 (4.8)</td>
<td>29.0 (4.7)</td>
<td>0.94</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>68.0</td>
<td>67.7</td>
<td>68.0</td>
<td>0.98</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>30.6</td>
<td>29.5</td>
<td>28.8</td>
<td>0.47</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>20.4</td>
<td>19.3</td>
<td>18.3</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 2. Relation of Lp-PLA₂ Activity to Cardiovascular Risk Factors at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Spearman Correlation Coefficient</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.020</td>
<td>0.44</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.038</td>
<td>0.15</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.079</td>
<td>0.003</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-0.068</td>
<td>0.01</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.050</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.281</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.191</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.044</td>
<td>0.09</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>0.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.021</td>
<td>0.42</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.012</td>
<td>0.65</td>
</tr>
<tr>
<td>CRP</td>
<td>-0.003</td>
<td>0.93</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>0.003</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Values shown for 1451 subjects at baseline.
tive CV event incidence difference for higher than lower levels of Lp-PLA2 was more pronounced for subjects with low HDL-C (comparing curve 2 with 4 in Figure 1B, adjusted HR of 1.47 [95% CI 1.06 to 2.04, \( P = 0.02 \]) than for subjects with high HDL-C (comparing curves 1 and 3, HR of 1.28 [95% CI 0.91 to 1.79, \( P = 0.15 \)), though the formal test for interaction was not statistically significant.

With gemfibrozil, on-trial levels of Lp-PLA2 activity were on average 6.6% lower than with placebo (\( P = 0.0001 \)), with no substantial change in this treatment difference throughout the range of Lp-PLA2 values (data not shown). In this study group of 1451, gemfibrozil increased HDL-C by an average of 6.3% compared to placebo (\( P = 0.0001 \) and decreased

Table 3. Values for Major Lipids, Apolipoprotein B, and Cardiovascular Events With Placebo Over a Quartile Distribution of Lp-PLA2 Values at Baseline

<table>
<thead>
<tr>
<th>Quartiles of Lp-PLA2</th>
<th>n</th>
<th>Lp-PLA2 (nmol/min/mL)</th>
<th>LDL-C (mg/dL) (SD)</th>
<th>HDL-C (mg/dL) (SD)</th>
<th>Triglyceride (mg/dL) (SD)</th>
<th>ApoB (mg/dL) (SD)</th>
<th>CV Events % (n event /n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>362</td>
<td>&lt;148.1</td>
<td>101.6 (22.0)</td>
<td>33.3 (5.4)</td>
<td>160.6 (74.4)</td>
<td>88.3 (19.7)</td>
<td>18.5 (29/157)</td>
</tr>
<tr>
<td>2</td>
<td>364</td>
<td>148.1–168.4</td>
<td>109.3 (20.6)</td>
<td>31.9 (4.9)</td>
<td>162.2 (70.3)</td>
<td>93.1 (20.4)</td>
<td>22.7 (42/185)</td>
</tr>
<tr>
<td>3</td>
<td>363</td>
<td>168.5–188.6</td>
<td>113.8 (19.2)</td>
<td>31.7 (4.7)</td>
<td>161.8 (62.2)</td>
<td>96.4 (19.2)</td>
<td>27.9 (51/183)</td>
</tr>
<tr>
<td>4</td>
<td>362</td>
<td>&gt;188.6</td>
<td>117.1 (19.0)</td>
<td>30.6 (5.2)</td>
<td>163.7 (61.6)</td>
<td>99.4 (20.1)</td>
<td>30.0 (60/200)</td>
</tr>
<tr>
<td>Column mean (SD)</td>
<td></td>
<td>169.0 (31.9)</td>
<td>110.4 (21.0)</td>
<td>31.9 (5.1)</td>
<td>162.1 (67.3)</td>
<td>94.3 (20.3)</td>
<td>25.1 (182/725)</td>
</tr>
</tbody>
</table>

Values for Lp-PLA2 activity, major lipid fractions, and apolipoprotein (apo)B are shown by quartiles of CAM for the entire study group (n = 1451) at baseline together with the No. and percentage of CV events (MI, CHD death, or stroke) after 5 years of placebo therapy. With increasing values of CAM the changes in LDL-C and HDL-C were each significant at \( P < 0.0001 \) and apoB was significant at \( P = 0.004 \), by a test for trends. The change in triglycerides was not significant (\( P = 0.94 \)). Over the quartile distribution of CAM there was an increased percentage of CV events (\( P = 0.03 \), by a test for trends).

Table 4. Risk of a Cardiovascular Event With Increasing Lp-PLA2 Activity

<table>
<thead>
<tr>
<th>Events</th>
<th>Events (n)</th>
<th>HR</th>
<th>95% CI</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Lp-PLA2 values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite CV events Model 1*</td>
<td>320</td>
<td>1.22</td>
<td>1.10 to 1.36</td>
<td>0.0003</td>
</tr>
<tr>
<td>Model 2†</td>
<td></td>
<td>1.17</td>
<td>1.04 to 1.32</td>
<td>0.007</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>191</td>
<td>1.25</td>
<td>1.09 to 1.43</td>
<td>0.002</td>
</tr>
<tr>
<td>Model 1*</td>
<td></td>
<td>1.20</td>
<td>1.03 to 1.39</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 2†</td>
<td></td>
<td>1.26</td>
<td>1.52 to 1.47</td>
<td>0.018</td>
</tr>
<tr>
<td>Model 2‡</td>
<td></td>
<td>1.23</td>
<td>1.01 to 1.50</td>
<td>0.047</td>
</tr>
<tr>
<td>Stroke</td>
<td>67</td>
<td>0.98</td>
<td>0.77 to 1.25</td>
<td>0.86</td>
</tr>
<tr>
<td>Model 1*</td>
<td></td>
<td>0.96</td>
<td>0.75 to 1.25</td>
<td>0.79</td>
</tr>
<tr>
<td>On-trial Lp-PLA2 values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite CV events Model 3‡</td>
<td>320</td>
<td>1.23</td>
<td>1.10 to 1.37</td>
<td>0.0003</td>
</tr>
<tr>
<td>Model 4§</td>
<td></td>
<td>1.18</td>
<td>1.05 to 1.33</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Adjusted hazard ratios (HR) and 95% confidence intervals (CI) from Cox proportional hazard models are shown for a 1 SD increase in Lp-PLA2 activity for the composite and single CV end points of nonfatal myocardial infarction, CHD death, and stroke. Values are for 1451 subjects at baseline or after 6 to 7 months on-trial, where indicated. All models adjusted for gemfibrozil therapy with all \( P \) values for gemfibrozil interaction 0.10 or greater.

* Cox models adjusted for age, hypertension, BMI, diabetes, active smoking.
† Cox models also adjusted for baseline values of LDL-C, HDL-C, triglycerides, and CRP.
‡ Cox models include age, hypertension, BMI, diabetes, active smoking, and other lipid-drug treatment.
§ Cox models also adjusted for on-trial values of LDL-C, HDL-C, triglycerides, and CRP.

Figure 1. Kaplan–Meier curves, adjusted for CVD risk factors, showing at baseline the cumulative incidence of CV events at lower and higher levels of Lp-PLA2 activity with lower and higher levels of LDL-C (A) and with lower and higher levels of HDL-C (B). Lower and higher values were defined by the median levels of Lp-PLA2 activity (168.5 nmol/min/mL); by the median value of LDL-C (112 mg/dL); and the median value of HDL-C (31.5 mg/dL). Mean values (in mg/dL) above and below the median were 126±11 and 94±15 for LDL-C and 36±3 and 28±3 for HDL-C. Overall, the log-rank test was significant for curves in A (\( P = 0.002 \)) and B (\( P = 0.01 \)). For A, by log-rank, the probability values for curve 1 versus curves 2, 3, and 4 were \( P = 0.03 \), \( P = 0.08 \), and \( P = 0.001 \), respectively. For B, by log-rank, the probability values for curve 1 versus curves 2, 3, and 4 were \( P = 0.79 \), \( P = 0.15 \), and \( P = 0.008 \), respectively.
that CV events or pathology associated with Lp-PLA2 have been generally correlated with higher levels of LDL-C\textsuperscript{21} and especially with numbers of small more atherogenic LDL particles.\textsuperscript{7} Although Lp-PLA2 is ordinarily associated with HDL particles in the circulation to a much lesser extent than with LDL, this enzyme has been shown to translocate from LDL to HDL particles in vitro\textsuperscript{6,8} and in the absence of plasma LDL, in abetalipoproteinemia, plasma Lp-PLA2 activity is not decreased but, instead, has been found to be entirely associated with HDL.\textsuperscript{8,22}

VA-HIT, in contrast to all other population studies in which increased plasma levels of Lp-PLA2 have been found to strongly correlate with an increase in CV events or vascular pathology, was a study designed to examine the effects of distinctly low levels of plasma HDL-C on CV events (and the potential for reducing CV events by increasing HDL-C) in the absence of a high LDL-C. Our finding in this population that low HDL-C as well as higher levels of LDL-C was associated with increased Lp-PLA2 activity and that low HDL-C together with increasing Lp-PLA2 activity independently predicted an increase in a combined end point of nonfatal MI, stroke, and CHD death would appear to provide strong support for the notion that a low plasma level of HDL may produce vascular injury.\textsuperscript{23}

A number of reports have shown that HDL may have an important cardioprotective role in suppressing early inflammatory changes in the vessel wall. Studies show that low HDL levels fail to adequately suppress vascular cell adhesion molecule (VCAM) formation and an early vascular inflammatory response that leads to foam cell development.\textsuperscript{13,14,24} In this process, the phosphatidylcholines of HDL appear to be the key components of the HDL particle that suppress adhesion molecule formation.\textsuperscript{15} From our present results one might speculate, therefore, that increased levels of a phospholipase such as Lp-PLA2 might so change the phosphatidylcholine composition of HDL as to decrease the capacity of this particle to inhibit a vascular inflammatory process.

Statins\textsuperscript{25,26} as well as fibrates\textsuperscript{26,27} have previously been shown to lower Lp-PLA2 levels. However, in studies of generally small groups of subjects, the percentage reduction in Lp-PLA2 has been highly variable. In one comparative study with statins\textsuperscript{25} the reduction in Lp-PLA2 with atorvastatin was relatively large (26%) but with simvastatin, fluvastatin, pravastatin, and lovastatin ranged from 1% to 10%, essentially in the same range as we have found with gemfibrozil. Although the reduction in Lp-PLA2 levels with gemfibrozil was the same at higher and lower levels of Lp-PLA2 activity (averaging 6.6%), we have found that the reduction in CV events with gemfibrozil compared to placebo was significantly greater when Lp-PLA2 activity was higher than lower (than the median value). This might be explained by our finding that at the highest levels of Lp-PLA2 values of HDL-C were lowest and values of LDL-C were highest (Table 3). As we have previously shown in the entire VA-HIT population,\textsuperscript{16} at both lowest levels of HDL-C and highest levels of LDL-C, gemfibrozil produced the largest reduction in CV events.

Although we have no more precise explanation for the relatively greater benefit of gemfibrozil at highest levels of Lp-PLA2, this might be linked to a reduction in LDL particle

**Figure 2.** Cardiovascular events (CV) in placebo and gemfibrozil treatment groups over a quartile range of Lp-PLA2 activity values at baseline. The percentage of nonfatal myocardial infarction, coronary heart disease death, or stroke (as a combined end point) is shown for subjects assigned placebo (n=725) by open bars and for subjects assigned gemfibrozil (n=726) by shaded bars. The treatment difference in CV events at each quartile level of Lp-PLA2 is shown by the percentages at each quartile division. A test for trends across these quartiles was significant for a gemfibrozil difference (P<0.03).

LDL-C by 5.6% (P=0.10). For all subjects, with a 1 SD increase in Lp-PLA2, the hazard ratio for a CV event with gemfibrozil of 1.08 (95% CI 0.90 to 1.29) was not significantly different than the hazard ratio for placebo of 1.28 (95% CI 1.09 to 1.49; P=0.15, for interaction). However, as shown in Figure 2, over a quartile distribution of increasing Lp-PLA2 values there was a progressive decrease in CV events with gemfibrozil compared to placebo (P=0.03, for the difference in trend), with a treatment difference that appeared especially pronounced at Lp-PLA2 values above the median. Indeed, with a 1-SD increase in Lp-PLA2 above the median value of Lp-PLA2 the hazard ratio for a CV event in the gemfibrozil-treated group was 0.97 (95% CI 0.63 to 1.50) and was significantly lower than in the placebo group (HR of 1.54 [95% CI 1.20 to 1.98] P=0.005).

**Discussion**

In the present study we have shown that an increase in the activity of the lipoprotein-associated phospholipase, Lp-PLA2, in the VA-HIT population predicted a significant increase in major CV events. Most particularly, we have found in analyses that were adjusted for a number of more established CV risk factors, that an increase in Lp-PLA2 activity was independently associated with a significant increase in nonfatal MI as well as CHD death; that an increase in Lp-PLA2 coupled with either highest levels of LDL-C or lowest levels of HDL-C was related to the highest rates of CV events; and that gemfibrozil therapy significantly reduced Lp-PLA2 and CV events at highest levels of Lp-PLA2 activity.

Although Lp-PLA2 had once been regarded as a phospholipase that might reduce inflammation and vascular injury, a number of studies have shown that high levels of plasma Lp-PLA2 are associated with an increase in CV events\textsuperscript{1-5} or vascular pathology.\textsuperscript{10,20} Consistent with a proatherogenic role of Lp-PLA2 in relation to lipoproteins are the observations that CV events or pathology associated with Lp-PLA2 have been generally correlated with higher levels of LDL-C\textsuperscript{21} and especially with numbers of small more atherogenic LDL particles.\textsuperscript{7} Although Lp-PLA2 is ordinarily associated with HDL particles in the circulation to a much lesser extent than with LDL, this enzyme has been shown to translocate from LDL to HDL particles in vitro\textsuperscript{6,8} and in the absence of plasma LDL, in abetalipoproteinemia, plasma Lp-PLA2 activity is not decreased but, instead, has been found to be entirely associated with HDL.\textsuperscript{8,22}

VA-HIT, in contrast to all other population studies in which increased plasma levels of Lp-PLA2 have been found to strongly correlate with an increase in CV events or vascular pathology, was a study designed to examine the effects of distinctly low levels of plasma HDL-C on CV events (and the potential for reducing CV events by increasing HDL-C) in the absence of a high LDL-C. Our finding in this population that low HDL-C as well as higher levels of LDL-C was associated with increased Lp-PLA2 activity and that low HDL-C together with increasing Lp-PLA2 activity independently predicted an increase in a combined end point of nonfatal MI, stroke, and CHD death would appear to provide strong support for the notion that a low plasma level of HDL may produce vascular injury.\textsuperscript{23}

A number of reports have shown that HDL may have an important cardioprotective role in suppressing early inflammatory changes in the vessel wall. Studies show that low HDL levels fail to adequately suppress vascular cell adhesion molecule (VCAM) formation and an early vascular inflammatory response that leads to foam cell development.\textsuperscript{13,14,24} In this process, the phosphatidylcholines of HDL appear to be the key components of the HDL particle that suppress adhesion molecule formation.\textsuperscript{15} From our present results one might speculate, therefore, that increased levels of a phospholipase such as Lp-PLA2 might so change the phosphatidylcholine composition of HDL as to decrease the capacity of this particle to inhibit a vascular inflammatory process.

Statins\textsuperscript{25,26} as well as fibrates\textsuperscript{26,27} have previously been shown to lower Lp-PLA2 levels. However, in studies of generally small groups of subjects, the percentage reduction in Lp-PLA2 has been highly variable. In one comparative study with statins\textsuperscript{25} the reduction in Lp-PLA2 with atorvastatin was relatively large (26%) but with simvastatin, fluvastatin, pravastatin, and lovastatin ranged from 1% to 10%, essentially in the same range as we have found with gemfibrozil. Although the reduction in Lp-PLA2 levels with gemfibrozil was the same at higher and lower levels of Lp-PLA2 activity (averaging 6.6%), we have found that the reduction in CV events with gemfibrozil compared to placebo was significantly greater when Lp-PLA2 activity was higher than lower (than the median value). This might be explained by our finding that at the highest levels of Lp-PLA2 values of HDL-C were lowest and values of LDL-C were highest (Table 3). As we have previously shown in the entire VA-HIT population,\textsuperscript{16} at both lowest levels of HDL-C and highest levels of LDL-C, gemfibrozil produced the largest reduction in CV events.

Although we have no more precise explanation for the relatively greater benefit of gemfibrozil at highest levels of Lp-PLA2, this might be linked to a reduction in LDL particle
numbers, which, as reflected by apoB levels, were shown to correlate with Lp-PLA₂ activity in this study (Table 2). We have previously demonstrated that gemfibrozil significantly reduced LDL particle numbers in VA-HIT, especially small LDL particles, which have been shown to be increased at higher levels of Lp-PLA₂. We have further shown that with lowest levels of LDL-C in VA-HIT, an increase in Lp-PLA₂ was associated with an especially high rate of CV events. Moreover, therapy with the fibrate, gemfibrozil, which only modestly reduced Lp-PLA₂ values, significantly reduced CV events in the presence of an increased Lp-PLA₂.

**Acknowledgments**

We thank diaDexus for performing the Lp-PLA₂ analysis and gratefully acknowledge the assistance of the VA-HIT study participants and study personnel.

**Sources of Funding**

Funding for this analysis was provided by a grant from GlaxoSmithKline. VA-HIT is supported by the Department of Veterans Affairs Office of Research and Development Cooperative Studies Program. Past additional funding was received from Parke-Davis, and an R03 grant, HL069111.

**Disclosures**

S.J.R., D.C., and B.F.A. have received grant funding from Glaxo-Smith Kline for an analysis of multiple biomarkers in VA-HIT. J.J.N. is an employee of GlaxoSmithKline.

**References**


Cardiovascular Events With Increased Lipoprotein-Associated Phospholipase A2 and Low High-Density Lipoprotein-Cholesterol: The Veterans Affairs HDL Intervention Trial

Sander J. Robins, Dorothea Collins, Jeanenne J. Nelson, Hanna E. Bloomfield and Bela F. Asztalos

Arterioscler Thromb Vasc Biol. 2008;28:1172-1178; originally published online March 20, 2008;
doi: 10.1161/ATVBAHA.107.160739
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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