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Atherosclerosis in children and young adults: An overview of the World Health Organization and International Society and Federation of Cardiology study on Pathobiological Determinants of Atherosclerosis in Youth study (1985—1995)

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KEYWORDS
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Summary

Background and methods Atherosclerosis begins in early in life, however, observations on the association of risk factors with atherosclerosis in childhood are limited. This multi-institutional study investigated 1277 subjects 5—34 years of age who had died from trauma. The pilot study covered 18 centers from 15 countries while the main study covered 11 centers in 11 countries.

Results About 87% of the aortas and 30% of coronary arteries in the youngest age group (5—14 years) had fatty streaks. The mean percent intimal surface with fatty streaks increased from 5 to 34 years, and raised lesions increased with age in the aorta and right coronary artery.

Differences in development and progression of atherosclerosis in different parts of the arterial system suggest possible importance of haemodynamic and other forces apart from risk factors in pathogenesis of atherosclerosis beginning at a very early age.

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Recognized risk factors for coronary heart disease are associated with lesion development and progression in the arteries of youth, already affecting coronary arteries and the aorta during the second and third decades of life and possibly earlier. Some risk factors affect one lesion type or one arterial segment more than another; Smoking and diabetes appears to be of particular importance in coronary arteries and the abdominal aorta, while hypertension increases risk of accelerated atherosclerosis in all parts of the arterial system.

Conclusions These findings provide strong justification for primary prevention of CHD in children and youth to prevent atherosclerosis and its clinical manifestations of coronary artery disease later in life.
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Introduction

The debilitating and often fatal complications of cardiovascular disease (CVD) are usually seen in middle aged or elderly people. However, atherosclerosis, the main pathological process leading to coronary heart disease (CHD) and cerebral vascular disease (CeVD) begins early in life and gradually progresses through adolescence and youth.

Early lesions of atherosclerosis known as fatty streaks, formed due to deposition of cholesterol and its esters in macrophages of the intima of large muscular and elastic arteries are themselves innocuous, however, in the presence of coronary risk factors lipid continues to accumulate, resulting in a lesion raised above the intima, called a fibrous plaque or a raised lesion. These lesions may increase in size and rupture exposing the underlying lipid-rich necrotic debris to blood. This may lead to thrombus formation and precipitate acute vascular events.

The Seven Countries Study, the first study that compared CVD frequency and risk factors in a systematic manner between defined populations reported a correlation between risk factors and CVD incidence.and population thresholds for fatty-artery diseases [1,2]. Modification of the predisposing factors of atherosclerosis such as elevated serum lipids, hypertension, smoking and diabetes is the major preventive strategy for CVD and CeVD. The effectiveness of this approach in the middle age and the elderly is now well established [3].

Previous studies have demonstrated that there are ranges for plasma cholesterol and blood pressure levels in children, and that there is tracking of blood pressures with increasing age [4–6]. However, observations on the association of risk factors with atherosclerosis in childhood are limited. Investigation of the factors that promote the development and progression of atherosclerosis in childhood and youth in different populations is vital in planning preventive strategies. In this context, two studies have documented the natural history of atherosclerosis in children and young adults and its relationship to risk factors. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study evaluated arteries for atherosclerosis lesions in about 2000 persons aged 15–34 years whose death was attributed to accidents, homicides and suicides [7–9]. In the Bogalusa Heart Study, atherosclerosis lesions were studied in 44 decedents, 6–27 years, who had previously been examined for coronary risk factors [10,11].

''The Pathobiological Determinants of Atherosclerosis in Youth'' (PBDAY) study, a 10 year multinational collaborative study investigated the structural changes in arteries which may precede the development of fatty streaks, the extent and prevalence of different atherosclerotic lesions, the association between structural changes and atherosclerosis lesions in arteries with coronary risk factors (smoking, hypertension and diabetes), and the influence of sociocultural setting, age and sex on the development and progress of atherosclerotic lesions in youth.

This study provides unique information about the determinants of atherosclerosis and structural changes in arteries and their progression from childhood in populations with vastly different life styles.

Materials and methods

1277 subjects (958 males and 319 females) from 18 centers in 15 countries for the pilot test and 11 centers in 11 countries for the main study from five WHO regions, with different economic, sociocul-

Table 1 Participating countries, collaborating centers and number of subjects by age and center of the Pathobiological Determinants of Atherosclerosis in Youth Study

Countries and collaborating centers	Pilot study	Number of subjects			Main study (*n*)
		5—14 years	15—24 years	25—34 years	
China, Beijing	P				
Hungary, Budapest	P	2	40	48	90
Nigeria, Ibadan	P				
Nigeria, Calabar	P				
India, Chandigarh	P	8	40	56	104
Cuba, Havana	P	1	22	22	55
Germany, Berlin	P	10	95	106	
Germany, Heidelberg	P	6	11	15	211
Hongkong	P				32
Hongkong, Shatin	P				
Lituania, Kaunas	P	21	28	17	66
Mexico, Mexico city	P	26	65	64	155
Sri Lanka, Peradeniya	P	34	156	234	424
Latvia, Riga	P	5	21	22	48
Italy, Siena	P	1	12	6	19
Uzbekistan, Tashkent	P				
Japan, Tokyo	P				
Cameroon, Yaounde	P	19	19	35	73
Total	18	133	509	635	1277

Eighteen centers from 15 countries participated in the survey and 1277 subjects were covered. There were 133, 509 and 635 subjects in the 5—14, 15—24 and 15—34 age groups, respectively. Mexico, Sri Lanka and Germany provided the highest number of cases.

tural and nutritional patterns participated in the study (Table 1). Standardized study protocols and manuals were prepared by a steering committee of principal investigators of study centers and other experts. Study subjects were persons 5—34 years of age who had died of external causes, 63.5% of whom suffered from fatal accidents, and who were autopsied within 24 h of death. Coronary arteries (CA), aortas, and other tissues were collected by researchers from postmortem forensic centers, coroners or medical examiners' laboratories and the data and materials were transmitted to the Receiving Processing and Distribution Centers.

An officially designated officer at each center recorded the general data, personal and family medical history, information on risk factors (smoking, diabetes and high blood pressure) and cause of death using standardized forms. Information was obtained from a close relative of the deceased as well as from death certificates and coroners reports. Specimens were analyzed at seven reference centers using various specialized techniques [12]. Table 2 shows the percentage of subjects providing information on educational status and risk factors by completing each related question.

Three different morphometric methods, visual grading, semiautomatic macroscopic and microscopic computer assisted grading and quantitative automatic computer-assisted grading, were used in atherosclerosis grading. The length density of myocardial capillaries (length of capillaries per unit volume of myocardium) was determined on tissue probes of the left ventricle as a parameter of myocardial blood supply [12]. Tissue probes of the left ventricle were taken at a constant distance from the septum between heart base and the apex. The nephrosclerosis index was determined according to Tracy and Tabares Toca [11]. Immunophenotype of intimal cells in early atherosclerotic lesions in 36 standard autopsies was performed in the Budapest Reference Center on deceased 20—34 years of age within 24 h of death [12]. In six cases additional samples from fatty streaks were harvested and examined by transmission electron microscopy to investigate the ultrastructure of intimal foam cells [12].

Definitions used for the purpose of this study were:

Fatty streaks: fat or slightly elevated intimal lesions that stained distinctively by Sudan IV and that does not show any other gross change.
Fibrous plaques: elevated intimal lesions which in the fresh state is pale gray, glistening and

Table 2 Number providing information on educational level and selected risk factors

Percentage of total sample providing information	Characteristics (percentage)
76.8	Educational level (n = 981) University 4.6; intermediate 11.5; secondary 54.9; primary 23.6; illiterate 5.4
36.9	Blood pressure (n = 472) Elevated 9.3; normal 90.7
69.6	Diabetes mellitus (n = 889) Diabetic 1.6; normal 98.4
72.4	Smoking status (n = 924) Smoker 40.3; non-smoker 56.6; ex smoker 3.1
72.8	Alcohol use (n = 930) Daily 6.9; regularly (less than daily) 6.9; occasionally 35.6; never 50.6

translucent. After staining, it may be partially or completely covered by sudanophilic deposits.
Calcified lesions: areas with calcium deposition detectable either visually or by palpation without overlying hemorrhage, ulceration or thrombosis.
Complicated plaques: lesions in which ulceration, hemorrhage is observed with or without calcium deposition.
Raised lesions: included fibrous plaques, calcified lesions and complicated lesions.

Statistical tests

A two-tailed Students t test, Wilcoxon test analysis and regression linear analysis was used to ascertain the significance of the data. Because of the paucity of right coronary artery samples for some populations only the seven most numerous populations were used for statistical analysis in order to compare the groups and determine the influence of age and sex.

Results

Initiation and progression of atherosclerosis; prevalence and extent with ages and effects of gender

Early atherosclerosis lesions showed a high prevalence in the aorta and CA of children and young adults, while raised lesions were less prevalent (Fig. 1). More than 80% of children 5—14 years of age had fatty streaks in the aorta. 29% had fatty streaks in the right CA (RCA) which increased to 78% by the third decade. The prevalence of raised lesions also increased during this period in the abdominal aorta (AA), thoracic aorta (TA) and CA. Seven subjects in the 25—34 age group showed complicated lesions.

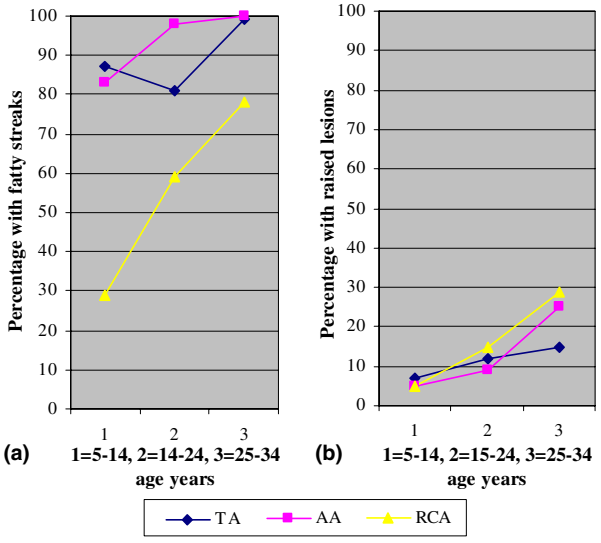

Figure 1 The prevalence of fatty streaks (a) and raised lesions (b), in the thoracic aorta (TA), the abdominal aorta (AA) and the right coronary artery (RCA) in different age groups. Fatty streaks were seen in 87% of the children in the youngest group in the thoracic aorta (TA) and 83% in the AA. In the RCA about 29% of children in the youngest age group (5—14 years) had fatty streaks increasing to 78% by the third decade. Raised lesions were less prevalent and they increased in the AA from 5% in the 5—14 age group to 25% in 25—34 age group. In the TA they increased from 7% to 15% during this period. In the RCA the rise in prevalence was similar; from 4% in 5—14 age group to 29% in 25—34 age group.

The extent of fatty streaks increased with age. The progression was most notable from 15 to 24 years and less marked from 25 to 34 years (Fig. 2). The RCA showed a more gradual and even increase.

The extent of raised lesions also increased with age during the first and the third 10-year period. In the RCA, it increased almost threefold with each successive 10-year period (Fig. 3).

Sex-dependent difference in the extent of fatty streaks appeared after the age of 14 years. Female showed greater involvement of the AA than the TA, while in males, it was the opposite. While the extent of fatty streaks in the RCA was greater in females, the extent of raised lesions in the RCA was greater in males. Raised lesions were more extensive in the AA than in the TA (Figs. 2 and 3).

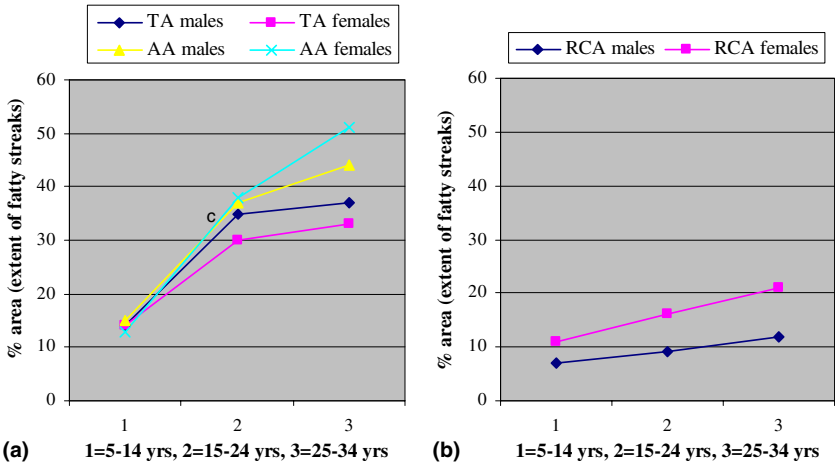

Figure 2 The extent of fatty streaks in the thoracic aorta (TA), the abdominal aorta (AA) (graph (a)) and the right coronary artery (RCA) (graph (b)) by age and sex. During the second 10-year period the extent of involvement with fatty streaks more than doubled: from 14% to 34% in the TA and from 14% to 37% in the AA. In the 25—34 year age group, it increased up to 36% and 46% in the TA and the AA, respectively. In the RCA more gradual and even increase were seen (about 2% in each 10-year period, reaching 14% by 25—34 years age). In the 5—14 age group male and female subjects had similar extent of fatty streaks in the TA and AA, however, after the age of 14 years, adolescent and young females had more extensive fatty streaks in the AA and males showed more extensive fatty streaks in the TA. In the RCA, fatty streaks were more extensive in females was higher in females than in males in all age groups.

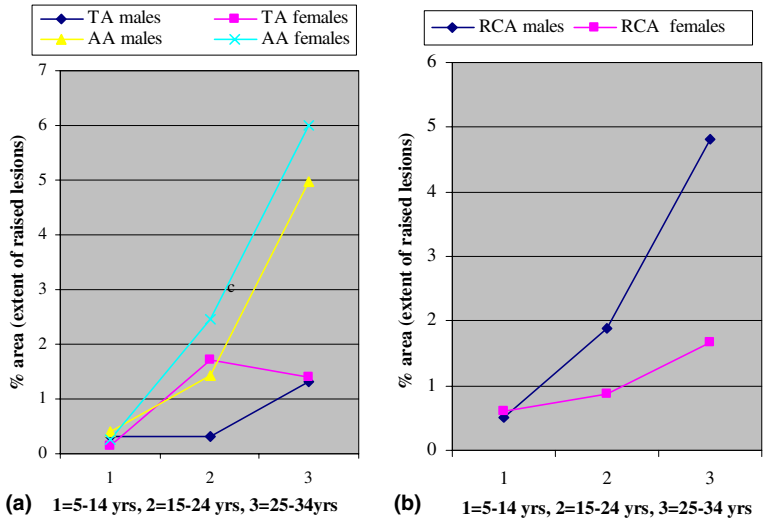

Figure 3 The extent of raised lesions in the thoracic aorta (TA), the abdominal aorta (AA) (graph (a)) and the right coronary artery (RCA) (graph (b)) by age and sex. The extent of raised lesions increased from 0.25% in the TA, 0.35% in the AA in 5—14 year age group to 1% in the TA and 5% in the AA in 25—34 age group. In the RCA the extent of raised lesions increased almost threefold with each successive 10-year period, the extent rising to 4% by the 25—34 age group.

Structural changes in arteries related to gender and physiological growth

In all arterial specimens except in the left circumflex CA males had a significantly greater intimal thickness than females ($p < 0.001$), which correlated positively with age ($r = 0.32$, $p < 0.05$). Cellular density of intima decreased with age ($r = 0.28$, $p < 0.05$). The mean medial thickness increased with age in both males and females.

Changes that represents physiological growth (the positive correlation between the heart weight and weight, height, vascular circumference and the thickness of the vascular wall and the intimal thickening associated with increase of coronary vascular circumference) were observed (data not shown).

The age related increase in intimal thickness and the age related reduction in intimal cellular density continued in males after physiological growth completed, but not in females.

The effect of geographic population group on progression of atherosclerosis

Although the extent of involvement of the RCA and aorta with fatty streaks was variable in different geographic groups, most teenagers regardless of the geographic area had fatty streaks in some part of their arteries. The extent of raised lesions in populations from different geographic areas did not parallel the age standardized cardiovascular disease mortality (Fig. 4).

Of the seven most numerous groups ($n \geqslant 20$) in the RCA Germany and Mexico were the least affected by raised lesions while Cuba, Sri Lanka and Lithuania showed the highest mean percentage of raised lesions (Cuba > Germany $p < 0.05$, Sri Lanka > Germany $p < 0.01$, Cuba > Mexico $p < 0.02$).

Using the ranking system advised by the International Atherosclerosis Project [16], arterial specimens from different geographic origins were ranked based on their mean intimal thickness [16]. The unweighted mean included two male groups (15—24 and 25—34 years) and their five arterial segments (one specimen each from a standard site from the thoracic aorta and left anterior descending coronary artery, two specimens from standard sites from the abdominal aorta and one non-standard specimen from thoracic fatty streaks). Ranking of geographic areas according to age-standardized mortality rates of cardiovascular disease paralleled unweighted mean intimal thickness (Fig. 5).

Variation among arteries

Raised lesions are observed at an earlier age and a higher prevalence in the aorta than in the coronary arteries (Fig. 1). The extent of raised lesions in the

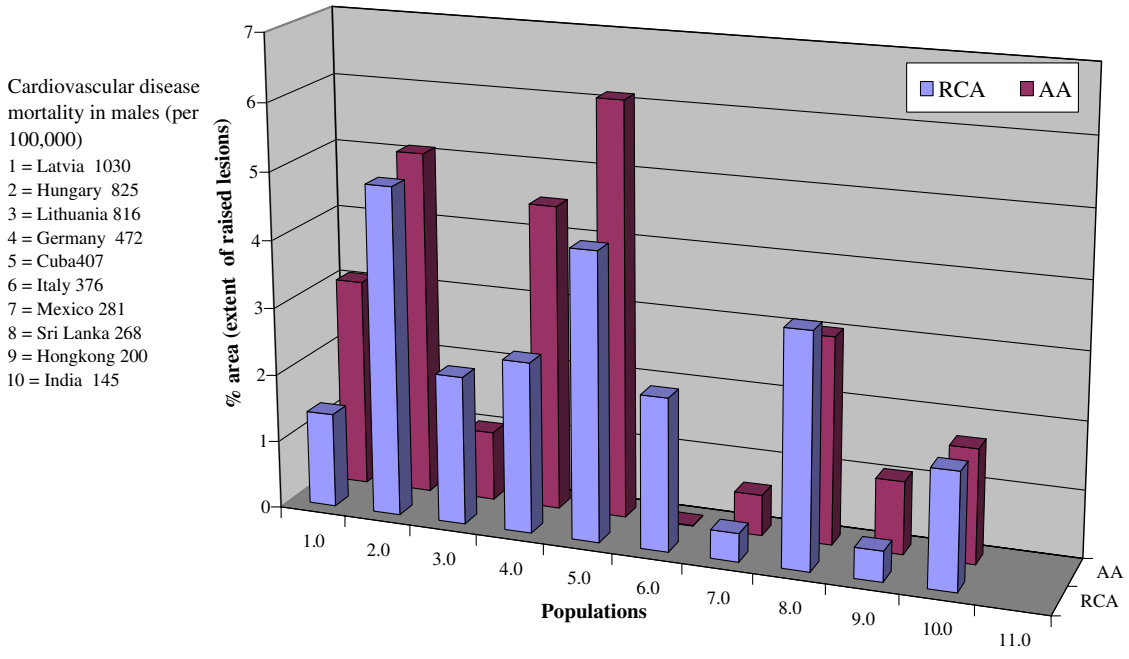

Figure 4 Populations ranked according to the cardiovascular mortality rates and extent of raised lesions in the right coronary artery (RCA) and the abdominal aorta (AA). The extent of fatty streaks in the RCA and aorta varied in different geographic groups, however, most teenagers of all groups showed fatty streaks in some parts of their arteries.

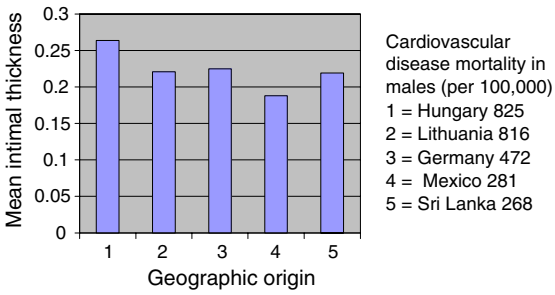

Figure 5 Populations ranked according to the cardiovascular disease mortality rates and mean intimal thickeness.

TA was less than in the RCA or the AA ($p < 0.001$). Although the extent of all lesions in the RCA is much less compared to the AA it had a greater proportion of raised lesions to total lesions compared to the AA.

In both males and females the AA has a significantly thicker intima and a thinner media than the TA ($p < 0.01$). Left anterior descending CA has a significantly greater mean intimal and medial thickness than the left circumflex coronary artery ($p < 0.01$).

Regional susceptibility to atherosclerosis in arterial segments

Lipid lesion distribution in blood vessels was similar and was independent of age, sex and geographic distribution. The term lipid lesion includes all lipid deposits that were distinctly stained with Sudan IV, on a macroscopic level. High-probability-of-occurrence-areas of lipid lesions were strictly related to branching regions.

In the TA, probability-of-occurrence of lipid lesions was low (0—33%) in the ventral surface and high (>50%) in the dorsal surface and extended laterally from the intercostal artery ostia, while in the AA, high-probability-of-occurrence-areas were found not only in the dorsal surface but also in the ventral surface caudal to the ostium of the celiac trunk and in between the mesenteric artery ostia.

Importantly in both the TA and AA areas with raised lesions differed from those with lipid lesions. However, in coronary arteries, high-probability-of-occurrence-areas of lipid lesions and raised lesions were greatly overlapping.

Regression analysis comparing the mean percentage of lipid lesions for each population in the RCA with that in the TA and AA did not show a significant correlation. Percentage of raised lesions in the RCA correlated with raised lesions in the TA when analyzed for 14 different geographic groups ($p < 0.05$, $n = 14$).

Effects of major coronary risk factors on atherosclerosis

Smokers comprised 40% of cases, ex-smokers 3% and non-smokers 57%. The extent but not the prevalence of fatty streaks in the TA and AA of smokers was significantly greater compared to non-smokers

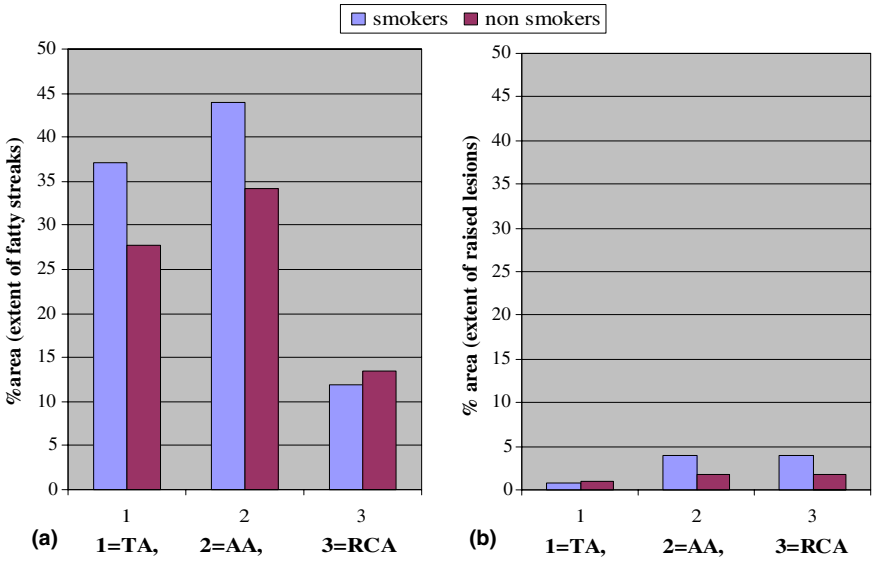

Figure 6 The extent of fatty streaks (a) and raised lesions (b) in the thoracic aorta (TA), the abdominal aorta (AA) and the right coronary artery (RCA) in smokers compared with non-smokers. The extent of involvement of the AA and RCA with raised lesions was greater ($p < 0.001$) in smokers (AA = 4%, RCA = 4%) than in non-smokers (AA = 2%, RCA = 2%). In the TA, no significant difference was seen.

(Figs. 6 and 7). There was a higher prevalence and extent of raised lesions in the AA and RCA ($p < 0.01$) in smokers compared to non-smokers (Figs. 6 and 7). There was no such difference in the TA.

The histomorphometric data showed that intimal thickness in the aorta and RCA was greater in smokers than in non-smokers in the 25—34 year age group ($p < 0.001$). The mean medial thickness did not significantly differ between smokers and non-smokers in either age group.

Prevalence of hypertension was 9.3%. In the RCA the prevalence and extent of raised lesions were significantly higher ($p < 0.001$) in hypertensives compared to normotensives (Figs. 7 and 8). Such an association was not seen in the aorta.

In males with hypertension the nephrosclerosis index, intimal thickness, mean width of myocardial fibers, heart weight and left ventricular thickness were higher ($p < 0.05$) and intimal cellular density and myocardial capillarization lower

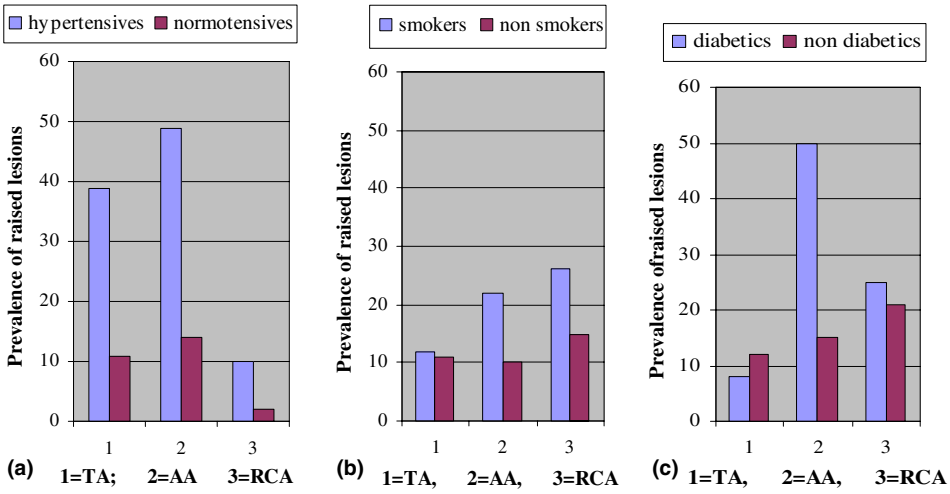

Figure 7 The prevalence of raised lesions in the thoracic aorta (TA), the abdominal aorta (AA) and the right coronary artery (RCA) in hypertensives (a), smokers (b) and diabetics (c) compared with normal subjects. (a) The prevalence of raised lesions in the TA, AA and RCA were remarkably higher ($p < 0.001$) in hypertensives compared to normotensives (TA 39% vs. 11%, AA 49% vs. 14% and RCA 54% vs. 20%). (b) The prevalence of raised lesions in the AA and RCA was greater ($p < 0.01$) in smokers (AA = 22%, RCA = 26%) compared to non-smokers (AA = 10%, RCA = 15%). In the TA there no significant difference was seen. (c) Raised lesions were more prevalent in the AA (50% vs. 15%, $p < 0.001$) and RCA (25% vs. 21%, $p < 0.05$) of diabetics.

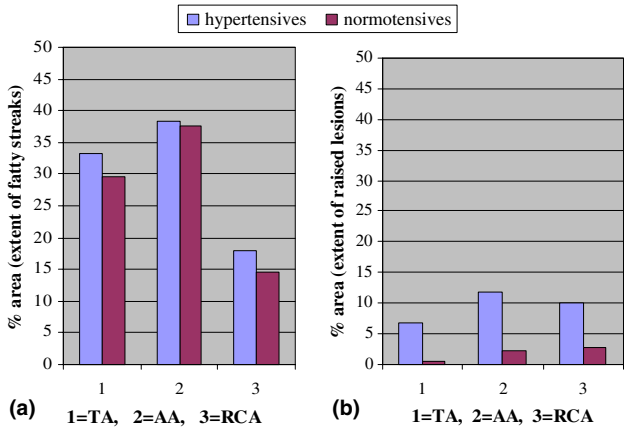

Figure 8 The extent of fatty streaks (a) and raised lesions (b) in the thoracic aorta (TA), the abdominal aorta (AA) and the right coronary artery (RCA) in hypertensives compared with normotensives. The extent of raised lesions was at least fivefold higher ($p < 0.001$) in hypertensives compared to normotensives (TA 7% vs. 1%, AA 12% vs. 2% and RCA 10% vs. 2%).

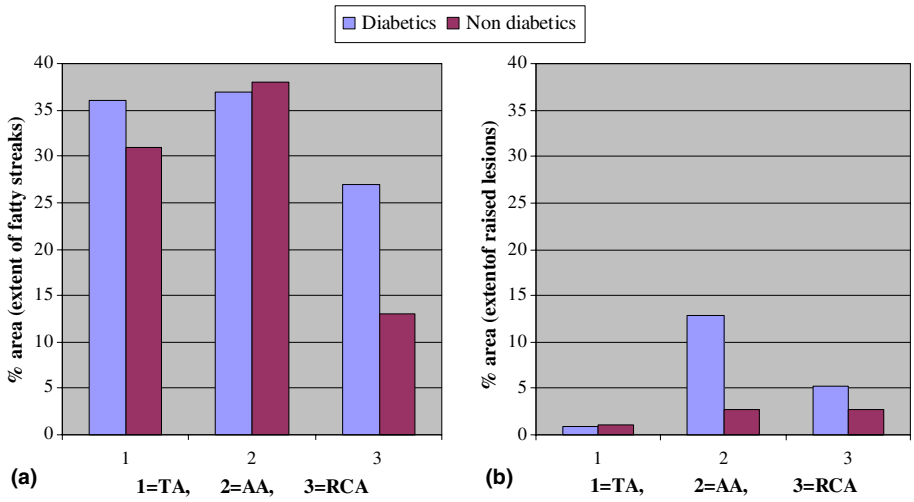

Figure 9 The extent of fatty streaks (a) and raised lesions (b) in the thoracic aorta (TA), the abdominal aorta (AA) and the right coronary artery (RCA) in diabetics compared with non-diabetics. The extent of raised lesions in the AA (13% vs. 3%, $p < 0.001$) and RCA (5% vs. 3%,$p < 0.05$) was higher in diabetic subjects than in non-diabetics.

compared to normotensives. The analysis was not done for females because of insufficient numbers.

Multiple regression analysis showed that age and hypertension are determinants of intimal thickness of left coronary arteries.

The prevalence and extent of fatty streaks were significantly higher among diabetics compared to non-diabetics ($p < 0.05$) in the TA and RCA (Figs. 7 and 9). Raised lesions were more prevalent and extensive in the AA and RCA of diabetics compared to non-diabetic (Figs. 7 and 9).

Statistical analysis also revealed that smoking, hypertension, and diabetes are strong risk factors of atherosclerosis in childhood and youth (Table 3).

Effect of age and sex on myocardial capillarization

Myocardial capillarization correlated positively with age ($r = 0.16$, $p < 0.05$) in both males and females and negatively with heart weight only in females ($r = -0.23$, $p < 0.01$).

Immunohistochemistry and ultrastructural studies of fatty streaks and earlier lesions of atherosclerosis

Fat staining on frozen sections was used to differentiate between different stages of fatty streak development; diffuse intimal thickening which is a very early stage (no fat in intima), initial lesion

Table 3 Multivariate analysis of fatty streaks (FS), fibrous plaques (FP) and severe plaques (SP) and coronary risk factors (smoking, hypertension and diabetes) in thoracic aorta, descending aorta and right coronary artery

Risk factor	Artery	FS (ANOVA *p*)	FP (ANOVA *p*)	SP (ANOVA *p*)	MANOVA (*p*)
Smoking	Thoracic aorta	0.0001	0.9854	0.3355	0.0001
Smokers ($n = 306$)	Abdominal aorta	0.0001	0.0092	0.3664	0.00001
Non-smokers ($n = 376$)	Right coronary	0.1157	0.0152	0.5218	0.03
Hypertension	Thoracic aorta	0.0077	0.0001	0.5601	0.00001
Hypertensive ($n = 37$)	Abdominal aorta	0.4555	0.0005	0.0001	0.00001
Normotensive ($n = 296$)	Right coronary	0.0006	0.0002	0.0001	0.00001
Diabetes	Thoracic aorta	0.0027	0.5739	0.4961	0.0265
Diabetes ($n = 12$)	Abdominal aorta	0.2134	0.0012	0.8825	0.0066
Non-diabetic ($n = 629$)	Right coronary	0.0109	0.5134	0.8345	0.0310

Data on fatty streaks, fibrous plaques and severe plaques in the AA, TA and RCA studied using the atherometric system were also statistically analyzed (ANOVA and MANOVA) in relation to the presence or absence of diabetes, smoking and hypertension. The results confirmed that these are strong risk factors of atherosclerosis in childhood and youth.

(scattered, fat positive cells in intima) and fatty streak (large areas of fat positive cells in intima).

Immunoreaction with factor VIII antibody showed an intact endothelium over the intima irrespective of lesion type. Each macrophage marker was positive in more intimal cells of initial lesions than those of diffuse intimal thickening ($p < 0.05$). A similar difference was found between fatty streak and initial lesion ($p < 0.05$).

Few OPD4 positive cells (T helper lymphocyte markers) were seen in each lesion, however, their number was greater in fatty streaks than in initial lesions.

Most foam cells expressed macrophage specific markers. Smooth muscle cell α-actin was positive in several non-foamy cells, however, it was negative in almost all foam cells. Numerous cells having some cytoplasmic lipid droplets showed ultrastructural characteristics of macrophages and smooth muscle cells.

In diffuse intimal thickening the rank of specific macrophage markers by immunoreaction scores was significantly different from each other (CD68(EBM11) > Leu M5 > Ber-MAC3 > CD68(KP1), $p < 0.05$ for each difference). In initial lesions there was no difference between immunoreaction scores for different macrophage markers but more cells were positive for macrophage markers. A similar, difference was found between fatty streaks and initial lesion: in fatty streaks more cells were positive for macrophage markers than in initial lesion ($p < 0.05$).

Discussion

Initiation and progression of atherosclerosis

These findings, like the PDAY study [7—9] and other similar studies [13—15] demonstrate that atherosclerosis starts in the first decade of life and undergo substantial changes between the ages 5 and 34. Progression of lesions were especially rapid between 15 and 34 yeas of age and severe lesions were unusual before the age of 34. Progression is different in males and females and in different arterial segments. The main trends in the prevalence of fatty streaks and advanced lesions as well as the findings above are in agreement with the results of the PDAY study [7—9].

In all arteries studied except in the left circumflex CA, the prevalence of fatty streaks increased rapidly with age and reached a plateau, while the number of raised lesions showed exponential growth. In males, well-developed atherosclerotic changes in coronary arteries are seen by the third decade of life. This is in keeping with high rates of coronary heart disease in middle aged males.

The continued age related increase in thickness and cellular density reduction in intima, was observed in men, but not in women, suggesting that there are sex-related differences in the response to growth stimuli. Further, the thickness and intimal cellular density may be suitable early morphometric parameters that estimate intimal fibrosis and atherosclerosis in males.

The effect of geographic population group on progression of atherosclerosis

The development, progression and the distribution of atherosclerosis lesions were similar in all populations. High-probability-of-occurrence-areas of atherosclerosis lesions in all populations were strictly related to branching regions where low shear stress and turbulent blood flow occur.

A substantial variation in the degree of atherosclerosis seen in different populations is most likely to be related to the variation in risk factor levels among different populations. In previous studies, prevalence and extent of atherosclerotic lesions have been shown to be associated with mortality rates from CHD [13]. In the present study, the age standardized mortality rates for CVD positively correlated with mean intimal thickness and extent of raised lesions in the RCA in some populations.

Variation of atherosclerosis among arteries

We have no reasonable hypothesis to explain greater propensity of the coronary artery to develop lesions of higher severity despite its lower total extent of involvement than the AA. These results suggest that atherosclerosis in the coronary arteries converts to clinically significant raised lesions at a more rapid rate despite its later onset. The severe involvement of coronary arteries with atherosclerosis despite the lower extent of lesions compared to the aorta has been reported in previous studies [16].

The present study demonstrated the greater extent of total lesions in the AA in young women compared to young men. The left anterior descending CA was the most susceptible coronary artery to atherosclerotic lesions and the AA was more affected than the TA. Similar findings have been reported in previous studies, [7—9,14,16], and are primarily attributed to hemodynamic factors [17]. However, they may also be partly re-

lated to structural differences. The greater intimal thickness of the AA reflects greater atherosclerotic involvement and/or greater susceptibility to disease compared to the TA. The greater intimal involvement and the thinner media of the AA also makes it vulnerable to aneurysmal changes in later life and is consistent with the higher prevalence of aneurysmal changes in the AA compared to the TA.

In accordance with other studies branching regions where low shear stress and turbulent blood flow occurs showed high-probability-of-occurrence-areas of lipid lesions [18—21].

Effects of risk factors on atherosclerosis

The present study is in agreement with other studies implicating smoking as a risk factor for the development of atherosclerosis [22]. The PDAY study documented the association of serum thiocyanate concentration with the prevalence of raised lesions in the AA and coronary arteries and the extent of raised lesions in the AA [23], whereas the present study also documented the higher extent of raised lesions in the RCA in smokers than in non-smokers. Smoking was associated with an increased intimal thickness more in the aorta than in the coronary arteries, and appears particularly to promote the development of raised lesions in the AA. Smoking appears to cause structural changes in blood vessels even before atherosclerosis develops and also make them more vulnerable to develop advanced atherosclerotic lesions. The reason why smoking affects one type of lesion and one arterial segment more than the other is unclear.

Diabetes is associated with increased prevalence and extent of raised lesions particularly in the abdominal aorta and coronary arteries.

Our results support the findings of the Bogalusa Heart Study that showed significant correlation with fatty streaks in the coronary arteries though not in the aorta with blood pressure [6,24]. Other studies have also reported a correlation between renal indices of hypertension and the extent of lesions in both the aorta and coronary artery [7,25].

The present study supports the association between cellular and connective tissue changes of initial pre-atheromatous conditions and hypertension and smoking. This suggests the possibility of faster development of atherosclerosis in children with elevated blood pressure. Lower levels of blood pressure and absence of smoking in early life are likely to be effective in retarding the process atherosclerosis. The higher intimal and medial thickness in the coronary arteries and aorta of hypertensives may be used as postmortem indices of hypertension.

Effect of age and sex on myocardial capillarization

Myocardial capillarization which has a bearing on myocardial ischaemia has been considered to depend on the degree of cardiac hypertrophy. The negative correlation between myocardial capillarization and heart weight seen only in females appears to support the recent evidence that other factors such as growth factors and hormones are more important determinants of myocardial capillarization than the degree of cardiac hypertrophy [26].

Histopathologic events preceding fatty streak formation

Although the exact sequences of pathologic events has not yet been fully understood, monocytes, macrophages, smooth muscle cells and lymphocytes play major roles in early atherosclerosis [27—30].

Greater number of T helper lymphocytes observed in fatty streaks than in initial lesions suggests a possible role of T helper lymphocytes in initial lesion-fatty streaks transformation [35].

Morphologically intact endothelium seen over intima irrespective of lesion type supports previous observations on the lack of endothelial denudation in early atherosclerotic lesions [31]. In accordance with previous studies [32—34], more intimal cells expressing HLA-DR were found in fatty streaks and initial lesions than in diffuse intimal thickening due to increasing number of foam cells in these lesions.

There is still some controversy in the literature concerning the origin of foam cells in atherosclerotic lesions [36,37]. In the present study, most foam cells expressed macrophage specific markers besides non-specific mesenchymal markers, such as vimentin, and was negative for smooth muscle cell α-actin, contradicting the results of ultrastructural studies which revealed smooth muscle cell characteristics in foamy cells. A reasonable explanation for this could be that in early lesions smooth muscle cells probably accumulate less lipid than macrophages and may appear to be foamy only on electron micrographs.

Most macrophage-specific-marker-positive intimal cells were foam cells. More and more cells were found to be positive for each macrophage marker as number of foam cells increased in early lesions, as previously reported [38]. In diffuse intimal thickening well-defined rank between different macrophage markers, however, in initial lesions, no difference was seen. In fatty streaks there were less cells positive for Ber-MAC3 than for any other macrophage specific markers, otherwise, all of them were expressed in the same number of cells. These observations suggest the presence of relative differences in macrophage specific marker expression during the progression of atherosclerosis from diffuse intimal thickening to fatty streaks.

The origin of fibrous plaques

The progress of fatty streaks to raised lesions has been questioned based on differences in topographic patterns. Some recent studies suggest conversion of fatty streaks into raised lesions, based on the later development of raised lesions in areas formerly occupied by fatty streaks. A decrease of fatty streaks in older age groups concomitant with a rise in raised lesions has also been reported [15]. Recent work by Stary [39] provide microscopic evidence that some fatty streaks do indeed convert to raised lesions. However, our results showed different distribution of raised lesions and lipid lesions and some high-probability-of-occurrence-areas for lipid lesions were spared from raised lesions, suggesting that not all of fatty streaks progress into raised lesions. Although fatty streaks appear to be a primary step in the process as yet undetermined local factors appear to influence the development of fatty streaks to raised lesions. These findings differ from those of the PDAY study that suggest that fatty streak and plaque development are in similar locations [7]. It is possible that there are several types of fatty steaks that differ in their ability to progress to more advanced lesions [13].

In conclusion, our results supplement the PDAY study [7—9] and the Bogalusa Heart Study [6,24] in providing information on the development for atherosclerosis in young people and the association of adult coronary risk factors with the early development of atherosclerosis.

This study has provided evidence that smoking, hypertension and diabetes influence the early stages of atherosclerosis, in childhood and youth, many years before clinical disease develops. These findings emphasize the need for effective prevention of atherosclerosis in the early stage of life by preventing the development of risk factors in the first place.

The increased extent of raised lesions by the second decade suggest that risk of modification should start by 15—20 years of age. Unfortunately, at this age young people are unlikely to be concerned about disease prevention. Although risk factors may only cause fatty streak development and not cause permanent changes in arteries in many subjects before 15 years of age it may be advisable to get children to adopt healthy lifestyles early so that these healthy behaviours may be continued into adolescence and young adulthood. Intervention programs for prevention of cardiovascular disease should address life style related preventable risk factors such as tobacco consumption, physical inactivity and unhealthy diet and such programs must necessarily focus on children and youth.

The fact that changes of atherosclerosis are seen in all ethnic groups reiterate the need to implement preventive action not only in western populations with high prevalence of clinical cardiovascular disease but in all populations. Indeed, it is particularly important to take timely action to prevent the emergence of risk factors in populations which have been hitherto relatively protected from exposure to these risk factors and to their social, economic and behavioural determinants.

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