Association of Coronary Heart Disease Risk Factors With Microscopic Qualities of Coronary Atherosclerosis in Youth

Henry C. McGill, Jr, MD; C. Alex McMahan, PhD; Arthur W. Zieske, MD; Richard E. Tracy, MD; Gray T. Malcom, PhD; Edward E. Herderick, BS; Jack P. Strong, MD; for the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group

Background—This study examined whether atherosclerosis in young people is associated with the risk factors for clinical coronary heart disease (CHD).

Methods and Results—Histological sections of left anterior descending coronary arteries (LADs) from 760 autopsied 15- to 34-year-old victims of accidents, homicides, and suicides were graded according to the American Heart Association (AHA) system and computerized morphometry. Risk factors (dyslipoproteinemia, smoking, hypertension, obesity, impaired glucose tolerance) were assessed by postmortem measurements. Approximately 2% of 15- to 19-year-old men and 20% of 30- to 34-year-old men had AHA grade 4 or 5 (advanced) lesions. No 15- to 19-year-old women had grade 4 or 5 lesions; 8% of 30- to 34-year-old women had such lesions. Approximately 19% of 30- to 34-year-old men and 8% of 30- to 34-year-old women had atherosclerotic stenosis $\geq 40\%$ in the LAD. AHA grade 2 or 3 lesions (fatty streaks), grade 4 or 5 lesions, and stenosis $\geq 40\%$ were associated with non-HDL cholesterol $\geq 4.14$ mmol/L (160 mg/dL). AHA grade 2 or 3 lesions were associated with HDL cholesterol $\leq 0.91$ mmol/L (35 mg/dL) and smoking. AHA grade 4 or 5 lesions were associated with obesity (body mass index $\geq 30$ kg/m$^2$) and hypertension (mean arterial pressure $\geq 110$ mm Hg).

Conclusions—Young Americans have a high prevalence of advanced atherosclerotic coronary artery plaques with qualities indicating vulnerability to rupture. Early atherosclerosis is influenced by the risk factors for clinical CHD. Long-range prevention of CHD must begin in adolescence or young adulthood. (Circulation. 2000;102:374-379.)

Key Words: atherosclerosis ■ coronary disease ■ risk factors ■ youth

The major strategy to prevent coronary heart disease (CHD) has been to control risk factors that predict the probability of developing clinical disease.1 The success of these efforts2 encourages continued efforts directed toward risk factor control,3 but the age at which such efforts should begin, particularly efforts to control serum lipid levels, remains controversial.4 The risk factors for CHD are associated with the extent and severity of atherosclerosis in adults $>35$ years old,5 but until recently, we did not know whether the risk factors were also associated with the early lesions of atherosclerosis in younger people. In 1985, investigators organized a multicenter cooperative study, Pathobiological Determinants of Atherosclerosis in Youth (PDAY), to determine the relation of cardiovascular risk factors to atherosclerosis in victims of accidents, homicides, or suicides who were 15 to 34 years old.6 Previous PDAY reports showed that the gross extent of lesions of the right coronary artery and aorta was associated with the risk factors for CHD (male sex, high non-HDL cholesterol, low HDL cholesterol, smoking, hypertension, obesity, and impaired glucose tolerance).7–9 However, each of these gross lesion classes is microscopically heterogeneous, and some lesion qualities are associated with the likelihood of plaque rupture and arterial thrombosis.10,11 The relations of the risk factors to the qualitative characteristics associated with lesion progression and vulnerability to rupture would provide additional information with which to assess the need for risk factor modification in young people.

Therefore, in 760 PDAY cases, we evaluated the microscopic qualities of lesions at a standard site in the left anterior descending coronary artery (LAD) by the American Heart Association (AHA) grading system12,13 and measured the cross-sectional area of the potential arterial lumen occupied by these lesions as an indicator of stenosis. The association of

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From the Department of Pathology, The University of Texas Health Science Center at San Antonio (H.C.M., C.A.M.) and Southwest Foundation for Biomedical Research (H.C.M.), San Antonio, Tex; the Department of Pathology, Louisiana State University Medical Center, New Orleans (A.Z.W., R.E.T., G.T.M., J.P.S.); and the Biomedical Engineering Center, Ohio State University, Columbus (E.E.H.).
Correspondence to Henry C. McGill, Jr, MD, Southwest Foundation for Biomedical Research, PO Box 760549, San Antonio, TX 78245-0549. E-mail hmcgill@icarus.sfbr.org
Reprint requests to Jack P. Strong, MD, Department of Pathology, Louisiana State University Medical Center, 1901 Perdido St, New Orleans, LA 70112-1393. E-mail jstron@lsumc.edu
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TABLE 1. Risk Factors, Samples, Analyses, Classification, and Prevalence in the PDAY Study

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Sample</th>
<th>Analysis</th>
<th>Classification</th>
<th>Prevalence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>High non-HDL cholesterol</td>
<td>Serum</td>
<td>Total cholesterol minus HDL cholesterol</td>
<td>≥4.14 mmol/L (≥160 mg/dL)</td>
<td>28.0</td>
</tr>
<tr>
<td>Low HDL cholesterol</td>
<td>Serum</td>
<td>Cholesterol after precipitation of apo B lipoproteins</td>
<td>&lt;0.91 mmol/L (&lt;35 mg/dL)</td>
<td>18.7</td>
</tr>
<tr>
<td>Smoking</td>
<td>Serum</td>
<td>Thiocyanate</td>
<td>≥90 μg/L</td>
<td>44.0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Renal arteries</td>
<td>Intimal thickness and algorithm to estimate mean arterial pressure</td>
<td>≥110 mm Hg</td>
<td>15.5</td>
</tr>
<tr>
<td>Obesity</td>
<td>Measured at autopsy</td>
<td>BMI=weight (kg)/height (m)²</td>
<td>≥30 kg/m²</td>
<td>14.3</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>Red blood cells</td>
<td>% Glycohemoglobin</td>
<td>≥8%</td>
<td>4.3</td>
</tr>
</tbody>
</table>

BMI indicates body mass index.

Methods

Study Design
Fifteen cooperating centers adopted a Standard Operating Protocol and Manual of Procedures to collect specimens and data and to submit them to central laboratories for analysis. A statistical coordinating center received all data pertaining to each case.

Subjects
Study subjects were persons 15 through 34 years of age who died of external causes (accidents, homicides, or suicides) within 72 hours of injury and were autopsied within 48 hours in one of the cooperating forensic laboratories. Age and race were obtained from the death certificate. We collected 2876 acceptable cases from June 1, 1987, to August 31, 1994. For 760 cases, we had measurements of all risk factors, histological sections of a standard segment of the LAD, and digitized images from these sections. Fifty-two percent of the subjects were black, and 26% were women. The Institutional Review Board of each cooperating center approved this study.

Risk Factor Measurements
Methods of measuring CHD risk factors and the limitations of these measurements have been presented in previous publications and are summarized in Table 1, which also shows the prevalence of each risk factor in the 760 PDAY subjects included in this study.

Preparation of the LAD
PDAY investigators ligated the left circumflex and right coronary arteries close to their origins and removed them for other studies. They perfused the left main coronary artery and the LAD with 10% buffered formalin at a pressure of ~100 mm Hg (130 cm H2O) for ≥30 minutes while the heart lay in a formalin bath. The left main coronary arteries and LADs were then dissected from the heart and shipped to a central laboratory.

A technician cut a 5-mm transverse block from the LAD bounded on its proximal edge by the distal flow divider of the left main and left circumflex arteries. The proximal half of the bisected block was sectioned on a freezing microtome and stained with oil red O, and the distal half was embedded in paraffin and stained with Gomori’s trichrome aldehyde fuchsin (GTAF). This site was selected because of its susceptibility to clinically significant atherosclerotic lesions, particularly in young persons. The AHA grade at this site was associated with the extent of lesions in the entire right coronary artery (Kendall’s τ=0.324 for association with right coronary artery fatty streaks, τ=0.311 with right coronary artery raised lesions, P<0.0001). This finding supports the use of this single site in the LAD as representative of atherosclerosis in the entire coronary artery system. Therefore, to have statistical power to detect associations with the risk factors, we quantified selected histological and structural characteristics of this single site of maximum susceptibility in a large number of individuals rather than measuring more points in a smaller number of individuals.

Grading Histological Characteristics
Two pathologists (H.C.M. and A.W.Z.) graded the GTAF- and oil red O-stained sections according to the AHA classification system. The criteria are described briefly in Table 2. Differences were resolved by discussion, and the consensus grade was used in the statistical analyses.

Computerized Morphometry
A central laboratory scanned the GTAF-stained sections at a resolution of 1024×1024 pixels by 24-bit full color and displayed the
image on a color monitor. The operator manually identified the luminal border, the internal elastic lamina (IEL), and the external elastic lamina (EEL). If the IEL or the EEL was broken, the operator drew a smooth curve between the 2 ends. The operator then measured the lengths of the EEL and IEL and the cross-sectional areas of the intima and media. Because some of the sections were flattened transversely during sectioning, we assumed that the EEL was a circle and calculated the total artery cross-sectional area. The maximum potential lumen area was calculated by subtracting the measured medial area from the calculated area within the EEL. A case was classified as having atherosclerotic stenosis if the ratio of measured intimal area to maximum potential lumen area was $40\%$ and the AHA grade was $3$. We chose $40\%$ as the definition of stenosis because compensatory remodeling is believed to occur up to this level. All cases with AHA grade 0, 1, or 2 were considered to have adaptive intimal thickening (a “normal” structure) or minimal atherosclerosis and not to have atherosclerotic stenosis.

**Statistical Analyses**

For statistical analysis, the AHA grades were placed into 3 combined categories. We analyzed the prevalence of grades 0 or 1, 2 or 3, and 4 or 5 using polytomous logistic regression. Using grade 0 or 1 as the reference group, we constructed logits for comparing grades 2 or 3 with 0 or 1 and for comparing grades 4 or 5 with 0 or 1. The regression model included the effects of sex, race, 5-year age group, non-HDL cholesterol, HDL cholesterol, smoking, hypertension, obesity, and impaired glucose tolerance. We analyzed the prevalence of cases with atherosclerotic stenosis $\geq 40\%$ using binary logistic regression with the same model.

**Results**

**Prevalence of Lesions by AHA Grade in the LAD**

Figure 1 shows the prevalence of each grade of LAD lesions by 5-year age groups and sex. No lesions with hemorrhage or thrombosis (AHA grade 6) were encountered. There were no significant differences by race (black versus white; results not shown). There was a progressive increase in the frequency of advanced lesions with age in both sexes. Grade 4 or 5 lesions were present in 2.4% of 15- to 19-year-old men and in 20.3% of 30- to 34-year-old men. There were no grade 4 or 5 lesions among 15- to 19-year-old women, and grade 4 or 5 lesions were present in 7.8% of 30- to 34-year-old women.

**Prevalence of Atherosclerotic Stenosis**

Figure 2 shows the prevalence of atherosclerotic stenosis $\geq 40\%$ in men and women by 5-year age groups. Men 15 to 19 years old occasionally (3.2%) had stenosis by this definition, and the prevalence increased with age to 18.8% in the 30- to 34-year age group. Women had no stenosis ($\geq 40\%$) before age 25, and had $\leq 50\%$ the prevalence of men in the 30- to 34-year age group.

**Prevalence of Risk Factors**

Table 1 gives the prevalence of each of the risk factors. Only 22.4% of these adolescents and young adults had no risk factors as defined in Table 1, whereas 42.7% had 1 risk factor and 34.9% had $\geq 2$ risk factors.

**Associations of Risk Factors With Lesion Grades and Stenosis**

Table 3 presents the odds ratios for the associations of the risk factors with coronary artery lesions, and Figures 3 and 4 illustrate the effects of these associations on the prevalence of lesions. The odds ratios do not depend on prevalence of lesions and could apply to a population with a prevalence of lesions different from that in the PDAY sample. The odds ratios for risk factor effects in Table 3 are presented for AHA grade 2 or 3 versus grade 0 or 1, AHA grade 4 or 5 versus grade 0 or 1, and stenosis $\geq 40\%$ versus stenosis $< 40\%$. The odds ratio for a grade 4 or 5 lesion versus a grade 2 or 3 lesion can be estimated by
dividing the odds ratio for a grade 4 or 5 lesion versus a grade 0 or 1 lesion (Table 3, column 4) by the odds ratio for a grade 2 or 3 lesion versus a grade 0 or 1 lesion (Table 3, column 2). Odds ratios for risk factor combinations can be estimated as the product of the odds ratios for the individual risk factors.

Men had greater odds of grade 2 or 3 lesions ($P = 0.0044$), grade 4 or 5 lesions ($P = 0.0236$), and stenosis ($P = 0.0285$) (Table 3, row 1). Men had an ≈60% higher prevalence of grade 4 or 5 lesions than women (Figure 1). The higher prevalence of advanced lesions in men could not be explained by differences in risk factors.

High non-HDL cholesterol concentrations were associated with greater odds of grade 2 or 3 lesions ($P = 0.0003$), grade 4 or 5 lesions ($P = 0.0054$), and stenosis ($P = 0.0013$) (Table 3, row 2) and with ≈60% higher prevalence of grade 4 or 5 lesions (Figure 3).

Low HDL cholesterol concentrations were associated with greater odds of grade 2 or 3 lesions ($P = 0.0341$) (Table 3, row 3) and with ≈30% higher prevalence of grade 4 or 5 lesions (Figure 3).

Smoking was associated with greater odds of grade 2 or 3 lesions ($P = 0.0475$) (Table 3, row 4) and higher prevalence of grade 2 or 3 lesions, whereas there was little difference in the

### Table 3. Odds Ratios for Risk Factor Effects Adjusted for Other Risk Factors and 5-Year Age Group

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>AHA Grade (2–3 vs 0–1)</th>
<th>AHA Grade (4–5 vs 0–1)</th>
<th>Atherosclerotic Stenosis ≥40%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>1.83 1.20–2.80</td>
<td>2.47 1.00–6.10</td>
<td>2.80 1.11–7.03</td>
</tr>
<tr>
<td>High non-HDL cholesterol</td>
<td>2.04 1.39–3.01</td>
<td>2.59 1.32–5.09</td>
<td>2.95 1.53–5.70</td>
</tr>
<tr>
<td>Low HDL cholesterol</td>
<td>1.63 1.03–2.58</td>
<td>1.81 0.84–3.91</td>
<td>1.32 0.61–2.88</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.44 1.01–2.06</td>
<td>1.12 0.53–2.38</td>
<td>0.87 0.45–1.66</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.30 0.80–2.13</td>
<td>2.18 0.96–4.93</td>
<td>1.29 0.56–2.96</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.35 0.82–2.23</td>
<td>2.66 1.18–6.03</td>
<td>2.27 1.08–4.74</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>1.68 0.78–3.58</td>
<td>2.33 0.53–10.33</td>
<td>1.33 0.35–5.12</td>
</tr>
</tbody>
</table>

Boldface numbers indicate 95% CIs that do not include 1.00.
prevalence of grade 4 or 5 lesions in smokers compared with nonsmokers (Figure 3).

Hypertension was associated with greater odds of grade 4 or 5 lesions ($P=0.0589$) (Table 3, row 5) and with a higher prevalence of grade 4 or 5 lesions (Figure 4).

Obesity was associated with greater odds of grade 4 or 5 lesions ($P=0.0138$) (Table 3, row 6) and of atherosclerotic stenosis ($P=0.0303$). Obese persons had a higher prevalence of grade 4 or 5 lesions at all ages (Figure 4).

Impaired glucose tolerance was not significantly associated with LAD lesion grade ($P=0.2043$; Table 3, row 7), although the odds ratios and prevalences (Figure 4) suggest a substantial effect. The lack of statistical significance is most likely a result of the low prevalence of impaired glucose tolerance in these young persons and the resulting small numbers of cases.

Discussion

Summary of Results

Severe atherosclerotic lesions (AHA grade 4 or 5) causing stenosis occur at a standard site of the proximal LAD in a few 15- to 19-year-old men and in $\approx20\%$ of 30- to 34-year-old men. The prevalence of similar lesions in 30- to 34-year-old women is $<50\%$ that of men. High non-HDL cholesterol concentrations are associated with a higher prevalence of atherosclerosis (both AHA grades 2 or 3 and 4 or 5) and atherosclerotic stenosis $\geq40\%$. Low HDL cholesterol levels and smoking are associated with greater prevalence of AHA grade 2 or 3 lesions. Hypertension and obesity are associated with greater prevalence of AHA grade 4 or 5 lesions, and obesity also is associated with atherosclerotic stenosis $\geq40\%$.

Comparison With Other Studies

The associations of risk factors with the histological qualities of LAD lesions are similar to the associations of risk factors with the extent of lesions in the right coronary artery and the abdominal aorta.\cite{7-9} Impaired glucose tolerance is not significantly associated with LAD lesion grade ($P=0.2043$; Table 3, row 7), although the odds ratios and prevalences (Figure 4) suggest a substantial effect. The lack of statistical significance is most likely a result of the low prevalence of impaired glucose tolerance in these young persons and the resulting small numbers of cases.

A detailed comparison of the PDAY results with atherosclerosis in Korean War battle casualties is not possible because Enos et al\cite{20} used different methods to assess atherosclerosis and did not report ages in detail. We would expect to find a lower prevalence of stenosis in PDAY cases, because we assessed stenosis at only 1 site. Stary\cite{21} reported the prevalence of coronary artery lesions in 1160 persons from birth to 29 years of age by a grading system similar to the AHA system. For comparable age and sex groups, the prevalence of grade 4 or 5 lesions was $\approx25\%$ to $50\%$ the prevalence reported by Stary in multiple coronary artery sections. As with the Korean War battle casualties, this difference may be partly a result of our having examined only 1 site. There also may have been a secular trend in the severity of atherosclerosis between the 3 periods of collection (Enos et al, circa 1950–53; Stary, 1979–86; PDAY, 1987–1994).

Significance of Gross Fatty Streaks and Grade 1 or 2 Microscopic Lesions

The ubiquity of the fatty streak\cite{22} has led some investigators to conclude that the fatty streak does not represent the initial stage of atherosclerosis.\cite{23} However, a continuous progression of lesions from grade 2 coronary artery lesions can be traced through successive age groups to clinically significant grade 4 or 5 plaques.\cite{8,12,13,21} Results presented here show that the prevalence of grade 2 lesions, corresponding to a gross fatty streak, is associated with the risk factors for clinical CHD, just as the gross extent of fatty streaks is associated with those risk factors. Therefore, it is reasonable to expect that control of risk factors in young persons would retard the progression of the fatty streak and thereby retard the occurrence of CHD.

Significance of Stenosis

Stenosis is measured in these fixed arteries as the percentage of potential lumen inside the IEL that is occupied by normal intima and atherosclerotic lesion(s). Our measure of stenosis may not correspond with stenosis measured by angiography. Furthermore, our measure of stenosis may not indicate true functional stenosis, because many of the grade 4 or 5 lesions are associated with distended arterial walls and appear to be undergoing the adaptive remodeling described by Glagov et al.\cite{16} The clinical significance of grade 4 or 5 lesions is probably more closely related to their susceptibility to plaque rupture and its sequelae.

Plaque Vulnerability

The major criterion for AHA grade 4 or 5 atherosclerotic plaques is the presence of a large core of lipid and necrotic debris lying beneath a fibromuscular cap.\cite{13} This core, which is softer than the fibrous cap,\cite{24} is also the hallmark of a plaque vulnerable to rupture and thrombotic occlusion.\cite{10,11,25,26} Other changes associated with plaque rupture and thrombosis are thinning and macrophage infiltration of the fibrous cap,\cite{27,28} both of which may be accelerated by continued lipid accumulation. Thus, a substantial proportion of these young individuals have coronary artery lesions that are vulnerable to rupture and thrombosis and have the potential to precipitate CHD.\cite{29,30}

Implications for Early Primary Prevention

There is little or no controversy regarding hygienic measures to control smoking, obesity, hypertension, and hyperglycemia in young persons, but there is controversy regarding how early in life serum cholesterol levels should be a matter of concern.\cite{1,31–34} The results presented here show that $\approx1$ of 5 young men between the ages of 30 and 34 has $\approx1$ advanced atherosclerotic plaque that has probably developed over the preceding decade and show that such lesions are associated with the CHD risk factors, including elevated non-HDL cholesterol. These observations indicate the need for risk factor modification early in life. If these susceptible individuals wait for the first CHD event, $\approx25\%$ or more will die unexpectedly and suddenly outside the hospital.\cite{35,36} The survivors will be at greatly increased risk of recurrence, and aggressive lipid lowering offers at best only $\approx40\%$ risk reduction over 5 years.\cite{37}
The high prevalence of risk factors in the PDAY sample (Table 1) and in living children and adolescents indicates that a large number of young people are at risk of precocious atherosclerosis and eventual CHD. Thus, there is considerable potential for risk factor reduction, with resulting prevention or retardation of severe atherosclerosis and clinical CHD. Although changing the behavior of young people is a difficult task, available information indicates that the earlier the task, available information indicates that the earlier the cardiovascular risk factors are modified, the greater the potential for deferring the onset of CHD. Prevention of CHD is, indeed, a pediatric problem.

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