Pathobiological determinants of atherosclerosis in youth (PBDAY Study), 1986–96*

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This article is a summary of the 10-year multinational collaborative WHO/ISFC Study of Pathobiological Determinants of Atherosclerosis in Youth (PBDAY Study). Details are provided of the study design, relevant results, conclusions, and recommendations, as formulated at a consultation of the heads of PBDAY Reference Centres, held in Budapest, Hungary, in October 1996. The WHO/ISFC study provides unique information about the determinants of atherosclerosis and structural changes in the arteries, especially during their early stages, and their progression from early life in populations with vastly different lifestyles. The pilot study covered subjects aged 5–34 years, of both sexes, from 18 centres in 15 countries, while the main study covered 11 centres in 11 countries. Included were both developed and developing countries with different economic, sociocultural and nutritional patterns from five WHO regions. Collected was background epidemiological information, information about cases, and special studies of arteries using various morphometric methods and specialized techniques. Atherosclerotic lesions start to develop early in life independently of race, sex or geographical origin. The rate of fatty streak development is higher between 15 and 25 years of age, while raised lesions begin developing slowly during the second decade of life, progressing steadily during the third and more rapidly during the fourth. Fatty streaks are more prevalent among females and raised lesions among males. The prevalence and extent of raised lesions were greater in countries with a high prevalence of known risk factors and high mortality rates for cardiovascular diseases, coronary heart disease, and cerebrovascular diseases.

Introduction

Atherosclerosis, with its complications, is the pathological process that underlies most cases of coronary heart disease, cerebrovascular disease, and aortic and peripheral vascular disease. In developed countries it is thus a major cause of preventable morbidity, disability, and premature death, and is also emerging as a problem in developing countries. It is a lifelong disease process: the initial stages occurring among children and young people often silent and without symptoms, with clinical manifestations appearing in middle age or later, mainly as sudden cardiac death, myocardial infarction, angina pectoris, stroke, aortic aneurysm, renovascular hypertension, and intermittent claudication (1–13).

This study, like the PDAY Study (14),* complements the International Atherosclerosis Project (15) and the WHO Study of Atherosclerosis of the Aorta and Coronary Arteries in Five Towns (9). Since 1982, many pathologists from different countries have been involved in the preparation and review of the PBDAY Study protocol (15–17).

Objectives

The objectives of the PBDAY Study are outlined below.

• To explore the structural changes in arteries which may determine the development of atherosclerosis, particularly its early stages and progression.
• To study the topographical relationships of different atherosclerotic lesions and their relation to possible causal factors.
• To explore the influence of contrasting socioeconomic settings and individual characteristics on the presence and progress of atherosclerotic lesions in youth.
• To assess the extent to which changes in the arterial wall of young individuals can be detected by morphometry, atherometric analysis, histo-

* Prepared by the authors for the PBDAY Principal Investigators. A full description of the study appears in the following: Report of the Joint WHO/ISFC Study Pathobiological Determinants of Atherosclerosis in Youth (PBDAY), a ten-year multinational collaborative study (unpublished document WHO/CVD/97.1).
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* PDAY Study, the North American multicentre cooperative research programme "Pathobiological Determinants of Atherosclerosis in Youth".
chemistry, immunohistochemistry, biochemistry and ultrastructural studies before the appearance of grossly visible atherosclerotic lesions.

- To study the differences in the lipid and/or lipoprotein composition and location of the arterial wall and its relation to pathological findings.

**Study design**

The management of the study was delegated to the PBDAV Steering Committee, which organized the administrative and technical work, as well as coordinating data collection and the receipt and distribution of specimens, in close collaboration with members of the PBDAV Steering Committee (15-17). Special analyses using various morphometric methods and specialized techniques were developed at seven reference centres and used to examine specimens collected and forwarded from 18 Collaborating Centres to the Receiving, Processing and Distribution Centres (see Annex).

The study included subjects of both sexes (age range, 5-34 years), with a preponderance of those who had suffered fatal accidents. The pilot study covered populations from 18 centres in 15 countries, while the main study covered 11 centres in 11 countries, including both developed and developing countries from five WHO regions, with different economic, sociocultural and nutritional patterns.

The data required to fulfill the study's objectives were obtained from three sources (15):

- background epidemiological information;
- subject/individual information; and
- collection, preparation and analysis of specimens.

The background epidemiological information was prepared at the management and data centre, using the most recent and reliable data from any available source. The subject/individual information was obtained from individual records, while family information was obtained from the questionnaire forms (forms 01 and 02) (16).

Specimens were collected, prepared and shipped by the Collaborating Centres. The core procedure included a general autopsy, a more detailed examination of thoracic and abdominal organs, and collection of the main specimens, which were forwarded frozen or formalin-fixed in plastic containers/bags to the Receiving, Processing and Distribution Centres (16-19), as follows:

- aorta (Ao), abdominal (AA), descending thoracic (DTA);
- coronary arteries (CA) and branches, right (RC), left main (LMC), left circumflex (LC), left anterior descending (LAD); and
- postmortem blood, kidney tissue, and myocardial tissue.

Specimens were analysed (17) using different morphometric methods in the following Reference Centres: Malmo (visual grading (9, 17-19)), Siena (semiautomatic macro- and microscopic computer-assisted grading (17, 20); and Havana (quantitative automatic computer-assisted grading) "atherosclerotic system" (17, 21, 22); and also using special techniques in Budapest (histochemistry and immunohistochemistry (17, 23-26), Geneva (blood and subcellular analysis (17)), Heidelberg (ultrastructural techniques (17, 27-29) and Moscow (biochemistry (17, 30-32)).

All analyses of specimens were performed blind. The results were forwarded to the data centre, using the appropriate record form (forms 04-21) for data processing and elaboration.

In 1987, a pilot study was undertaken in 18 centres to assess the feasibility of carrying out the PBDAV Study in each site and to perfect the data recording instruments.

**Results**

**Background epidemiological information**

Atherosclerosis is a major cause of morbidity, disability and premature mortality in developed countries, and is becoming so in many developing countries.

Age-standardized mortality rates for all cardiovascular diseases, coronary heart disease, and cerebrovascular diseases were higher in Latvia, Lithuania and Hungary, and lower in Italy, Mexico, Hong Kong Special Administrative Region of China, and Sri Lanka.

Clinical and epidemiological data were processed from 1277 cases (958 males and 319 females); 133 were aged 5-14 years, 509 were aged 15-24 years, and 635 were aged 25-34 years.

The prevalence of cardiovascular risk factors (arterial hypertension, diabetes mellitus, and smoking) and alcohol consumption were, in general, higher in Lithuania, Hungary, and Germany. The prevalence of smoking and hypertension were also high in Cuba.

Analysis by clinical and epidemiological characteristics of the pool of all cases of atherosclerosis indicates that the rate was high among people with high blood pressure, diabetes mellitus, a smoking habit and regular-to-heavy alcohol consumption; among individuals aged 25-34 years; and among males and in the WHO European Region and in the Region of the Americas.

**Morphometric studies**

Atherosclerotic lesions begin to develop early in life, independently of race, sex or geographical origin. The proportion of affected subjects and the mean percentage of intimal surface affected by athero-

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b This method also provides the pondeometric indices of obstruction, stenosis, and benignity — which make it possible to estimate the severity of atherosclerosis.
sclerotic lesions exhibit considerable inter-individual and inter-vessel variability (see Fig. 1–3). Although there was a good correlation between the results of the three methods used, the two computer-assisted methods proved more reliable and also more suitable for data elaboration.

**Morphometric visual grading (Malmo).** Data from 134 cases were studied: 94 males (70.1%) and 40 females (29.9%); 16.4% in the age group 5–14 years, 41.1% in the age group 15–24 years and 42.5% in the age group 25–35 years. Fatty streaks were found in the Ao of all cases, being more prevalent for thoracic Ao (96%) and less for right coronary artery tissue. Raised lesions (fibrous plaques plus calcified and complicated plaques) were seen in a small proportion of cases (7.1% in the AA and 11.1% in the RC).

**Macroscopic and microscopic computer-assisted grading (Siena).** Macroscopic study of Ao and RC. Fatty streak lesions in Ao were found in 100% of the 355 cases and in 68% of the 319 RC specimens. The occurrence of fatty streaks was low (0–33%) on the whole ventral surface and high (>50%) on the dorsal surface of Ao and was strictly related to branching regions, where fatty streaks are highly likely to occur. Raised lesions occurred in a low proportion of cases (7.05% of DTA specimens, 25.75% of AA specimens and 22.05% of RC specimens). Most fatty streaks and raised lesions completely overlapped.

**Histomorphometric study of Ao and CA.** The prevalence of fatty streaks increased with age in both sexes until a plateau was reached (m >1), while the prevalence of raised lesions in males increased exponentially with age (m >1) and was higher in LAD and AA specimens.

Mean intimal and medial thickness were greater for males and in AA and LAD specimens. Both these thicknesses increased with age, with great variability among different vessels and by sex; and both were greater among smokers and those with hypertension.

**Atherometric system (Havana).** Fatty streak lesions were observed in 99.6% of the 996 DTA specimens, in 95.8% of the 947 AA specimens, and in 38.65% of the 958 RC specimens, with the mean proportion of intimal surface affected comprising 32.6% in DTA, 38.6% in AA and 12.05% in RC specimens. Raised lesions were found in 12.6% of DTA, 16.4% of AA and 2.6% of RC specimens, with the mean proportion of intimal surface affected being 0.95% in DTA, 3.2% in AA and 2.6% in RC specimens (Fig. 1).

Fatty streaks were found in all three age groups; although raised lesions were seen in a small proportion of cases, their development progresses slowly from the second decade of life and accelerates during the fourth. The thoracic aorta (TA) is the main site for fatty streaks during the first 15 years of life, but thereafter the AA is predominant (Fig. 2). Females were most affected by fatty streaks in all vessels. For all age groups, males developed fibrous plaques earlier than females in both aortas and in RC specimens. The mean percentages of subjects and the area affected by raised lesions were higher among smokers.

Atherosclerotic lesions, mainly fatty streaks, were observed in cases from all participating countries, developed and developing (Fig. 3). The extent of the area affected by raised lesions and the pondeorative atherometric indices for obstruction and stenosis were higher in countries with a high prevalence of well-known risk factors and high mortality rates for cardiovascular diseases, coronary heart disease and cerebrovascular diseases (Cuba, Germany, Hungary, and Lithuania).

**Special studies**

**Morphometric-quantitative lesion analysis (Heidelberg); structure of coronary arteries and myocardial capillarization in youth and nephrosclerosis index.** The mean values of the most relevant variables in the cases studied (717 males and 241 females) are shown in Table 1.

Cellular density gives an indication of the extent of the extracellular matrix and is more closely related to epidemiological data on cardiovascular mortality than intimal thickness.

The nephrosclerosis index is positively correlated with blood pressure.

Hypertension and smoking have a considerable influence on intimal thickness and intimal cellular density.

**Histology, histochemistry and immunohistochemistry of atherosclerotic lesions (Budapest).** Histology and histochemistry of atherogenic lesions. The
Fig. 2. Percentage of affected subjects and mean percentage of intimal surface affected by atherosclerotic lesions, by age group (FS = fatty streak, RS = raised lesions, A. sub = % affected subjects, A. area = % affected area).

Fig. 3. Mean percentage of intimal surface affected by atherosclerotic lesions by country (see Annex codes).

Table 1. Mean values of the key study variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density of intima cells</td>
<td>1 696.2</td>
<td>1 889.4</td>
</tr>
<tr>
<td>Density of media cell</td>
<td>942.0</td>
<td>1 082.8</td>
</tr>
<tr>
<td>Intimal thickness (µm)</td>
<td>336.6</td>
<td>247.1</td>
</tr>
<tr>
<td>Medial thickness (µm)</td>
<td>178.2</td>
<td>149.5</td>
</tr>
<tr>
<td>Nephrosclerosis index</td>
<td>28.8</td>
<td>28.7</td>
</tr>
<tr>
<td>Capillary length density of myocardium</td>
<td>193.3</td>
<td>185.7</td>
</tr>
</tbody>
</table>

Mean intima/media (I/M) ratio of the 214 cases studied was greater in LAD specimens than in either AA or TA specimens. The prevalence of atheromatous lesions (type III–VI lesions) was the same in LAD and AA, but less common in TA specimens.

Immunohistochemistry of early atherosclerotic lesions. The immunohistochemistry of intimal cells was studied using 36 standard aortic samples. The intensity of expression of HLA-DR in endothelial cells was the same for each type of lesion. The number of HLA-DR-positive cells was significantly greater in intimal lesions and fatty streaks than in cases of diffuse intimal thickening, owing to the increased number of foam cells. Immunoreactions using different macrophage-specific markers suggested the presence of specific marker expression during the progression of early lesions.

Chemistry of arterial wall. Lipid determination of arterial wall (Moscow, Havana, Malmo). The lipid fractions of the intimal and medial wall of 191 TA specimens and 180 AA specimens were studied. Dried de-fatted mass is the major component of the arterial wall (919.7 mg per g of dried tissue). The three commonest lipid components were phospholipid (52.0 mg per g), esterified cholesterol (27.1 mg per g) and free fatty acids (15.1 mg per g), while free cholesterol and triglycerides were present in low concentrations. There was no difference in the lipid fractions by sex: the concentrations of free and esterified cholesterol in TA tend to increase with age.
The planned special studies with frozen specimens (Geneva RC) could not be completed owing to inadequate specimen transportation and preservation.

Conclusions

- Atherosclerotic lesions and early arterial changes can be detected by various morphometric methods before the appearance of grossly visible atherosclerotic lesions.
- Atherosclerotic lesions begin their progressive development early in life, independently of an individual's race, sex or geographical origin.
- Development of fatty streak is higher for the age group 15-25 years, while raised lesions begin developing slowly during the second decade of life, progress steadily during the third, and more rapidly during the fourth. Fatty streaks are more prevalent among females, and raised lesions among males and smokers.
- In most cases there is overlap between localization of fatty streaks and raised lesions in the arterial wall.
- The prevalence and extent of raised lesions were higher in countries that had a high prevalence of well-known risk factors and high mortality rates for cardiovascular diseases, coronary heart disease, and cerebrovascular diseases.
- There were marked differences in vascular structure, especially intimal cellular density and thickness, and myocardial capillarization in different populations and risk factors.
- There were structural, histochemical and immunohistochemical variations in early atherosclerotic lesions in different populations.
- These findings reiterate the need to promote a healthy lifestyle from very early childhood, as well as the primary and secondary prevention of cardiovascular diseases.

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Résumé

Détecteurs physiopathologiques de l'athérose chez les jeunes (Étude PBDAY), 1986-1996

Cet article rend brièvement compte de l'étude collective multinationale conduite sur 10 ans par l'OMS et la Société et Fédération internationale de Cardiologie sur les déterminants physiopathologiques de l'athérosclérose chez les jeunes. Il donne des indications détaillées sur le plan de l'étude, ses résultats, et les conclusions et recommandations qui ont été formulées à Budapest (Hongrie) en octobre 1996 lors d'une consultation des chefs des centres de référence ayant participé à l'étude.

Cette étude a fourni des données uniques sur les déterminants de l'athérosclérose et les modifications de la structure des artères, en particulier à leurs premiers stades, et sur leur progression, depuis le plus jeune âge, au sein de populations caractérisées par des modes de vie très différents. L'étude pilote a porté sur des sujets des deux sexes âgés de 5 à 34 ans dans 18 centres de 15 pays cependant que l'étude principale a couvert 11 centres de 11 pays, soit des pays développés et en développement appartenant aux cinq Régions de l'OMS et caractérisés par des schémas économiques, socioculturels et nutritionnels très divers. On a recueilli des données épidémiologiques de base, des informations sur les cas et les résultats d'études spéciales sur les artères faites à l'aide de diverses méthodes anatomométriques et de techniques spécializées.

Les lésions athérosclérotiques apparaissent au début de la vie indépendamment de la race, du sexe ou de l'origine géographique. L'épaisseur des fibres est plus rapide entre 15 et 25 ans cependant que l'athérome commence à se développer lentement entre 10 et 20 ans, progressant régulièrement entre 20 et 30 ans, puis plus rapidement après 30 ans. L'épaississement des fibres est plus fréquent chez les sujets de sexe féminin et l'athérome chez ceux de sexe masculin. La prévalence et l'étendue des athéromes sont plus grandes dans les pays où la fréquence des facteurs de risque connus est élevée, de même que la mortalité due aux maladies cardiovasculaires, aux cardiopathies coronariennes et aux maladies cérébrovasculaires.

Resumen

Determinantes biopatológicos de la aterosclerosis en la juventud (Estudio PBDAY), 1986-1996

En el presente artículo se resume el Estudio OMS/IFSC sobre los Determinantes Biopatológicos de la Aterosclerosis en la Juventud (Estudio PBDAY), estudio colaborativo multinacional de diez años de duración. Se informa sobre el diseño del estudio, los resultados de mayor interés, las conclusiones y las recomendaciones
formuladas en una reunión consultiva celebrada por los jefes de los Centros de Referencia PB/DAY en Budapest (Hungría) en octubre de 1996.

El estudio OMS/ISFC aporta información singular sobre los determinantes de la aterosclerosis y de los cambios estructurales sufridos por las arterias, especialmente en las primeras fases del proceso, así como sobre su evolución desde los primeros años de la existencia en poblaciones con muy distintos modos de vida. En el estudio piloto participaron sujetos de 5 a 34 años de ambos sexos de 18 centros de 15 países, mientras que el estudio principal abarcó 11 centros de 11 países. Se incluyeron países tanto desarrollados como en desarrollo, con distintos niveles económicos, socioculturales y nutricionales, de cinco regiones de la OMS. Se procedió a reunir información epidemiológica, información sobre casos, y datos obtenidos mediante la aplicación de diversos métodos morfométricos y técnicas especializadas al estudio de las arterias.

Las lesiones ateroscleróticas empiezan a aparecer en fases tempranas de la vida, con independencia de la raza, el sexo o el origen geográfico. El ritmo de aparición de las estrías adiposas es mayor entre los 15 y los 25 años, y las lesiones protuberantes empiezan a desarrollarse lentamente durante la segunda década de la vida, siguiendo progresando durante la tercera, y luego hacen aún más rápidamente durante la cuarta. Las estrías adiposas son más frecuentes en las mujeres, y las lesiones protuberantes en los varones. La prevalencia y extensión de estas últimas era mayor en los países con alta prevalencia de factores de riesgo conocidos y altas tasas de mortalidad por enfermedades cardiovasculares, cardiopatía coronaria y enfermedades cerebrovasculares.

References


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