Natural History and Risk Factors for Early Human Atherogenesis

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A multi-institutional study, Pathobiological Determinants of Atherosclerosis in Youth (PDAY), was initiated to document the natural history of atherosclerosis, its relationship to risk factors, and the pathobiology of lesion development in young subjects. Pathology laboratories in nine centers collected arteries and tissues from >2000 persons, ages 15–34 years, whose deaths were attributed to homicides, accidents, or suicides. Arteries were evaluated for lesions, and risk factors were analyzed in a central laboratory. Postmortem risk factors include serum lipoproteins, serum thiocyanate (smoking), glychomeglobin (diabetes), thickness of panniculus adiposus (obesity), changes in small renal arteries (hypertension), and apoprotein isoforms. This PDAY study documents the development of atherosclerosis at an early age and shows that the recognized risk factors for coronary heart disease are associated with lesion development in the arteries of these young subjects. The findings provide a strong justification for reducing risk factors in young persons.

Indexing Terms: atherosclerosis/adolescents' health/lipoproteins/apoproteins/smoking/diabetes/hypertension/obesity

Elsewhere, I have described the origin of atherosclerosis in childhood and have shown that the early arterial lesions of atherosclerosis progress to more advanced plaques in some subjects during adolescence and young adulthood (1). The early onset of atherosclerosis and recent emphasis on preventing clinically significant atherosclerotic disease in adults have led to interest in the relationship of known risk factors for coronary heart disease (CHD) to the early lesions of atherosclerosis in young human subjects.⁶

Recent reviews of the natural history of atherosclerosis summarize the evidence on the development of lesions in young people (1–3). The early lesions of atherosclerosis, fatty streaks, are intracellular and extracellular deposits of cholesteryl esters and other lipids and are visible as streaks and spots in the intima of the aorta, coronary arteries, and other large muscular arteries. These fatty streaks may progress with further lipid deposition and connective tissue proliferation into fibrous plaques, the next grossly visible stage of lesion development. Further progression to more advanced or "complicated lesions" occurs with hemorrhage, ulceration, and thrombosis, and finally, ischemia and clinically manifest disease.

Early studies of the natural history of atherosclerotic lesions were limited to the relationship of lesions to age, sex, ethnic origin, geographic population group, and clinical disease associated with the cause of death or autopsy findings. Relationships of lesions to suspected risk factors were limited to characteristics of the population from which the autopsied persons were studied (4). Longitudinal epidemiologic studies showed that most of the major risk factors for CHD were also associated with atherosclerosis in adult men (5). Little or no information existed on the relationship of the concentrations of serum lipoproteins in individual subjects and arterial lesions in young people (except for reports on advanced atherosclerosis in young persons with homozygous familial hypercholesterolemia) until reports from the Bogalusa Heart Study were published (6–8). These reports demonstrated positive relationships between serum concentrations of total cholesterol and low-density lipoprotein cholesterol (LDL-C) and the extent of aortic fatty streaks, and between total cholesterol, LDL-C, and very-low-density lipoproteins (VLDL-C) and fatty streaks in the coronary artery.

Postmortem Risk-Factor Variables

Because preexisting information on risk factors in young deceased subjects is almost never available, investigators in this laboratory developed surrogates (markers) for preexisting risk factors, based on analysis of postmortem specimens at autopsy. These markers for risk factors were used in a community pathology study of atherosclerosis in New Orleans to examine the relationship among risk factors and arterial lesions in men

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² Nonstandard abbreviations: CHD, coronary heart disease; HDL-C, LDL-C, VLDL-C, high-, low-, and very-low-density lipoprotein cholesterol; PDAY, Pathobiological Determinants of Atherosclerosis in Youth; RFLP, restriction fragment length polymorphism; IAP, International Atherosclerosis Project; apo, apoprotein.
ages 25 to 44 (9, 10). Some of these postmortem risk-factor variables measured in blood obtained at autopsy were serum concentrations of lipoproteins, thiocyanate (as an indicator for smoking), and glycohemoglobin (as an indicator of glucose intolerance or diabetes). Other markers were morphometry of small arteries in the kidney as a reflection of blood pressure during life, and measurements of height, weight, and thickness of panniculus adiposus at autopsy to determine the degree of obesity. Fatty acids in adipose tissue were analyzed as a marker for dietary and metabolic variables (11, 12). The various methods and assumptions used in drawing inferences from this postmortem material have been studied carefully as discussed in detail elsewhere (13, 14).

Study of Pathobiological Determinants of Atherosclerosis in Youth

In the mid 1980s a group of scientists organized a study to investigate directly the associations of CHD risk factors with precocious atherosclerosis in adolescents and young adults. In this study, Pathobiological Determinants of Atherosclerosis in Youth (PDAY), 14 laboratories in the US are collaborating to evaluate coronary arteries, aortas, and postmortem blood samples and other tissues from >2000 subjects, ages 15 to 34, who died of trauma and were examined at forensic pathology centers (15). The PDAY scientists adapted and expanded the methods used in a community pathology study in New Orleans to study the relationship of postmortem risk-factor variables to the prevalence, extent, and severity of arterial lesions in young people. In addition to those risk factors listed above, DNA restriction fragment length polymorphisms (RFLPs) were analyzed in liver samples. Central laboratories analyzed the blood and tissues and graded the arteries for the extent and severity of atherosclerosis, and a central statistical center analyzed the data. Methods for evaluating atherosclerosis and procedures for estimating the postmortem risk factors have been described in detail (14–18).

Three pathologists, blinded to clinical or pathological observations or demographic data, independently evaluated the right coronary artery and left half of the aorta, which had been stained with Sudan IV. Using procedures developed in the International Atherosclerosis Project (IAP) (19), they visually estimated the extent of intimal surface involved with fatty streaks, fibrous plaques, and complicated and calcified lesions. The sum of the percentages of surface involved with fibrous plaques, complicated lesions, and calcified lesions was designated “raised lesions.”

The procedures for risk factor measures were recently published in detail (13, 14). Briefly, blood collected from the aorta, heart, or vena cava was centrifuged, and both cells and serum were frozen and shipped to the central laboratory for analysis. Serum cholesterol and high-density lipoprotein cholesterol (HDL-C), after precipitation of serum by heparin/MnCl₂, were determined by the cholesterol oxidase method. The non-HDL-C concentration (or VLDL + LDL-C) was obtained by subtrac-

tion. The CV between results for blind duplicates for serum cholesterol was <1.5%; for HDL-C it was <6.5%.

The procedure used for thiocyanate measured the color produced by the thiocyanate–ferric nitrate complex after ferric nitrate treatment of trichloroacetic acid filtrates of serum. The CV between blind duplicates was <6%. Serum thiocyanate ≥90 μmol/L was taken to indicate that the subject had been a smoker.

Because emergency medical technology teams often administer large quantities of intravenous fluids to some individuals just before death, we excluded all measures of serum analytes from an individual from the statistical analysis when the result for serum cholesterol was <2.59 mmol/L. The percentage of cases in which acceptable serum data (cholesterol ≥2.59 mmol/L) were obtained was not associated with race, sex, age group, or cause of death.

Glycohemoglobin was determined by affinity column chromatography after an aliquot of thawed “cell hemolysate solution” was mixed with hemolysate reagent to assure complete lysis. The column used was packed with an insoluble cellulose resin bound to dihydroxyboryl groups with an affinity for the cis-diol groups in glucose. This method separates all the glycohemoglobin from the nonglycosylated hemoglobins and is not interfered with by “labile” glycohemoglobin.

Thickness of panniculus (subcutaneous adipose tissue) was determined at the time of autopsy by measuring subcutaneous fat to the nearest millimeter with a straight-edged ruler. The measurement was made at a point halfway between the xiphoid process and the umbilicus and included the subcutaneous tissue from the inner edge of the rectus sheath. This measurement was made immediately after the primary incision in the abdominal wall and before the viscera were removed.

Natural History of Aortic and Coronary Atherosclerotic Lesions

The results of this systematic quantitative study of lesions in the thoracic and abdominal aortas and right coronary arteries of 1532 subjects ages 15–34 years confirm the early appearance of lesions in human subjects and compare the prevalence and extent among black and white men and women in this age group (16). As for the prevalence of lesions (the percentage of specimens having any fatty streaks or raised lesions), all of the aortas and about half of the coronary arteries in the youngest age group (15–19 years) had lesions. The prevalence of lesions in coronary arteries increased to about 75% in the 30–34-year-old age group and was greater in men than in women. The extent of atherosclerosis, expressed as mean percent intimal surface covered by lesions in 5-year age groups, increased from age 15 to 34. Raised lesions increased with age in both extent and prevalence in the aorta and in the right coronary arteries.

Figure 1 from the PDAY Research Group (16) shows the mean extent of fatty streaks and raised lesions in the thoracic aorta, abdominal aorta, and right coronary artery by sex, race, and 5-year age group. Fatty streaks are well established in both segments of the aorta by age
right coronary had the least percentage of intimal surface involved with all types of lesions but the greatest proportion of raised lesions among total lesions.

These results confirm the origin of atherosclerosis in childhood and adolescence and show that the prevalence and extent of fatty streaks and fibrous plaques increase rapidly during this 15–34-year age span. Also, several of these subjects showed a progression toward clinically significant lesions (including complicated lesions, calcified lesions, and stenosis) in young adulthood.

Risk Factors and Arterial Lesions

Reports from the PDAY study have provided unique observations on the relationship of risk factors for adult CHD to the prevalence and extent of atherosclerotic lesions in these young subjects. Table 1 contains the results of a multiple regression analysis of percent intimal surface involved with lesions in the abdominal aorta of male subjects by risk-factor variables. The predictor variables were age, race, VLDL+LDL-C, HDL-C, smoking (thiocyanate concentration ≥90 μmol/L), apoprotein (apo) B, and apo A-I. The effects of each of these variables were estimated while holding all other variables constant; all were significant at \( P < 0.005 \). For each 5-year increase in age, there is a 5.6% increase in intimal surface involvement with lesions. Blacks on the average have ~6% more surface involvement with lesions than whites. Surface involvement accompanying an increase of ~1 SD of VLDL+LDL-C concentration increases by 5%, whereas that accompanying a corresponding increase of HDL-C decreases by 3%. The effect of apo B is approximately the same as that of VLDL+LDL-C, and the effect of apo A-I is about the same as that of HDL-C. Smokers have ~6% more surface involvement with lesions than do nonsmokers.

Figure 2 shows the predicted average extent of total

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**Table 1. Effects** on percentage of total surface involvement of abdominal aorta, adjusted for other variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit or cutoff</th>
<th>Effect, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>5 years</td>
<td>5.6</td>
</tr>
<tr>
<td>Race</td>
<td>Black–white</td>
<td>6.3</td>
</tr>
<tr>
<td>VLDL+LDL-C</td>
<td>1.16 mmol/L</td>
<td>4.9</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.52 mmol/L</td>
<td>-3.0</td>
</tr>
<tr>
<td>Smoking</td>
<td>Smoker–nonsmoker</td>
<td>6.2</td>
</tr>
<tr>
<td>Apo B</td>
<td>0.40 g/L</td>
<td>4.5</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>0.35 g/L</td>
<td>-3.2</td>
</tr>
</tbody>
</table>

* Estimated from multiple regression analysis of 611 cases (except for apo B and A-I, n = 292 cases), all males.

*All significant at \( P < 0.005 \).
lesions (fatty streaks plus raised lesions) in the abdominal aorta of male subjects by age for two combinations of risk profiles after adjustment for race: high VLDL+LDL-C, low HDL-C, and smoker; and low VLDL+LDL-C, high HDL-C, and nonsmoker. The lipoprotein values used in this analysis were 1 SD above and below the respective means for this study and are not extreme concentrations. Those subjects with the high-risk profile (based on risk factors for CHD) had about three times as much of the abdominal aortic intimal surface involved with all lesions as did those with the low-risk profile. This difference extended across the 15- to 34-year age group.

Tables 2 and 3 show some preliminary results for men for two additional risk factors: glycohemoglobin content as a surrogate for glucose intolerance or diabetes, and thickness of the panniculus adiposus as a measure of obesity. In preliminary analyses, glycohemoglobin (Table 2) significantly affected both total and raised lesions in the coronary artery but not in the aorta. The measure of obesity also showed a positive and significant effect for both total lesions and raised lesions in the coronary artery but no effect on the aorta (Table 3).

Figure 3 shows results of preliminary analyses in which the subjects were classified as normotensive, borderline hypertensive, and hypertensive according to morphometric measures of their small renal arteries as described by Tracy et al. (20, 21). The principal difference among these categories is a stepwise increase in the mean percentage of the surface area involved with raised lesions. The involvement of raised lesions was significantly different between these groups ($P = 0.0001$). Although there is some increase in total lesions, this increase was mainly from raised lesions.

A number of genotypes related to apoproteins have been determined. Numerous publications have described relationships with clinical manifestations of CHD, but only two describe significant differences in arterial lesions according to these apoprotein genotypes (22, 23). Figs. 4 and 5 show the extent of lesions among the genotypes of apo E (E2, E3, E4) and among the insert–delete genotypes of the apo B signal peptide. Analyses of the apo E genotypes indicate a significant relation to lesions in the abdominal aorta: Genotypes containing E2 have the least, E3 intermediate, and E4 the greatest extent of lesions. For the apo B signal peptide genotypes, one combination, apo B signal peptide D/D, is associated with significantly more lesions in the black men only.

As the PDAY study accumulates more specimens to include a greater number of females, more definitive analyses for age, race, sex, and other risk factors will be performed. Although the more-definitive pathobiological studies of the arterial wall included in the PDAY program are not the subject of this report, those studies that have been published (13, 24) and those under way will yield a rich harvest of valuable information on the cellular and humoral arterial wall reactions in both sexes and these two races.

In conclusion, as we learn more about the natural history and progression of atherosclerosis in the aorta and coronary arteries, we find that not only are practically all of the known risk factors for clinically manifest CHD related to the prevalence and extent of the arterial lesions of atherosclerosis in adults, but also many of these CHD risk factors are related to the prevalence and extent of lesions (fatty streaks, or raised atherosclerotic lesions, or both) in one or more of the arterial segments.
Fig. 4. Percentage of the surface area of the abdominal aorta involved with total lesions according to apo E isoform genotype for men, adjusted for age and race (P = 0.001). Shaded bars indicate genotypes with large N. Drawn from results reported by Hixson et al. (22).

Fig. 5. Percentage of the surface area of the abdominal aorta involved with total lesions by insert (I)/delete (D) polymorphisms of signal peptide apo B for white (○) and black (□) men, adjusted for age (P = 0.008). Drawn from results reported by Hixson et al. (23).

...in youth. Some risk factors affect one lesion type or one arterial segment more than another, and it is increasingly clear that the known risk factors do not explain nearly all of the variability in lesions. Nonetheless, the results provide a rationale for programs to reduce major risk factors (e.g., cholesterol and smoking) in young persons and suggest that such programs will help prevent clinical manifestations of disease later in life.

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