High-Density Lipoprotein Subpopulation Profile and Coronary Heart Disease
Prevalence in Male Participants of the Framingham Offspring Study

Bela F. Asztalos, L. Adrienne Cupples, Serkalem Demissie, Katalin V. Horvath, Caitlin E. Cox, Marcelo C. Batista, Ernst J. Schaefer

Objective—High-density lipoprotein (HDL) is a heterogeneous lipoprotein class and there is no consensus on the value of HDL subspecies in coronary heart disease (CHD) risk assessment. We tested the hypothesis whether specific HDL subpopulations are significantly associated with CHD-prevalence.

Methods and Results—ApoA-I concentrations (mg/dL) in HDL subpopulations were quantitatively determined by native 2d gel electrophoresis, immunoblotting, and image analysis in male participants in the Framingham Offspring Study (FOS). CHD cases (n=169) had higher preβ-1 and α-3 particle and lower α-1, preα-3, and preα-1 particle levels than either all (n=1277) or HDL cholesterol-matched (n=358) controls. α-1 and preα-3 levels had an inverse association, whereas α-3 and preα-1 particle levels had a positive association with CHD prevalence after adjusting the data for established CHD risk factors. Standardized logit coefficients indicated that α-1 HDL was most significantly associated with CHD prevalence. Moreover, each mg/dL increase in α-1 particle level decreased odds of CHD by 26% (P<0.0001), whereas each mg/dL increase in HDL cholesterol decreased odds of CHD by 2% in a model including all established CHD risk factors.

Conclusion—Specific HDL subpopulations were positively correlated, whereas others were inversely correlated with CHD prevalence in male subject in the FOS, indicating that the various HDL particles might have different roles in the cause of CHD. (Arterioscler Thromb Vasc Biol. 2004;24:1-7.)

Key Words: lipoproteins—high-density lipoprotein cholesterol—high-density lipoprotein subpopulations—apolipoprotein A-I—coronary heart disease

High-density lipoprotein (HDL) is a heterogeneous class of lipoprotein particles with subspecies that differ in apolipoprotein and lipid composition, size, density, and charge; the different subspecies appear to have different physiological functions. Traditionally, HDL has been separated into major subclasses by polyanion precipitation, ultracentrifugation (HDL2 and HDL3), or by the apoA protein content, distinguishing particles containing only apoA-I (LpA-I), the major apolipoprotein of HDL, from particles containing both apoA-I and apoA-II (LpA-I:A-II). None of these techniques has provided any convincing evidence that 1 kind of HDL subfraction has any greater cardioprotective function than another. The lack of agreement among these studies is probably related to the fact that all of these HDL subfractions are themselves heterogeneous, containing a variety of different HDL subspecies with possibly different physiological functions.

Our laboratory uses native 2-dimensional gel electrophoresis, immunoblotting, and image analysis to separate HDL subpopulations quantitatively from plasma with high-resolution based on electrophoretic charge and particle size. We determine apoA-I content, not cholesterol, in these particles. This method has been useful in studies of HDL metabolism and cholesterol transport from cells because it separates intermediates in these processes. A small case-control study indicated that coronary heart disease (CHD) patients not only had HDL deficiency but also had a major rearrangement in the apoA-I-containing HDL subpopulations with significantly lower levels of the large α-1 and preα-1 (≈11 nm), and higher levels of the small α-3 (≈8.4 nm) and preβ-1 (≈5.6 nm) HDL particles than controls. Among these particles, α-3 contains both apoA-I and apoA-II; the rest contain apoA-I but no apoA-II. Moreover, an HDL intervention study indicated that changes in the concentration of α-1 were significantly and inversely correlated with the rate of coronary artery stenosis in CHD patients after treatment with simvastatin–niacin versus placebo for 2 years. These data suggest that specific HDL subpopulations...
Selection criteria for the CHD cases included a history of acute kidney disease, thyroid dysfunction, and drug or alcohol abuse. Participants had a standardized medical history, physical examination, and fasting lipid measurements. Exclusion criteria for the participants were a possible confounding or modifying effect by the use of cholesterol lowering medication, age, smoking, hypertension, diabetes, body mass index (BMI), as well as LDL cholesterol, HDL cholesterol, and TG levels. In these analyses, each HDL subpopulation was used as a dependent variable, CHD status as a primary independent variable, and the potential confounding factors listed were used as covariates. In addition, we used the generalized estimating equation approach (Proc Genmod in SAS) to account for correlated data caused by familial relations and matched design.

To evaluate the association between the HDL subpopulations and the odds of CHD prevalence, we used logistic regression models with CHD status as a dependent variable and the HDL subpopulations as independent variables. In these analyses, we used the generalized estimating equation approach (Proc Genmod in SAS) to account for correlated data caused by familial relations and conditional logistic regression to analyze the matched data. For each HDL subspecies and HDL cholesterol, we considered 3 models. In model 1, data were unadjusted; in model 2, data were adjusted for lipid-lowering medication, familial relations, age, smoking, hypertension, BMI, and presence of diabetes; in model 3, data were adjusted for HDL cholesterol (for HDL subpopulations), LDL cholesterol, and TG, in addition to all variables in model 2, to evaluate whether the HDL subspecies provided information beyond that contributed by traditional lipid measures. Because the HDL subpopulations are highly correlated with each other and with HDL cholesterol, we also evaluated all of these variables jointly. To assess the relative importance of these variables, we calculated the standardized logit coefficients and the corresponding odds ratios (ie, odds ratios computed after standardizing the variables to 0 mean and unit variance). All analyses that involved HDL cholesterol were performed in the unmatched data set only. For further examination of a possible confounding or modifying effect by the use of cholesterol medications, we repeated the aforementioned analyses among subjects who were not taking cholesterol medications at examination 6 (85 subjects with CHD and 1134 subjects without CHD). The results have significant roles in the development of CHD. Based on these results, we tested the hypothesis that specific HDL subpopulations were significantly associated with CHD prevalence in male participants in the Framingham Offspring Study (FOS).

**Methods**

**Study Population**

Participants in the FOS, a long-term, community-based, prospective, observational study of risk factors for cardiovascular disease, are the offspring and spouses of the original Framingham Heart Study (FHS) cohort. During cycle 6 of the FOS (1995 to 1998), participants had a standardized medical history, physical examination, and fasting lipid measurements. Exclusion criteria for the controls were: any evidence of heart or vascular disease, hepatic or kidney disease, thyroid dysfunction, and drug or alcohol abuse. Selection criteria for the CHD cases included a history of acute coronary insufficiency and myocardial infarction. We performed our analyses on all available plasma samples from male participants in cycle 6, which comprised 169 subjects with CHD and 1277 subjects without CHD.

**Laboratory Measurements**

Total cholesterol, triglyceride (TG), and HDL cholesterol concentrations were determined by standard enzymatic methods. HDL cholesterol was isolated from the supernatant after dextran–sulfate magnesium precipitation. Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula. Total plasma apoA-I concentrations were measured with a turbidimetric immunoassay (Wako Chemicals, Richmond, Va.).

Two-dimensional nondenaturing gel electrophoresis, immunoblotting, and image analysis were performed on plasma stored at −80°C, as described (Figure 1). Briefly, 4 μL of plasma were separated on agarose gel in the first dimension by charge into preβ−, α−, and preα-mobility subclasses. The agarose strips were excited and transferred to the top of nondenaturing 3% to 35% concave gradient polyacrylamide gels, and HDL was separated by size in the second dimension, followed by electrophoretic transfer to nitrocellulose membranes. Membranes were then incubated with monospecific anti-human apoA-I first and 125 I-labeled second antibodies. Quantification was performed in a PhosphorImager with the use of an ImageQuant software package (Molecular Dynamics). Each HDL subpopulation was delineated at the peaks were determined by integrating the designated areas. The program automatically measured the volume of each area and the percent distribution of each of the encircled HDL subpopulations. Data were expressed as pixels linearly correlated with the disintegrations per minute of the 125 I bound to the antigen–antibody complex. Absolute concentrations (in milligrams apoA-I per deciliter plasma) were calculated by multiplying the plasma total apoA-I concentration (mg/dL) by the percent area of each subpopulation. The effects of long-term storage on HDL subspecies were investigated and no significant changes in the values obtained after the measurement of the same samples fresh and from short-term and long-term storage were observed.

The interassay and intra-assay coefficients of variation were <5% for the lipid measurements and <10% for the apoA-I and HDL subpopulations determinations.

**Study Design and Statistical Analysis**

HDL subpopulations were measured in 1446 male participants of the FOS: 169 men with CHD and 1277 without CHD, at examination 6. To study the association between HDL subpopulations and CHD prevalence, we used data from all 1446 men, 169 men with CHD and 1277 without CHD; and from all 169 men with CHD and 358 HDL cholesterol-matched men without CHD.

Descriptive statistics, means±SD for continuous variables, or proportions for categorical variables were computed for all study variables and all study groups. The distribution of the variables was compared between subjects with and without CHD using 2-sample t tests for continuous variables and χ2 tests for categorical variables. For HDL subpopulations, we calculated adjusted means (and standard errors) for CHD and CHD-free subjects using analysis of covariance techniques that adjusted for lipid-lowering medication, age, smoking, hypertension, diabetes, body mass index (BMI), as well as LDL cholesterol, HDL cholesterol, and TG levels. In these analyses, each HDL subpopulation was used as a dependent variable, CHD status as a primary independent variable, and the potential confounding factors listed were used as covariates. In addition, we used the generalized estimating equation approach (Proc Genmod in SAS) to account for correlated data caused by familial relations and matched design.

![Image](http://atvb.ahajournals.org/)
Results

Table 1 shows major characteristics of subjects with and without a history of CHD in the FOS. CHD cases were compared with all controls (P1) and to HDL cholesterol-matched (±1 mg/dL) controls (P2). CHD patients were significantly more likely to have hypertension and diabetes and were older than controls. They had significantly lower LDL cholesterol levels than either of the control groups, probably because of the use of diets and lipid-lowering medications in this group; 50% of CHD patients and 11% of controls were on such medications. Although plasma apoA-I levels were similar, the apoA-I–containing HDL subpopulation profiles of CHD patients were significantly different from those of the control groups after adjusting for possible confounders (lipid-lowering medication, age, smoking, hypertension, diabetes, BMI, as well as LDL cholesterol, HDL cholesterol, and large TG levels). CHD cases had significantly lower mean levels of the large, cholesterol-rich, LpA-I 1 and preα-mobility particles, whereas they had significantly higher mean levels of the small, lipid-poor preβ-1 and α-3 particles than HDL cholesterol-matched controls.

To test which of these variables were significantly associated with CHD prevalence, the odds ratios of CHD were calculated (Table 2). The model that evaluated HDL cholesterol with adjustment for risk factors indicated that HDL cholesterol had an inverse association (odds ratio [OR] = 0.98, P = 0.04). In this model, age, diabetes, and cholesterol treatment were also significantly associated with CHD preva-
TABLE 2. Odd Ratios of CHD as Calculated for Each Parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All CHD vs All Controls</th>
<th>All CHD vs HDL-C-Matched Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C</td>
<td>0.98*</td>
<td>0.98</td>
</tr>
<tr>
<td>LDL-C</td>
<td>1.00</td>
<td>0.98</td>
</tr>
<tr>
<td>Large TG</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>Age</td>
<td>1.08*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.37</td>
<td>0.30</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.27</td>
<td>0.26</td>
</tr>
<tr>
<td>BMI</td>
<td>0.99</td>
<td>0.63</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.84*</td>
<td>0.01</td>
</tr>
<tr>
<td>Lipid Rx</td>
<td>5.15*</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Significantly associated with CHD prevalence.

TABLE 3. Odd Ratios as Calculated by a Model Including all HDL Particles and the Traditional Lipid and Non-Lipid CHD Risk Factors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All CHD vs All Controls</th>
<th>All CHD vs HDL-C-Matched Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preβ-1</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>Preβ-2</td>
<td>1.15</td>
<td>0.18</td>
</tr>
<tr>
<td>α-1</td>
<td>0.73*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>α-2</td>
<td>0.99</td>
<td>0.44</td>
</tr>
<tr>
<td>α-3</td>
<td>1.15*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Preα-1</td>
<td>1.61*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Preα-2</td>
<td>1.11</td>
<td>0.24</td>
</tr>
<tr>
<td>Preα-3</td>
<td>0.60*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.02</td>
<td>0.48</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.99</td>
<td>0.08</td>
</tr>
<tr>
<td>Large TG</td>
<td>0.27*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age</td>
<td>1.10*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.36</td>
<td>0.41</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.00</td>
<td>0.99</td>
</tr>
<tr>
<td>BMI</td>
<td>0.97</td>
<td>0.37</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.85*</td>
<td>0.04</td>
</tr>
<tr>
<td>Lipid Rx</td>
<td>5.00*</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Significantly associated with CHD prevalence.

Ors were calculated for one unit increase in each determined parameter or yes or no for the other parameters.

Discussion

The present study confirmed that the concentrations of the large lipid-rich LpA-I particles, especially α-1, were signifi-

Figure 2. Frequency distribution of α-1 HDL particle (a) and HDL cholesterol (b) levels of CHD cases (n=169) and controls (n=1277) in the FOS.

(OR=1.10, P<0.01) and diabetes (OR=1.85, P=0.04) remained significantly associated and large TG (OR=0.27, P<0.01) became inversely associated with CHD prevalence. Similar ORs were obtained when CHD cases were compared with HDL cholesterol-matched controls.

When standardized variables were used to evaluate the relative importance of the HDL particles, α-1 particle level had the strongest association with CHD followed by preα-1, α-3, and preα-3, with standardized logit coefficients of -2.78, 1.6, 1.34, and -0.99, respectively.

The frequency distribution of α-1 (Figure 2), HDL cholesterol, and LDL cholesterol indicated that α-1 differentiated CHD cases from controls more clearly than either HDL cholesterol or LDL cholesterol.

HDL cholesterol versus α-1 levels of all controls and CHD cases were plotted separately in Figure 3. The regression line of the CHD group had a significantly (P<0.001) different slope than that of controls. Each mg/dL increase in HDL cholesterol resulted in a significantly (P<0.001) higher level of α-1 in controls (0.55 mg/dL) than in CHD cases (0.31 mg/dL).

Figure 3. Plots of α-1 HDL particle levels versus HDL cholesterol levels of CHD (n=169) and control (n=1277) subjects.
cantly lower in male CHD patients compared either to all or to HDL cholesterol-matched controls. In addition, CHD patients had significantly higher mean α-3 and preβ-1 particle levels than either all or HDL cholesterol-matched controls.

Because HDL cholesterol level has a significant positive correlation with α-1 particle level, we examined whether the differences in α-1 levels reflected only the differences in HDL cholesterol levels between control and CHD subjects. The results indicated that CHD subjects had significantly lower mean level of α-1 particles compared either to all or to HDL cholesterol-matched controls after adjusting the data for established CHD risk factors. Moreover, each mg/dL increase in HDL cholesterol resulted in a mean 0.31 mg/dL increase in α-1 level in CHD patients and a mean 0.55 mg/dL increase in control subjects (Figure 3).

Based on these and earlier findings, we hypothesized that the concentrations of specific HDL particles were significantly associated with CHD prevalence independent of other lipid and nonlipid risk factors. The data generated on the FOS cohort supported this hypothesis. Increases in α-1 and preα-3 particle levels were significantly associated with decreased odds of CHD, whereas increases in α-3 and preα-1 particle levels were significantly associated with increased odds of CHD in a model including all established lipid and nonlipid-risk factors (Table 3). In crude analysis (unadjusted for covariates) or in analysis that adjusted for all covariates and HDL subpopulations except α-1, lower levels of preα-1 were associated with CHD prevalence. However, in models that evaluated α-1 and preα-1 simultaneously, higher levels of preα-1 were associated with CHD prevalence. Our results indicate that this discrepancy is caused by the strong positive correlation between α-1 and preα-1 particle levels (r=0.76, P<0.001) and the strong inverse association of α-1 with CHD prevalence. To affirm that the association in the model that jointly evaluated the 2 correlated variables was not a statistical artifact (eg, because of collinearity), we evaluated the association using a 2-stage analysis; first, we computed the standardized residuals of preα-1 from a linear regression model by regressing preα-1 on α-1. Then, we used the residuals (which are now uncorrelated with α-1) and other covariates as independent variables and CHD prevalence as a dependent variable in a logistic regression model. The 2 approaches, the direct adjustment for α-1 and the residual analyses, produced similar results. A similar investigation of HDL cholesterol, large TG, and α-1 also suggested that the inverse association between HDL cholesterol and CHD prevalence and the positive association between large TG and CHD prevalence in crude models or adjusted models that did not include HDL subpopulations is probably confounded by these subpopulations.

Although it is hypothesized that an increased level of preβ-1 HDL is a significant risk factor for CHD,18 and significantly higher preβ-1 levels were observed in CHD cases compared either to all or to HDL cholesterol-matched controls in this study, preβ-1 level was not significantly associated with CHD prevalence. There were no associations between the concentrations of preβ-2, α-2, and preα-2 particles and CHD prevalence, either.

We have evaluated which HDL particle was most highly associated with CHD prevalence by using standardized HDL variables in the analysis. The results indicated that α-1 was most strongly associated with CHD prevalence. These data indicate that the various HDL particles might have different roles in the cause of CHD.

In accordance with other studies, HDL cholesterol level also decreased odds of CHD prevalence significantly by 2% to 3% after adjusting data for traditional risk factors. However, when α-1, α-3, preα-1, or preα-3 were included in the model, HDL cholesterol level was not significantly associated with CHD prevalence (data not shown). As we have noted, we postulate that a strong positive correlation between α-1 and HDL cholesterol levels13 and a strong correlation of α-1 with CHD might have decreased the association of HDL cholesterol with CHD prevalence to nonsignificant. The frequency distributions clearly indicated that α-1 distinguishes cases from controls more clearly than HDL cholesterol and LDL cholesterol.

The inverse association between HDL cholesterol and CHD is partially because of the key role HDL plays in reverse cholesterol transport (RCT). In this pathway, HDL transports cholesterol from the periphery to the liver for biliary secretion. However, the extent to which plasma HDL concentration regulates RCT is not fully understood.19 We know that specific HDL subspecies are involved in certain steps of RCT.20 It has recently been shown that intravenous injection of apoA-I/phosphatidylcholine discs into humans increases plasma preβ-1 HDL concentration, which can stimulate RCT.21 Also, HDL, in the form of recombinant human apoA-I-liposomes infused into hypercholesterolemic humans, has stimulated net cholesterol excretion from the body, directly demonstrating the stimulation of RCT.22 Moreover, recombinant apoA-I Milano/phospholipid complex produced significant regression of coronary atherosclerosis as measured by intravascular ultrasound.23

It is assumed that preβ-1 HDL is involved in the first step of RCT by mediating cellular cholesterol efflux. The small lipid-poor preβ-1 then matures into large HDL, which is believed to deliver cholesterol to the bile via scavenger receptor-B1 (SR-B1) in the liver, the last step of RCT. We have shown that preβ-1 level correlates positively with ABCA-1–mediated and inversely with SR-B1–mediated cell cholesterol efflux.24 We have also documented that α-1, a large cholesterol-rich subpopulation, was converted into significantly smaller particles in 5 hours in human SR-B1 transgenic mice, indicating that α-1 particles successfully delivered cholesterol to SR-B1.25 Data obtained in this study suggest that in CHD subjects, the lipid-poor preβ-1 maturation into larger discoidal particles is impaired, resulting in inadequate RCT. Moreover, decreased levels of the large lipid-rich HDL particles may influence the antioxidant capacities of HDL,26 indicating that not only RCT but also other important antiatherogenic functions of HDL are impaired in subjects with low α-1 level.

Studying the HDL subpopulation profile of subjects with and without CHD improves our understanding of the association between HDL and CHD prevalence. Moreover, these studies might be helpful in selecting therapies for reducing or
managing CHD risk. We have demonstrated that statin monotherapies selectively increased α-1 and preα-1 HDL levels; however, different statins in various concentrations had significantly different capacities to increase the concentration of these particle levels independently of their capacity of changing HDL cholesterol and other lipid levels.\cite{27,28} In the HDL Atherosclerosis Treatment Study, 2 laboratories using different methods showed a significant inverse correlation between changes in the large Lp(a)-1 HDL particle levels and in the progression of coronary artery stenosis after simvastatin–niacin versus placebo treatment for 2 years.\cite{15,29} Subjects in the top tertile of α-1 level had no progression of stenosis, whereas subjects in the bottom tertile had 2.1% narrowing in the examined coronary artery lumens.\cite{15}

This study had several limitations. We studied only male subjects, and the results might not be applicable for females. Our preliminary data on females have indicated similar differences, but on a different scale between cases and controls. These findings have to be confirmed in a significantly larger study population.

Although FOS controls were selected without a history of CHD, any of these subjects could have had asymptomatic CHD. This assumption is supported by the fact that a large number of control subjects had 1 or more CHD risk factors: 34% had low HDL cholesterol (≤40 mg/dL), 16% had high LDL cholesterol (≥160 mg/dL), 32% had high TG (≥150 mg/dL), 22% had low HDL cholesterol and high TG, and 5% had low HDL cholesterol, high LDL cholesterol, and high TG levels.

Data on lipid levels in CHD cases might have been influenced by the use of lipid-lowering medication (50% of CHD and 11% of control subjects used such medications). We have no information about the specific drug use, but based on the general practice at the time period of cycle 6 (1995 to 1998), probably most of the subjects were on statin monotherapies. A subanalysis on subjects not taking any lipid-lowering medication (85 CHD cases and 1134 controls) indicated that such therapies did not influence our overall results (data not shown).

The method we use to assess HDL subfractions is not widely available for clinical practice; however, the information we gain by these studies help us to understand HDL metabolism, drug-induced modifications in HDL composition, and the role of HDL in CHD.

In this study, we confirm that male CHD patients have a significantly different HDL subpopulation profile than controls. Moreover, we document that specific HDL subpopulations are significantly associated with CHD prevalence, with α-1 being the one most significantly associated with CHD prevalence.

We conclude that the measurement of HDL subpopulations provides useful information about CHD risk beyond that obtained from traditional CHD risk factors, especially in subjects with normal LDL cholesterol and TG levels. An optimal HDL subpopulation profile marked with high α-1 and low α-3 and preβ-1 levels should be a target for lifestyle and drug interventions.

Acknowledgments

We thank the participants of the Framingham study. This study was supported by the National Institutes of Health (NIH)/National Heart, Lung, and Blood Institute (NHLBI) to Bela F. Asztalos (HL-64738). The work is also supported by the NHLBI’s Framingham Heart Study (NIH/NHLBI Contract N01-HC-38038 and HL-54776). The Framingham Heart Study is conducted and supported by the NHLBI in collaboration with Boston University.

References


High-Density Lipoprotein Subpopulation Profile and Coronary Heart Disease. Prevalence in Male Participants of the Framingham Offspring Study

Bela F. Asztalos, L. Adrienne Cupples, Serkalem Demissie, Katalin V. Horvath, Caitlin E. Cox, Marcelo C. Batista and Ernst J. Schaefer

Arterioscler Thromb Vasc Biol, published online September 23, 2004; Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 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/early/2004/09/23/01.ATV.0000146325.93749.a8.citation