ApoE4 Polymorphism Increases the Risk for Exercise-Induced Silent Myocardial Ischemia in Older Men

Leslie I. Katzel, Jerome L. Fleg, Michael Paidi, Nyla Ragoobarsingh, Andrew P. Goldberg

The apolipoprotein (apo) E4 polymorphism is associated with increased risk for symptomatic coronary artery disease (CAD). This study examines whether the apoE4 allele is associated with an increased risk for exercise-induced silent myocardial ischemia (SI) in healthy, older (62 ± 7 years; mean ± SD), normocholesterolemic, nonsmoking male volunteers. The apoE4 allele was present in 20 of 45 (44%) men with SI on graded exercise treadmill testing compared with 22 of 127 (17%) men of comparable age with normal exercise tests (P < .001), resulting in a crude relative risk of 2.57 (95% confidence limits, 1.57 to 4.23) for SI in men with the apoE4 allele compared with those without the e4 allele. Although the lipoprotein lipid levels did not differ between men with normal exercise tests and those with SI, the men with the apoE4/3 phenotype had higher total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels than those with the apoE2/3 and 3/3 phenotypes (P < .05). Men with SI and the apoE4/3 phenotype were older (64 ± 5 versus 57 ± 8 years, P < .01) and leaner (P < .01) than the normal non-SI men with the apoE4/3 phenotype. The older age of the men with SI and the apoE4/3 phenotype is consistent with a progression of atherosclerosis over time. Men with SI and the apoE3/3 phenotype were of comparable age and body composition to apoE3/3 phenotype men with normal exercise tests. Thus, even in the presence of normal LDL-C levels, the apoE4 allele may predispose older men to SI. (Arterioscler Thromb. 1993;13:1495-1500.)

KEY WORDS • apoE • silent ischemia • atherosclerosis • aging • exercise treadmill test

Apolipoprotein (apo) E modulates the metabolism of apoB-containing lipoproteins. The gene locus for apoE is polymorphic, having three common alleles, e2, e3, and e4, which encode three major isoforms. Each individual has two copies of the allele, resulting in six common genotypes. The apoE locus accounts for approximately 7% of the interindividual variance in total cholesterol; this is more than any other commonly characterized genetic locus. Compared with individuals homozygous for the e3 allele, individuals with the e4 allele have higher total plasma cholesterol levels and higher low-density lipoprotein cholesterol (LDL-C) levels. The higher LDL-C levels in individuals with the apoE4 allele may in part explain their increased risk for the development of coronary artery disease (CAD). Furthermore, the prevalence of the apoE4 allele is lower in older than in younger people, suggesting that individuals with the apoE4 allele may have a reduced life expectancy.

Before participation in an intensive exercise training program, graded exercise treadmill tests were administered to healthy middle-aged and older men to screen for asymptomatic CAD. More than 25% of the men had asymptomatic exercise-induced electrocardiographic (ECG) ST segment depression consistent with silent myocardial ischemia (SI). Some of the men with SI had abdominal obesity and reduced high-density lipoprotein cholesterol (HDL-C) levels, whereas in others no apparent risk factors for CAD other than male gender and increased age were identified. We hypothesized that the presence of the apoE4 allele might predispose these men to atherosclerosis. The results of this study suggested that even with normal LDL-C levels, the apoE4 allele is associated with an increased risk for the development of SI.

Methods

Healthy, nonsmoking middle-aged and older male volunteers between 46 and 79 years of age with a wide range of body mass index (BMI) and maximal aerobic capacity (VO2max) were recruited from local athletic clubs and community centers, and from competitors in the Maryland Senior Olympics for participation in the Fitness After Fifty research program. This program is a prospective intervention study that examines the effects of weight loss and exercise training on cardiovascular and metabolic function in healthy older men. Based on a review of their medical histories, men with a history of angina, myocardial infarction, congestive heart failure, hypertension requiring antihypertensive...
medications, diabetes mellitus, and hyperlipidemia were excluded from the program. Eligible subjects underwent a physical examination, laboratory blood studies, and treadmill exercise test. Men with hyperlipidemia (LDL-C >160 mg/dL and triglycerides [TGs] >400 mg/dL) or diabetes mellitus (fasting glucose >140 mg/dL) were excluded. Data are reported on the first 172 consecutive men who completed metabolic testing. All of the men were Caucasian. This study was approved by the University of Maryland, Francis Scott Key Medical Center, and Johns Hopkins University Institutional Review Boards, and all men gave informed consent before participating in the research protocol.

An exercise treadmill test to >85% of the predicted age-adjusted maximal heart rate (220−age in years) was performed according to the Bruce protocol. On a subsequent visit, a maximal treadmill test was performed with determination of the VO2max by using a modified Balke protocol. The Minnesota code criteria of ≥1 mm horizontal or downsloping ST segment depression for at least 0.08 second after the J-point was used to define exercise-induced ischemia. Because of the ethical difficulty of performing cardiac catheterizations in asymptomatic volunteers, ECG responses during the Bruce protocol and Balke protocol exercise tests (see below) exercise tests were used to categorize the men; all control subjects were required to have normal Bruce protocol and Balke protocol exercise tests, and all men with SI had asymptomatic ST segment depression on the Bruce and Balke protocol treadmill tests. Men with ischemic changes on their exercise ECG were referred to their private physicians for further cardiac evaluation and care and are being followed by us longitudinally. We recognize that the use of these criteria for a diagnosis of SI may result in the misclassification of some of the men.

**Measurement of VO2max**

Maximum oxygen uptake capacity (ie, VO2max) was measured on a treadmill by using a modified Balke protocol. The initial treadmill speed was set to produce approximately 75% of the subject’s peak VO2 obtained during the screening treadmill test. The elevation of the treadmill was then increased every 1 to 2 minutes until the subject was exhausted and could not continue to exercise. Respiratory rate, minute ventilation, O2 consumption, and CO2 production were measured every 30 seconds as previously described, and the respiratory exchange ratio was then calculated. A 12-lead ECG was recorded every minute during the test. In three men the VO2max tests were stopped prematurely because of ST segment depression >2 mm during exercise, and a peak VO2 was obtained as a measure of their ischemic threshold. Other VO2max tests fulfilled the following three criteria: (1) heart rate at maximal exercise was >85% of the age-predicted maximal heart rate; (2) respiratory exchange ratio was >1.0; and (3) the VO2 reached a plateau during the final stage of exercise, ie, the increase in VO2 was ≤0.2 L/min during the final increase in work rate. The VO2max is expressed as milliliters per kilogram per minute.

**Measurement of Body Composition**

BMI was calculated as the body weight in kilograms/(height in meters)2. Percent body fat was determined by hydrostatic weighing and was calculated by using the Siri equation with correction for the residual lung volume. The waist-to-hip circumferential ratio (WHR), an index of body fat distribution, was calculated as the ratio of the maximal circumference of the abdomen at the waist to that of the buttock measured at the maximal gluteal protuberance.

**Measurement of Lipoprotein Lipids**

All subjects were instructed over a 6-week period on an American Heart Association (AHA) Step I diet and were weight stable on a constant composition, ad lib outpatient diet for at least 4 weeks before metabolic testing. For the 5 days immediately preceding and during metabolic testing, subjects consumed isocaloric weight-maintaining meals prepared by our metabolic kitchen. Body weight varied by <1 kg during the period of metabolic testing.

Blood samples were drawn after a 12- to 14-hour overnight fast for the determination of lipoprotein lipid levels. Reported lipoprotein lipids are the mean of at least two determinations. Plasma TGs, total cholesterol, and HDL-C were measured enzymatically as previously described, and LDL-C was calculated by using the Friedewald equation. The apoE phenotype was determined by using immunochemical techniques. Known apoE phenotype standards were run in the calibration lanes.

**Statistical Analysis**

The data were analyzed by using sas. Duncan’s multiple-range test was used to compare the mean values in men of different apoE phenotypes. The apoE phenotype distributions were compared by using the χ2 analysis. All results are expressed as mean±SD. A two-tailed probability value of ≤0.05 defined statistical significance.

**Results**

Forty-five of the 172 men (26%) had exercise-induced SI. The men with SI and the men with nonischemic exercise tests (normal subjects) were of similar age, percent body fat, WHR, and VO2max (Table 1). Plasma TGs, total cholesterol, HDL-C, and LDL-C levels were the same in the men with SI and in the normal subjects (Table 2).
The overall apoE allele frequencies in the 172 men were ε2, .067; ε3, .800; and ε4, .134. The apoE phenotype distribution in the men with SI was markedly different than in the normal subjects (Table 3). The apoε4 allele was present in 20 of the 45 (44%) men with SI compared with only 22 of 127 (17%) normal subjects ($\chi^2=12.0, P<.001$). Similarly, the apoE 2/3 phenotype was less prevalent in the men with SI (4%) than in the controls (11%). The crude relative risk for SI for men with the apoε4 allele was 2.57 (95% confidence limits, 1.55 to 4.23) compared with those without the apoε4 allele.

The relations between apoE phenotype, lipoprotein lipid levels, and presence or absence of ischemia was examined in the 172 men. The 35 men with the apoE 4/3 phenotype had higher total cholesterol (185±23 versus 175±24 mg/dL, respectively; $P<.05$) and LDL-C (126±21 versus 117±21 mg/dL, respectively; $P<.05$) levels than the 112 men with the apoE 3/3 phenotype (Table 4), whereas the lipoprotein lipid levels in men with the apoE 2/3 phenotype were comparable to those of men with the apoE 3/3 phenotype. There were insufficient numbers of men with the apoE 2/2, 4/4, and 4/2 phenotypes to allow meaningful comparisons of lipid levels in men with these phenotypes.

The 17 men with SI and the apoE 4/3 phenotype were significantly older (64±5 years) than the 18 men with the apoE 4/3 phenotype and normal exercise tests (57±8 years, $P<.01$; Table 5). Despite the consistently higher plasma levels of total cholesterol and LDL-C in the men with the apoE 4/3 phenotype, the lipoprotein profiles in the men with SI and the apoE 4/3 phenotype were not significantly different from their apoE 4/3 counterparts with normal exercise tests. However, the men with SI and the apoE 4/3 phenotype were significantly leaner than the nonischemic men with the apoE 4/3 phenotype ($P<.05$). The lower BMI ($P<.01$) and percent body fat ($P<.05$) in ischemic men with the apoE 4/3 phenotype may in part account for the similarity in their plasma levels of TGs, total cholesterol, LDL-C, and HDL-C to the nonischemic men with the apoE 4/3 phenotype. Nevertheless, the older age of the men with SI and the apoE 4/3 phenotype is consistent with a progression of atherosclerosis over time despite leanness, a slightly higher VO2max, and lipoprotein lipid levels comparable to the men with the apoE 4/3 phenotype and normal exercise tests.

In contrast to the men with the apoE 4/3 phenotype, the apoE 3/3 phenotype men with SI and the apoE 3/3 phenotype men with normal exercise tests were of similar age and body composition (Table 5). Although there was a trend for HDL-C levels to be lower in the ischemic men with the apoE 3/3 phenotype compared with the nonischemic apoE 3/3 control subjects ($P<.06$), TG, LDL-C, and HDL-C levels were the same in the two groups.

**ApoE Phenotype Distribution, Lipoprotein Lipid Levels, and SI**

The overall apoε allele distribution in these 172 men was not a randomly selected population of older men, as the conventional lipid levels were the same in the men who had hypertension or diabetes or smoked cigarettes. This suggests that the apoε allele predisposes older men to atherosclerosis independent of the major traditional risk factors for CAD. This is in agreement with studies by Nieminen et al,5 who have demonstrated that plasma levels of HDL-C, apoB, and the apoE phenotype were independent discriminants of angiographically verified CAD. Similarly, after adjustment for age, BMI, HDL-C, cigarette use, and diastolic blood pressure, apoε was an independent risk factor for the subsequent development of CAD, particularly fatal myocardial infarction, in the Multiple Risk Factor Intervention Trial (MRFIT). The mechanism by which apoε increases risk for CAD, particularly in those with normal LDL-C levels, requires further investigation.

**Table 2. Triglyceride and Lipid Levels in Men With Normal Exercise Tests and Men With Silent Ischemia**

<table>
<thead>
<tr>
<th>Triglyceride/Lipid</th>
<th>Normal Exercise Test (n=127)</th>
<th>Silent Ischemia (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>117±50</td>
<td>117±49</td>
</tr>
<tr>
<td>TC</td>
<td>175±25</td>
<td>179±24</td>
</tr>
<tr>
<td>HDL-C</td>
<td>37±9</td>
<td>37±8</td>
</tr>
<tr>
<td>LDL-C</td>
<td>117±22</td>
<td>120±19</td>
</tr>
</tbody>
</table>

**Table 3. Distribution of ApoE Phenotypes in Men With Normal Exercise Tests and Men With Silent Ischemia**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Normal Exercise Test (n=127)</th>
<th>Silent Ischemia (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE 2/2</td>
<td>2 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ApoE 2/3</td>
<td>14 (11%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>ApoE 3/3</td>
<td>89 (70%)</td>
<td>23 (51%)</td>
</tr>
<tr>
<td>ApoE 4/3</td>
<td>18 (14%)</td>
<td>17 (38%)</td>
</tr>
<tr>
<td>ApoE 4/2</td>
<td>3 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ApoE 4/4</td>
<td>1 (1%)</td>
<td>3 (7%)</td>
</tr>
</tbody>
</table>

Apo indicates apolipoprotein. The apoε allele was present in 20 of the 45 (44%) men with SI compared with 22 of 127 (17%) normal subjects ($\chi^2=12.0, P<.001$).
The prevalence of both overt CAD and SI increases with aging. Therefore, if one selects individuals solely on the basis of the presence or absence of CAD, one would expect that individuals with CAD to be on average older than those without CAD. In our study, there was a trend for men with SI to be older than those with normal tests (62±7 versus 59±6 years, P.<.08). The men with SI and the apoE 4/3 phenotype were on average 7 years older than the men with the apoE 4/3 phenotype and normal exercise tests, suggesting that there was a progression of atherosclerosis over time in this at-risk population. This is in agreement with the MRFIT data that demonstrates that age and the apoE4 allele are independent risk factors for CAD.

Based on the results of our previous studies in obese older men, one would have predicted that the men with the apoE 4/3 phenotype and SI would have a higher WHR and lower HDL-C and HDL-C levels than the control subjects, similar to the trend in HDL-C levels noted in the men with SI and the apoE 3/3 phenotype. Instead, the men with the apoE 4/3 phenotype and SI were leaner than the men with the apoE 4/3 phenotype with the normal exercise tests, and there was a trend toward higher, not lower, levels of HDL-C in the men with SI. These unexpected findings may be due to the subject selection criteria for entry into the study. The association between abdominal obesity, hyperinsulinemia, glucose intolerance, hypertension, and low HDL-C levels and increased risk for CAD is well established. It is therefore likely that a significant percentage of older men with abdominal obesity, low HDL-C levels, and the apoE 4/3 phenotype may already have symptomatic CAD. The exclusion of the obese older men with symptomatic CAD and the apoE 4/3 phenotype would lead to lower BMI and percent body fat in the apoE 4/3 men with SI, and it would also lead to lower HDL-C and HDL-C levels.
account for the observed lack of significant differences in plasma lipoprotein lipid profiles between the normal subjects and the men with SI. The obese men with the apoE 4/3 phenotype and normal exercise treadmill tests were being followed longitudinally to determine their rate of progression to asymptomatic and symptomatic CAD.

To control for the effects of dietary composition on lipoprotein lipid levels, all participants were instructed on the principles of the AHA Step I diet, and during periods of metabolic testing they were supplied with isocaloric weight-maintaining AHA Step I diets prepared by our kitchen. In agreement with the results of other studies, men with the apoE 4/3 phenotype had higher LDL-C levels than the men with either the apoE 3/3 or apoE 2/3 phenotypes while on the AHA Step I diet. The apoE phenotype appears to modulate both the efficiency of intestinal cholesterol absorption and the clearance of exogenous dietary fat. This has led some investigators to hypothesize that individuals with the apoE 4 allele may be more responsive to lipid-lowering drugs and to changes in dietary composition than those with the apoE 3/3 phenotype. Tikkanen et al demonstrate that when apoE4 homozygotes were switched from a low-fat to a high-fat diet, they increased their LDL-C levels more than individuals with the apoE 2/3 and 3/3 phenotypes. Therefore, the interaction between apoE polymorphism and dietary composition may confound the interpretation of the data in the present study. First, it is likely that the differences in LDL-C levels between the men with the apoE 4/3, 3/3, and 2/3 phenotypes would have been larger if the men had consumed a diet higher in saturated fat and cholesterol than the AHA Step I diet. Second, given the secular trend toward the consumption of diets lower in saturated fat and cholesterol, the lipoprotein lipid levels measured with subjects consuming an AHA Step I diet may not reflect their "true" lifelong cholesterol levels. Presumably, the total cholesterol and LDL-C levels were higher in the men for the majority of their lifespan, particularly in those with the apoE4 allele. This would lead to an underestimate of the effects of elevated LDL-C on the pathogenesis of atherosclerosis in the men with the apoE4 allele.

Apparently healthy individuals with SI have increased cardiac morbidity and mortality. In healthy asymptomatic volunteers of the Baltimore Longitudinal Study on Aging, individuals with concordant positive exercise ECG and abnormalities on thallium-201 scintigraphy were at a 3.6-fold relative risk for subsequent coronary events, independent of conventional cardiac risk factors. In a study of asymptomatic, normotensive factory workers, individuals with >1 mm exercise-induced ST segment depression on their exercise ECG were at a 5.5-fold increased relative risk for cardiac events solely on the basis of the exercise ECG results. A combination of ischemic ST segment depression, age greater than 55 years, and HDL-C <40 mg/dL was associated with a 17-fold increase in cardiac morbidity and mortality in the Lipid Research Clinics (LRC) study. Even when adjusted for standard CAD risk factors, individuals with ECG evidence of ischemia on an exercise test had a relative risk of 5 compared with LRC participants with normal treadmill exercise tests. In the MRFTT, usual-care men who had exercise-induced ST segment depression at baseline were at a threefold higher risk for death from CAD over the ensuing 6 to 8 years. In a subgroup analysis, CAD mortality was 57% lower in the special-care intervention group with SI than in the usual-care group with SI (P=.002). This suggests that aggressive risk factor intervention may substantially reduce cardiac morbidity in some men with SI.

Limitations in the cardiac evaluation of the volunteers in the present study need to be considered. Cardiac catheterization was not performed in these asymptomatic volunteers; instead, ECG responses during graded exercise treadmill testing was used to identify subjects with exercise-induced SI. Since the sensitivity and specificity for exercise ECG in the diagnosis of CAD are estimated to be 65% and 85%, respectively, and the prevalence of CAD was low in the study population, we recognize that relying on the exercise ECG would result in the misclassification of some of the men. However, this potential bias would cause the distribution of the apoE4 allele to be less skewed and result in an underestimation of the relative risk for SI in older men with the apoE4 allele.

In summary, these results demonstrated an association between the apoE4 allele and exercise-induced SI in normocholesterolemic older men. Furthermore, the men with SI and the apoE4 allele were on average 7 years older than those men with the apoE4 allele with normal exercise tests, consistent with a progression of atherosclerosis over time in men with the apoE4 allele. Population studies will be required to determine whether screening older individuals with SI for the apoE4 allele is warranted, and conversely, whether physicians should screen older individuals with the apoE4 allele for SI. This may permit an earlier identification of subjects at high risk for progression to symptomatic CAD and its complications. Longitudinal studies are in progress to examine these possibilities.

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References


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