

Smoking is associated with advanced coronary atherosclerosis in youth

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Abstract

Smoking is linked to atherosclerosis and coronary heart disease (CHD) in older adults. However, evidence that smoking affects coronary atherosclerosis in young people is incomplete. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study collected arteries, blood, and other tissues from persons 15 to 34 years of age dying of external causes and autopsied in forensic laboratories. Lesions in the proximal left anterior descending coronary arteries (LAD) from 1127 subjects were graded microscopically according to the American Heart Association criteria. Among individuals with advanced lesions (Grade 4 or 5), smokers had a greater prevalence of Grade 5 lesions than non-smokers (odds ratio 9.61, 95% confidence interval 2.34–39.57), a difference suggesting that smoking accelerates the transition from Grade 4 to Grade 5 lesions. This association occurred among both men and women, and among persons with and without other CHD risk factors. The difference in qualities of advanced lesions suggests that smoking possibly accelerates the transition from Grade 4 to Grade 5 lesions by promoting thrombosis and accretion on the intimal surface of the plaque.

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1. Introduction

Smoking was identified as a risk factor for coronary heart disease (CHD) shortly after the middle of the 20th century [1] and later was shown to be associated with atherosclerosis in adults over age 35 [2]. Although atherosclerosis begins in childhood and progresses during adolescence and young adulthood [3], whether smoking was associated with pre-clinical atherosclerosis in persons under age 35 was not

known. In 1985, investigators organized a multicenter cooperative study, Pathobiological Determinants of Atherosclerosis in Youth (PDAY), to determine the relation of CHD risk factors to atherosclerosis in young people. We previously reported a strong association of smoking with atherosclerosis in the abdominal aorta, and a weaker and less consistent association of smoking with atherosclerosis of the coronary arteries [4–6].

In this report, we present results on the association of smoking with the microscopic features of atherosclerosis in the proximal left anterior descending coronary artery (LAD) in a larger number of cases (1127) than previously reported (760) [6]. This larger number of cases makes possible the

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identification of a strong association of smoking with advanced atherosclerotic lesions in young persons.

2. Methods

2.1. Study design and subjects

Fifteen cooperating centers followed standardized procedures to collect specimens and data, and to submit them to central laboratories for analysis.

Study subjects were persons 15 through 34 years of age who died of external causes (accidents, homicides, suicides) within 72 h after injury and were autopsied within 48 h after death in a cooperating forensic laboratory. The Institutional Review Board of each participating center approved this study. All data were available for 1127 subjects (386 white men; 454 black men; 144 white women; 143 black women).

2.2. Arteries and lesions

Pathobiological Determinants of Atherosclerosis in Youth (PDAY) investigators perfused the left main coronary artery and LAD with 10% buffered formalin at a pressure of about 100 mmHg (130 cm H₂O) and dissected them from the heart. A central laboratory technician cut a 5 mm transverse block from the fixed LAD distal to the flow divider of the left main and left circumflex arteries, a site known to be susceptible to clinically significant atherosclerosis [6]. The proximal half of the block was sectioned in a cryostat and stained with Oil Red O (ORO); and the distal half of the block was sectioned in paraffin and stained with Gomori-Trichrome Aldehyde Fuchsin (GTAF).

Two pathologists (AWZ and HCM) blindly and independently graded the GTAF and ORO-stained sections, using the American Heart Association (AHA) classification [7]. Differences were resolved by discussion and a consensus grade was determined. The two pathologists agreed on lesion grade in 63.6% of cases. Of the disagreements, only 6.7% were by 2 or more grade categories. The consensus grade was the lower score in 78.7% of the disagreements and the higher score in 21.3% of disagreements. Among cases that had a consensus grade of 4 or 5, the agreement of the two pathologists in the initial classification was 58.7%.

Grade 0 designated a normal artery with no intimal lipid and with or without adaptive intimal thickening. A Grade 1 lesion, corresponding to a gross fatty streak, contained isolated macrophage foam cells. A Grade 2 lesion contained numerous macrophage foam cells and fine particles of extracellular lipid, but no pools of extracellular lipid, and also corresponded to a gross fatty streak. A Grade 3 lesion contained numerous macrophage foam cells and one or more pools of extracellular lipid, but no well-defined core of lipid, and corresponded to a raised fatty streak, also known as an in-

termediate or transitional lesion. A Grade 4 lesion contained numerous macrophage foam cells plus a well-defined core of extracellular lipid covered by normal intima. A Grade 5 lesion showed one or more cores of extracellular lipid plus a reactive fibrous cap, vascularization, or calcification. Grades 4 and 5 lesions corresponded to gross raised lesions and are susceptible to rupture and thrombosis [7]. Grade 6 lesions, which corresponded to complicated lesions, were not encountered in the PDAY sample.

2.3. Risk factor measurements

To assess smoking status, we measured color produced by the thiocyanate-ferric nitrate complex after treatment of trichloroacetic acid filtrates of serum with ferric nitrate [4]. A smoker was defined as having a serum thiocyanate concentration of ≥ 90 $\mu\text{mol/L}$.

We measured total serum cholesterol and HDL cholesterol (after precipitation of other lipoproteins) by a cholesterol oxidase method and calculated non-HDL cholesterol by subtraction [4]. A non-HDL concentration ≥ 4.14 mmol/L (160 mg/dL) was considered elevated and an HDL cholesterol concentration < 0.91 mmol/L (35 mg/dL) was considered low. Hypertension was identified when the intimal thickness of small renal arteries indicated a mean blood pressure ≥ 110 mmHg [8]. Adiposity was assessed by the body mass index (BMI) computed from the weight and body length measured at autopsy [9]. BMI > 30 kg/m² indicated obesity. A red blood cell glycated hemoglobin $\geq 8\%$ indicated hyperglycemia [9].

2.4. Statistical analysis

The prevalence of smoking was analyzed by binary logistic regression [10]. Overall association of AHA grade with smoking was analyzed using a chi-squared test for independence [11]. To examine the details of the association utilizing the ordered nature of the AHA grade [11], the data were partitioned into five 2×2 contingency tables. This partitioning, corresponding to continuation ratios [11], was appropriate because it is generally accepted that lesions progress sequentially through the AHA grades. Thus, individuals at a given grade (severity) of lesion had passed through all less severe levels of the lesion at an earlier age. We present odds ratios (OR) representing, among subjects who have attained a certain level of severity of lesions (grade *j* or higher), the odds of having a lesion of severity greater than *j* for smokers relative to the odds of having a lesion of severity greater than *j* for non-smokers. For contingency tables having a cell frequency of zero, we used the median unbiased estimate of the odds ratio [12] and exact one-sided (because the upper limit is not finite) confidence intervals [13]. To analyze the association of smoking with AHA grade adjusting for other variables, we used multivariable logistic regression analysis for ordinal data [10,11], again using continuation ratios.

3. Results

3.1. Smoking prevalence

The prevalence of smoking (Table 1) increased with age ($P=0.0001$) and was greater in whites than in blacks ($P=0.0320$). Smoking prevalence did not differ between men and women ($P=0.6438$).

3.2. Smoking and LAD lesions—unadjusted analyses

The prevalence of AHA grades by smoking status in all cases is shown in Table 2. There was a significant association between AHA grade and smoking status ($\chi^2=24.701$, d.f. = 5, $P=0.0002$). To further examine the relation of AHA grade to smoking, we partitioned the data into five 2×2 contingency tables (Table 3). The first 2×2 table (results for this table are given in the rows of Table 3 specified by 1–5 versus 0) was obtained by collapsing AHA grades 1 through 5. The prevalence of any lesion (Grades 1–5) among non-smokers was 45.6% and the prevalence among smokers was 53.0% (OR = 1.34, 95% CI 1.06–1.70). The confidence interval did not include 1.00 and indicated that smokers were more likely to have an LAD lesion than a non-smoker. Because a Grades 2–5 lesion was at a younger age a Grade 1 lesion, we interpreted this finding as indicating that smoking accelerated the transition from the Grade 0 (normal) tissue to the Grade 1 lesion.

The second 2×2 contingency table was obtained by ignoring cases with AHA Grade 0, and collapsing Grades 2 through 5. The prevalence of a Grades 2–5 lesion among non-smokers with at least a Grade 1 lesion was 60.9% and the prevalence among smokers was 68.4% (OR = 1.39, 95% CI 0.98–1.98). This odds ratio suggested that the transition from Grade 1 to Grade 2 was accelerated, although not significantly, in smokers compared to non-smokers. Other 2×2 tables (Table 3) were constructed in a similar fashion and the odds ratios indicated that there was a consistent trend for smoking to be associated with accelerated transitions, although the values were not statistically significant [Grades 2 and 3 (OR = 1.25, 95% CI 0.82–1.91); Grades 3 and 4 (OR = 1.09, 95% CI 0.57–2.10)].

However, among cases with a Grade 4 or 5 lesion (rows of Table 3 specified by 5 versus 4), the prevalence of Grade

Table 2
Number and prevalence of cases by AHA grade and smoking status

AHA grade	Non-smokers		Smokers	
	<i>n</i>	Prevalence (%)	<i>n</i>	Prevalence (%)
0	356	54.4 ± 1.9	222	47.0 ± 2.3
1	117	17.9 ± 1.5	79	16.7 ± 1.7
2	112	17.1 ± 1.5	96	20.3 ± 1.9
3	37	5.6 ± 0.9	38	8.1 ± 1.3
4	27	4.1 ± 0.8	14	3.0 ± 0.8
5	6	0.9 ± 0.4	23	4.9 ± 1.0

5 lesions among the 33 non-smokers was 18.2%, whereas the prevalence of Grade 5 lesions among the 37 smokers was 62.2% (OR = 7.39, 95% CI 2.45–22.35). This odds ratio indicated a strong and significant association. Again, because a Grade 5 lesion was at a younger age a Grade 4 lesion, we interpreted this substantial and significant odds ratio as suggesting that smoking accelerated the transition from the Grade 4 to the Grade 5 lesion. This association occurred in both men (OR = 6.65, 95% CI 1.98–22.39) and women (OR = 12.00, 95% CI 0.80–180.97).

To determine whether the presence of other risk factors was required for smoking to affect LAD lesions, we analyzed the prevalence of AHA Grades 4 and 5 by smoking status separately for cases without and with other risk factors measured in this study (Table 4). Smoking was associated with increased prevalence of Grade 5 lesions among cases with Grade 4 or Grade 5 lesions both in the cases without other risk factors [OR = 17.28 (97.5% one-sided CI 1.81)] and in cases with at least one other risk factor [OR = 4.67 (95% CI 1.44–15.13)]. For the cases without other risk factors, Grade 5 lesions occurred only in smokers.

3.3. Smoking and LAD lesions—multivariable adjusted analyses

Odds ratios for the effect of smoking after multivariable adjustment for sex, race, age, and other risk factors are given in Table 5. After adjusting for other risk factors as well as sex, race, and age, smoking was associated with increased prevalence of Grade 5 lesions among cases with Grade 4 or Grade 5 lesions (OR = 9.61, 95% CI 2.34–39.57). Other odds ratios were not statistically significant.

The significant odds ratio for the normal (Grade 0) to lesion (Grade 1) transition was not confirmed by the preferred

Table 1
Smoking prevalence (%) by sex, race, and 5-year age group

Sex	Race	Age (Years)							
		15–19		20–24		25–29		30–34	
		<i>n</i>	Prevalence	<i>n</i>	Prevalence	<i>n</i>	Prevalence	<i>n</i>	Prevalence
Men	White	89	22.5 ± 4.4	98	45.9 ± 5.0	111	49.5 ± 4.7	88	54.5 ± 5.3
	Black	114	23.7 ± 4.0	129	45.0 ± 4.4	116	41.4 ± 4.6	95	55.8 ± 5.1
Women	White	23	43.5 ± 10.3	37	37.8 ± 8.0	45	42.2 ± 7.4	39	64.1 ± 7.7
	Black	34	20.6 ± 6.9	34	23.5 ± 7.3	42	45.2 ± 7.7	33	48.5 ± 8.7

Table 3
Prevalence of AHA grades among sets of cases defined by increasing severity of lesion grade, by smoking status

Lesion comparison	Lesion	Non-smokers		Smokers		Odds ratio (95% CI)
		n	Prevalence (%)	n	Prevalence (%)	
1, 2, 3, 4, 5 vs. 0	Grades 1–5	299	45.6	250	53.0	1.34 (1.06–1.70)^a
	Grade 0	356		222		
2, 3, 4, 5 vs. 1	Grades 2–5	182	60.9	171	68.4	1.39 (0.98–1.98)
	Grade 1	117		79		
3, 4, 5 vs. 2	Grades 3–5	70	38.5	75	43.9	1.25 (0.82–1.91)
	Grade 2	112		96		
4, 5 vs. 3	Grades 4–5	33	47.1	37	49.3	1.09 (0.57–2.10)
	Grade 3	37		38		
5 vs. 4	Grade 5	6	18.2	23	62.2	7.39 (2.45–22.35)^a
	Grade 4	27		14		

^a Bold typeface indicates 95% confidence intervals (CI) that do not include 1.00.

Table 4
Prevalence of lesions among cases with Grades 4 or 5 lesions, by smoking status and by presence or absence of risk factors other than smoking (high non-HDL cholesterol concentration, low HDL cholesterol concentration, hypertension, obesity, hyperglycemia)

Other risk factors ^a	Lesion comparison	Lesion	Non-smokers		Smokers		Odds ratio (95% CI)
			n	Prevalence (%)	n	Prevalence (%)	
None	5 vs. 4	Grade 5	0	0.0	7	77.8	17.28 (1.81)^{b,c}
	–	Grade 4	6	–	2	–	
At least 1	5 vs. 4	Grade 5	6	22.2	16	57.1	4.67 (1.44–15.13)^c
	–	Grade 4	21	–	12	–	

^a High non-HDL cholesterol concentration, low HDL cholesterol concentration, hypertension, obesity, hyperglycemia.

^b Median unbiased estimate of odds ratio and one-sided 97.5% confidence interval.

^c Bold typeface indicates confidence intervals (CI) that do not include 1.00.

Table 5
Odds ratio for smoking and AHA grade, adjusted for sex, race, and age, and other CHD risk factors (high non-HDL cholesterol concentration, low HDL cholesterol concentration, hypertension, obesity, hyperglycemia)

Lesion comparison	Odds ratio for smoking effect (95% CI)
1, 2, 3, 4, 5 vs. 0	1.08 (0.83–1.39)
2, 3, 4, 5 vs. 1	1.18 (0.81–1.73)
3, 4, 5 vs. 2	1.13 (0.72–1.78)
4, 5 vs. 3	1.09 (0.52–2.25)
5 vs. 4	9.61 (2.34–39.57)^a

^a Bold typeface indicates 95% confidence intervals (CI) that do not include 1.00.

multivariable adjusted analysis. The significant unadjusted odds ratio was primarily due to increase with the age of both lesions and smoking prevalence (OR adjusted for age = 1.06, 95% CI 0.82–1.36).

4. Discussion

In both young men and young women having advanced atherosclerosis (Grade 4 or 5 lesions) in the proximal LAD, smoking was strongly associated with greater prevalence of Grade 5 lesions; that is, smokers were more likely to have more advanced disease than non-smokers. This association remained after adjustment for other risk factors and was

present in individuals with, and in individuals without other risk factors. Indeed, the only cases without other risk factors having Grade 5 lesions were smokers. We interpret the consistency of the association of smoking and prevalence of Grade 5 lesions in men and women, and in those with and without other risk factors, as strong evidence supporting the validity of this association.

The AHA classification system is based on a temporal sequence that is deduced from lesions taken from standard locations in many persons of different ages [7]. These observations indicate that lesions of a given severity have passed through all lower levels of the lesion at an earlier age. Thus, a Grade 5 lesion at the time of death had at an earlier age passed through the Grade 4 lesion. The significant odds ratios (both unadjusted and adjusted) show that smoking was associated with higher prevalence of Grade 5 lesions among cases with either Grade 4 or 5 lesions. This strong association—odds ratio >7—indicates that smoking accelerates the transition from Grade 4 to Grade 5 lesions. Smoking accelerates the Grade 4 to Grade 5 transition more strongly than it accelerates the progression through earlier stages, where the odds ratios are consistently greater than 1 but none is statistically significant.

The transition from Grade 4 to Grade 5 lesion could proceed by recurrent and transient episodes of thrombosis on the surface of the Grade 4 plaque, and subsequent organization

to increase the thickness of the fibromuscular cap, as suggested by the AHA grading scheme [14]. These small thrombotic accretions would be present only briefly, and would be difficult to detect by the method used in this study. The association of smoking with changes in fibrinogen levels and other hemostatic factors contributing to coronary thrombosis is well established [15]. However, the mechanism through which smoking accelerates transition of Grade 4 lesions to Grade 5 lesions cannot be determined with certainty in this study.

The association of smoking with Grade 5 lesions in the LAD is consistent with the established strength of smoking as a predictor for CHD [1] and particularly with the association of smoking with sudden cardiac death in younger persons [16]. It is also consistent with the reports of the association of smoking with coronary atherosclerosis in persons over 35 years of age [2,17].

The odds ratios in Table 5 suggest a weak ($OR \approx 1.2$, not statistically significant) association of smoking with the transition from Grade 1 to Grade 2. Previously, we reported an association of smoking with combined Grades 2 and 3 versus combined Grades 0 and 1 lesions ($OR = 1.44$) in the proximal LAD [6]. Although the additional 367 cases included in the present report were slightly younger than the original 760 cases, we were unable to explain the weaker association of smoking with earlier stages of atherosclerosis by the distribution of cases by age, sex, or risk factors, and concluded that the originally reported odds ratio was spuriously large.

We also previously reported that smoking was not associated with the mean extent of intimal surface involvement by raised lesions in the right coronary artery (RCA), but was associated with prevalence of cases having $\geq 5\%$ involvement with raised lesions in men [4]. In cases with extensive involvement of the RCA (such as $\geq 5\%$), we would expect many of the raised lesions to be the microscopic Grade 5 lesions. Thus, finding an association of smoking with prevalence of cases having extensive involvement in the RCA is consistent with the LAD results.

Smoking is associated with extent of fatty streaks, intermediate lesions, and raised lesions in the abdominal aorta [4,18], even in individuals with a favorable lipoprotein profile [19]. Smoking is also strongly associated with abdominal aortic aneurysms in older persons [20]. The abdominal aorta is more sensitive than the coronary arteries to smoking for unknown reasons.

The Seven Countries Study found that smoking did not predict risk of CHD in parts of southern Europe and Japan where serum cholesterol levels were low relative to serum cholesterol levels in other industrialized countries [21]. This observation, together with the contrast between the high prevalence of smoking and the low rate of CHD in Japanese men, led to the hypothesis that smoking augmented atherosclerosis and increased risk of CHD only in the presence of elevated serum cholesterol levels [22,23]. However, a 25-year followup of the Seven Countries Study partici-

pants found no evidence of an interaction between smoking and serum cholesterol levels [24]. Furthermore, several analyses of large numbers of subjects found that smoking increases risk of CHD in individuals with low serum cholesterol levels [25–27]. Smoking is also associated with coronary thrombosis in both young men [28] and young women [29] independently of the other risk factors. The present findings are consistent with these more recent observations that indicate hypercholesterolemia, as defined by current guidelines, is not necessary for smoking to accelerate the progression of atherosclerosis and thereby increase the risk of CHD.

Approximately 43% of the PDAY subjects 20–29 years of age and about 56% of those 30–34 years of age were smokers (Table 1). The higher prevalence of smoking in PDAY subjects compared to results of surveys of living subjects that relied on self-reported smoking status may be due to the association of smoking with deaths from suicide and accidents and the use of an objective indicator of smoking [4].

The results reported here emphasize the importance of preventing cigarette smoking in youth, even in the absence of the other established risk factors.

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