

## Smoking and atherosclerosis in youth

A.W. Zieske \*, H. Takei, K.B. Fallon, J.P. Strong

*Department of Pathology, Louisiana State University Medical Center, 1901 Perdido St., New Orleans, LA 70112, USA*

Received 28 July 1998; received in revised form 6 November 1998; accepted 1 December 1998

### Abstract

Coronary heart disease is the most common cause of death in the US. Studies have demonstrated that smoking is a major risk factor for coronary heart disease and that a positive relationship occurs between smoking and aortic and coronary atherosclerosis in adults. In 1985, a multicenter cooperative study, Pathobiological Determinants of Atherosclerosis in Youth (PDAY), was organized to study atherosclerosis in trauma victims 15–34 years of age. Reports from this study have demonstrated that smoking is strongly associated with the prevalence and extent of grossly visible raised lesions in the abdominal aorta but only weakly associated with similar lesions in the right coronary artery. Coronary arteries from 50 smokers and 50 non-smokers were classified microscopically using a system developed by the American Heart Association in order to determine the stage at which smoking affects atherosclerosis. Smokers had over twice as many advanced lesions, types IV and V, as non-smokers (32 vs 14%) and fewer early lesions, types I, II, III, as non-smokers (38 vs 62%). The prevalence of advanced or types IV and V lesions (32%) was over twice that of intermediate or type III lesions (14%) in smokers. The opposite relationship was observed in non-smokers (14 vs 26%). This observation suggest that intermediate lesions progress rapidly into advanced lesions in smokers and that intima formerly having early lesions is replaced by intima with raised lesions. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

*Keywords:* Atherosclerosis; Smoking; Pathobiological Determinants of Atherosclerosis in Youth (PDAY); Coronary artery; Intermediate lesion; Fibrous plaque

### 1. Introduction

Atherosclerosis begins in childhood with deposits of lipid in macrophages and smooth muscle cells located in the intima to form fatty streaks [1–3]. In young adulthood, some fatty streaks become raised lesions when intracellular and extracellular lipid accumulates and a fibromuscular cap forms above the intima [4–7]. In middle aged individuals raised lesions continue to progress to complicated lesions, which may lead to ischemic clinical events [1].

Epidemiologic studies have identified numerous risk factors that are associated with the clinical events resulting from atherosclerosis [8]. These studies provide strong evidence that smoking is a major risk factor for

coronary heart disease (CHD) and in 1983, the Public Health Service Report on the Health Consequences of Smoking stated that, “cigarette smoking is considered the most important known modifiable risk factor for coronary heart disease in the United States” [9].

Several autopsy studies have demonstrated a positive relationship between smoking and atherosclerotic lesions in the aorta and coronary arteries of adults [10–15]. Both the 1983 Surgeon General’s report [9] and the 1983 review by Strong and Solberg [15] concluded that smoking is associated with more severe coronary artery lesions and that the effect of smoking on atherosclerosis is more pronounced in the aorta than in the coronary arteries of adults. At that time the effect of smoking on atherosclerosis in young subjects was unknown.

In 1985, a multicenter cooperative study titled Pathobiological Determinants of Atherosclerosis in Youth

\* Corresponding author. Tel.: +1-504-568-6031; fax: +1-504-568-6037.

(PDAY) was organized to study atherosclerosis in victims of traumatic death 15–34 years of age. A preliminary report demonstrated that smoking is positively associated with the prevalence and extent of raised lesions in the abdominal aorta and positively associated with the prevalence of raised lesions in the right coronary arteries of 390 young men [16]. Completion of the collection phase of this study provided a larger number of cases and allowed study of atherosclerosis and risk factors in both men and women. The results confirmed the preliminary report by demonstrating that smoking is associated with more extensive fatty streaks and raised lesions in the abdominal aorta in both men and women and with the prevalence of raised lesions involving 5% or more of the intimal surface in the right coronary arteries [17].

Most studies addressing the relationship of smoking and atherosclerosis in autopsied subjects evaluated lesions grossly; microscopic examination was limited. Microscopic examination of a subset of the PDAY material by Wissler et al. demonstrated a higher prevalence of fibrous plaques in coronary arteries of smokers [18].

This report presents the results of microscopic studies of coronary arteries from young smokers and non-smokers in order to further clarify the relationship of smoking to atherosclerosis.

## 2. Methods

### 2.1. Subjects studied

The PDAY archive at the Louisiana State University Medical Center contains over 3000 subjects, black and white, male and female, 15 to 34 years who died from external causes. The subjects used for this study were randomly selected and consisted of 50 white male smokers and 50 white male non-smokers aged 25–34 years. These subjects were chosen because the prevalence of raised lesions is highest in this age group [1].

### 2.2. Serum thiocyanate levels

Serum thiocyanate levels were measured colorimetrically by the thiocyanate–ferric nitrate complex after treatment of trichloroacetic acid filtrates of serum with ferric nitrate. A subject was considered a smoker if his postmortem serum thiocyanate level was  $\geq 90$   $\mu\text{mol/l}$  [16,17].

### 2.3. Preparation of arteries

The arterial segment comprising the left main and left anterior descending (LAD) coronary arteries was fixed at autopsy by perfusion with 10% neutral buffered

formalin under 100 mmHg pressure. Standardized PDAY core samples from either of two regions on the LAD coronary artery were used for this study. The first region was a 5-mm length of artery located immediately distal to the bifurcation of the left main coronary artery. The second region was a 5-mm length of artery located adjacent and distal to the first region. The second 5-mm region was used only if the first 5-mm length was not available. The right coronary arteries were preserved for gross visual evaluation of lesions [16,17].

### 2.4. Histochemical staining

Two 6- $\mu\text{m}$  sections were cut from each formalin fixed, paraffin embedded tissue block. One section was stained with hematoxylin–eosin and one with Gomori's trichrome aldehyde fuchsin [19]. Adjacent formalin fixed frozen sections were cut at 20  $\mu\text{m}$  on a cryostat and stained for lipid using Oil Red O with Lillie hematoxylin as a counterstain [18,20].

### 2.5. Classification of lesions

The stained sections were evaluated independently by three pathologists (without knowledge of smoking status), and each case was classified using a system developed by the Committee on Vascular Lesions of the Council on Arteriosclerosis of the American Heart Association [6,7,21]. This classification system includes adaptive intimal thickening of the coronary artery (a normal, self-limited phenomenon not considered as a lesion), early lesions that correspond grossly to fatty streaks (types I, II, and III), and advanced or raised lesions that are clinically significant (types IV, V, and VI). A summary of this classification scheme is illustrated in Fig. 1 [21].

### 2.6. Statistical analysis

Differences in the distribution of lesion types between the non-smoking and smoking groups were determined using  $\chi^2$  test analysis.

## 3. Results

Results of the classification of the histological sections of the coronary arteries are summarized in Table 1. The prevalence of type IV (atheroma) and type V (fibroatheroma) lesions is increased (32 vs 14%) and the prevalence of type III (intermediate) lesions is decreased (14 vs 26%) in smokers compared to non-smokers. Type II fatty streaks are less prevalent in the smoking group. There were no type VI (complicated lesions) identified in this subset of coronary arteries.

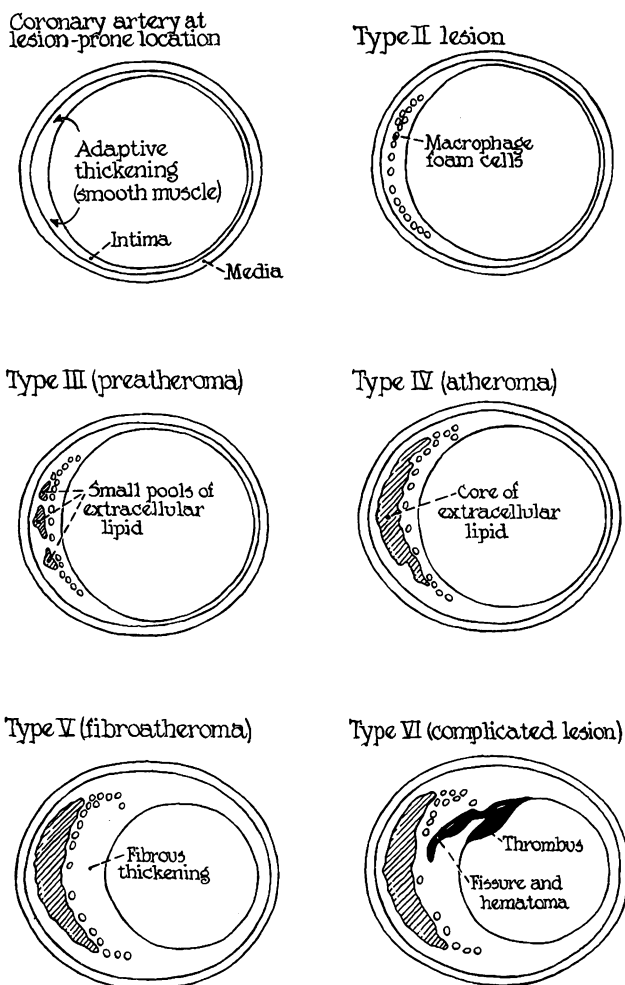


Fig. 1. American Heart Association classification system for atherosclerotic lesions. The type I (initial) lesion, which consists of small, isolated groups of macrophages containing lipid droplets, is not shown in this figure (with permission from Stary [21]).

Fig. 2 shows a significant difference ( $P = 0.0345$ ) between smokers and non-smokers in the distribution of lesions grouped as none (no lesion and adaptive intimal thickening), early lesions (types I, II, and III),

Table 1  
Histological classification of atherosclerotic lesions of left anterior descending coronary arteries from 100 white men age 25–34 years using the AHA system

Lesion type	Non-smokers (n = 50)		Smokers (n = 50)	
	n	%	n	%
None	12	24	15	30
Type I (initial)	1	2	1	2
Type II (fatty streak)	17	34	11	22
Type III (intermediate)	13	26	7	14
Type IV (atheroma)	1	2	5	10
Type V (fibroatheroma)	6	12	11	22

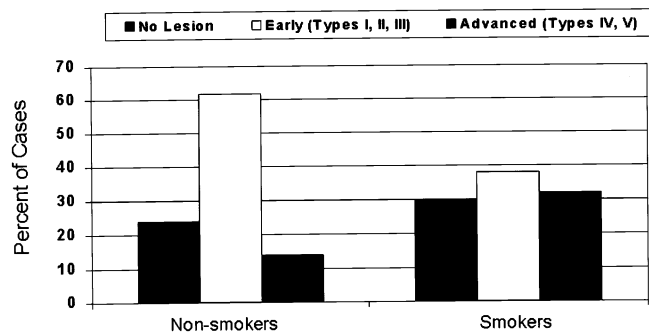


Fig. 2. Distribution of left anterior descending coronary arteries from 100 white men aged 25–34 years by type of atherosclerotic lesion in smokers and non-smokers.  $P = 0.0345$ .

and advanced lesions (types IV and V). Smokers have twice as many advanced lesions (associated with proliferative activity) and fewer early lesions (predominantly associated with lipid accumulation) when compared to the non-smokers.

Fig. 3 shows associations between intermediate lesions (type III), advanced lesions (types IV and V), and smoking status. The twofold increased prevalence of advanced lesions compared to intermediate lesions in smokers markedly contrasts with the nearly twofold decreased prevalence of advanced lesions when compared to intermediate lesions in non-smokers.

#### 4. Discussion

The results show that the proximal LAD coronary artery sections from smokers have twice as many advanced lesions and half as many intermediate lesions compared to non-smokers. This observation suggests that smoking begins to affect atherosclerosis at the type IV lesion stage. The observation also suggests that in smokers there is a rapid progression from intermediate lesions into advanced lesions and that intima formerly containing early lesions has been replaced by raised lesions. This stage in progression occurs at the point

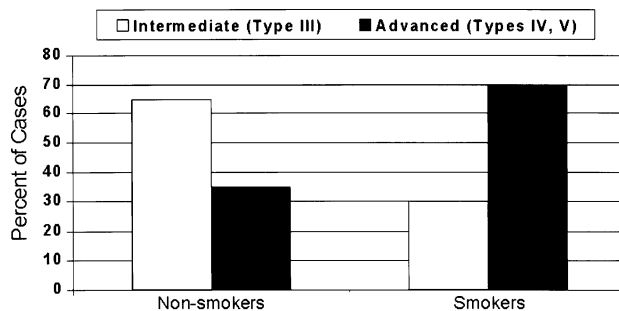


Fig. 3. Distribution of intermediate lesions (type III) versus advanced lesions (types IV and V) by smoking status in the LAD coronary arteries.  $P = 0.0337$ .

between lesions predominantly associated with lipid accumulation (intermediate or type III lesions) and those associated with proliferation or increased fibrous tissue (advanced or types IV and V lesions).

The type III lesion, known as the preatheroma or intermediate lesion (intermediate lesion is the term used in this study), indicates progression from a fatty streak to an advanced lesion. Since sampling the same lesion over time is impossible, this classification represents a deduced temporal sequence that is based on lesions taken from standardized locations from many persons of different ages. Therefore, it is impossible to determine if these lesion types truly represent the progression seen in an individual lesion. However, this classification scheme provides a way to circumstantially extrapolate the evolution of lesions in a systematic, reproducible manner.

The results of our study are similar to those based upon a different microscopic classification system designed by Wissler et al. [18], which showed a higher prevalence of advanced lesions in smokers when compared to non-smokers. They divided the intermediate atherosclerotic lesion into four categories based on intracellular lipid localization (macrophages vs. smooth muscle cells), extracellular lipid deposition, and the presence of lymphocytes. The gross counterpart for the microscopic intermediate lesion defined by Wissler et al. is the 'fatty plaque', which represents a distinct subtype of fatty streak. Results from both studies support the findings of the previous PDAY studies of lesions, which were graded by gross visual evaluation [16,17].

Another study by Wissler et al. of PDAY cases showed that 72% of type V lesions occurred in smokers, a higher proportion than for any other risk factor [22].

The effects of smoking on the cardiovascular system include increased coronary vascular resistance, reduction of oxygen delivery, increased viscosity of blood, enhancement of platelet aggregation, increased fibrinogen concentration, depression of HDL cholesterol concentration, and possibly acute increases in blood pressure [23]. The specific mechanisms by which cigarette smoking accelerates atherosclerotic lesion progression are not known. Of the more than 400 different compounds identified in tobacco smoke, nicotine and carbon monoxide are the constituents most often cited as having both supporting data and a rational role in the pathogenesis of atherosclerosis [24]. Free radicals have also been implicated as having a potential role in smoking-related atherogenesis [23].

Studies reviewed by Stein et al. demonstrated an association between smoking and oxidized LDL [25]. Scanlon et al., using aorta samples from the PDAY archives, demonstrated that oxidized LDL deposits are more extensive in fatty streaks and fatty plaques (intermediate lesions) in smokers than in non-smokers [26].

Advanced glycosylation end products (AGEs) were linked to atherosclerotic cardiovascular disease by the observation that AGEs were deposited in lesions of adult diabetics [27]. A previous PDAY study demonstrated that elevated glycohemoglobin levels were associated with accelerated atherogenesis in the third and fourth decades of life [28]. Reactive glycation products are present in aqueous extracts of tobacco and in tobacco smoke in a form that can rapidly react with proteins to form advanced glycosylated end products [29]. AGE modification of Apo B prevents the normal uptake of LDL by tissue LDL receptors, thereby increasing circulating LDL levels [30], and AGE-apolipoprotein B and serum AGE levels are higher in smokers when compared to non-smokers [29]. These tobacco 'glycotoxins' may prove to play a central role in the progression of atherosclerosis associated with smoking.

Whether primary prevention of adult coronary heart disease should begin in youth with diet modifications has been a controversial issue because of the possibility of adverse effects. Promoting the avoidance of tobacco usage to reduce the risk of cardiovascular disease poses no hidden risks for young or adult individuals. Therefore, evidence linking smoking with the progression of atherosclerosis and CHD in youth strongly supports a mandate for intervention to prevent smoking in youth.

## 5. Conclusion

Microscopic studies of perfusion fixed left anterior descending coronary artery show that smokers have a higher prevalence of advanced lesions than non-smokers. Previous reports from the PDAY study, based on gross evaluation alone, have underestimated the effects of smoking on the coronary arteries.

## Acknowledgements

The authors would like to thank Dr Gray T. Malcom and Dr Henry C. McGill, Jr. for their expertise in the preparation and review of this manuscript. The authors would also like to acknowledge the scientific staff at the 14 PDAY centers, where the important work of collecting the cases, processing, preserving, and distributing the samples as well as the determination of risk factors, etc. was performed (Appendix A). A.W. Zieske and H. Takei contributed equally to this work.

## Appendix A. The PDAY Research Group

The investigators cooperating in the multicenter study 'The Pathobiological Determinants of Atherosclerosis in Youth,' and the grants supporting their activities are listed below.

### 1. Program Director

Jack P. Strong, M.D., 1996–date, and Robert W. Wissler, Ph.D., M.D., 1985–1996.2. *Steering Committee*

Fredrick Cornhill, D.Phil.; Henry C. McGill, Jr., M.D.; C. Alex McMahan, Ph.D.; Gray T. Malcom, Ph.D.; Margaret C. Oalman, Dr. P.H.; Jack P. Strong, M.D.; Robert W. Wissler, Ph.D., M.D.3. *Participating centers, principal and coinvestigators, and supporting grants from the National Heart, Lung, and Blood Institute:*

*University of Alabama*, Birmingham, AL: Department of Medicine, Steffen Gay, M.D. (HL-33733); Department of Biochemistry, Edward J. Miller, Ph.D. (HL-33728).

*Albany Medical College*, Albany, NY: Assad Daoud, M.D.; Adriene S. Frank, Ph.D. (HL-33765).

*Baylor College of Medicine*, Houston, TX: Louis C. Smith, Ph.D. (HL-33750).

*University of Chicago*, Chicago, IL: Robert W. Wissler, Ph.D., M.D.; Dragoslava Vesselinovitch, D.V.M., M.S.; Akio Komatsu, M.D., Ph.D.; Yoshiaki Kusumi, M.D.; Toshinori Oinuma, M.D.; Alyna Chien, M.A.; Alexis Demopoulos, M.D.; Gertrud Friedman, B.A.; R. Timothy Bridenstine, M.S.; Robert J. Stein, M.D.; Robert H. Kirschner, M.D.; Manuela Bekermeier, ASCP; Blanche Berger, ASCP; Laura Hiltcher, ASCP (HL-33740; HL-45715).

*University of Illinois*, Chicago, IL: Abel L. Robertson, Jr., M.D., Ph.D.; Robert J. Stein, M.D.; Edmund R. Donoghue, M.D.; Robert J. Buschmann, M.D.; Yoshihisa Katsura, M.D. (HL-33758).

*Louisiana State University Medical Center*, New Orleans, LA: Jack P. Strong, M.D.; Gray T. Malcom, Ph.D.; William P. Newman, III, M.D.; Margaret C. Oalman, Dr. P.H.; Richard E. Tracy, M.D., Ph.D.; Sulochana Y. Bhandaru, M.D., M.P.H.; Cynthia S. Zsembik, B.S.; DeAnne G. Gibbs, B.S.; Dana A. Troxclair, M.S. (HL-33746; HL-45720).

*University of Maryland*, Baltimore, MD: Wolfgang Mergner, M.D., Ph.D.; Catherine Cole, Ph.D.; J. Smialek, M.D. (HL-33752; HL-45693).

*Medical College of Georgia*, Augusta, GA: A. Bleakley Chandler, M.D.; Raghunatha N. Rao, M.D.; D. Greer Falls, M.D.; Ross G. Gerrity, Ph.D.; Benjamin O. Spurlock, B.A.; Kalish B. Sharma, M.D.; Joel S. Sexton, M.D. (HL-33772).

*University of Nebraska Medical Center*, Omaha, NE: Bruce M. McManus, M.D., Ph.D.; Jerry W. Jones, M.D. (HL-33778).

*The Ohio State University*, Columbus, OH: J. Fredrick Cornhill, D.Phil.; William R. Adrion, M.D.; Patrick M. Fardel, M.D.; Brian Gara, M.S.; Edward Herderick, B.S.; Larry R. Tate, M.D. (HL-33760; HL-45694).

*Southwest Foundation for Biomedical Research*, San Antonio, TX: James E. Hixson, Ph.D. (HL-39913).

*The University of Texas Health Science Center at San Antonio*, San Antonio, TX: C. Alex McMahan, Ph.D.; Henry C. McGill, Jr., M.D.; Yolana Martinez, M.A.; Thomas J. Prihoda, Ph.D. (HL-33749; HL-45719).

*Vanderbilt University*, Nashville, TN: Renu Virmani, M.D.; James B. Atkinson, M.D., Ph.D.; Charles W. Harlan, M.D. (HL-33770; HL-45718).

*West Virginia University Health Sciences Center*, Morgantown, WV: Singanallur N. Jagannathan, Ph.D.; James Frost, M.D. (HL-33748).

### References

- [1] Strong JP, McGill HC Jr. The natural history of coronary atherosclerosis. *Am J Pathol* 1962;40:37–49.
- [2] Stary HC. Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. *Arteriosclerosis* 1989;9:19–32.
- [3] McGill HC Jr, Geer JC, Strong JP. Natural history of human atherosclerotic lesions. In: Sandler M, Bourne GH Jr, editors. *Atherosclerosis and Its Origin*. New York: Academic Press, 1963:39–65.
- [4] Robertson WB, Geer JC, Strong JP, McGill HC Jr. The fate of the fatty streak. *Exp Mol Pathol* 1963;Suppl 1:28–39.
- [5] Geer JC, McGill HC Jr, Robertson WB, Strong JP. Histologic characteristics of coronary artery fatty streaks. *Lab Invest* 1968;18:565–70.
- [6] Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W Jr, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1994;89:2462–2478.
- [7] Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Guyton JR, Insull W Jr, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler Thromb Vasc Biol* 1995;15:1512–1531.
- [8] Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and ECK abnormalities to incidence of major coronary events: final report of the Pooling Project. *J Chron Dis* 1978;31:201–306.
- [9] U.S. Department of Health and Human Services. *The Health Consequences of Smoking: Cardiovascular Disease*, A report of the Surgeon General. Rockville, MD: Office of Smoking and Health. DHHS (PHS) 1983;63–156.
- [10] Strong JP, Richards ML, McGill HC Jr, et al. On the association of cigarette smoking with coronary and aortic atherosclerosis. *J Atheroscler Res* 1969;10:303–17.
- [11] Patel YC, Eggen DA, Strong JP. Obesity, smoking and atherosclerosis: A study of interassociations. *Atherosclerosis* 1980;36:481–90.
- [12] Patel YC, Kodlin D, Strong JP. On the interpretation of smoking risks in atherosclerosis. *J Chron Dis* 1980;33:147–55.
- [13] Tracy RE, Toca VT, Strong JP, et al. Relationship of raised atherosclerotic lesions to fatty streaks in smokers. *Atherosclerosis* 1981;38:347–57.

- [14] Auerback O, Garfinelk L. Atherosclerosis and aneurysms of the aorta in relation to smoking habits and age. *Chest* 1980;78:805–9.
- [15] Solberg LA, Strong JP. Risk factors and atherosclerotic lesions. A review of autopsy studies. *Arteriosclerosis* 1983;3:187–98.
- [16] A preliminary report from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Relationship of atherosclerosis in young men to serum lipoprotein cholesterol concentrations and smoking. *J Am Med Assoc* 1990; 264(23):3018–3024.
- [17] McGill HC Jr, McMahan A, Malcom GT, Oalman MC, Strong JP. Effects of serum lipoproteins and smoking on atherosclerosis in young men and women. *Arterioscler Thromb Vasc Biol* 1997;17:95–106.
- [18] Wissler RW, Hiltcher L, Oinuma T. The lesions of atherosclerosis in the young from fatty streaks to intermediate lesions. In: Fuster V, Ross R, Topol EJ, editors. *Atherosclerosis and Coronary Artery Disease*. Philadelphia, PA: Lippincott-Raven, 1996:475–89.
- [19] Pearse AGE. *Histochemistry, Theoretical and Applied*, 2nd edn. London: Churchill, 1960:846.
- [20] Wissler RW, Komatsu A, Kusumi Y, Curi E, Hiltcher L, Vesselinovitch D. Qualitative and quantitative distribution of stainable lipid in the intimal atheromatous lesions of 100 young people, aged 15–34 years. Presented at the IXth International Symposium on Atherosclerosis, Rosemont, IL, October 1991.
- [21] Stary HC. The histological classification of atherosclerotic lesions in human coronary arteries. In: Fuster V, Ross R, Topol EJ, editors. *Atherosclerosis and Coronary Artery Disease*. Philadelphia, PA: Lippincott-Raven, 1996:463–74.
- [22] Wissler RW, Hiltcher L, Wahden M. Early evolution of coronary artery lesions in young people: from fatty streaks to fibrous plaque—implications for imaging. In: Fuster V, editor. *Syndromes of Atherosclerosis: Correlations of Clinical Imaging and Pathology*. Mount Kisko, NY: Futura, 1996:65–80.
- [23] Stafford RS, Becker CG. Cigarette smoking and atherosclerosis. In: Fuster V, Ross R, Topol EJ, editors. *Atherosclerosis and Coronary Artery Disease*. Philadelphia, PA: Lippincott-Raven, 1996:303–25.
- [24] U.S. Public Health Service. *The Health Consequences of Smoking. A Report of the Surgeon General: 1971*. U.S. Department of Health, Education, and Welfare, Public Health Service, Health Services and Mental Health Administration, DHEW Publications No. (HSM) 71-71-7513:3, 1971;15–135.
- [25] Stein Y, Harats D, Stein O. Why is smoking a major risk factor for coronary heart disease in hyperlipidemic subjects? *Ann New York Acad Sci* 1993;686:66–9.
- [26] Scanlon OC, Berger B, Malcom GT, Wissler RW. Evidence for more deposits of epitopes of oxidized low density lipoprotein in aortas of young people with elevated serum thiocyanate levels. *Atherosclerosis* 1996;121:23–33.
- [27] Nakamura Y, Horii Y, Nishino T, Shiiki H, Sakaguchi Y, Kagoshima T, Dohi K, Makita Z, Vlassara H, Bucala R. Immunohistochemical localization of advanced glycosylation endproducts in coronary atheroma and cardiac tissue in diabetes mellitus. *Am J Pathol* 1993;143:1649–56.
- [28] McGill HC Jr, McMahan AC, Malcom GT, Oalman MC, Strong JP, and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Relation of Glycohemoglobin and Adiposity to Atherosclerosis in Youth. *Arterioscler Thromb Vasc Biol* 1995;15:431–440.
- [29] Cerami C, Founds H, Nicholl I, et al. Tobacco smoke is a source of toxic reactive glycation products. *Proc Natl Acad Sci USA* 1997;94:13915–20.
- [30] Bucala R, Mitchell R, Arnold K, Innerarity T, Vlassara H, Cerami A. Identification of the major site of apolipoprotein B modification by advanced glycation endproducts blocking uptake by the low density lipoprotein receptor. *J Biol Chem* 1995;270:10828–32.