LDL Subclass Phenotypes and Triglyceride Metabolism in Non–Insulin-Dependent Diabetes

Kenneth R. Feingold, Carl Grunfeld, Miyin Pang, William Doerrler, and Ronald M. Krauss

Plasma low density lipoprotein (LDL) comprises multiple discrete subclasses differing in size, density, and chemical composition. A common, heritable phenotype characterized by the predominance of small, dense LDL particles (LDL subclass phenotype B) is associated with relatively increased concentrations of plasma triglycerides, reduced levels of high density lipoprotein, and increased risk of coronary artery disease in comparison with subjects with larger LDL (LDL subclass phenotype A). Population studies have indicated that approximately 20–30% of adult men have phenotype B, and another 15–20% have LDL of intermediate size. The lipid changes in phenotype B are similar to those that have been observed in patients with non–insulin-dependent diabetes mellitus (NIDDM). In the present study, we have assessed LDL subclass phenotypes in normolipidemic men with NIDDM and in age-matched control subjects who had similar lipid levels. There was a greater than twofold increase in the percentage of individuals with the LDL B phenotype in the NIDDM subjects. The LDL B phenotype was associated with higher plasma triglyceride levels and a trend toward lower high density lipoprotein cholesterol levels compared with the LDL A phenotype in the NIDDM subjects, as has been previously observed in control groups. Indices of diabetic control, such as fasting and hemoglobin A1c levels, were similar regardless of LDL phenotype pattern, suggesting that glycemic control was not likely to account for the increase in the LDL B phenotype. In both control and NIDDM subjects, the clearance of triglyceride-rich lipoproteins was slowed in the subjects with the LDL phenotype B compared with those with the A phenotype. Multiple regression analysis demonstrated that the diagnosis of NIDDM is an independent predictor of the LDL B phenotype. In summary, the present study demonstrates that NIDDM is associated with an increased prevalence of the LDL subclass phenotype B, even in the absence of frank hyperlipidemia. Thus, genetic and metabolic factors leading to the predominance of small, dense LDL may contribute to the increased risk of vascular disease in patients with NIDDM. (Arteriosclerosis and Thrombosis 1992;12:1496–1502)

KEY WORDS • non–insulin-dependent diabetes mellitus • low density lipoprotein subclasses • triglyceride metabolism

From the Department of Medicine (K.R.F., C.G., M.P., W.D.), University of California, San Francisco, and the Metabolism Section, Medical Service, Department of Veterans Affairs Medical Center, San Francisco; and the Donner Laboratory (R.M.K.), University of California, and the Molecular Medicine Research Program, Research Medicine and Radiation Biophysics Division, Lawrence Berkeley Laboratory, Berkeley, Calif.

Supported by grants from the Bernard Bird Memorial Fund, the Jewish Community Endowment Fund, Department of Veterans Affairs, and the National Institutes of Health (grants DK-40990 and HL-18754). The work was conducted in part at the Lawrence Berkeley Laboratory through the US Department of Energy under contract No. DE-AC03-76SF00098 to the University of California.

Address for correspondence: Kenneth R. Feingold, MD, Metabolism Section (11IF), Department of Veterans Affairs Medical Center, 4150 Clement Street, San Francisco, CA 94121.

Received June 18, 1992; revision accepted September 4, 1992.
tension, cigarette smoking, etc. Furthermore, patients with NIDDM commonly have a type of dyslipidemia characterized by elevations of plasma triglycerides and reductions in HDL cholesterol similar to those seen in conjunction with the LDL subclass phenotype B. Therefore, in the present study, we determined LDL subclass phenotypes in subjects with NIDDM but without severe dyslipoproteinemia.

Methods

Patients

All subjects in the study were male. The subjects with diabetes (n=29) had a history of two fasting serum glucose values greater than 140 mg/dl and were being treated with either oral hypoglycemic agents or insulin. None of the diabetic subjects had a history of ketoacidosis, and they were classified as NIDDM on the basis of clinical criteria. NIDDM subjects with fasting plasma triglyceride levels greater than 325 mg/dl were excluded from this study. Control subjects (n=87) did not have diabetes, kidney failure, nephrotic syndrome, active liver disease, or other disorders that would affect lipid metabolism. None of the control subjects or diabetics were receiving hypolipidemic therapy or were taking drugs that might affect lipid metabolism. The control subjects were age matched to those with NIDDM.

Analytical Methods

Plasma triglyceride, cholesterol, HDL cholesterol, glucose, and hemoglobin A, levels were determined by standard laboratory procedures. LDL cholesterol content was calculated by the method of Friedewald et al. Nondenaturing gradient gel electrophoresis of whole plasma was performed on 2-16% polyacrylamide gradient gels and analyzed by using a lipid stain (oil red O) followed by scanning with a Transynet RFT scanning densitometer. Particle diameters were calculated from calibration curves by using protein standards of known size. Each subject's scan was classified into one of three patterns (type A, type B, or intermediate) by one author (R.K.) using criteria previously described without knowledge of the clinical history or classification of the patient.

Triglyceride Clearance

All of the NIDDM subjects and an equal number of control subjects had the clearance of triglyceride-rich particles determined by the intravenous fat-tolerance test as described previously. In brief, after fasting plasma samples were drawn, the subjects were given 20% Liposyn II intravenous fat emulsion (Abbott Laboratories, North Chicago, Ill.) at a dose of 0.1 g/kg body wt. Plasma samples were then taken every 5 minutes for 30 minutes and then every 10 minutes for the next 20 minutes. Clearance was measured by nephelometry. Regression of a semilog plot of clearance was linear for each patient, and the half-time (t½) was determined by using SIGMA-PILOT software. The t½ for clearance by this method is not influenced by triglyceride levels in disorders with VLDL overproduction (reviewed in Reference 17).

Statistics

Differences between groups were analyzed by Student's t test. Prevalence was compared by the χ² test. Multiple regression analysis and analysis of variance were performed with the STATVIEW II statistical package.

Results

Demographic Data and Plasma Lipid Levels

Table 1 presents the demographic data and plasma lipid levels of control and NIDDM subjects. As expected, body mass index is increased in the subjects with NIDDM. In these NIDDM subjects, plasma triglyceride and HDL cholesterol levels are similar to those of the control group, whereas small but statistically significant decreases in total plasma cholesterol and LDL cholesterol levels were seen. The absence of increases in plasma lipid levels in the NIDDM compared with control subjects is most probably due to the fact that we specifically excluded from this study NIDDM subjects with elevated triglyceride levels (plasma triglyceride levels >325 mg/dl) and those who were being treated with hypolipidemic drugs.

LDL Phenotype in Control and Diabetic Subjects

As shown in Table 2, in these NIDDM subjects there is an increase (116%) in the percentage of individuals with the LDL B phenotype and a decrease (41%) in the LDL A phenotype compared with control subjects. The percentage of individuals with the intermediate phenotype is similar in NIDDM and control subjects. Thus, diabetes, a disorder that can cause alterations in triglyceride metabolism, is associated with an increased prevalence of the LDL subclass phenotype B even when hypertriglyceridemic subjects are excluded.

<p>| TABLE 1. Demographic Data and Plasma Lipid Levels in Control and NIDDM Subjects |
|-------------------------------|-------------------------------|-------------------------------|-------------------------------|</p>
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=87)</td>
<td>60±0.86</td>
<td>25±0.33</td>
<td>123±6.4</td>
<td>207±3.0</td>
<td>46±1.3</td>
</tr>
<tr>
<td>Diabetic (n=29)</td>
<td>62±2.3</td>
<td>28±0.82*</td>
<td>123±12.1</td>
<td>190±5.5*</td>
<td>42±2.0</td>
</tr>
</tbody>
</table>

NIDDM, non-insulin-dependent diabetes mellitus; BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein. All triglyceride and cholesterol measurements are in milligrams per deciliter. *p<0.01 vs. control; †p<0.05 vs. control.

<p>| TABLE 2. LDL Phenotypes in Control and NIDDM Subjects |
|-------------------------------|-------------------------------|-------------------------------|</p>
<table>
<thead>
<tr>
<th>A (%)</th>
<th>Intermediate (%)</th>
<th>B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=87)</td>
<td>47</td>
<td>29</td>
</tr>
<tr>
<td>Diabetic (n=29)</td>
<td>28</td>
<td>21</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein; NIDDM, non-insulin-dependent diabetes mellitus.
Demographic Data and Plasma Lipid Levels According to LDL Phenotype

Table 3 presents the demographic data and plasma lipid levels in control subjects divided by LDL phenotypes. As was seen in previous studies, there is an increase in plasma triglyceride levels and a decrease in HDL cholesterol levels in control subjects with the LDL B phenotype compared with subjects with the LDL A phenotype. Control subjects with the intermediate LDL phenotype have plasma triglyceride and HDL cholesterol levels that are between those for the A and B phenotypes. Thus, the presence of the LDL B phenotype is associated with an atherogenic lipid profile.

Table 4 presents the demographic data and plasma lipid levels in NIDDM subjects within each LDL phenotype. As observed in control subjects, the LDL B phenotype is associated with higher plasma triglyceride levels and a decrease in HDL cholesterol levels in NIDDM subjects with the intermediate phenotype. Reduced plasma HDL levels also predict LDL B phenotype. Most importantly, the diagnosis of NIDDM is also an independent predictor of the LDL B phenotype. Similar regression coefficients for the lipid variables were found for the control subjects when considered separately, whereas none of the coefficients was significant for the NIDDM subjects considered separately (data not shown).

Triglyceride Clearance and LDL Phenotype

In the NIDDM subjects and in a subset of the control subjects, the clearance of triglyceride-rich particles was determined by the intravenous fat-tolerance test. In these control subjects, as shown in Figure 1A, mean plasma triglyceride levels are elevated in subjects with the LDL B phenotype compared with the LDL A phenotype. Importantly, indices of diabetic control, such as fasting glucose and hemoglobin A1c levels, are similar regardless of LDL phenotypic pattern, and thus glycemic control is not likely to account for the increase in the LDL B phenotype.

Table 3. Demographic Data and Plasma Lipid Levels in Controls Subjects According to LDL Phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=41)</td>
<td>60±1.3</td>
<td>24±0.45</td>
<td>87±4.6</td>
<td>202±4.1</td>
<td>52±1.8</td>
<td>132±3.9</td>
</tr>
<tr>
<td>Intermediate (n=25)</td>
<td>58±1.6</td>
<td>26±0.70</td>
<td>127±5.3</td>
<td>211±6.4</td>
<td>43±2.3</td>
<td>139±5.2</td>
</tr>
<tr>
<td>B (n=21)</td>
<td>61±1.6</td>
<td>26±0.80</td>
<td>187±16.3</td>
<td>214±4.9</td>
<td>37±1.6</td>
<td>139±5.5</td>
</tr>
<tr>
<td>A vs. intermediate</td>
<td>NS</td>
<td>p&lt;0.05</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>p&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>A vs. B</td>
<td>NS</td>
<td>p&lt;0.01</td>
<td>p&lt;0.001</td>
<td>NS</td>
<td>p&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Intermediate vs. B</td>
<td>NS</td>
<td>NS</td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein; BMI, body mass index; HDL, high density lipoprotein. All triglyceride and cholesterol measurements are in milligrams per deciliter.

Table 4. Demographic Data and Plasma Lipid Levels in NIDDM Subjects According to LDL Phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=8)</td>
<td>60±4.1</td>
<td>26±1.6</td>
<td>92±15.9</td>
<td>190±7.0</td>
<td>48±6.00</td>
<td>124±6.0</td>
</tr>
<tr>
<td>Intermediate (n=6)</td>
<td>58±5.9</td>
<td>28±1.3</td>
<td>73±9.1</td>
<td>166±12.8</td>
<td>47±5.2</td>
<td>105±10.4</td>
</tr>
<tr>
<td>B (n=15)</td>
<td>61±3.2</td>
<td>29±1.2</td>
<td>160±16.8</td>
<td>200±7.6</td>
<td>36±2.05</td>
<td>132±6.48</td>
</tr>
<tr>
<td>A vs. intermediate</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>A vs. B</td>
<td>NS</td>
<td>NS</td>
<td>p&lt;0.01</td>
<td>NS</td>
<td>p&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Intermediate vs. B</td>
<td>NS</td>
<td>NS</td>
<td>p&lt;0.001</td>
<td>p&lt;0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Fasting glucose (mg/dl) | HbA1c* (%)
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=8)</td>
<td>217±24.1</td>
</tr>
<tr>
<td>Intermediate (n=6)</td>
<td>204±26.0</td>
</tr>
<tr>
<td>B (n=15)</td>
<td>201±21.0</td>
</tr>
<tr>
<td>A vs. intermediate</td>
<td>NS</td>
</tr>
<tr>
<td>A vs. B</td>
<td>NS</td>
</tr>
<tr>
<td>Intermediate vs. B</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Normal range, 4.3–7.7.
TABLE 5. Multivariate Predictors of LDL Subclass Phenotype B

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Regression coefficient</th>
<th>SEM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.014</td>
<td>0.018</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.007</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.004</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.018</td>
<td>0.006</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diagnosis*</td>
<td>-0.399</td>
<td>0.148</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein; BMI, body mass index; HDL, high density lipoprotein.

*Presence (0) or absence (1) of non-insulin-dependent diabetes mellitus.

phenotype, but there is considerable overlap between the two groups. As shown in Figure 1B, the $t_{1/2}$ of triglyceride clearance is delayed in subjects with the LDL B phenotype compared with subjects with the LDL A phenotype (LDL B, 24.5±2.4 minutes versus LDL A, 12.3±0.6 minutes; $p<0.001$). Subjects with the intermediate LDL phenotype have a clearance midway between these values (LDL intermediate, 17.4±1.6 minutes; $p<0.05$ versus LDL B, $p<0.01$ versus LDL A).

Of note is that except for a single individual with LDL B, there is no overlap in clearance rates between the LDL B and LDL A groups, suggesting that the clearance rate of triglyceride-rich particles may be a strong marker for the LDL B phenotype. After adjustment for fasting plasma triglyceride by analysis of covariance the relation of fat clearance and LDL phenotype remained significant at $p<0.05$.

Figure 2A shows the plasma triglyceride distribution in the NIDDM subjects divided according to LDL subclass phenotype. As expected, plasma triglyceride levels are increased in subjects with the LDL B phenotype, but again, there is considerable overlap between groups. Similar to our observations in control subjects, the clearance of triglyceride-rich particles (Figure 1B) is delayed in NIDDM subjects with the LDL B phenotype compared with those with the LDL A phenotype (LDL B, 25.3±2.7 minutes versus LDL A, 13.8±1.8 minutes;
Discussion

The factors that determine the size and composition of LDL particles are uncertain. Studies have demonstrated that the phenotypic pattern of LDL is inherited.\(^7\)\(^8\) In addition, environmental factors or other disease states may also influence the size and composition of LDL. First, the penetrance of the expression of the LDL B phenotype increases with age.\(^7\)\(^8\) Second, studies have shown that weight loss induced by exercise decreases the concentration of small, dense LDL.\(^8\) Lastly, we have recently found that in subjects with acquired immunodeficiency syndrome, an infection associated with hypertriglyceridemia,\(^7\)\(^8\)\(^9\) the prevalence of the LDL B phenotype increases 2.5-fold (K.R. Feingold, et al, unpublished observations). Thus, both genetic and environmental factors affect LDL phenotypic pattern expression.

The LDL B phenotype is associated with increased plasma triglyceride levels.\(^7\)\(^8\)\(^10\) Whether hypertriglyceridemia itself leads to the formation of small, dense LDL or whether those who inherit the LDL B phenotype are more susceptible to the development of hypertriglyceridemia is unresolved. The transfer of triglyceride from triglyceride-rich lipoproteins to LDL in exchange for cholesterol has been postulated to alter the metabolism of LDL and thereby ultimately lead to the formation of small, dense LDL.\(^7\)\(^8\)\(^11\) However, small, dense LDL may arise from distinct precursors whose levels are influenced by variations in triglyceride metabolism.\(^22\)

Diabetes is associated with alterations in triglyceride metabolism. In studies including more subjects with diabetes, plasma triglyceride levels correlate directly with indices of glycemic control, and improvements in glycemic control by oral hypoglycemic agents or insulin therapy lead to decreases in plasma triglyceride concentrations.\(^7\)\(^8\)\(^23\)\(^29\) Studies have shown that the clearance of triglyceride-rich lipoproteins is decreased and the production of triglyceride-rich lipoproteins is increased in diabetes.\(^30\)\(^31\) The decrease in the clearance of triglyceride-rich lipoproteins may be due to decreased activity of lipoprotein lipase, an enzyme whose synthesis is dependent on insulin.\(^32\)\(^34\) The increase in hepatic triglyceride production is thought to be secondary in part to the increased delivery of free fatty acids to the liver in individuals who are in poor metabolic control.\(^30\)\(^31\)

In the present study, we demonstrate that the frequency of the LDL B phenotype is increased approximately twofold in normolipidemic subjects with NIDDM, whereas the frequency of the LDL A phenotype is decreased by 41%. The percentage of diabetic subjects with the intermediate phenotype is similar to that of control subjects. Studies by other investigators using different techniques to examine LDL size and composition have demonstrated that diabetic LDLs have increased heterogeneity, with a tendency to greater quantities of small, dense LDL.\(^35\)\(^36\) To the best of our knowledge the present study is the first to classify a group of NIDDM subjects according to LDL subclass phenotype.

There were no differences in fasting plasma glucose and hemoglobin A\(_1c\) levels (indices of glycemic control) in the diabetic subjects with either the LDL A, B, or intermediate phenotype. This suggests that poor glycemic control is not a major factor in determining LDL phenotypic pattern. As expected, plasma triglyceride levels are increased and HDL cholesterol levels decreased in NIDDM subjects with the LDL B phenotype compared with NIDDM subjects with the LDL A phenotype. Of note is that despite the exclusion of diabetics with increased plasma triglyceride levels (triglyceride values \(>325\) mg/dl) and those on hypolipidemic therapy, the present study still demonstrated an increased prevalence of the LDL B phenotype. It is likely that a study of diabetic subjects with hyperlipidemia, especially hypertriglyceridemia, would demonstrate an even higher prevalence of the LDL B phenotype.

Although the LDL B phenotype was related to plasma triglyceride values within both the control and NIDDM groups, plasma triglyceride levels in these NIDDM subjects and the control subjects were identical. Yet the NIDDM subjects had a higher prevalence of the LDL B phenotype, suggesting that rather than being simply related to increased plasma triglyceride levels per se, the development of the LDL B phenotype may be dependent on underlying changes in triglyceride metabolism. As shown in Figures 1A and 2A, there is considerable overlap in plasma triglyceride levels between individuals with the LDL A and B phenotypes. In contrast, in control subjects the clearance of triglyceride-rich particles is delayed approximately twofold in subjects with the LDL B phenotype, and there is little overlap between the individuals with the LDL A and B phenotypes. Only a single individual with the LDL B phenotype has a clearance rate within two standard deviations of the mean rate of clearance of the LDL A group (Figure 1B). These results suggest that the slowed clearance rate of triglyceride-rich particles may be a better marker for the LDL B phenotype in controls than are plasma triglyceride levels. In NIDDM, there is greater variability in the clearance of triglyceride-rich lipoproteins, which may reflect the underlying metabolic disorders. As a consequence there is a greater overlap in clearance rates between the LDL A and LDL B groups, although the degree of overlap of the clearance rates is still somewhat less than that observed with plasma triglyceride levels.

Interestingly, the diagnosis of diabetes is also an independent determinant for the presence of the LDL B phenotype. Whether this indicates a direct relation between NIDDM and the LDL B phenotype or whether this is merely due to NIDDM causing disturbances in lipid metabolism that are not taken into account in our multivariate analysis is unclear. Both NIDDM and the LDL B phenotype are genetically determined, and it is possible that they are linked. Insulin resistance is a characteristic abnormality in NIDDM,\(^41\) and preliminary studies have suggested that the prevalence of the LDL B phenotype is increased in nondiabetic subjects who have insulin resistance.\(^42\) Thus, in NIDDM the...
situation is potentially very complex, with the increased prevalence of the LDL B phenotype being secondary to a variety of factors, including hypertriglyceridemia, alterations in lipid metabolism not reflected in plasma lipid levels, and perhaps to a linkage between two common genetic disorders.

Regardless of the cause of the increased prevalence of the LDL B phenotype in NIDDM, these changes have important clinical implications. The LDL B phenotype is associated with an approximately threefold increased risk of myocardial infarction in nondiabetic subjects.6–10 This risk may be due to associated lipid abnormalities, such as increased serum triglyceride levels and decreased HDL cholesterol concentrations.6–10 However, studies have also suggested that small, dense LDL (LDL B) per se may be more atherogenic. Small, dense LDL may bind to the arterial wall to a greater degree.43 Additionally, for any level of cholesterol there are more apoprotein B-containing particles, which may increase the risk of vascular disease. Lastly, small, dense LDL have been shown to have increased susceptibility to oxidation.44,45

In summary, the present study demonstrates that NIDDM is associated with an increased prevalence of the LDL B phenotype, even in the absence of frank hyperlipidemia. This may account for some of the increased independent risk of vascular disease in patients with NIDDM.

Acknowledgments

We thank P. Herranz, for excellent editorial assistance. We thank Laura Holl and Dulce Poyaoan for performing the gradient gel analysis and Bonnie Millar for assistance with statistical analysis. The cooperation and support of our patients and the staff of the Special Diagnostic and Treatment Unit are greatly appreciated.

References

40. Brunzell JD, Porte D Jr, Bierman EL: Reversible abnormalities in post hepatic lipase activity during the late phase of release in diabetes mellitus. Metabolism 1975;24:1123–1138
LDL subclass phenotypes and triglyceride metabolism in non-insulin-dependent diabetes.
K R Feingold, C Grunfeld, M Pang, W Doerrler and R M Krauss

doi: 10.1161/01.ATV.12.12.1496

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
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:
http://atvb.ahajournals.org/content/12/12/1496

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org//subscriptions/