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Special Article

Risk Factors and Progression of Atherosclerosis in Youth

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"The Pathobiological Determinants of Atherosclerosis in Youth" (PDAY), a unique research program initiated in 1985, has now developed into a detailed and comprehensive 15-center investigation of the arteries of more than 3,000 young persons who died between 15 and 34 years of age (Appendix A). The main objectives of this highly organized multicenter study are to compare the quantitatively evaluated risk factors for coronary heart disease including blood lipid values, evidence of smoking, indices of hypertension, tendency toward diabetes, etc., with the results of macroscopic and microscopic quantitation of severity and with microscopic components of developing atherosclerotic lesions in these young people.[1–5]

This commentary will summarize some of the results which have been published in more than 75 full-length reports, with emphasis on the results obtained because of the unusual and frequently unique features of the study's protocol, which was developed during a 10-year period before the study began.[6–17]

Reported findings can be divided into two categories. Results in the first category are derived from the gross evaluation of the extent and severity of lesions.[18–28] Computer-assisted mapping was also applied to raised lesions traced out by the pathologists.[28,29] The second category of reported results comes from detailed micromorphometric, microchemical, and immunohistochemical quantitative data based on the major microscopic components and the classification of each lesion.[30–39] In fact, as the study progressed, it became possible to classify the four major types of intermediate lesions that are associated with different rates of progression of the atherosclerotic process in the aortas and coronary arteries of these young people as well as with certain risk factors.[40,41]

Because lesions tend to increase in extent, numbers, and severity with age, it is assumed that this in-depth quantitative analysis of the atherosclerosis found in each of these cases from the 15–34 age group may give new insights into why some young people's plaques seem to progress rapidly with age while others' remain almost stationary, as well as a comparison of progression in various parts of the arterial tree.[20,22,30,42]

Results of the study reflect the state of atherosclerosis development in young people living in the USA late in the 20th century. All case material came from forensic autopsies on young individuals who had no evidence of chronic debilitating disease and who succumbed suddenly to traumatic or other fatal episodes. The intervals between death and refrigeration of the body, as well as performance of the autopsy, were generally short. This has made it possible for the tissues and cells to be studied using a number of pathobiological methods appropriate for well-preserved human tissues.[31–41]

In order to insure an adequate and representative autopsy population, nine of the centers selected, approved, and funded functioned as "collecting centers." All nine are geographically and/or organizationally related to forensic laboratories where state and local regulations make it possible to collect and utilize small samples of tissue for research purposes. Most of the principal investigators leading the 15 centers have a long-standing interest and a record of productivity in the field of atherosclerosis research with an emphasis on the artery wall and the pathogenesis of atherosclerotic lesions.[5]

Centers were selected during a period of approximately two years (1983–85) of intensive preliminary meetings of potential principal investigators. The overall plans took shape over a 10-year period (1975–85) during which a carefully prepared and detailed protocol as well as a detailed manual of procedures for the study were developed.[5,34,36] Furthermore, during this period the steering committee developed a standardized sampling

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strategy and procedures based on the results of an NIH-funded preliminary study by Dr. Frederick Cornhill, Director of the Biomedical Engineering Center at Ohio State University, and Dr. Herbert Stary, Professor of Pathology at Louisiana State University. This preliminary study established the patterns of lesion location most likely to develop in the aorta[5,29] and helped establish the best sites to study in the proximal coronary arteries.

This commentary will conclude with a list of major opportunities for future studies afforded by the accumulated data and the currently unused PDAY samples of arteries and other tissues.

Major Revelations from Quantitative Gross Observations

Initiation and Progression of the Atherosclerotic Plaque

The PDAY protocol makes it possible to survey and evaluate both grossly and microscopically the presence of fatty streaks and fatty plaques, or intermediate lesions, in the aorta and the major coronary arteries. The results of the PDAY study further document the often-demonstrated fact that most teenagers in the United States have fatty streaks in some part of their arterial system. There is, on the other hand, a wide variation in the age at which intermediate lesions (fatty plaques) develop from fatty streaks in the lesion-prone sites of both the aorta and coronary arteries. This evidence of variable progression is definitely related to the risk factors measured quantitatively. Figure 1 illustrates the type of comparisons that are being made regarding the development of aortic lesions as compared to coronary lesions over the 20-year age span by gross evaluation of the opened, flattened, and Sudan-stained gross specimens from 1532 cases and relating the results to the age of the individuals.[24,43] Raised lesions are observed earlier and at a higher prevalence in the aorta than in the coronary arteries, and lipid-containing lesions are seen in a much higher percentage of the aortas, starting as early as the 15- to 20-year age group. Because the same criteria were used in each of these arterial beds for identifying raised lesions, the results appear to indicate that much more sustained deposit of lipid in the aortic intima is necessary before cell proliferation and other reactions to the lipid lead to the typical raised appearance.[26–28]

The unique visual as well as computer-assisted methodologies used in quantitative measurements of lesions grossly made it possible to evaluate the extent as well as the elevation of the developing lesions.[18,29,30,43,44]

The quantitation of the risk factors in numerical terms as well as the forensic origin of the cases were important factors contributing to the success of the study. They give additional confidence that the results relating risk factor data to lesion size and severity will be applicable to a large segment of the youthful population of the United States in this part of the millenium.

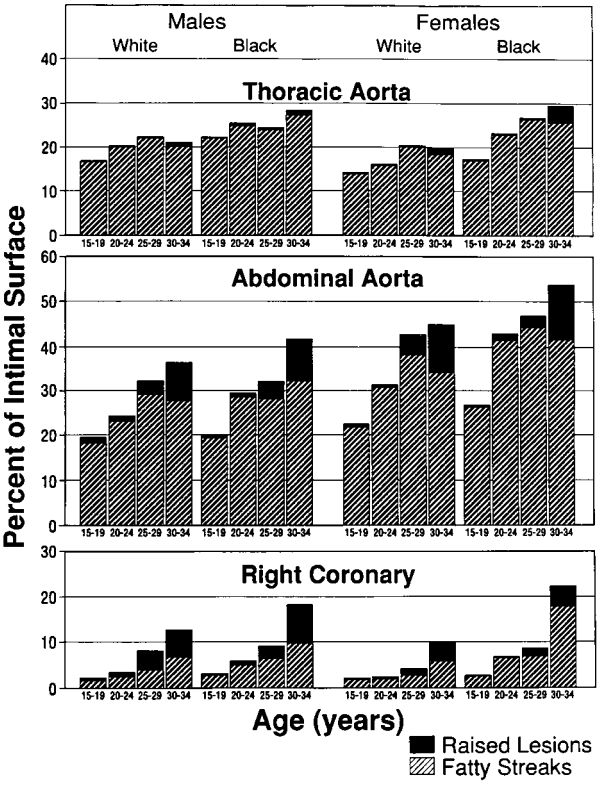

Figure 1. Bar graphs showing percent of intimal surface area involved with fatty streaks and raised lesions for both the thoracic and the lower abdominal aortas as well as the right coronary artery. The effects of age (by five-year groups), sex, and race are shown. (Reprinted[43] from PDAY Research Group. Arterioscler Thromb 1993, 13:1291–1298, with permission).

Progression of Lesions Related to Age

In this and other recent studies, age was found to be the most important risk factor in terms of mean percentage of intimal surface involved by lipid. The extent of lesions increased steadily from 15 to 34 years in the 5 year age groups.[43,45–47] Evidence of the relationship of age to the quantitative assessment of lesion extent made it possible to relate lesion progression to race and sex in 1532 individuals.[42,43]

Effects of Race and Sex

Early observations indicated that black subjects showed more extensive lesions than did white subjects in all arterial segments measured. They also revealed that young women had more extensive fatty streaks in the abdominal aorta while young men had more in the thoracic aorta. Male subjects had more extensive lesion distribution and a higher prevalence of raised lesions in the right coronary artery than did female subjects.[19,20,24,43]

Progression of Coronary Artery Disease in Males

Similarly, as is illustrated in Figure 1, the further work necessary to obtain tissues from a sufficient number of

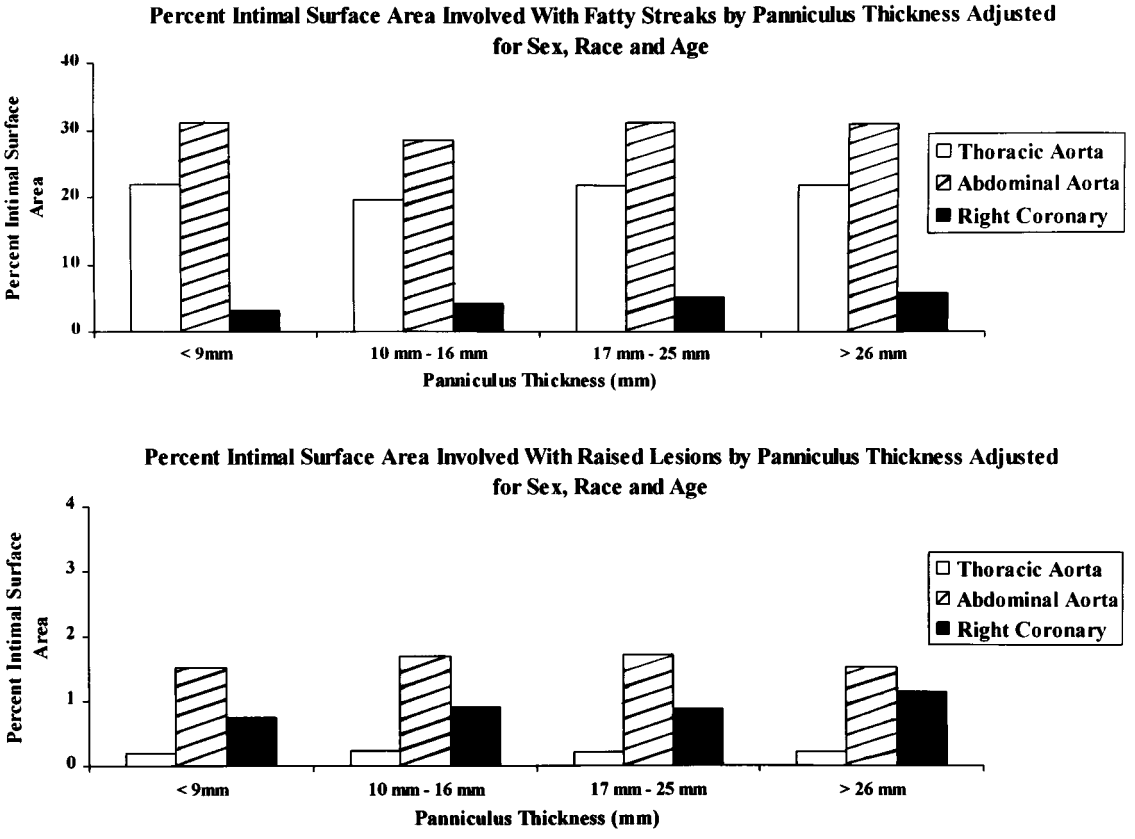

Figure 2. Bar graphs showing percentage of intimal surface area involved with lesions by panniculus thickness, adjusted for sex, race, and age. Top: fatty streaks; Bottom: raised lesions. (Adapted[27] from Arterioscler Thromb Vasc Biol 1995, 15:431–440, with permission.)

females evenly distributed in age was definitely worthwhile. It made it possible to demonstrate rather clearly that although the prevalence of sudanophilic coronary artery lesions was rather similar in the two sexes when over 1400 cases were analyzed, the raised lesions were much more prevalent in the coronary arteries of 30- to 34-year-old males.[40] So it appears that it is not the prevalence or even the extent of lipid, but the kind of lipid deposits and/or the arteries' reaction to them that leads to the progression of raised coronary lesions.[43]

The strong association of the progression of the extent of disease with hyperlipidemia as reflected by the postmortem analyses of serum lipids including elevated total cholesterol, LDL cholesterol, low HDL cholesterol level in the serum is enhanced when these risk factors are combined with the elevation of thiocyanate values reflecting cigarette smoking.[18,23,24,28]

Obesity as a Risk Factor

A number of studies of the effects of obesity on heart disease have yielded inconsistent results whether judged by mortality or extent of arterial disease.[13,48–50] The PDAY results based on analysis of 1420 cases have yielded a definite indication that obesity is a positive risk factor when body mass index measurements are used.[27]

The evidence for the augmenting effect of obesity, judged by panniculus adiposus measurements, on the development of coronary atherosclerosis and based on the analysis of 1532 cases is also shown in Figure 2. It indicates that in obese subjects almost twice as much coronary surface is occupied by lesions in the right coronary artery as in nonobese subjects.

The ability to evaluate obesity by either body mass index or the thickness of the panniculus adiposus and the similarity of the results obtained with either of these measurements demonstrates another unique contribution of the forensic autopsy, in which special attention is given to these types of measurements.

Genetic Studies of Apolipoprotein Phenotypes on Lesion Extent

The splendid one-of-a-kind demonstration by Hixson et al using PDAY cases has made it possible for the first time to relate the influence of genetically determined serum lipoprotein types and patterns, especially the E2E2, E2E3 and the E3E4, E4E4 phenotypes, to the extent of the gross lesions.[51] More recently, Hixson has discovered important effects of Apo B genetic deletions on the progression of abdominal aorta lesions.[52] He is exploring

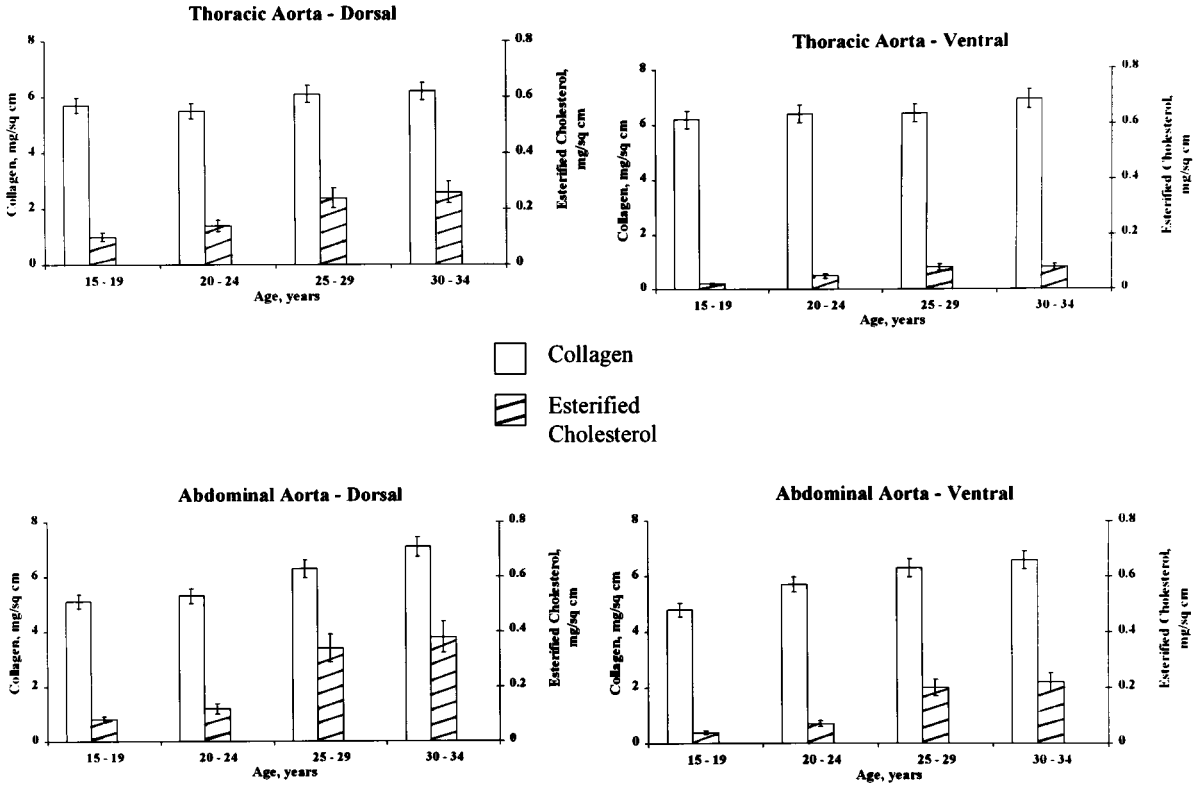

Figure 3. Quantitative analyses of collagen and esterified cholesterol in square centimeters of the standardized dorsal and ventral samples of the thoracic and lower abdominal aortas plotted by age groups. The height of the bars indicates geometric means and the ninety-five percent confidence intervals are also indicated. (Reprinted[42] from Matrix 1993, 13:294, with permission.)

other effects in the Apo C3[53] and Apo A–IV[54] genetic deviations.

Aortic Regional Susceptibility to Atherosclerosis

Although not completely explained, the remarkable differences in the development of lower abdominal aortic atherosclerosis compared to the thoracic involvement are strikingly demonstrated by the results of the recently published study of Miller et al (Figure 3).[42] The unique sampling strategy resulted from the demonstration by Cornhill, Herderick, and Stary[29] that there is a repetitive pattern in human descending aortic lesion development. This made it possible to express in quantitative terms the remarkable contrasts which are evident between the extent of lesion development in the lower abdominal aorta and the thoracic aorta where lesions rarely progress substantially in this 20-year period. Similarly, this study led to a sampling strategy in which the lesions of the ventral and dorsal areas of the aorta could be quantitated separately because their study confirmed that progression of lesions with time as well as the development of raised fatty plaques are largely limited to the dorsal parts of the aorta. The graphs in Figure 3 give a very good reflection of these contrasts over the 20-year period between 15 and 34 years of age. The esterified cholesterol analyses are particularly revealing of the differences between dorsal and ventral parts of the arterial samples as well as the thoracic and abdominal aortic samples.

Effects of Smoking as Reflected by Serum Thiocyanate Levels

Excellent comprehensive data implicating smoking as a risk factor for extensive involvement of the abdominal aorta have recently been published.[28] They indicate that both black and white men and women develop much more extensive lipid deposition when their thiocyanate levels are high than when they are low and that this is accompanied by a remarkably increased prevalence of raised lesions in the abdominal aorta, especially in the 25–34 age group. The additive effect of hyperlipidemia as a risk factor combined with smoking was also illustrated again for both sexes.[18] It is notable that the raised lesion data showed no difference between blacks and whites compared to the excess of fatty streaks in blacks with high serum thiocyanate levels.

Effects of Hypertension on the Extent of Atherosclerosis

Hypertension as related to the renal indices developed and validated at LSU by Tracy and co-workers[26,55] has been reported to affect the extent of lesions in both the

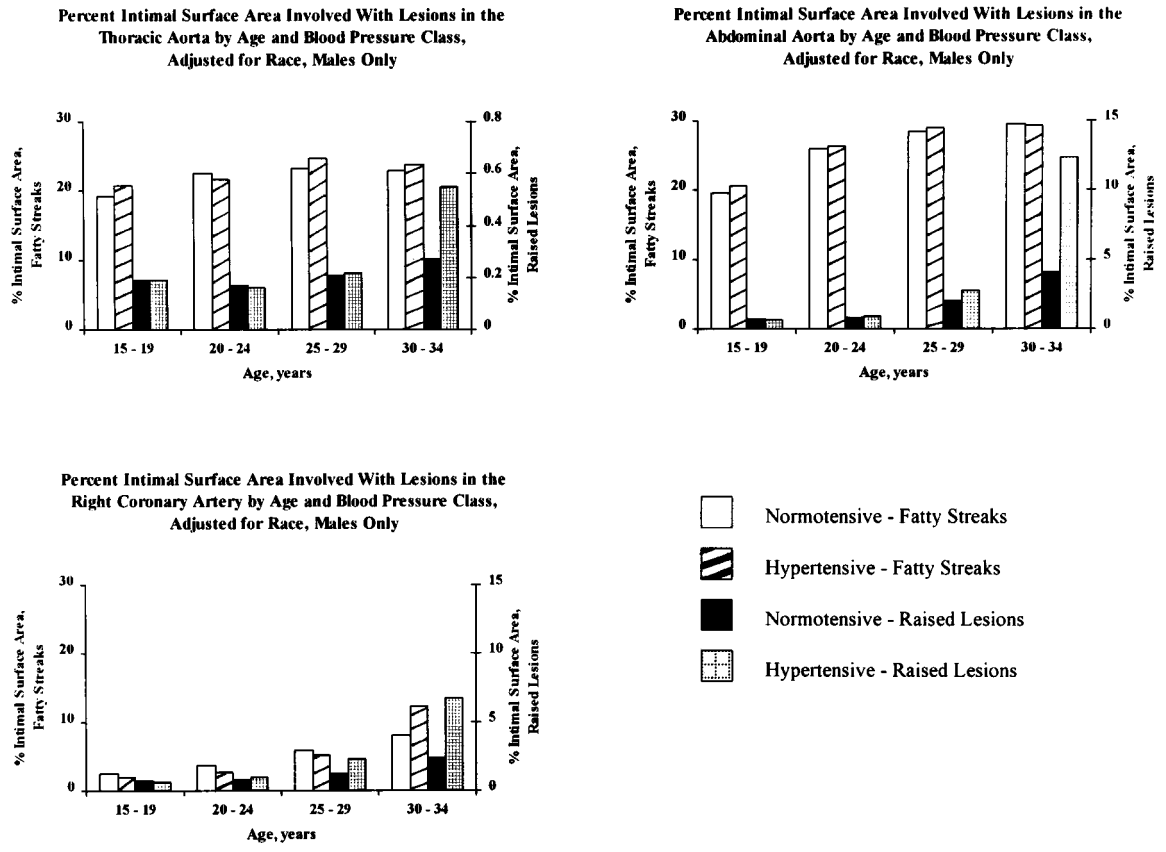

Figure 4. Bar graphs showing percentage of intimal surface area involved with lesions by age and blood pressure group, adjusted for race (males only). (Adapted[26] from Arterioscler Thromb Vasc Biol 1995, 15:2222–2228, with permission.)

aortas and the coronary arteries of PDAY cases. The effects are quite definite and have been reported recently (Figure 4). They show especially striking effects of elevated hypertension indices on raised lesion extent in both the abdominal aorta and right coronary in the 25–34 age group. These observations have been expanded and published recently.[56] In this upcoming report, the computer-assisted records of the effects of hypertension on some aspects of the severity of the lesions have also been included.

Gross Effect of Low HDL Levels

There is one published report of the effects of HDL cholesterol levels on the extent of PDAY lesions recorded by means of computer mapping and quantitation of the areas involved in sudanophilic *en face* staining.[24] It contains results that demonstrate the definite beneficial effects of higher serum HDL cholesterol levels on the extent of lesions.

Major Revelations from Quantitative Microscopic Observations

Classification of Raised Fatty Plaque Intermediate Lesions

Table 1 summarizes the results of classifying the lesions in the standard (core) samples of thoracic and abdominal

Table 1. Analysis of all available cases with gross and microscopic lesion classification using the new criteria for intermediate lesion microscopic types

			Fatty plaques				
Sample no.	No microlesions	Micro fatty streaks	Intracellular lipid predom.: 1	Extracellular lipid >50%: 2	Mϕ foam cell >20%: 3	Rich in lymphocytes: 4	Fibrous plaques
01	235	174	175	117	121	5	2
18	264	128	216	155	102	0	7
16	250	53	164	225	89	1	38
45	388	47	58	206	69	4	102

Reprinted from Chapter 27, Atherosclerosis and Coronary Artery Disease, 1995, Philadelphia, Lippincott-Raven, with permission.[35]

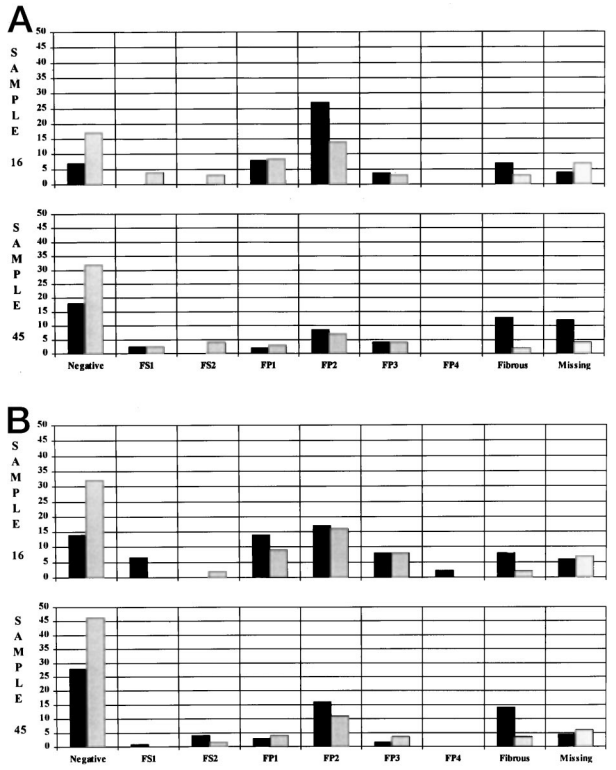

Figure 5. A: Observed microscopic classifications of the 55 cases with the highest postmortem serum thiocyanate levels (solid black bars) as compared to the 50 cases with the lowest postmortem serum thiocyanate levels in the 500 cases for which these analyses were available in this study. (Reprinted[35] from Chapter 27 in *Atherosclerosis and Coronary Artery Disease,* 1995, Lippincott-Raven, with permission). B: Bar graphs showing the quantitative analyses on the 75 cases with the high extremes of the renal indices for hypertension as compared to the 75 cases with the lowest renal indices from this total group of 750 PDAY cases on which these renal indices were available at the time of this study. Negatives indicate that no microscopic lesions were seen. FS_1 is a fatty streak in which most of the lipid is intracellular and in FS_2, it is mostly extracellular. The FP numbers 1–4 correspond to the four types of intermediate (fatty plaque) lesions and the term "fibrous" refers to the typical fibrous plaque with a fibrous cap and a fat-filled necrotic center. "Missing" means that a few microscopic sections were not available.

aorta and the coronary arteries using the gross and microscopic criteria which were developed for this purpose. These results indicate that intermediate lesions are the most common category seen in these 15- to 34-year-old individuals. These lesion-derived data demonstrate the effects of elevated thiocyanate levels as an index of smoking (Figure 5a) or the effects of elevated renal indices for hypertension (Figure 5b) on the types of lesions which develop. In the lower abdominal aorta and the proximal left anterior descending coronary artery, several different trends are demonstrated. The positive serum thiocyanate indications of smoking are much more likely to correlate with intermediate raised lesions, which have a predominance of extracellular lipid, than are the lesions developing in people who have high indices of hypertension. This appears to be an important finding which results from the application of the new classification system of intermediate lesions which has been developed using PDAY cases.[35] In general, it supports the other evidence that the predominance of extracellular lipid in a lesion is a strong indicator for progression of the lesion.[32,34,37] The study also demonstrates that each of these risk factors greatly reduces the number of cases with no lesions and each of them increases the number of cases with advanced atheromas (fibrous plaques). These data indicate that cigarette smoking and hypertension seem to have the greatest effect on the development of advanced plaques in the young, resulting in a sixfold and a nearly fourfold increase respectively in advanced coronary lesions in this large group of PDAY cases.

The unique ability to obtain high quality microscopic sections stained for lipid and connective tissue elements through the center of core samples that have been classified and recorded by close-up color macrophotography of the fresh unfixed artery specimens has turned out to be a very advantageous element in the unique contributions the study can make. These are likely to increase as more positive effects of individual risk factors are identified.

Hypertension's Effect on the Cellular and Lipid Components of Lesions

Sullivan and co-workers have recently reported a detailed quantitative biochemical and microscopic study of the thoracic and abdominal aortas in 118 randomly selected white males aged 15–34 from the PDAY study.[38] About half of them showed high renal artery indices for hypertension and about half were in the lower hypertension indices group. The ventral and dorsal portions of the samples were evaluated separately and the results indicated a remarkable increase compared to the nonhypertensive cases in the collagen and stainable lipid per unit area which was especially prominent in the dorsal segments of the abdominal aorta where lesions usually progress most rapidly. This topographic effect was greatly amplified in individuals with high renal indices of hypertension.[42] Sullivan and co-workers also reported a remarkable increase in extracellular lipid in the abdominal aortas of individuals with high renal indices of hypertension, with this being especially prominent in black males. As might be expected, the intimal thicknesses were much greater in the abdominal and thoracic aortas of the individuals with high renal indices of hypertension as compared with individuals with low renal artery indices, especially in the dorsal parts of the samples.

Microscopic Effects of High Serum Thiocyanate on Lesion Components

In a report published recently by Botti and co-workers, it is evident that a high index of smoking is accompanied by a much larger proportion of macrophages in the lesions as well as by a much larger intimal area being occupied by apolipoprotein E.[39] In a separate report published recently by Scanlon and co-workers, it was demonstrated that a high index of smoking as reflected in high serum thiocyanate levels is accompanied by much more immunohistochemical evidence of epitopes of oxidized LDL deposition in the developing atherosclerotic lesions, even though the extent of apo B deposition is almost identical in the lesions examined from smokers to

those in nonsmokers. This type of information may lead ultimately to a better understanding of the mechanism by which smoking leads to very accelerated progression of atherosclerosis.[57]

More recent studies of nearly 1,000 PDAY cases with quantitatively evaluated and classified gross and microscopic analyses have revealed that most of the advanced true atheromas found in this series of cases have evidence of smoking as one of their major risk factors. It is also significant that 10% of the young people under 35 in this study, which might be considered to represent a random sample of United States youth, have advanced atherosclerosis consisting of plaques with soft, necrotic, fat-filled centers and fibrous caps of varying thickness. Almost 80% of these young people with advanced plaques were smokers, as indicated by high thiocyanate serum levels. So far, the risk factor data do not explain why about half of these advanced coronary artery plaques have very thick fibrous caps, while the others have thin fibrous caps. The latter are likely to be the most unstable and dangerous, because their thin fibrous caps are more easily fractured, leading to coronary thrombosis and a heart attack.[40,41]

New Insights into the Effects of Serum Lipid Levels

Among other new facts that the PDAY study has furnished about lesions are the effects of Lp(a) on the development of lesions and the demonstration of its presence in the arteries of young people.[58] Similarly, it is becoming increasingly evident that the location of lipid in the artery wall and type of distribution relative to the cells that are present may be related specifically to the combination of risk factors present.[33–35]

Additional New Observations from PDAY Studies

Other original observations resulting from the PDAY studies and not necessarily related to what is known about risk factors at present are the quantitative results of the PDAY research program. Briefly, these are as follows:

1) Macrophages make up a larger proportion of the developing lesions in areas of the arterial tree where the atherosclerosis progresses slowly, which usually results in few dangerous advanced plaques.[31,32,36,37]
2) The predominance of extracellular lipid in developing lesions of atherosclerosis is an indicator of progression.[31,32,36,37]
3) The numbers of mast cells demonstrable in the artery wall is correlated with the progression of lesions in the PDAY cases.[59]
4) The substantial presence of circulating immune complexes is correlated with the development of coronary artery lesions with a concentric microarchitecture, as well as increased numbers of inflammatory cells in the lesions and more medial involvement than is usually seen in developing plaques in youth.[47,60–62] The PDAY study confirms this relatively new concept about the pathogenesis of certain lesions, which have been described previously as being correlated with elevated serum immune complexes in the arteries of cynomolgus monkeys.[63–65] Results from the study of the PDAY specimens provide the opportunity to ascertain whether the deleterious effects of lowering the serum low-density lipoprotein levels on the lesions with the concentric architecture will result in the hoped-for expansion of the artery lumen. The regression studies carried out in cynomolgus monkeys demonstrated that the concentric atheroarteritis produced by high blood lipid levels and circulating immune complexes resulted in increased stenosis and smaller lumens when the lipid was removed from the lesions.[63–65]

Future Investigations Using PDAY Arterial Samples and Risk Factor Data

Many important problems remain regarding the quantitative risk factor effects on the quantitative measurements of lesion extent, lesion components, and lesion severity which can be readily studied in the future utilizing PDAY arterial samples and risk factor data.

1) In addition to extending the preliminary work that has been reported on the use of the new classification system for intermediate lesions, it should be possible to link additional risk factors such as the presence of high levels of glycosylated hemoglobin, some genetically determined risk factors, lesions resulting from high levels of Lp(a), and high levels of homocysteine to the types, components, and severity of developing intermediate lesions.
2) With greater use of the risk factor data, the factors responsible for the greatest number of the most advanced atherosclerotic plaques in these young people should become more thoroughly identified and evaluated.
3) It may be possible to define the factors that influence the thickness of the fibrous cap overlying advanced atheromas.
4) The study material lends itself to an expanded study using the classification system for intermediate lesions to ascertain which combinations of risk factors are most likely to support the accelerated development of plaques that are clinically severe and dangerous.
5) Although extensive PDAY data are available on the fatty acid composition of cholesterol esters in the lesions and the lipids in the perirenal adipose tissue, the opportunity to correlate the components of the lesions with some of the fatty acid patterns that have been so well demonstrated in experimental animals, has not yet been undertaken in the lesions of these young people.[60,66–71]

These are just a few examples of studies which could be pursued productively with PDAY's standardized samples of young people's arteries. More than 50,000 are available, preserved in many different ways from fresh snap-frozen to fixation in a number of fixatives, including Carnoy's, glutaraldehyde, and neutral buffered formalin.

Fortunately, an immense amount of study material is readily available for use by qualified investigators. They may apply to use samples or data by contacting the current PDAY administrative center at Louisiana State University's Medical Center in New Orleans. In addition to the anatomically standardized artery samples and the quantitatively expressed risk-factor data on most of the more than 3,000 PDAY cases, there are also a large number of gross *en face* photographs and a large library of standardized microscopic sections of the core samples of the aorta and the left anterior descending pressure-perfusion-fixed coronary artery stained both for lipid and for the major connective tissue cells and their major fiber proteins, collagen and elastin. These and the paraffin-embedded blocks lend themselves to further detailed studies of the components of developing atherosclerotic lesions in young people.

Important Prevention Procedures for the Pediatrician and the Primary Care Physician Derived from PDAY Observations

This research program is far from complete and is likely to yield many more publishable contributions to our understanding of the development of atherosclerosis during youth. Nevertheless, the results thus far have produced a number of important guidelines that can support national and international efforts to prevent the development of coronary heart disease starting with young people in their mid- to late teens. These efforts include:

1) stepped-up organized efforts to prevent smoking in childhood and adolescence and to prevent and overcome addiction to tobacco;
2) speeded-up development of programs to systematically check for serum hypercholesterolemia and low HDL cholesterol levels in people as young as their late teens;
3) expanded efforts to prevent and treat obesity and to encourage and support organized exercise for young people;
4) increased support for research on atherogenic genetic traits to develop practical ways to identify and to reveal the mechanisms by which these factors influence atherogenesis, including the special augmenting effects of Lp(a) and homocysteine on the artery walls;
5) intensified efforts to identify and control hypertension in youth;
6) encouragement of effective testing for a tendency toward hyperglycemia and glucose intolerance in young people; and
7) greater vigilance and sensitivity to the evidence indicating that disorders with sustained circulating immune complexes or with frequent hyperstimulation of the release of vasoactive amines may constitute important accelerating influences on atherogenesis.

It is also the responsibility of the entire biomedical scientific community to encourage and support the development of new noninvasive methods to identify, measure, and evaluate the developing plaque components in the coronary artery wall and abdominal aorta of young people even when lesions are at the intermediate plaque stage.

Value of the PDAY Study for the Anatomical Pathologist and Forensic Pathologist

The PDAY Research Program demonstrates the value of the autopsy as a powerful research tool and establishes forensic centers as valuable generators of research data and the forensic pathologist as an important medical research scientist. This is particularly true if one wishes to obtain accurate and much needed information about the development of our most important and most serious disease processes, such as cardiovascular disease.

Bear in mind that the forensic center generally deals with the victims of sudden, unexpected, and unattended deaths. At present, these victims of vehicular accidents, homicides, suicides, etc., seem to come from all groups in our population. In our society, these external forces often represent the most frequent causes of death among our young people. Studying the arteries of 3000 of these individuals can give us a reflection of the risk factors at work in the development of our country's number one killer, atherosclerosis, which is responsible for most fatal heart attacks and strokes: two clinical events which, combined, cause more fatalities in our population than any other serious disease.

To correlate many of the most important risk factors for atherosclerosis, measured quantitatively and numerically, with quantitative computer-generated measures of lesion severity and extent, is real progress. To apply many of the modern microchemical, microscopic, histochemical, immunohistochemical, cellular, and pathobiological approaches to the artery walls of a large number of these victims is a huge step forward from previous studies of atherogenesis in human arteries.

Standardized artery samples and risk factor data gained from this study should be valuable research resources for qualified pathologists and medical scientists worldwide who have questions or ideas to which they think the substantial output of the PDAY study may be applied.

Appendix: Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group

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