

PASSIVE SMOKING AND IMPAIRED ENDOTHELIUM-DEPENDENT ARTERIAL DILATATION IN HEALTHY YOUNG ADULTS

DAVID S. CELERMAJER, PH.D., MARK R. ADAMS, M.B., B.S., PETER CLARKSON, M.B., B.S.,
JACQUI ROBINSON, R.N., ROBYN MCCREDIE, B.Sc., ANN DONALD, AND JOHN E. DEANFIELD, M.B., CH.B.

Abstract Background. Passive smoking has been linked to an increased risk of dying from atherosclerotic heart disease. Since endothelial dysfunction is an early feature of atherogenesis and occurs in young adults who actively smoke cigarettes, we hypothesized that passive smoking might also be associated with endothelial damage in healthy young-adult nonsmokers.

Methods. We studied 78 healthy subjects (39 male and 39 female) 15 to 30 years of age (mean \pm SD, 22 ± 4): 26 control subjects who had never smoked or had regular exposure to environmental tobacco smoke, 26 who had never smoked but had been exposed to environmental tobacco smoke for at least one hour daily for three or more years, and 26 active smokers. Using ultrasonography, we measured the brachial-artery diameter under base-line conditions, during reactive hyperemia (with flow increase causing endothelium-dependent dilatation), and after sublingual adminis-

tration of nitroglycerin (an endothelium-independent dilator).

Results. Flow-mediated dilatation was observed in all control subjects (8.2 ± 3.1 percent; range, 2.1 to 16.7) but was significantly impaired in the passive smokers (3.1 ± 2.7 percent; range, 0 to 9; $P<0.001$ for the comparison with the controls) and in the active smokers (4.4 ± 3.1 percent; range, 0 to 10; $P<0.001$ for the comparison with the controls; $P=0.48$ for the comparison with the passive smokers). In the passive smokers, there was an inverse relation between the intensity of exposure to tobacco smoke and flow-mediated dilatation ($r=-0.67$, $P<0.001$). In contrast, dilatation induced by nitroglycerin was similar in all groups.

Conclusions. Passive smoking is associated with dose-related impairment of endothelium-dependent dilatation in healthy young adults, suggesting early arterial damage. (N Engl J Med 1996;334:150-4.)

©1996, Massachusetts Medical Society.

PASSIVE smoking, which includes exposure to both sidestream smoke from burning cigarettes and exhaled mainstream smoke, has been associated with increased respiratory symptoms in children¹ and excess deaths from lung cancer in adults.² However, the greatest morbidity and mortality related to passive smoking have been attributed to atherosclerotic heart disease in middle and old age; this factor may account for up to 20,000 deaths annually in nonsmokers in the United States alone.³⁻⁶

Studies in laboratory animals have shown that passive smoking may increase atherosclerosis in cholesterol-fed rabbits⁷ and in cockerels,^{8,9} but few studies have assessed the effects of passive smoking on the arterial wall in humans. Since endothelial dysfunction is an early feature of atherogenesis *in vitro*,¹⁰ in laboratory animals,¹¹ and in humans,^{12,13} it may represent an important marker of early vascular damage. We therefore studied endothelial function in the arteries of healthy teenagers and young adults (mean age, 22 years) who had no known risk factors for atherosclerosis other than prolonged exposure to environmental tobacco smoke.

METHODS

Subjects

We studied 78 subjects 15 to 30 years of age; all had normal blood pressure, did not have diabetes, and had no history of hyperlipidemia

From the Department of Cardiology, Royal Prince Alfred Hospital (D.S.C., M.R.A., J.R., R.M.), and the Heart Research Institute (D.S.C., J.R., R.M.), Sydney, Australia; and the Cardiothoracic Unit, Hospital for Sick Children, London (P.C., A.D., J.E.D.). Address reprint requests to Dr. Celermajer at the Department of Cardiology, Royal Prince Alfred Hospital, Missenden Rd., Camperdown 2050, Sydney, Australia.

Supported by grants from the National Health and Medical Research Council of Australia (to Dr. Celermajer), the National Heart Foundation of Australia (to Dr. Adams), the Royal Australasian College of Physicians, the British Heart Foundation (to Dr. Deanfield and Dr. Clarkson), and the Coronary Artery Disease Research Association (to Ms. Donald).

or family history of premature vascular disease. They were clinically well and taking no regular cardiovascular medications. Subjects were recruited from among friends, families, hospital staff, and other community volunteers. All subjects gave informed consent, and the study was approved by the institutional committees on ethical practice.

The control subjects were 26 lifelong nonsmokers who had never been regularly exposed to environmental tobacco smoke at home (both parents or any other cohabitants or both had been nonsmokers while the subjects had been living with them) or in the workplace. The passive-smoking group consisted of 26 lifelong nonsmokers with self-reported histories of exposure to environmental tobacco smoke at home or at work or both for at least one hour per day for at least three years, and the active-smoking group consisted of 26 subjects who were active smokers, with self-reported smoking histories of at least two pack-years. One pack-year was defined as 20 cigarettes per day for one year, or the equivalent. In the passive-smoking group, the average intensity of exposure to environmental tobacco smoke was assessed by questionnaire as light (one to three hours per day), moderate (four to six hours per day), or heavy (more than six hours per day).

Study Design

Each subject made one visit to the study hospital, during which a medical history was taken, the supine resting blood pressure was measured, saliva was collected for later analysis of the cotinine concentration by rapid gas-liquid chromatography,¹⁴ and the vascular reactivity of the brachial artery was analyzed. Except in the control subjects, the amount of time since the last exposure to active or passive smoke was less than 24 hours in every case. Total cholesterol was measured in 70 of 78 subjects (the other 8 had not consented to venesection) at the same time as or within 12 months of the arterial studies, with the use of a Hitachi 747 AutoAnalyzer. In cases in which fasting samples were available, triglycerides were measured with the AutoAnalyzer, high-density lipoprotein was measured after precipitation with dextran sulfate magnesium, and low-density lipoprotein was calculated by means of the Friedewald formula.¹⁵

The ultrasound method for measuring endothelium-dependent and endothelium-independent arterial dilatation has been described previously.^{13,16} The brachial-artery diameter was measured on B-mode ultrasound images, with the use of a 7.0-MHz linear-array transducer and a standard Acuson 128XP/10 system (Mountain View, Calif.). In all studies, scans were obtained with the subject at rest, during reactive hyperemia, again with the subject at rest, and after sublingual administration of nitroglycerin. The subjects lay quietly for at least 10 minutes before the first scan. The brachial artery was scanned in lon-

gitudinal section 2 to 15 cm above the elbow, and the center of the artery was identified when the clearest picture of the anterior and posterior intimal layers was obtained. The transmit (focus) zone was set to the depth of the near wall, because of the greater difficulty of evaluating the "m" line (the interface between media and adventitia) of the near wall as compared with that of the far wall.¹⁷ Depth and gain settings were set to optimize images of the interface between the lumen and the arterial wall, images were magnified with the use of a resolution box function (leading to a video line width of approximately 0.065 mm), and machine-operating settings were not changed during any study.

When a satisfactory transducer position was found, the skin was marked and the arm remained in the same position throughout the study. A resting scan was obtained, and the velocity of arterial flow was measured with a pulsed-Doppler signal at a 70-degree angle to the vessel, with the range gate (1.5 mm) in the center of the artery. Increased flow was then induced by the inflation of a pneumatic tourniquet placed around the forearm (distal to the scanned part of the artery) to a pressure of 250 mm Hg for 4.5 minutes, followed by release. A second scan was performed continuously for 30 seconds before and 90 seconds after deflation of the cuff, including a repeated recording of flow velocity for the first 15 seconds after the cuff was released. Thereafter, 10 to 15 minutes was allowed for recovery of the vessel, after which an additional resting scan was performed. Sublingual nitroglycerin spray (400 µg) was then administered, and three to four minutes later the last scan was performed.

Data Analysis

The diameter of the vessel was measured in every case by two independent observers who were unaware of the results of the questionnaire, the smoking status of each subject, and the stage of the experiment. Flow-mediated dilatation and nitroglycerin-induced dilatation were calculated by each observer, and the average results of the two observations were recorded. We have previously shown that this method is accurate and reproducible for measuring small changes in arterial diameter,¹⁸ with low rates of interobserver error in measuring flow-mediated dilatation.^{13,16}

The arterial diameter was measured at a fixed distance from an anatomical marker (such as a fascial plane or a vein seen in cross section) with the use of ultrasonic calipers. Measurements were taken from the anterior to the posterior "m" line at end-diastole, coincidentally with the R wave on a continuously recorded electrocardiogram. For the reactive-hyperemia scan, measurements of diameter were taken 50 to 60 seconds after deflation of the cuff. Four cardiac cycles were analyzed for each scan, and the measurements for each observer were averaged. The vessel diameter in scans obtained after reactive hyperemia and the administration of nitroglycerin was expressed as a percentage of the average diameter of the artery in the two resting (or control) scans (considered as 100 percent). Volume flow was calculated by multiplying the velocity-time integral of the Doppler flow signal (corrected for angle) by the heart rate and the cross-sectional area of the vessel ($\pi \times r^2$). The flow velocity used in our calculation was measured in the center of the artery; absolute flow may therefore be overestimated, but relative flow values before and after cuff inflation are accurate.¹⁹ Reactive hyperemia was calculated as the maximal flow recorded in the first 15 seconds after cuff deflation divided by the flow during the first resting (base-line) scan.

Statistical Analysis

Descriptive data are expressed as means ±SD. An analysis of variance for the three groups was performed, followed by Scheffé's test for multiple comparisons, to allow pairwise testing for significant differences between the groups.²⁰ In the control subjects and passive smokers, the relation between

flow-mediated dilatation and intensity of exposure to environmental tobacco smoke (0 = none, 1 = light, 2 = moderate, and 3 = heavy, as defined above) was assessed by one-way analysis of variance. The determinants of flow-mediated dilatation were then assessed by multiple linear regression analysis, with flow-mediated dilatation as the dependent variable and age, sex, blood pressure, total cholesterol concentration, vessel size, and intensity of exposure to environmental tobacco smoke as the independent variables. Similar analyses were also performed with nitroglycerin-induced dilatation as the dependent variable. Statistical significance was inferred at a two-tailed P value of less than 0.05.

RESULTS

Base-Line Characteristics

The subjects had an average age of 22±4 years (range, 15 to 30), with similar ages in the three groups studied (Table 1). There were 13 male subjects and 13 female subjects in each of the control, passive-smoking, and active-smoking groups. Other important base-line characteristics — such as blood pressure; total, low-density lipoprotein, and high-density lipoprotein cholesterol levels; and vessel size and flow at rest — were also similar in all three groups.

Among the passive smokers, exposure to environmental tobacco smoke was light for nine subjects, moderate for nine, and heavy for eight. The duration of passive smoking was 16±8 years (range, 3 to 28); 17 of 26 subjects (65 percent) had been exposed to environmental tobacco smoke throughout childhood. For the active smokers, cigarette consumption was 7±4 pack-years (range, 2 to 19), and the intensity of exposure was 19±8 cigarettes per day (range, 8 to 35).

Vascular-Study Results

The degree of reactive hyperemia produced by cuff inflation and release was similar in the three groups studied (Table 2). In response to this increase in flow, arterial dilatation was 8.2±3.1 percent (range, 2.1 to 16.7) in the controls.

In the passive smokers, flow-mediated dilatation was

Table 1. Base-Line Characteristics of 26 Control Subjects, 26 Passive Smokers, and 26 Active Smokers.*

CHARACTERISTIC	CONTROLS	PASSIVE SMOKERS	ACTIVE SMOKERS	P VALUE†
Age (yr)	22±4	23±5	22±3	0.39
Sex (% male)	50	50	50	1.0
Systolic blood pressure (mm Hg)	108±9	114±14	112±10	0.19
Diastolic blood pressure (mm Hg)	72±8	75±9	75±8	0.28
Total cholesterol (mg/dl)‡	178±28	189±32	171±32	0.12
Low-density lipoprotein cholesterol (mg/dl)‡	100±28	114±26	100±20	0.20
High-density lipoprotein cholesterol (mg/dl)‡	61±11	55±14	55±15	0.31
Vessel size at rest (mm)	3.53±0.58	3.60±0.63	3.65±0.52	0.85
Salivary cotinine (ng/ml)	1.2±1.5	3.7±3.6	170±102	<0.001§
Flow at rest (ml/min)	99±65	108±78	114±69	0.66

*Plus-minus values are means ±SD.

†P values are for comparisons of the three groups by analysis of variance (see the Methods section).

‡To convert values for cholesterol to millimoles per liter, multiply by 0.02586.

§By pairwise comparison with the use of Scheffé's test, salivary cotinine levels were significantly higher in active smokers than in passive smokers or control subjects (P<0.001 for each comparison), but there was no significant difference between passive smokers and controls (P=0.68).

significantly reduced (3.1 ± 2.7 percent; range, 0 to 9; $P < 0.001$ for the comparison with the controls) (Fig. 1A). Flow-mediated dilatation was impaired in both the male passive smokers (3.2 ± 2.5 percent, vs. 7.3 ± 1.9 percent in the controls; $P < 0.001$) and the female passive smokers (3.0 ± 2.9 percent, vs. 9.1 ± 3.9 percent in the controls; $P < 0.001$). Flow-mediated dilatation was 4.1 ± 3.3 percent in the subjects with light exposure to environmental tobacco smoke, 3.1 ± 2.2 percent in those with moderate exposure, and 1.8 ± 2.0 percent in those with heavy exposure (Fig. 2).

In the group of 52 nonsmokers, flow-mediated dilatation was inversely related to the intensity of exposure to environmental tobacco smoke on both univariate regression analysis ($r = -0.67$, $P < 0.001$) and multiple regression analysis (partial $r = -0.72$, $P < 0.001$). Even in the group of passive smokers only, flow-mediated dilatation was inversely related to the intensity of exposure to environmental tobacco smoke ($r = -0.39$, $P = 0.04$). Salivary cotinine levels were not significantly correlated with flow-mediated dilatation on either univariate or multivariate analysis.

In the active smokers, flow-mediated dilatation was 4.4 ± 3.1 percent (range, 0 to 10), which was significantly less than that in the controls ($P < 0.001$) but similar to the value in passive smokers ($P = 0.48$). If the group of passive smokers was excluded, there was an inverse correlation between flow-mediated dilatation and the number of cigarettes smoked daily ($r = -0.57$, $P < 0.001$).

In the controls, nitroglycerin-induced dilatation was 18.5 ± 5.2 percent (range, 9.5 to 31.8). This response was not impaired in the passive smokers, at 16.4 ± 5.1 percent (range, 8.1 to 26.5), or in the active smokers, at 17.2 ± 5.4 percent (range, 8 to 28; $P = 0.33$) (Fig. 1B and Table 2). On multivariate analysis of the group as a whole, nitroglycerin-induced dilatation was inversely related to vessel size ($P < 0.001$) but was not significantly related to age, sex, blood pressure, or total cholesterol level.

DISCUSSION

Active cigarette smoking has long been known to predispose people to atherosclerotic vascular disease,²¹ but it has recently become evident that exposure to environmental tobacco smoke may also have deleterious cardio-

vascular effects, with enormous public health implications.³⁻⁶ This study shows that endothelial dysfunction, an important early feature of the atherogenic process, may occur in the systemic arteries of healthy teenagers and young adults as a result of passive smoking. The impairment of endothelium-dependent dilatation is dose-related and may be equivalent to the degree of vascular abnormality found in age-matched active smokers.

Environmental tobacco smoke consists of approximately 85 percent sidestream smoke (from the burning ends of cigarettes) and 15 percent exhaled mainstream smoke.⁵ Since cigarettes burn at higher temperatures during inhalation, combustion is more complete, and some toxic components of tobacco smoke are broken down or filtered out before inhalation. Consequently, many toxic constituents, such as carbon monoxide and benzopyrene, are found in higher concentrations in sidestream than in inhaled smoke,⁴ and more than 4000 chemicals are contained in environmental tobacco smoke.⁵ One or more of these compounds may be injurious to the arterial wall; in laboratory animals, exposure to environmental tobacco smoke is associated with endothelial dysfunction and with accelerated atherosclerosis.⁷⁻⁹ Environmental tobacco smoke in both low and high doses increases the percentage of the aorta covered by atheroma in cholesterol-fed rabbits,⁷ and exposing cockerels to levels of environmental tobacco smoke routinely encountered by people in smoke-filled environments is associated with an increase in the size of aortic atheroma plaques.^{8,9} Sun et al.²² have shown that dietary supplementation with L-arginine (the precursor of nitric oxide, or endothelium-derived relaxing factor) protects cholesterol-fed rabbits from the endothelial dysfunction associated with exposure to environmental tobacco smoke, suggesting that impaired endothelial production of nitric oxide may be pathogenetically important in this animal model of atherosclerosis related to environmental tobacco smoke.

We have previously reported impaired endothelium-dependent dilatation in young cigarette smokers.¹⁶ In humans, however, there have been few data to associate passive smoking with damage to the arterial wall. Short-term exposure to environmental tobacco smoke is associated with an increase in circulating damaged endothelial cells and also with a tendency toward enhanced platelet aggregation.²³⁻²⁶ It may also be associated with mild coronary-artery vasoconstriction in non-smoking adults.²⁷ Passive smoking may have adverse effects on lipid profiles,^{28,29} but in this study the impairment of vascular reactivity observed in the passive smokers was not related to the lipid levels, which were similar to those of the control subjects. Passive smoking may also be associated with an increased thickness of the intima-media layer of the common carotid artery,³⁰ by an unknown mechanism.

In this study, we have shown that passive smokers have significantly impaired arterial endothelial function. Impaired bioavailability of nitric oxide, the endothelium-derived relaxing factor, may be particularly

Table 2. Vascular-Study Results for 26 Control Subjects, 26 Passive Smokers, and 26 Active Smokers.*

VARIABLE	CONTROLS	PASSIVE SMOKERS	ACTIVE SMOKERS	P VALUE†
Flow-mediated dilatation (%)	8.2 ± 3.1	3.1 ± 2.7	4.4 ± 3.1	< 0.001 ‡
Nitroglycerin-induced dilatation (%)	18.5 ± 5.2	16.4 ± 5.1	17.2 ± 5.4	0.33
Hyperemia (%)	550 ± 204	466 ± 159	505 ± 215	0.16

*Plus-minus values are means \pm SD.

†P values are for comparisons of the three groups by analysis of variance (see the Methods section).

‡By pairwise comparison with the use of Scheffé's test, flow-mediated dilatation was significantly lower in passive smokers and in active smokers than in control subjects ($P < 0.001$ for both comparisons), but there was no significant difference between active and passive smokers ($P = 0.48$).

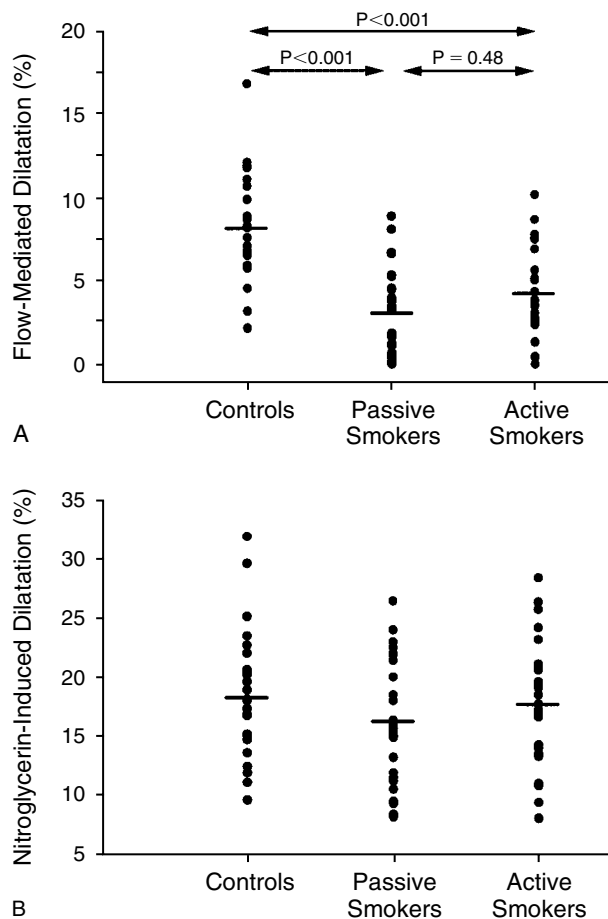


Figure 1. Comparison of Flow-Mediated Dilatation (Panel A) and Nitroglycerin-Induced Dilatation (Panel B) in 26 Controls, 26 Passive Smokers, and 26 Active Smokers.

Horizontal lines represent the mean values for each group. Flow-mediated dilatation was significantly impaired in the passive and active smokers as compared with the control subjects, whereas nitroglycerin-induced dilatation was similar in all three groups.

important, since nitric oxide acts to inhibit platelet aggregation, the adhesion of monocytes to the arterial wall, and proliferation of smooth-muscle cells.³¹ Dilatation mediated by brachial-artery flow is endothelium-dependent³² and is mediated in large part by the release of nitric oxide.³³ Therefore, our results suggest that the activity of endothelial nitric oxide may be impaired in young passive smokers as well as in active smokers. In vitro work has also suggested that decreased nitric oxide bioactivity might be implicated in smoke-related endothelial dysfunction.³⁴ The actual mechanism responsible for this arterial damage is not known but may be related to the effects of tobacco smoke on interactions between platelets and the vessel wall or on oxidation products or lipid components that change with long-term exposure to smoke.^{26,29,35,36} The toxic substance or substances involved appear to be present in both environmental and inhaled cigarette smoke.

All the passive smokers in this study were exposed to

environmental tobacco smoke for at least one hour daily. The intensity of exposure to environmental tobacco smoke depends on a large number of variables, such as the number of hours of exposure per day, the proximity to the active smoker (or smokers), the number of active smokers in the home or the workplace, and the size and ventilation of the rooms where passive smoking occurs. The relation between the extent of exposure to environmental tobacco smoke and endothelial physiology was assessed by structured questionnaire rather than by salivary cotinine levels. The latter proved useful in indicating that there were probably no active smokers in the passive-smoking group and helped substantiate exposure to environmental tobacco smoke in these subjects. The values reflect only recent exposure over a short time (two to four days) to only one component of smoke, however, and are thus of limited value in quantifying overall exposure to environmental tobacco smoke. There was, nevertheless, an inverse relation between an intensity score for passive smoking, based on the number of hours of exposure per day, and endothelium-dependent arterial dilatation. This dose-dependent relation between passive smoking and endothelial dysfunction is similar to that between active smoking and arterial injury¹⁶ and is consistent with (but does not prove) a causative role for environmental tobacco smoke in the early stages of atherosclerosis.

We deliberately studied passive smokers with a heavy environmental exposure, and the active smokers were young, with light-to-moderate smoking histories. In this study, the degree of impairment of endothelium-dependent responses was similar in the active and the passive smokers. This clearly does not imply equivalent exposure to smoking-related products, however, nor does it allow comparison of the susceptibility of the arterial wall to damage from active smoking with that from passive smoking.

We have previously described this noninvasive method for the *in vivo* assessment of endothelium-dependent and endothelium-independent arterial dilatation in children and young adults and have found the method to be accurate and reproducible.^{13,16,18} Because we studied healthy young adults without known atherogenic risk factors, such as diabetes or hypertension, which have been shown to cause impaired vascular reactivity,^{37,38} we were able to investigate the effects of passive and active smoking themselves on endothelial physiology. Although it is possible that some unidentified risk factor was present in the passive or active smokers but not in the controls, the frequency of the major known atherogenic risk factors was similar in all three groups. Although only superficial systemic arteries can be studied with this ultrasound-based method, endothelial dysfunction in the brachial artery appears to be well correlated with both coronary endothelial physiology³⁹ and coronary atherosclerosis.⁴⁰

Large-scale epidemiologic studies have consistently linked passive smoking to an excess risk of atherosclerotic heart disease, and some authors have suggested that

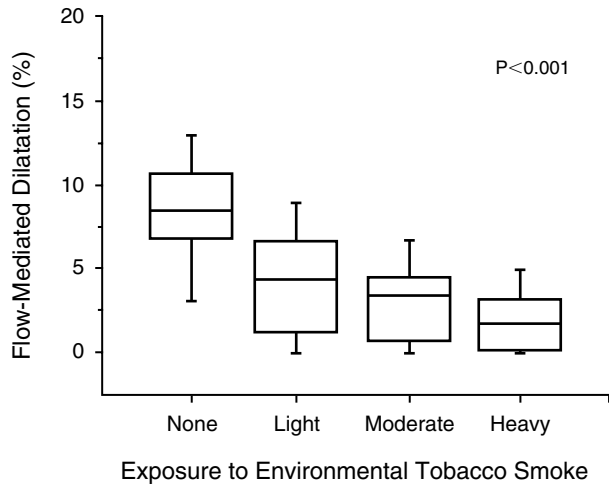


Figure 2. Relation between the Intensity of Exposure to Passive Smoking (None, Light, Moderate, or Heavy) and Flow-Mediated Dilatation in 52 Healthy Nonsmoking Teenagers and Young Adults.

For each category of intensity of exposure to environmental tobacco smoke, the box represents the interquartile range (between the 25th and 75th percentiles), with the mean shown as a horizontal bar within each box. The bars outside each box show the range of 95 percent of all values.

tens of thousands of premature deaths in nonsmokers may be related to passive smoking, with the large majority due to cardiac ischemia.³⁻⁶ We have now shown that passive smoking is associated, in a dose-dependent manner, with significant endothelial dysfunction, a key early event in atherogenesis, in healthy teenagers and young adults.

We are indebted to Robyn Richmond and Abilio de Almeida Neto for assistance with the recruitment of subjects for this study.

REFERENCES

- Colley JR, Holland WW, Corkhill RT. Influence of passive smoking and parental phlegm on pneumonia and bronchitis in early childhood. *Lancet* 1974;2:1031-4.
- Repace JL, Lowrey AH. A quantitative estimate of nonsmokers' lung cancer risk from passive smoking. *Environ Int* 1985;11:3-22.
- Wells AJ. An estimate of adult mortality from passive smoking. *Environ Int* 1988;14:249-65.
- Glantz SA, Parmley WW. Passive smoking and heart disease: epidemiology, physiology, and biochemistry. *Circulation* 1991;83:1-12.
- Taylor AE, Johnson DC, Kazemi H. Environmental tobacco smoke and cardiovascular disease: a position paper from the Council on Cardiopulmonary and Critical Care, American Heart Association. *Circulation* 1992;86:699-702.
- Steenland K. Passive smoking and the risk of heart disease. *JAMA* 1992;267:94-9.
- Zhu B-Q, Sun Y-P, Sievers RE, Isenberg WM, Glantz SA, Parmley WW. Passive smoking increases experimental atherosclerosis in cholesterol-fed rabbits. *J Am Coll Cardiol* 1993;21:225-32.
- Penn A, Snyder CA. Inhalation of sidestream cigarette smoke accelerates development of arteriosclerotic plaques. *Circulation* 1993;88:1820-5.
- Penn A, Chen L-C, Snyder CA. Inhalation of steady-state sidestream smoke from one cigarette promotes arteriosclerotic plaque development. *Circulation* 1994;90:1363-7.
- Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801-9.
- Moore S. Thromboatherosclerosis in normolipemic rabbits: a result of continued endothelial damage. *Lab Invest* 1973;29:478-87.
- Fish RD, Nabel EG, Selwyn AP, et al. Responses of coronary arteries of cardiac transplant patients to acetylcholine. *J Clin Invest* 1988;81:21-31.
- Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340:1111-5.
- Feyerabend C, Russell MAH. A rapid gas-liquid chromatographic method for determination of cotinine and nicotine in biological fluids. *J Pharm Pharmacol* 1990;42:450-2.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
- Celermajer DS, Sorensen KE, Georgakopoulos D, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 1993;88:2149-55.
- Wendelhag I, Gustavsson T, Suurkula M, Berglund G, Wikstrand U. Ultrasound measurement of wall thickness in the carotid artery: fundamental principles and description of a computerized analysing system. *Clin Physiol* 1991;11:565-77.
- Sorensen KE, Celermajer DS, Spiegelhalter DJ, et al. Non-invasive measurement of endothelium-dependent arterial responses in man: accuracy and reproducibility. *Br Heart J* 1995;74:247-53.
- Chauveau M, Levy B, Dessanges JF, Savin E, Bailliart O, Martineaud JP. Quantitative Doppler blood flow measurement method and in vivo calibration. *Cardiovasc Res* 1985;19:700-6.
- Hogg RV, Craig AT. *Introduction to mathematical statistics*. 4th ed. New York: Macmillan, 1978.
- Department of Health and Human Services. The health consequences of smoking: cardiovascular disease: a report of the Surgeon General. Washington, D.C.: Government Printing Office, 1983. (DHHS publication no. (PHS) 84-50204.)
- Sun Y-P, Zhu B-Q, Sievers RE, Glantz SA, Deedwania PC, Parmley WW. L-arginine preserves endothelial dependent relaxation during environmental tobacco smoke in lipid fed rabbits. *Circulation* 1994;Suppl:1-459. abstract.
- Davis JW, Shelton L, Watanabe IS, Arnold J. Passive smoking affects endothelium and platelets. *Arch Intern Med* 1989;149:386-9.
- Davis JW, Shelton L, Zucker ML. A comparison of some acute effects of smoking and smokeless tobacco on platelets and endothelium. *J Vasc Med Biol* 1990;2:289-93.
- Sinzinger H, Virgolini I. Besitzen Passivraucher ein erhöhtes Thromboserisiko? *Wien Klin Wochenschr* 1989;101:694-8.
- Burghuber OC, Punzengruber C, Sinzinger H, Haber P, Silberbauer K. Platelet sensitivity to prostacyclin in smokers and non-smokers. *Chest* 1986;90:34-8.
- Brown RE, Nahser PJ Jr, Rossen JD, Winniford MD. Passive exposure to environmental tobacco smoke causes coronary vasoconstriction in humans. *Circulation* 1993;Suppl:1-260. abstract.
- Feldman J, Shenker IR, Etzel RA, et al. Passive smoking alters lipid profiles in adolescents. *Pediatrics* 1991;88:259-64.
- Moskowitz WB, Mosteller M, Schieken RM, et al. Lipoprotein and oxygen transport alterations in passive smoking preadolescent children. *Circulation* 1990;81:586-92.
- Howard G, Burke GL, Szklo M, et al. Active and passive smoking are associated with increased carotid wall thickness: the Atherosclerosis Risk in Communities Study. *Arch Intern Med* 1994;154:1277-82.
- Cooke JP, Tsao RS. Is NO an endogenous antiatherogenic molecule? *Arterioscler Thromb* 1994;14:653-5.
- Pohl U, Holtz J, Busse R, Bassenge E. Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension* 1986;8:37-44.
- Lieberman EH, Knab ST, Creager MA. Nitric oxide mediates the vasodilator response to flow in humans. *Circulation* 1994;90:Suppl:1-138. abstract.
- Raj L, Nagy J, Jaimes E, Shultz P, DeMaster EG. Mechanisms of cigarette smoke induced impairment of endothelium dependent modulation of vascular tone. *Circulation* 1994;90:Suppl:1-575. abstract.
- Duthie GG, Arthur JR, James WP. Effects of smoking and vitamin E on blood antioxidant status. *Am J Clin Nutr* 1991;53:Suppl:1061S-1063S.
- Jendryczko A, Szpyrka G, Gruszczynski J, Kozowicz M. Cigarette smoke exposure of school children: effect of passive smoking and vitamin E supplementation on blood antioxidant status. *Neoplasma* 1993;40:199-203.
- Johnstone MT, Creager SJ, Scales KM, Cosco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 1993;88:2510-6.
- Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990;323:22-7.
- Anderson TJ, Uehata A, Gerhard MD, et al. Close relationship of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol* 1995;26:1235-41.
- Vogel RA, Vaitkevicius PV, Plotnick GD. Ultrasound assessment of brachial artery endothelium-dependent vasoactivity as a means for diagnosing coronary artery disease. *J Am Coll Cardiol* 1993;21:345A. abstract.