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ORIGINAL ARTICLE

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Declining density of intimal smooth muscle cells as a precondition for atheronecrosis in the coronary artery

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Abstract As arteries move from a healthy youth toward the atheronecrotic state of later life, they maintain a record which can be read by measuring the declining densities of intimal smooth muscle cells. Atheronecrosis is found when the artery is marked by expanded collagenous matrix, which dilutes the resident smooth muscle cells to a critically low cell density. Aging produces atheronecrosis through effects that are associated with diminishing cell density, without need to consider any other mechanism. Male-female differences in atheronecrosis could, in a statistical sense, be fully explained by a faster decline of cell density in men. Arteries with low cell densities foster inception of atherosclerotic "plaques" when they are focally infiltrated by collections of foam cells. These findings emerge from morphometric assessment of hematoxylin and eosin stained paraffin sections of coronary arteries obtained at autopsy in a series of forensic cases.

Key words Atherosclerosis · Arteriosclerosis · Aging

Introduction

The necrotic core of atherosclerosis is distinguished by the presence of fluid, lipid rich plaque material, often affected by dystrophic calcification. The lesion is recognized in histological sections by the presence of atheronecrosis, identified by its typical features [5, 6]. Arteries having this diagnostic feature (YesA arteries) tend to differ in several conspicuous ways from arteries lacking this feature (NoA arteries). A low tissue density of vascular smooth muscle cells, and infiltration by collections of foam cells are especially prominent intimal characteristics of YesA arteries [3, 4].

Three processes that are regularly observed in the aging coronary artery are emphasized here [5]. Intimal smooth muscle cells increase in number to a peak level around age 40–50 years and then decline. The intima grows thicker because collagenous matrix materials accumulate, and this fibroplasia continues at least to age 69 years. The density of cells steadily declines throughout adult life as the expanding matrix spreads the cells apart from each other and this decline in cell density was recently shown to hold an especially important relationship to atheronecrosis [3]. Atheronecrosis tends to occur in arteries with cell densities below a well defined threshold, and this threshold is similar in the aorta, coronary arteries and basilar artery.

Ongoing studies in this laboratory now seek to add further details to the understanding of atheronecrosis and the kind of artery that it tends to affect. The effort hopes to yield fresh insight especially into the way that age records its effects upon the coronary intima. The present report undertakes, statistically speaking, to explain the effects of age upon the emergence of atheronecrosis by reference to observed variables, and therefore to eliminate age from the multivariate analyses. Similar efforts are made to explain the sex and racial group effects. A successful outcome would greatly enhance our understanding of age as a risk factor for coronary heart disease.

Materials and methods

The specimens of right coronary artery used in this study were obtained from forensic autopsies performed at the request of coroners in several parishes of Southeastern Louisiana, encompassing most of the New Orleans metropolitan area. In the age range 15–54 years, all females and white males, and all cases of coronary heart disease or related conditions, were retained in the series during the period March 1993 to February 1996. Black males were retained, when time and resources permitted, in numbers sufficient to equal or exceed those of white males of similar ages. In the ages 55 years or greater, all basal cases were retained, excluding cases with atherosclerosis related conditions. For some analyses, a basal group was constructed by retaining only cases with deaths from violence or natural causes having no known relationship to atherosclerosis. This basal group offers an approximation to a representative sample of the living population [2].

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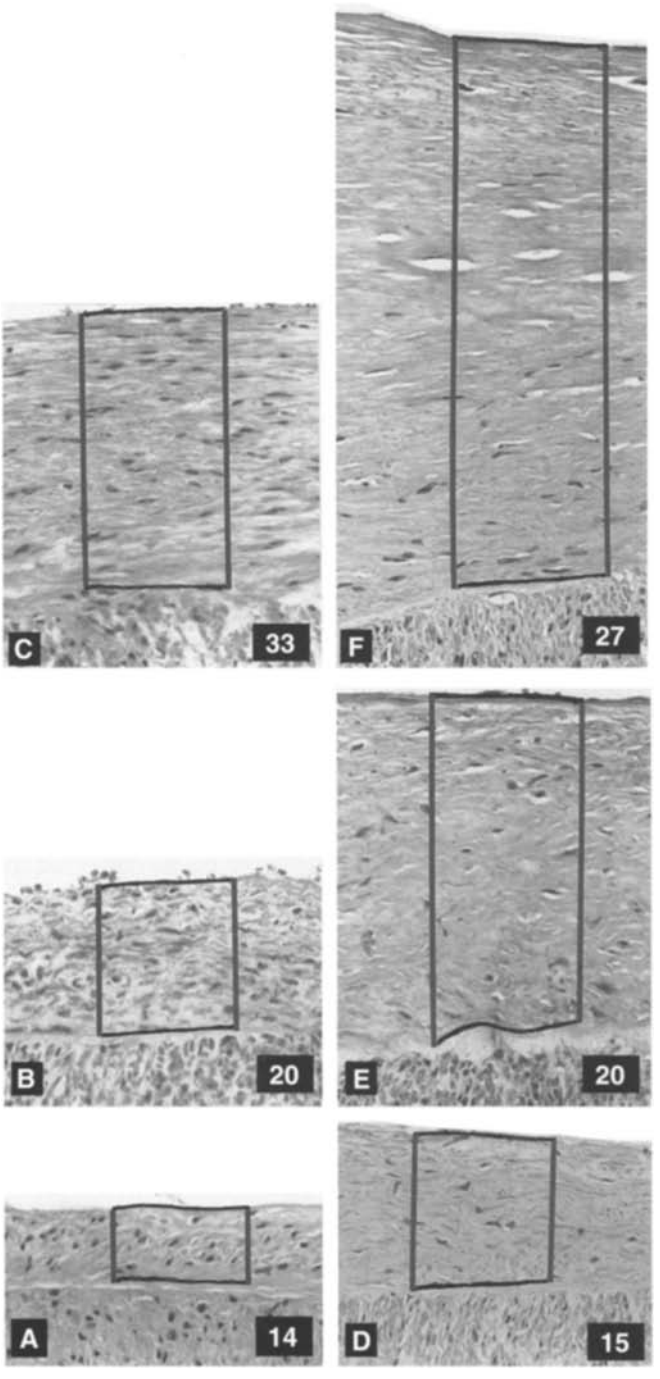

Fig. 1 Typical appearances of a NoA (**A, B, C**) and a YesA (**D, E, F**) artery are shown. Rectangular areas are 100 μm wide, and subsume the full intimal thickness (internal elastica is placed in the media); numbers of nuclear profiles, counted in these areas under the ×40 objective lens (*lower right corners*), estimate the intimal smooth muscle cell numbers and define the variable C_T. Areas chosen for side by side comparisons have similar estimates of C_T. Hematoxylin and eosin

Previously detailed techniques of processing [5], were used with little alteration. The first 9 cm of the right coronary artery were opened longitudinally, removed from the heart by dissection away of adventitial fat, and flattened for fixation in acetate buffered 3% formaldehyde. Three longitudinal segments of 3 cm length, oriented to display a plane parallel to the arterial axis and perpendicular to the luminal surface, were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. Ink marks were placed on the cover slips to define nine equally spaced positions along the length of the specimen.

Portions of the artery affected by atheronecrosis, operationally defined as having cholesterol clefts easily discerned under the 40× objective lens, were colored with black ink; the percentage of the length of the specimen colored positive for atheronecrosis was called P_A. Portions of specimen infiltrated by easily identified collections of foam cells were colored with green ink; the percentage of the nonnecrotic length colored positive for foam cells was called P_F. Intimal and medial thicknesses were measured, under the 10× objective lens, at each of the nine previously marked positions, using a ruler inserted into the eyepiece; under the 40× objective lens, smooth muscle cells were counted using an eyepiece grid. If the marked position was unsuitable for measurement, then the nearest suitable position was substituted; the presence of necrosis or foam cell infiltration was cause for substitution, as were artifacts such as tearing or folding of the section. The mean nonnecrotic, fibroplastic intimal thickness, averaged over all measurements, is F, and the mean medial thickness is M. The number of smooth muscle cells, counted in a band of 100 μm width through the full intimal thickness, is C_T, and the cell density $(C_D)=C_T/(0.01F)$. Descriptive characteristics of these variables are given in Appendix 1.

The molding of collagenous matrix materials (fibrous tissue) into masses of increased intimal bulk is described as "fibroplasia". This is distinguished from another intimal structure the necrotic core which is not composed of collagenous matrix. The total fibroplasia at a locus in the artery can be measured by the intimal thickness when necrotic cores are absent. (Measurements omit the core when it is present, and sum the fibrous cap and base [5, 6]; the resulting variable F_2, is not used in this study.) Dividing the intimal thickness by the number of smooth muscle cells to yield matrix per cell measures the "relative" or "proportional" fibroplasia. The inverse of this ratio, cells per matrix, $C_D=C_T/0.01F$, is used in this report, because the quantity, cell density, is familiar while the matrix per cell is an oddity to most readers. Total cell numbers, C_T, did not distinguish YesA from NoA arteries in this or other studies [8, 10]. Figure 1 compares some representative appearances of a NoA artery with those in a YesA artery. The side by side pairs of photos are chosen to match for nearly equal numbers of smooth muscle cells, allowing a visual impression of the diluted cells that populate zones of excessive fibroplasia.

Results

YesA arteries are those with at least one example of atheronecrosis somewhere within the examined specimen, while NoA arteries have no such example. When the sites of atheronecrosis are excluded from the assessed sample of tissue, then other features of the arteries can be evaluated.

Features that distinguish YesA from NoA coronary arteries include fibroplastic intimal thickness (F), medial thickness (M), foam cell infiltration (expressed as the square root $\sqrt{P_F}$) and density of intimal smooth muscle cells (C_D; Table 1), and do not include total cell numbers (data not shown). (Interactions in the ANOVA show that, with one exception, these characteristics of YesA arteries are equally substantial at all ages; the one exception is that total fibroplasia, F, differs most between YesA and NoA arteries at ages 30–44 years.)

Table 1 Mean of selected variables by age and presence or absence of atheronecrosis in the coronary artery (*NoA* arteries lacking the diagnostic features of atheronecrosis, *YesA* arteries having the diagnostic features of atheronecrosis)

Age, years	Intima (F) μm		Media (M) μm		Foam cells (P_F) √%		Cell density (C_D) N/10,000 μm²		Number of cases	
	NoA	*YesA*	*NoA*	*YesA*	*NoA*	*YesA*	*NoA*	*YesA*	*NoA*	*YesA*
15–29	99	122	173	157	2.3	4.0	19.6	12.4	51	5
30–44	133	328	179	210	1.9	5.1	17.1	10.3	48	31
45–59	198	333	182	217	2.3	4.0	15.5	9.8	21	35
60–88	216	306	213	245	2.8	3.8	12.5	9.8	5	13
Unweighted means	161	272	187	207	2.3	4.2	16.2	10.6	125	84
ANOVA	F	*P*	F	*P*	F	*P*	F	*P*		
Age	8	0.00	6	0.00	0	0.85	6	0.00		
Necrosis	24	0.00	5	0.00	21	0.00	59	0.00		
Interaction	4	0.01	2	0.18	2	0.10	1	0.23		
R^2	0.43		0.21		0.24		0.55			

Table 2 Numbes of cases and mean extent of atheronecrosis (√% units) in the coronary artery by tertiles of foam cell infiltration according to tertiles of intimal smooth muscle cell density

Cell density;[a] C_D Number/ 10,000 μm²		Foam cells (P_F; %)[a]			Unweighted mean
		T1 (low)	T2 (mid)	T3 (high)	
T1 (low)	mean[b]	1.8Aa	3.6Ba	4.6Ca	3.3
	number	6	29	35	70
T2 (mid)	mean[b]	0.4Ab	1.0Ab	2.0Bb	1.2
	number	29	13	26	68
T3 (high)	mean[b]	0.0Ab	0.1Ab	0.2Ac	0.1
	number	38	24	10	72
Unweighted	mean	0.8	1.5	2.3	
	number	73	66	71	210
ANOVA		F[c]	*P*		
	Intima	44	0.00		
	Foam cells	10	0.00		
	Interactions[d]	2	0.10		
	R^2		0.543		

[a] Cutpoints for P_F are 2.0 and 4.3 √%, for C_D are 11.5 and 16.8 N/10,000 μm²
[b] Means within a *row* that share an upper case letter (A, B, or C), do not differ significantly ($P>0.05$) by PROC GLM *t*-test; means within a *column* that share a lower case letter (a, b, or c) also do not differ significantly
[c] When intimal fibroplasia (variable F in Table A1) is put into the model as an additional covariate, it significantly improves the model (F=48.7, $P=0.00$), but age does not (F=1.1, $P=0.29$)
[d] Interactions are of borderline significance, which raises some doubt about the contrasts between rows 1 and 3 and columns 1 and 3 seen in the *t*-tests

Relationship of foam cells to smooth muscle cell density

Foam cell infiltration is measured by the percentage of specimen affected (expressed here as the square root). This infiltration shows a strong tendency to accompany diminished cell density from one coronary artery to another ($r=-0.43$, age adjusted, Table A1). When cases are grouped into tertiles of cell density, and also grouped into tertiles of foam cell infiltration, the nine resulting subgroups are shown in Table 2. The 70 cases with the lowest cell density (first row of Table 2) include 35 of the 71 cases with the greatest foam cell percentage (third column). The 72 cases with the highest cell density (third row of Table 2) include 38 of the 73 cases with the least foam cell percentage (first column).

Relationship of atheronecrosis to cell density and foam cells

The average extent of atheronecrosis rises substantially with increasing foam cell infiltration, and this tendency is best seen when cell densities are low (first row of Table 2). Atheronecrosis increases greatly with declining

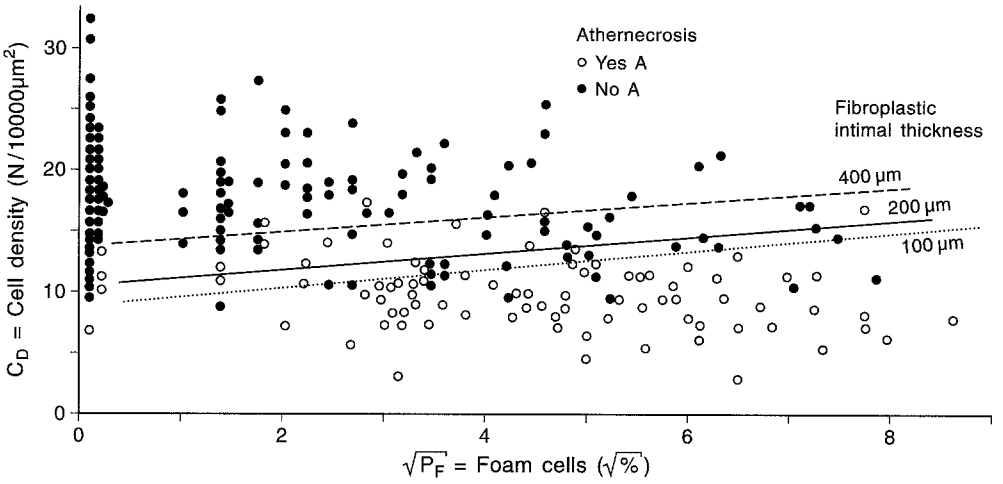

Fig. 2 Mean values of intimal smooth muscle cell density are plotted against the extent of infiltration by foam cells (percent expressed as square root) in 84 YesA (*circles*) and 125 NoA (*spots*) arteries observed at nonnecrotic sites. *Diagonal solid* and *dashed lines* represent the discriminant function given in Eq. 1, which optimally separates the circles from the spots, when mean fibroplastic intimal thickness takes the specified values and the value of the discriminant function is set to zero

Table 3 Means of selected variables, by race and sex, age adjusted by regression to average age 39.2 years (basal cases only) (*M* male, *F* female)

Variable	Racial group				ANOVA[a]				R^2
	Black		White		Race		Sex		
	M	F	M	F	F	*P*	F	*P*	
P_A ($\sqrt{\%}$)	1.4AB	0.6B	1.7A	0.9AB	1	0.34	7	0.01	0.040
P_F ($\sqrt{\%}$)	3.3A	2.0B	2.8AB	2.4AB	0	0.78	5	0.02	0.042
F (µm)	185B	117C	234A	156BC	6	0.01	16	0.00	0.113
M (µm)	199A	161B	200A	181AB	0	0.51	17	0.00	0.094
C_T (total)	22.1A	18.0A	31.2B	23.0A	26	0.00	14	0.00	0.188
C_D (N/10,000 µm²)	14.4A	16.3B	15.5AB	16.8AB	1	0.25	5	0.03	0.040
Disc (weighted average)[b]	–0.4A	–2.0C	–0.7AB	–1.8BC	0	0.86	11	0.00	0.059
Number of cases	81	25	56	25					

[a] Interactions between race and sex effects are not significant ($P>0.05$) and are therefore excluded from the Table

[b] Disc is the variable discussed in Appendix 2

[c] See Table 2 for explanation of letters A, B, and C

cell density, and this is best seen when foam cell infiltrates are extensive (third column of Table 2). Necrosis preferentially affects arteries that have low cell densities coexisting with extensive foam cell infiltration, and this coexistence is a common occurrence, affecting 35 cases (third column of Table 2). Low cell density is rarely seen with few foam cells, affecting only 6 cases (first column of Table 2); these 6 cases have only modestly increased atheronecrosis. Foam cells have no significant effect on necrosis in arteries with high cell densities (third row). (see Appendix 3 for details).

Presence or absence of necrosis in relation to cell density and foam cells

In Figure 2, the circles which represent YesA cases tend to be rightward and below the spots which represent NoA cases. The solid diagonal line marked 200 µm (average total fibroplasia) represents the discriminant function that best distinguishes the two kinds of case (Appendix 2). This line is nearly horizontal, showing that cell density holds the dominant influence. Yet, those YesA cases that fall below the line also typically have extensive infiltration by foam cells, because low cell density implies the likely coexistence of abundant foam cells (although the reverse is not true). (In Equation 1, C_D has nearly three-fold greater weighting than P_F, because the coexistence of large P_F with the YesA condition is already known from the measurement of C_D).

Sex effects

All variables assessed here display conspicuous differences between men and women, equally for black and white racial groups (Table 3). Women show the aging changes measured by fibroplastic intimal thickness and cell numbers in a pattern that resembles what is seen in men, but with a lag of about 15 years (Fig. 3, upper frame). Cell densities decline with age faster in men, and the average woman experiences a 12–15 year delay in reaching the threshold for atheronecrosis (the horizontal line in the lower frame of Fig. 3 is crossed at age 42 by the curve for men, and at age 56 for women). The multivariate analysis of Appendix 2 concludes that the lag in declining cell density, together with lesser amounts of foam cells, fully explain, statistically, the lower values of

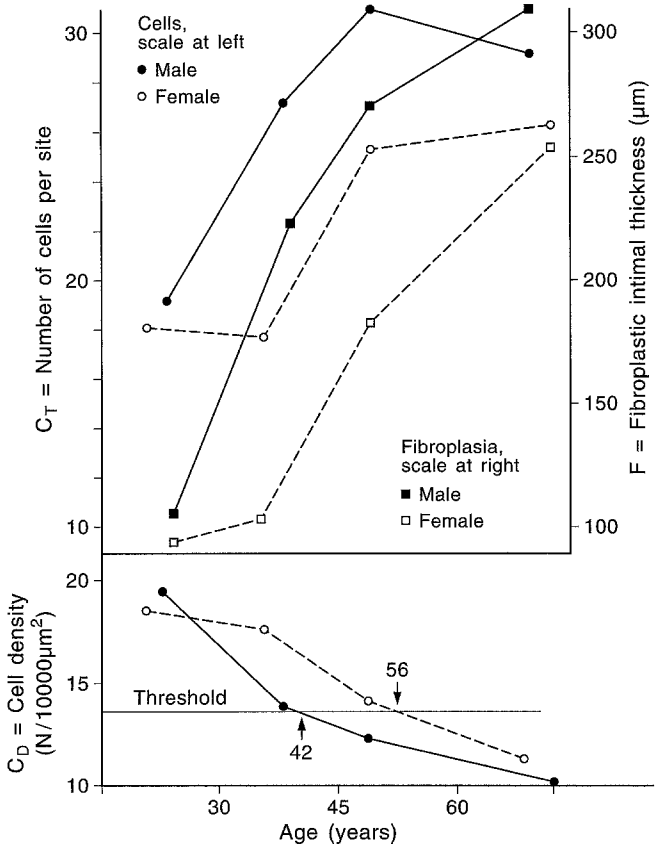

Fig. 3 Means of selected variables observed within broad age ranges for males and females (racial groups combined) are plotted, using the observed mean age within each age range on the horizontal. Threshold for atheronecrosis, represented as a *solid horizontal line* in the lower frame, depicts the discriminant function given in Eq. 1 when means of F and P_F from Table A1 are substituted into that equation, and Disc is set to zero

atheronecrosis in women, with no need to look for other mechanisms.

Race effects

Black and white racial groups are generally similar to each other in most measurements (Table 3). The only exceptions are lower values of total fibroplasia and total cell numbers in blacks, equally for both men and women. The ratio of these values determines cell density (C_D=C_T/0.01F), and this ratio does not differ between racial groups. The similarity of atheronecrosis in the two racial groups is appropriate to the equality in cell density, and not appropriate to the greatly differing total intimal thickness or cell numbers. The similarity is also appropriate to the equality in foam cells. Again, as for sex comparisons, the racial comparisons do not raise a need to look for other mechanisms to explain atheronecrosis beyond the predisposition that relates to cell density and foam cells.

Discussion

Among the risk factors for atherosclerosis and coronary heart disease, age holds a special and dominating position. The results of the study reported here offer fresh insights into the way that age registers its effects upon the intima of the coronary arteries. The bulk of fibroplastic collagenous matrix material expands at varying rates in various persons, but with an average tendency that proceeds relentlessly from adolescence into old age. Atheronecrosis preferentially intrudes into an artery that has expanded matrix, diluting the resident smooth muscle cells to a critically low cell density. The value of this threshold density shows a small variation from one subject to another, governed by the extent of foam cell infiltration (a measure of lipid deposition) and total intimal thickness (another measure of fibroplasia), but not by age. There is no need to look for a further explanation of how age affects the initial development of atheronecrosis.

The results of this study suggest that an observer who knows the cell density in the coronary intima of living subjects, would require no knowledge about the ages of the subjects when predicting risk for atherosclerosis or coronary events; those below the line are at great risk while those above are largely spared of risk, irrespective of age. Moreover, it would also not be necessary to know the sex or racial group membership of the subjects. As the artery evolves from healthy youth toward the acquisition of the atherosclerotic state later in life, its morphology provides a record of the stage of evolution at each point in time. This record can be read by measuring the reduced densities of intimal smooth muscle cells. Individuals or groups of subjects will move across the threshold for atheronecrosis, sooner or later.

Foam cells in the arterial intima interact with the diminished density of smooth muscle cells when promoting the intrusion of an atheronecrotic focus. This finding is reminiscent of similar conclusions reported in a recent review published by the American Heart Association (AHA) Committee on Lesions. They define a type II lesion, which corresponds roughly to the idea of "fatty streak" [1]: "Whether a lesion is type II is determined by its microscopic composition ... They consist primarily of macrophage foam cells stratified in adjacent layers ... Intimal smooth muscle cells, in addition to macrophages, now also contain lipid droplets ... Of the many type II lesions generally present in a person who has average levels of atherogenic lipoproteins, a smaller subgroup will be the first to proceed to type III lesions, and then to advanced lesions, if advanced lesions are to develop at all. This smaller subgroup of type II lesions, which collocates with specific adaptive intimal thickenings in predictable locations, is called progression-prone, advanced lesion prone, or type IIa."

The entity called "adaptive thickening" by the AHA Lesions Committee corresponds broadly to the intimal fibroplasia as measured here in F, C_D, or a weighted average of the two variables. Foam cell infiltrates, operationally defined here in the variable P_F, correspond

closely to the type II lesion as defined by the Lesions Committee. The collocating of these two quantities, fibroplasia (i.e. adaptive thickening) and foam cells, is called "progression-prone, advanced lesion-prone, or type IIa lesions" by the Lesions Committee, reflecting their view that simultaneously high values of the two conditions is an especially ominous circumstance. Table 2 is a quantitative statistical statement of these concepts.

The two measures of fibroplasia, total intimal thickness (F) and cell density (C_D), both jointly help to discriminate arteries with a focus of atheronecrosis, in a multivariate context (Appendix 2). As noted elsewhere, excessive intimal thicknesses can sequester great quantities of lipid [9], and offer the lipid prolonged opportunity for turning toxic by oxidation or other means [7]. The protective effect of high cell densities, seen here, raises the possibility that smooth muscle cells may prevent sequestering of lipid, or may delay its turning toxic.

Evidence reviewed here indicates that intimal fibroplasia and foam cell infiltration may have central positions in the early alterations of coronary arteries leading toward atherosclerosis. It would therefore be useful to know how these early changes relate to known coronary risk factors. The Bogalusa Heart Study has recently reported, for the first time, data of this kind [11]. Prospectively measured risk factor data were compared with histological arterial features in children and young adults. Intimal foam cells and lipid deposits were found to correlate with blood pressure and lipoprotein patterns. Intimal thicknesses and smooth muscle cell numbers were weakly and inconsistently related to observed risk factors. These scraps of information offer an impetus to continue this approach.

The findings reported here reaffirm the results previously published for the basilar artery, coronary arteries and aorta [3]. To the previous conclusions, the present report adds quantitative detail about the relationships of relevant variables to age, race, and sex. Results given here are in keeping with the recent statement by the AHA Lesions Committee, indicating that fatty streaks are often progression resistant, but that they can become progression prone when collocated with adaptive intimal thickening (fibroplasia). The findings reviewed here raise, for the first time, interesting questions about what etiologic factors might related in some way to the declining density of intimal smooth muscle cells in some individuals and groups of individuals. Since the questions have not previously been raised, few possibilities are ready at hand for testing. Perhaps this new line of questioning can now begin.

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Appendices

1 Description of variables

The variables used in this report are listed in Table A1, which gives descriptive information about them. The upper triangle at the right gives raw product moment correlation coefficients, and the lower triangle the age adjusted partial correlations. This Table provides the information needed to carry out multivariate analyses such as correlation and regression. Square root transform is used for P_F and P_A to reduce the severe skewness in the distributions of these variables.

2 Discriminant functions

The division of cases as YesA or NoA was explored by discriminant function analysis. The end product of this analysis is an equation (Eq. 1) giving a weighted average of selected variables which, when set equal to zero, provides an optimal division of cases into the two classes. Using a stepwise backward elimination routine, variables offered for retention in the function were those in Table A1, excluding P_A, the variable used for classification. Retaining only the terms with significant coefficients ($P<0.05$) the result was:

$$\text{Disc}=0.98F-2.03\,C_D+0.72P_F \quad (T^2=4.70,\ \%=85)$$

The coefficients apply to variables measured in units of standard deviations (SDs); these can be converted to raw form through division by the SDs in Table A1 and adding the constant term 2.76. T^2 is the distance between the two groups, YesA and NoA, in terms of the means of the variable "Disc", measured by the number of SDs squared. The quantity "%" is the percentage of cases correctly classified by the equation.

The unstandardized form of Eq. 1, with Disc=0, is plotted in Fig. 2, using three substitute values for F. The variable Disc, calculated in its unstandardized form, is reported in Tables 3 and A2, and in Fig. A1. The dashed line in Fig. A1, which represents the equation that optimally separates the YesA from the NoA cases, is

Table A1 Descriptive characteristics of selected variables, including correlation coefficients, raw (*upper triangle*) and age adjusted (*lower triangle*) in 210 specimens of coronary artery (*SD* standard deviation)

Variable	Symbol	Units	Mean	SD	Correlation coefficients					
					P_A	P_F	F	M	C_T	C_D
Age	A	Years	39.8	14.7	0.41*	0.17**	0.45*	0.39*	0.31*	–0.55*
Atheronecrosis	P_A	√%	1.7	2.4	–	0.47*	0.70*	0.37*	0.36*	–0.67*
Foam cells	P_F	√%	3.1	2.3	0.45*	–	0.40*	0.44*	0.25*	–0.45*
Intima (fibroplasia)	F	μm	209	155	0.64*	0.37*	–	0.35*	0.74*	–0.64*
Media	M	μm	193	49	0.25*	0.41*	0.21*	–	0.23*	–0.42*
Cell total	C_T		25.3	11.9	0.26*	0.21*	0.71*	0.12	–	–0.18*
Cell density	C_D	/10,000 μm²	14.6	5.4	–0.58*	–0.43*	–0.53*	–0.27*	–0.13	–

* $P<0.01$; ** $P<0.05$

Table A2 Means of selected variables, by sex, according to predicted status of bearing a focus of atheronecrosis, age adjusted by regression to average age 39.2 years (basal cases only)

Variable	Predicted status				ANOVA				R^2
	Negative		Positive		Status		Sex		
	M	F	M	F	F	*P*	F	*P*[a]	
P_A ($\sqrt{\%}$)	0.3A	0.4A	3.0B	2.1B	38	0.00	2	0.18	0.327
P_F ($\sqrt{\%}$)	1.9A	1.5A	4.5B	4.9B	47	0.00	0	0.94	0.265
F (μm)	144.5AB	122.0A	281.0C	200.0B	25	0.00	8	0.01	0.285
M (μm)	186.9B	155.4C	213.9A	223.2A	29	0.00	2	0.15	0.208
C_T	25.0AB	20.8A	27.1B	19.0A	0	0.93	9	0.00	0.061
C_D (N/10,000 μm²)	18.0A	17.6A	11.0B	11.9B	72	0.00	0	0.69	0.419
Disc (units) (weighted average)	−2.5A	−2.6A	1.8B	1.1B	108	0.00	2	0.19	0.520
Number of cases	78	37	59	13					

[a] The interaction term is significant in the ANOVA for M (F=7.52, $P<0.00$); all other ANOVAS are not significant ($P>0.05$)
[b] See Table 2 for explanation of letters A, B and C

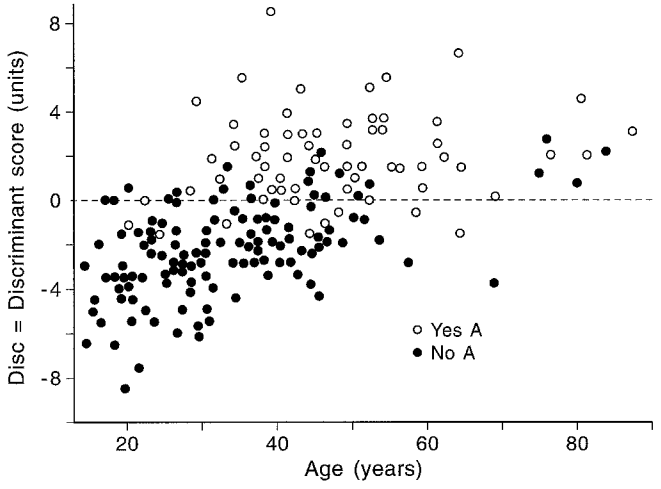

Fig. A1 Values of Disc calculated from the unstandardized form of Eq. 1 are plotted against age for the 84 YesA (*circles*) and 125 NoA (*spots*) arteries. The *horizontal dashed line* represents Eq. 1 with Disc set to zero, and depicts the optimal separation between circles and spots

exactly horizontal (age is rejected from Eq. 1). This result illustrates that the function operates exactly the same at all ages, and that the effect of age upon the coronary artery is fully measured by the observed anatomical features, so far as age affects the emergence of atheronecrosis.

Table 3 shows that women tend to have less atheronecrosis than men, and that males and females differ substantially in all three of the variables on the right side of Eq. 1. It is useful to ask how much of the sex difference in P_A can be attributed to each of the three discriminator variables that make up the weighted average, "Disc". The matter is examined in Table A2. Using unstandardized Eq. 1 to place each individual into a positive or negative group, on the basis of a positive or negative sign for Disc, a two way ANOVA is carried out.

After classification into negative and positive groups, women no longer differ from men in the levels of atheronecrosis (no sex effect in the ANOVA for P_A in Table A2). The large sex difference in P_A seen in Table 3 has been fully measured by the sex difference in Disc. This outcome suggests that women have less atheronecrosis (atherosclerosis) than men entirely because of the preconditions described by the discriminator variables.

Looking in Table A2 at the variables P_F and C_D, the positive men resemble the positive women, just as the negative men resemble the negative women (no sex effect is present in the ANOVA). Hence, the sex differences in these two variables (Table 3) have been used up when classifying more women into the negative group. The variable F, however, retains a significant sex difference even after classification. Before classification (Table 3) men have 69.5 μm greater average F than women; after classification (Table A2) they have 52.0 μm greater F, showing that this variable was applied almost exactly alike to men and women and that the sex difference in F was not used up in the classification process. The women are placed into the negative group more often than the men because of lower P_F and higher C_D values and not because of smaller F values.

3 Multiple regression

This was done with P_A taken as dependent variable using a backward stepwise elimination process. Independent variables offered were A, P_F, C_D, F and M. Retaining only the variables with significant coefficients ($P<0.05$) gave us Eq. 2:

$$P_A=0.42F-0.32C_D+0.15P_F \quad (R^2=0.595)$$

Variables are those listed in Table A1, and are expressed in standardized form (raw coefficients can be calculated using the SDs in the table). When C_T was also offered, this variable entered with a negative sign ($P=0.01$) and a rise of R^2 to 0.607; this outcome offers a weak suggestion that necrosis slightly favors the arteries with the least cellularity. Equation 1 reveals a weaker relative importance for F than in Eq. 2; this result suggests that F has less impact upon the first appearance of an atheronecrotic core than it does upon the later emergence and extension of necrosis.

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