USA Multicenter Study of the Pathobiology of Atherosclerosis in Youth[a]

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Multicenter Study of the Pathobiological
Determinants of Atherosclerosis in Youth
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INTRODUCTION

The main purpose of this unique study is to learn more about progression of atherosclerosis in the aortas and coronary arteries of young people aged 15-34 inclusive. More specifically, its main objective is to ascertain how the generally recognized risk factors for adult atherosclerotic cardiovascular disease influence atherosclerosis in young persons. The study uses modern tools of gross and microscopic morphometry and relates these measures to the major risk factors for adult coronary heart disease. In addition, a number of special studies that utilize electron microscopy, immunohistochemistry, microchemistry, and a number of molecular and genetic probes are being applied to standard samples of these arteries. They are designed to answer numerous fundamental questions about human atherogenesis at the cellular and molecular level as outlined below:

- The effects of passenger and/or pathogenic viruses on lesion development;
- The role of immunological and inflammatory elements in plaque development;
- The localization of apolipoproteins [apo B, apo E, apo Lp(a)] and other lipoprotein components in the lesions and the correlation of these distributions with levels of these apoproteins in the blood, and with intracellular and extracellular microscopic fat (oil red O) in the developing plaque;
- The effects and the frequency of distribution of microthrombi in standard samples of aorta and the left anterior descending coronary artery prepared for scanning electron microscopy as related to evident endothelial damage and to blood lipids, use of tobacco, and other risk factors;
- The correlations of a number of the risk factors with the severity of the inflammatory response as judged by T-lymphocyte populations and macrophage infiltration into the developing plaque;

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- The quantitation of the percentage of total cells (nuclei) in each lesion identifiable as arterial smooth muscle cells as compared to the percentage identifiable as macrophages;
- The correlation of the localization and the severity of the early fatty streak and fatty plaque lesions with race, sex, and age data with special emphasis on some of the paradoxes observed in earlier studies, such as the unusual predominance of fatty lesions in black and white females and the lesser occurrence of advanced lesions in older black and white females;
- The usefulness of phenotypic modulation of smooth muscle cells (conversion to a synthetic form) as an important indication of atherosclerotic plaque development as distinguished from diffuse intimal thickening;
- The influence of mast cells, histamine, and other vasoactive amines on progression of atheromatous lesions and their potential effects on artery spasm and their relationships to risk factors;
- The relationship of immune complexes in the serum and deposited in the developing atheromatous lesions to the various risk factors and to the composition and progression of the atherosclerotic plaque;
- The relation of *trans-cis* fatty acids in adipose tissue to lesion development and lesion components;
- The correlation of the proteoglycan types to the risk factors and to the evidence of lesion development under the influence of various risk factor(s);
- The quantitation of collagen using microchemical and microimmunochemical probes to assess the types and quantities of collagen and its distribution during lesion development;
- The tissue and blood lipid chemistry as correlated with the histological features of lesions known to result from the consumption of various food fats (coconut oil, peanut oil, olive oil, fish oil, etc.).

All of these special studies are being actively pursued at one or more of the 10 special study centers utilizing designated samples from carefully selected and balanced groups of cases which are complete as far as fundamental data, risk factor data, and the necessary samples are concerned. They are also selected on the basis of their suitability for these types of study as established by preliminary studies during the feasibility phase of the PDAY program.

METHODOLOGY

This multicenter study, which is now in its sixth year, and which is nearing the completion of its second phase, has from the beginning involved acquisition of standardized samples of arteries of young people who die from accidental and traumatic causes. The cases must be without evident postmortem autolysis. These samples are collected at nine "collection centers" which are all related to forensic pathology laboratories where such sampling for research purposes is legal, where there is sufficient interest, and where there are the resources to make this type of interface possible.

The manual of procedures for PDAY gives instructions for collecting samples from both coronary arteries and aortas and for preservation, preparing forms, photography, sample distribution, etc. It has been developed by a committee for this research program and tested during the feasibility phase. The artery samples are preserved both frozen

(−70° C) and fixed (formalin, glutaraldehyde, or Carnoy's solutions) and are processed for distribution to the various study centers.

The research program in its definitive form is carried out in 14 research centers. Five of these are designated as core centers where most of the sample processing, tissue preparation, risk factor assessment, histotechnical slide production, gross and microscopic morphometry, chemical analyses, and the analyses of data including the risk factor data processing from the analyses on postmortem samples of blood, liver, adipose tissue, and other basic information, are performed. These 5 centers and the rest of the collection and study centers are given in TABLE 1. The principal investigators at the 5 core centers serve as the steering committee for the program, interacting intensively with each other and with the program director as well as with the appro-

TABLE 1. Performance Sites, Principal Investigators, and Steering Committee Members for the Multicenter Cooperative Study of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY)

Scientist and Site	Collection Center	Steering Committee	Special Study Center	Laboratory for Specified Core Function
Bleakley Chandler Profession and Chairman Dept of Pathology Medical College of Georgia Augusta, Georgia	*		*	
J. Frederick Cornhill Professor and Chairman Biomedical Engineering Center Ohio State University Columbus, Ohio	*	*		Morphometry center
Assad Daoud Profession of Pathology Albany Medical College Veterans Admin Hospital Albany, New York	*			
Steffan Gay Assoc Prof of Medicine U of Alabama Birmingham, Alabama			*	
Singanallur Jagannathan Assoc Professor of Pathology W Virginia U Med Center Morgantown, West Virginia	*		*	
Henry McGill Professor of Pathology and Scientific Director of the Southwest Foundation for Biomedical Research San Antonio, Texas		*		Statistical center

TABLE 1 continued.

Scientist and Site	Collection Center	Steering Committee	Special Study Center	Laboratory for Specified Core Function
Alex McMahan Professor of Pathology University of Texas Health Sciences Center San Antonio, Texas		*		Statistical center
Bruce McManus Professor of Pathology U of Nebraska Med Center Omaha, Nebraska	*			
Wolfgang Mergner Professor of Pathology Univeristy of Maryland Baltimore, Maryland	*			
Edward Miller Professor of Biochemistry University of Alabama Birmingham, Alabama			*	
Abel Robertson Professor of Pathology University of Illinois College of Medicine Chicago, Illinois	*	*	*	Associated administration center
Louis Smith Prof of Surgery & Pathology Baylor College of Medicine Houston, Texas			*	
Jack Strong Professor and Chairman Department of Pathology Louisiana State University New Orleans, Louisiana		*		Risk factor and fixed tissue center
Renu Virmani Assoc Prof of Pathology Vanderbilt University School of Medicine Nashville, Tennessee	*		*	
Robert W. Wissler Professor of Pathology University of Chicago Medical Center Chicago, Illinois	*	*	*	Administration, histotechnical preparation center, frozen tissue center

priate research staff members of the Heart and Vascular Diseases Division and the Atherosclerosis, Lipid Metabolism, and Hypertension Section of the National Heart, Lung, and Blood Institute.

ORGANIZATIONAL PATTERN AND SAMPLING STRATEGY

As the program has developed, a number of satellite study centers have also developed to make use of the research materials and opportunities that this study affords. Of special interest is the identification and study of genetic markers for candidate genes for atherosclerosis, several of which have not been previously identified and which carry with them an increased or a lesser susceptibility to atherogenesis. The organizational pattern and the interrelationships among the centers involved are diagrammatically presented in FIGURE 1. Here the most frequent interactions among the centers and the relationships among the core centers are portrayed.

A special preliminary investigation conducted by Drs. Herbert Stary and Frederick Cornhill showed that there was a recurring topographical pattern in the localization of atheromatous lesions in the aortas and the coronary arteries of these young people.[1] Some of these patterns had been strongly suggested by earlier studies,[2-4] but doubts remained in the minds of some investigators as to the continuity of these patterns during the development of atherosclerosis from fatty streak to fibrous plaque as the disease progresses with age. The preliminary studies in this research program showed that there was a pattern of lesion localization as the disease process developed between ages 15-34 inclusive. It also became increasingly evident that the lesions in the aorta are symmetrical[1,5] so that it has been possible to use one half of the aorta for topographical analyses and to use the other half for histopathological and chemical studies. This sampling strategy is presented diagrammatically in FIGURE 2 where it is evident that each of the samples of the aorta and, to a lesser extent, of the left circumflex coronary artery goes through a "lesion-prone" and a "lesion-resistant" area. Therefore, quantitation of the fundamental topography has increased the value of the information that this study is generating.

The sampling strategy for the coronary arteries also makes maximal use of the material available and enhances the value of the samples. The plan permits topographical analysis of the open right coronary artery, chemical and special cellular and histopathological studies of the left circumflex coronary artery and study of the perfusion-fixed left anterior descending coronary artery.

In addition to the core centers and the core data being generated, and as indicated in the introduction to this report, various PDAY centers are engaged in a number of special studies.[6] These centers are now generating data from arterial samples from selected cases so that the data from these studies can be related to data from other special study centers. Furthermore, centers are taking care to be sure that these groups of cases are balanced in terms of race, age, and sex and that each case utilized for special studies has complete core and risk factor information.

The methods being utilized to evaluate risk factors in autopsied persons have been developed under the leadership of Professor Jack Strong during the community studies investigations conducted on forensic pathology cases at Louisiana State University during the past 20 years.[7-9] Their feasibility and applicability to the PDAY study have been established for this research program during the feasibility phase.

EARLY RESULTS

Among the most revealing gross morphometric results from this study thus far are the core center data indicating the relationship between serum lipoprotein levels and the development of surface-occupying, lipid-containing, as well as raised, lesions in the aortas of white nonsmoking males. An example of this type of data as it is related to age is given in FIGURE 3. These preliminary results are now being strengthened by extension of the study to additional cases of black and white males and females.

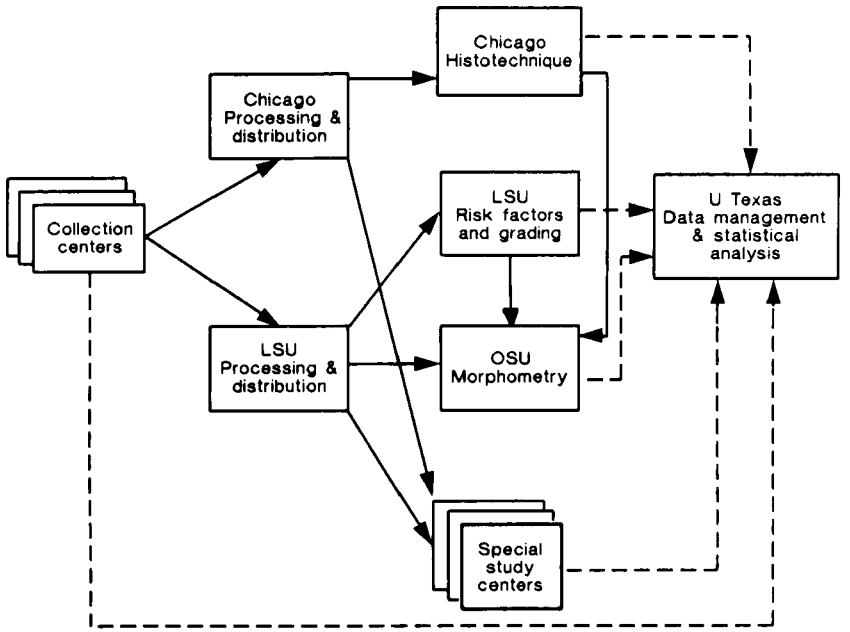

FIGURE 1. PDAY materials and data flow. This diagram indicates how the collection centers interact with the various core centers in preserving and transmitting samples for processing, for risk factor measurements, for statistical evaluation of data, for gross and microscopic morphometry, and for use in the various special studies that are being carried out at a number of centers. This system, which was demonstrated to be workable during a "phase 1 feasibility trial" involving samples, data, and information generation and utilization on more than 200 cases, has now been used successfully in more than 1500 cases.

A more revealing relationship between risk factors and the development of lipid-containing gross lesions in the aorta in relationship to age is shown in FIGURE 4 in which elevated blood lipids are considered for a group of more than 300 cases of males which had been studied when these results were presented to the National Heart, Lung, and Blood Institute Council in early 1990.

An additive relationship between blood lipids in postmortem blood, smoking as indicated by thiocyanate concentrations in postmortem blood, and surface involvement

of the right coronary artery of white males is given in FIGURE 5. All of these relationships are significant for white males. At present the number of cases has been greatly augmented and the data are being analyzed to include black and white males. Furthermore, an effort is being made to extend the support for the research program so that adequate numbers of black and white females can be included.

TABLE 2 lists the statistical significance of some of these effects and their interactions on total surface aortic involvement. It serves as an indicator of the power that these

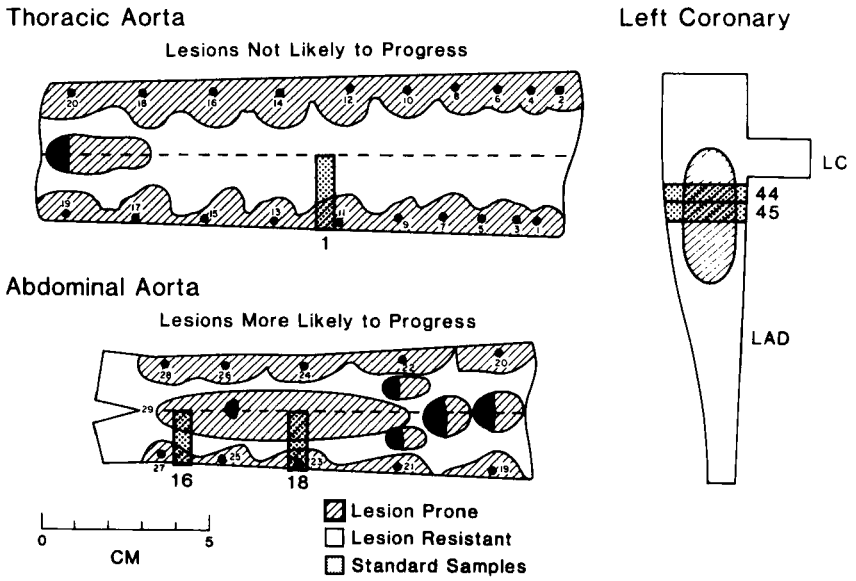

FIGURE 2. This figure illustrates in diagrammatic form some of the strengths and advantages of the core sampling strategy that PDAY is employing on all of its cases. As can be seen, these samples make it possible to study and to evaluate quantitatively the thoracic aorta core sample area (no. 1) as compared to the standard abdominal aorta areas (nos. 16 and 18). It also makes it possible to evaluate similarly the left anterior descending coronary artery (LAD) standard samples (nos. 44 and 45) and to compare lesion-prone and lesion-resistant areas in each of these standard samples. The arteries are oriented and marked so that under the microscope it is relatively easy to distinguish lesion-prone and lesion-resistant areas and to match up the sections stained for lipid with the sections stained for connective tissue components. These are less than a millimeter from each other because of the way samples are prepared for microscopic examination. (As modified from Wissler, R. W., D. Vesselinovitch, A. Komatsu & R. T. Bridenstine. 1988. The arterial wall and atherosclerosis in youth. *In*: Biology of the Arterial Wall: 265-274. CIC Edizioni Internazionali. Rome, Italy.)

measurements can generate as the study progresses. Other correlations of lesions with established and emerging risk factors are beginning to be observed as this study progresses. The power of the data and the contributions that these data will make to our understanding of the pathogenesis and histogenesis of progressive atherosclerosis in young people will increase as more cases are acquired. Furthermore, the important differences in the responses of females and males as well as the responses of the two

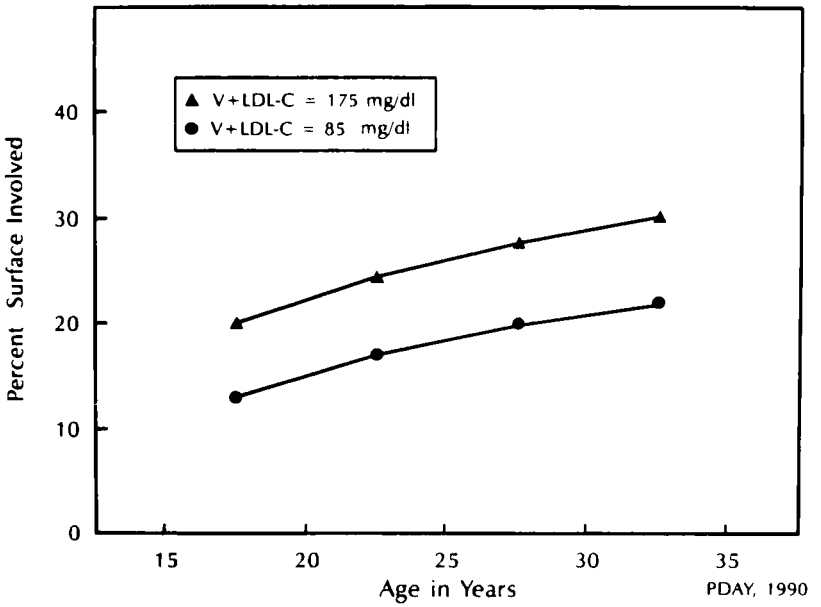

FIGURE 3. Graph based on multiple regression analysis of the total percent surface involved with both fatty streaks and raised lesions in the abdominal aortas of 329 males aged 15 to 34 inclusive. The values for percent of surface involved are adjusted for white males at two levels of lower density lipoprotein cholesterol—the upper one standard deviation above the mean, and the lower one standard deviation below the mean.

major racial groups being studied will become much more firmly established as the numbers of available cases and samples to be compared are more adequate.

The special studies being performed at the University of Chicago have been planned so that equal numbers of cases from black and white males and from black and white females would be utilized. The cases selected for the University of Chicago studies by the statistical center consist of complete cases with data available on all risk factors.

TABLE 2. Significance of Main Effects and Two-Way Interactions on Total Surface Involvement in 329 Abdominal Aortas[a]

Predictor Variable	*p* Value
Age	0.0001
Race	0.0011
V + LDL-C	0.0001
HDL-C	0.0025
Smoking	0.0012
Race × V + LDL-C	0.0442
Age × smoking	0.0340

[a]V + LDL-C is very low and low-density lipoprotein cholesterol. HDL-C is high-density lipoprotein cholesterol.

Furthermore, these cases are complete because all core samples of arteries, blood, etc., are present, and the cases are evenly distributed among the four age cells, each consisting of a five-year interval. Also from the beginning it was recognized that most of the special studies would have to be performed in phase 3 of the research program, i.e., from July 1990 through July 1992. It is our intent to complete these special studies with 150 cases in each of the race and sex categories and with approximately 35-40 cases in each of the five-year age groups.

The preliminary results from the pathobiological studies being performed at the University of Chicago indicate a number of interesting correlations which have been partially delineated in two previous recent publications.[6,10] Of the numerous evident quantitative observations that appear to be emerging from these pathobiological studies, the following would seem to have the most important implications relative to atherogenesis:

- Extracellular lipid in lesions appears to be one of the most important indicators of the potential for progression of the atherosclerotic process.
- Quantitation of the lipid-laden macrophage population appears to indicate far greater numbers of these cells in the lesion-prone parts of the thoracic aorta where lesions are least likely to progress as compared to the much sparser population of

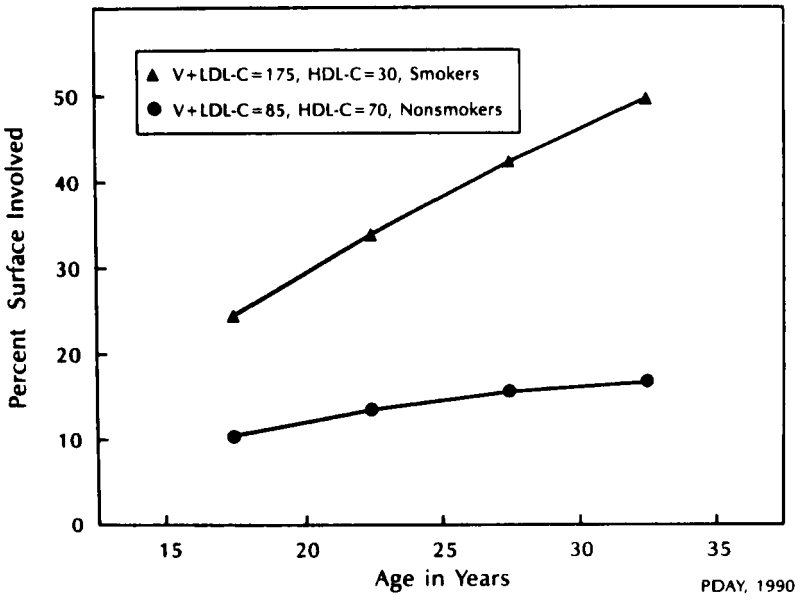

FIGURE 4. Graph based on multiple regression analysis of total percent surface involved with both fatty streaks and raised lesions in the abdominal aortas of 329 males aged 15 to 34 inclusive. The values are adjusted for white males at two risk levels—high lower-density cholesterol concentration, low high-density cholesterol concentration, and smoking; and low lower-density cholesterol concentration, high high-density cholesterol concentration, and not smoking. The effects of each risk factor are additive and their combined effect is greater with increasing age.

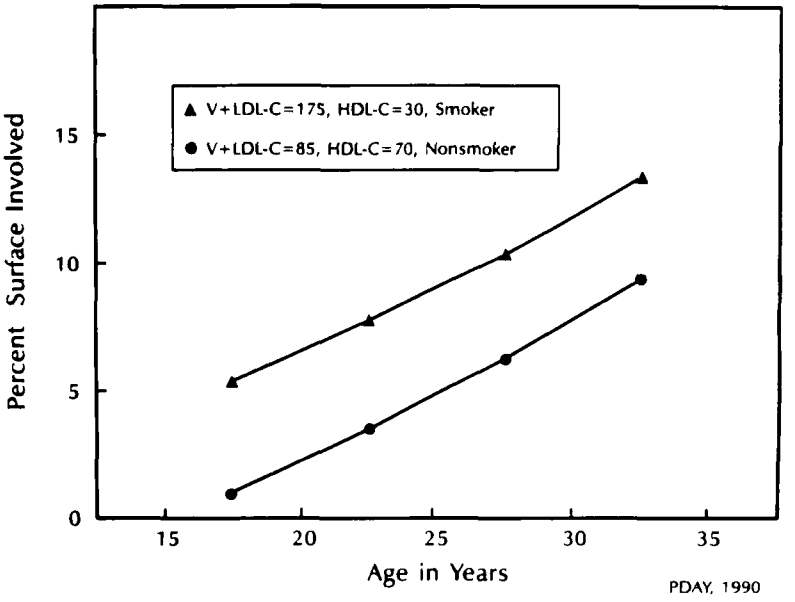

FIGURE 5. Graph of total percent surface involved with fatty streaks and raised lesions in the right coronary arteries of 329 males aged 15 to 34 inclusive, adjusted for white males and the two risk levels described in FIGURE 4. The additive effect of the three risk factors is apparent in these preliminary results.

these cells in the lower abdominal aorta, lesion-susceptible parts of the aorta where the disease process progresses most rapidly.

- In contrast to the results of at least four previous publications from four different laboratories on the localization of Lp(a) as compared to the localization of apo B in advanced atherosclerotic lesions in older individuals, this apolipoprotein appears to have a very different localization than does apo B in young people's lesions when compared by means of quantitative micromorphometry.
- When concentric atheroarteritis is observed in the proximal left anterior descending artery it is frequently correlated with high levels of immune complexes in the serum.[11]

SUMMARY AND SIGNIFICANCE

The results of these studies are likely to emphasize the need for intervention to prevent atherosclerosis by the age of 15, and possibly earlier. If sufficient support is available to collect adequate numbers of female cases and cases with advanced lesions, it is likely that a number of subtle new risk factors involving immunological and other

types of injury to the artery cells and the artery wall will be correlated with accelerated lesion development in young individuals.

The study has already indicated that the well-known risk factors of hyperlipidemia and smoking are associated with more rapid development of lesions with age in black and white males in the United States and that the interactions of these factors are likely to show a progressive effect and to be highly additive between the ages of 15 and 35.

These and many other observations that we are making in this cooperative multicenter study portend a very rich harvest of new facts which should help to understand the pathobiological determinants of atherosclerosis in youth. They illustrate the continuing value of research using the human autopsy.

ACKNOWLEDGMENTS

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APPENDIX
Pathobiological Determinants of Atherosclerosis in Youth Research Group

The investigators cooperating in the multicenter study "The Pathobiological Determinants of Atherosclerosis in Youth" are listed below.

Program Director

Robert W. Wissler, PhD, MD, University of Chicago; Associate Director: Abel L. Robertson, Jr., MD, PhD, The University of Illinois

Steering Committee

J. Fredrick Cornhill, D Phil, The Ohio State University; Henry C. McGill, Jr., MD, and C. Alex McMahan, PhD, The University of Texas Health Science Center at San Antonio; Abel L. Robertson, Jr., MD, PhD, The University of Illinois; Jack P. Strong, MD, Louisiana State University Medical Center; Robert W. Wissler, PhD, MD, University of Chicago

Chair of Protocol and Manual of Operations Committee

Margaret C. Oalmann, Dr PH, Louisiana State University Medical Center

Internal Advisory Committee

Jack C. Geer, MD, University of Alabama; James E. Grizzle, PhD, University of North Carolina; Paul E. Lacy, MD, Washington University School of Medicine; Robert W. Prichard, MD, Wake Forest University; Robert Selzer, MS, Jet Propulsion Laboratory, California Institute of Technology.

Participating Centers

University of Alabama, Birmingham, Ala. *Department of Medicine:* Principal Investigator: Steffen Gay, MD; Coinvestigators: Renate E. Gay, MD, Guo-quiang Huang, MD [HL-33733]; *Department of Biochemistry:* Principal Investigator: Edward J. Miller, PhD; Coinvestigators: Donald K. Furuto, PhD, Margaret S. Vail, Annie J. Narkates [HL-33728].

Albany Medical College, Albany, N.Y. Principal Investigator: Assaad Daoud, MD; Coinvestigators: Adrienne S. Frank, PhD, Mary A. Hyer, E. Carol McGovern [HL-33765].

Baylor College of Medicine, Houston, Tex. Principal Investigator: Louis C. Smith, PhD; Coinvestigator: Faith M. Strickland, PhD [HL-33750].

University of Chicago, Chicago, Ill. Principal Investigator: Robert W. Wissler, PhD, MD; Coinvestigators: Dragoslava Vesselinovitch, DVM, MS, Akio Komatsu, MD, PhD, Yoshiaki Kusumi, MD, R. Timothy Bridenstine, MS, Robert J. Stein, MD, Robert H. Kirschner, MD, PhD, Manuela Bekermeier, ASCP, Blanche Berger, ASCP, Laura Hiltscher, ASCP [HL-33740].

The University of Illinois, Chicago, Ill. Principal Investigator: Abel L. Robertson, Jr., MD, PhD; Coinvestigators: Robert J. Stein, MD, Eugene E. Emeson, MD, Luna Ghosh, MD, Herbert M. Yamashiroya, PhD, Robert J. Buschmann, PhD; Assistant Investigator: John Gabrovsek, DDS. *Collection:* Edmund R. Donoghue, Jr., MD, Robert H. Kirschner, MD, PhD, Tae Lyong An, MD, Michael Chambliss, MD, Eupil Choi, MD, Nancy Jones, MD, Mary I. Jumbelic, MD, Mitra S. Kalelkar, MD, Uuksel Konakci, MD, Barry Lifschultz, MD, Michael I. Schaffer, PhD, Shaku Teas, MD, Margarita Arruza, MD, James Dianovsky, BA, Donald Waterford, BA, *Special Studies:* Mei-Ling Shen, PhD, Richard Yang, MS, Frances Norris, HTL (ASCP), Dana Gylys, HT (ASCP) [HL-33758].

Louisiana State University Medical Center, New Orleans, LA. Principal Investigator: Jack P. Strong, MD; Coinvestigators: Gray T. Malcom, PhD, William P. Newman III, MD, Margaret C. Oalmann, Dr PH, Paul S. Roheim, MD, Ashim K. Bhattacharyya, PhD, Miguel A. Guzman, PhD, Ali A. Hatem, MD, Conrad A. Hornick, PhD, Carlos D. Restrepo, MD, Richard E. Tracy, MD, PhD [HL-33746].

University of Maryland, Baltimore, MD. Principal Investigator: Wolfgang J. Mergner, MD, PhD; Coinvestigators: Margaret L. Couture, PhD, James H. Resau, PhD, Robert D. Vivigorito, MS, PA, Q.-C. Yu, MD, PhD [HL-33752].

Medical College of Georgia, Augusta, GA. Co-Principal Investigators: A. Bleakley Chandler, MD, Raghunatha N. Rao, MD; Coinvestigators: D. Greer Falls, MD, Benjamin O. Spurlock, BA; Associate Investigators: Kailash B. Sharma, MD, Joel S. Sexton, MD; Research Assistants: K. K. Smith, HTASCP, G. W. Forbes [HL-33772].

University of Nebraska Medical Center, Omaha, Nebr. Principal Investigator: Bruce M. McManus, MD, PhD; Coinvestigators: Jerry W. Jones, MD, Todd J. Kendall, MS, Jerrold A. Remmenga, BS, William C. Rogler, BS [HL-33778].

The Ohio State University, Columbus, Ohio. Principal Investigator: J. Fredrick Cornhill, D. Phil; Coinvestigators: William R. Adrion, MD, Patrick M. Fardel, MD, Brian Gara, MS, Edward Herderick, BS, John Meimer, MS, Larry R. Tate, MD [HL-33760].

Southwest Foundation for Biomedical Research, San Antonio, Tex. Principal Investigator: James E. Hixson, PhD [HL-39913].

The University of Texas Health Science Center at San Antonio, San Antonio, Tex. Principal Investigator: C. Alex McMahan, PhD; Coinvestigators: George M. Barnwell,

PhD, Henry C. McGill, Jr., MD, Yolan N. Marinez, MA, Thomas J. Prihoda, PhD, Herman S. Wigodsky, MD, PhD [HL-33749].

Vanderbilt University, Nashville, Tenn. Principal Investigator: Renu Virmani, MD; Coinvestigators: James B. Atkinson, MD, PhD, Charles W. Harlan, MD [HL-33770].

West Virginia University Health Sciences Center, Morgantown, W.Va. Principal Investigator: Singanallur N. Jagannathan, PhD; Coinvestigators: Bruce Caterson, PhD, James L. Frost, MD, K. Murali K. Rao, MD, Syamala Jagannathan, BS, Peggy Johnson, MS, Nathaniel F. Rodman, MD [HL-33748].