

Tobacco Smoke Exposure Is Associated With Attenuated Endothelial Function in 11-Year-Old Healthy Children

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Background—Passive smoking is associated with early arterial damage in adults, but its effect on endothelial function in children is unknown.

Methods and Results—Serum cotinine concentration was measured annually in children between 8 and 11 years of age who had participated since infancy in a randomized, prospective atherosclerosis prevention trial (Special Turku Coronary Risk Factor Intervention Project for children [STRIP]). At age 11, endothelium-dependent flow-mediated vasodilatory responses of the brachial artery were examined with high-resolution ultrasound in 402 children. These children were divided into 3 groups according to serum cotinine concentrations: the noncotinine group (nondetectable cotinine, n=229), the low cotinine group (cotinine between 0.2 and 1.6 ng/mL, n=134), and the top decile cotinine group (cotinine \geq 1.7 ng/mL, n=39). Longitudinal cotinine data in children aged 8 to 11 years and ultrasound studies were available in 327 children. At age 11, the increase in cotinine concentration was associated with attenuated peak flow-mediated dilation response (mean \pm SD: the noncotinine group 9.10 \pm 3.88%, the low-cotinine group 8.57 \pm 3.78%, and the top-decile cotinine group 7.73 \pm 3.85%; $P=0.03$ for trend). Similarly, total dilation response (the area under the dilation response versus time curve between 40 and 180 seconds after hyperemia) was affected by the cotinine level ($P=0.02$ for trend). These trends were not explained by traditional atherosclerosis risk factors. Arterial measures and passive smoking showed even stronger associations when longitudinal cotinine data were used (peak flow-mediated dilation, $P=0.01$ for trend; total dilation response, $P=0.008$ for trend).

Conclusions—Exposure to environmental tobacco smoke confirmed by serum cotinine concentrations impairs endothelial function in a dose-dependent manner in 11-year-old children. (*Circulation*. 2007;115:NA;-)

Key Words: smoking, passive ■ endothelium ■ pediatrics ■ atherosclerosis ■ ultrasonics

Exposure to tobacco smoke has adverse effects on vascular biology, causing endothelial dysfunction, arterial stiffness, platelet activation, inflammation, oxidative stress, and a decreased antioxidant defense, unfavorable lipid profile, insulin resistance, and atherosclerosis.¹⁻³ The mechanisms involved interact and are considered to be due to toxins present in secondhand smoke. Surprisingly, the impact of passive smoking on the cardiovascular system is more extensive than expected when doses of tobacco smoke exposure are compared between active and passive smokers.² Serum concentration of cotinine, a metabolite of nicotine, is a sensitive and specific estimate of exposure even when levels are as low as those encountered in children.⁴

Endothelial dysfunction is an early feature of atherogenesis, and conventional atherosclerosis risk factors such as

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hypercholesterolemia, diabetes mellitus, and obesity have already been associated with endothelial dysfunction in childhood.⁵⁻⁷ In adults and in children, endothelial function may modify the association between conventional risk factors and atherosclerosis.^{6,8} Impaired endothelial function is also related to the prevalence of coronary atherosclerosis⁹ and predicts coronary events in patients with coronary artery disease.¹⁰ Active and passive smoking are associated with impaired endothelium-dependent dilation in young adults and teenagers.^{11,12} However, because studies analyzing the association between reliably quantified passive smoke exposure and vascular function in children are scarce, we measured arterial endothelial function and smoke exposure in school-

Received November 7, 2006; accepted April 17, 2007.

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Clinical trial registration information—URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT00223600.

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Circulation is available at <http://www.circulationaha.org>

DOI: 10.1161/CIRCULATIONAHA.106.674804

aged children who participate in an ongoing randomized, prospective atherosclerosis prevention trial, the Special Turku Coronary Risk Factor Intervention Project for children (STRIP).

Methods

Design

The design of the STRIP project has been published previously.^{13,14} Briefly, families of 5-month-old infants were voluntarily recruited in 1990 through 1992 at the well-baby clinics in the city of Turku, Finland. At the age of 7 months, 1062 infants were randomized to an intervention group (n=540) or to a control group (n=522). Biannually, the intervention families received individualized and detailed lifestyle counseling aimed at reducing exposure of the intervention children to known environmental cardiovascular risk factors. At 9 years of age, prevention of the onset of smoking was introduced to the intervention group. Venous blood samples were drawn yearly. Serum cotinine measurements were performed annually beginning at 8 years of age, and smoking habits were assessed with a questionnaire beginning at 9 years of age. Ultrasound studies were performed at the age of 11 years. The study protocol was approved by the local ethics committee. Informed consent was obtained from the parents.

Subjects

Children's serum cotinine concentrations were determined from every obtained blood sample between the ages of 8 and 11 years. Consequently, cotinine values of 625, 561, 555, and 548 healthy children were available at the ages of 8, 9, 10, and 11 years, respectively, comprising 96%, 91%, 94%, and 95% of the participating age cohort. Serum cotinine values from all 4 age points were available in 441 children. None of the children reported active smoking during follow-up, but because the suggested serum cotinine cutoff point for active smoking is 15 ng/mL,¹⁵ we excluded one 10-year-old child who had a cotinine concentration of 100.8 ng/mL.

At age 11 years, of the 548 children with cotinine measurements, 418 (76%) participated in the ultrasound study, and successful brachial artery measurements were available in 402 children (73%). Participation rates in ultrasound studies were similar in the intervention and control groups. The main reasons given for nonparticipation were child's unwillingness to participate, lack of time, fear of the study, and transportation problems.¹⁴ For the longitudinal analyses, all children who had cotinine values at all 4 age points and brachial artery measurements at age 11 were included (n=327).

Background Data

At age 11, weight was measured with an electronic scale to the nearest 0.1 kg (Soehnle S10; Soehnle, Leifheit AG, Nassau, Germany) and height to the nearest 0.1 cm with a Harpenden stadiometer (Holtain, Crymych, United Kingdom), and the body mass index was calculated. Children's sexual maturation was classified by Tanner staging.¹⁶ Blood pressure was measured 3 times from the right arm with a standard sphygmomanometer, and the average was used in the analyses. Serum cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations were analyzed by standard methods.¹⁷ Serum low-density lipoprotein (LDL) cholesterol values were calculated with the Friedewald formula.¹⁸ Serum high-sensitivity C-reactive protein (hsCRP) concentrations were assayed by an immunoturbidimetric method, as described previously.¹⁹ During each child's 11-year-old visit, all attending parents (331 mothers and 239 fathers) answered a self-administered questionnaire about their smoking habits.

Ultrasound Studies

All studies were performed by an experienced vascular sonographer using an Acuson Sequoia 512 mainframe (Acuson, Mountain View, Calif) with a 13.0-MHz linear-array transducer. As described previously,²⁰ brachial artery diameter was determined from B-mode ultrasound images at rest, during reactive hyperemia, and after

administration of sublingual nitrate. A resting scan was performed, and arterial flow velocity was measured with a Doppler signal. Increased flow was then induced by inflation of an adult-sized blood pressure cuff around the forearm to a pressure of 250 mm Hg for 4.5 minutes, followed by release. A second scan was taken continuously 40 to 180 seconds after cuff deflation. The same reader blinded to the subject's details analyzed all ultrasound scans. The percentage of dilation from baseline in 10-second intervals was assessed. From these data, peak flow-mediated dilation (FMD) and total dilation, defined as the area under the dilation response versus time curve between 40 and 180 seconds after hyperemia (AUC), were determined. The brachial artery dilation response induced by this method is mainly mediated by nitric oxide (NO) released from arterial endothelial cells, because the response can be blocked by simultaneous infusion of arginine analogues.²¹ AUC was measured to assess more comprehensively the vasodilatory response. This variable correlated significantly with FMD ($R=0.9$, $P<0.001$).

Maximum artery diameter 5 minutes after 2 sublingual doses (50 μg plus 200 μg) of nitroglycerin administered 5 minutes apart was used to calculate nitrate-mediated (endothelium-independent) dilation. The dilation response to exogenous nitrates is thought to be endothelium independent and to reflect vascular smooth muscle responsiveness.²² In our laboratory, the interobserver coefficient of variation (CV) of FMD measurements was 8.6%, and the between-study CV was 9.3%.²³

Serum Cotinine Measurement

Serum samples were stored at -70°C until analyzed. Cotinine was extracted into dichloroethane from 0.2 mL of serum to which 0.2 mL of 5-methylcotinine (0.1 $\mu\text{g}/\text{mL}$ in 0.01 mol/L HCl) was added by the method of Feyerabend and Russell.²⁴ Concentrated extract (0.2 μL) was injected into the Hewlett Packard FFAP silica capillary column of the Shimadzu model GC-17 gas chromatograph, equipped with a nitrogen-sensitive flame-thermionic detector.²⁵ The analytical sensitivity of the method was 0.16 ng/mL. The intra-assay and interassay CVs at a cotinine concentration of 22 ng/mL were 4.4% and 11.7%, respectively. The interassay CV at a cotinine concentration of 1 ng/mL was 23.3%.

Statistical Methods

The distribution of the STRIP children's serum cotinine concentrations is highly skewed,²⁵ with the majority of the children having undetectable cotinine concentrations and a large number of the children having very low cotinine concentrations, indicative of rare exposure to tobacco smoke. In addition, children with the highest cotinine concentrations have been exposed only modestly. Thus, to analyze the effect of passive smoking on the ultrasound measures, children were divided into 3 groups. Because the functional sensitivity of the chromatographic method (interassay CV $<20\%$) was between 1 and 2 ng/mL, the top-decile cotinine group (n=39) was composed of 11-year-old children representing the highest 10th percentile of cotinine concentrations (serum cotinine ≥ 1.7 ng/mL). The noncotinine group (n=229) comprised children with cotinine concentrations under the detection limit of the assay (57% of the 11-year-old children), and the low-cotinine group (n=134) comprised the remainder of the children.

To study whether prolonged exposure of children to tobacco smoke is associated with endothelial function, longitudinal data from a subgroup of children with all 4 cotinine measurements between the ages of 8 and 11 years (n=327) were analyzed. In line with the aforementioned grouping, children with serum cotinine concentrations in the highest decile in at least 2 of the annual measurements (n=20) constituted the group of children with repeatedly high cotinine values, and the other extreme group comprised children with cotinine concentrations that were never in the highest decile (persistently low cotinine values, n=211). The remainder of the children constituted the group of children with remittent cotinine values. For comparison, only 1 child had a cotinine value in the highest decile 4 times during follow-up.

Results are expressed as mean (SD). Cotinine, triglyceride, and hsCRP values were log-transformed for the analyses. The nonpara-

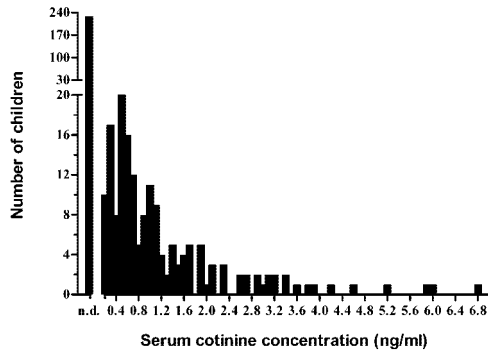


Figure 1. Distribution of serum cotinine concentrations of 11-year-old children with both cotinine and ultrasound variables measured ($n=402$). n.d. indicates nondetectable cotinine values ($n=229$). Children with measurable cotinine concentrations were divided into 2 groups: the top-decile cotinine group (the highest 10th percentile of cotinine concentrations, serum cotinine between 1.7 and 6.8 ng/mL, $n=39$) and the low-cotinine group (serum cotinine between 0.2 and 1.6 ng/mL, $n=134$). Vertical line shows cotinine concentration cutoff point for the top decile.

metric Mann-Whitney U test was used to compare serum cotinine values of children with and without ultrasound data. The Pearson correlation coefficient was used to study the association between ultrasound measures. The associations between serum cotinine levels and ultrasound variables were examined with single-predictor regression models. The effect of possible confounding factors was controlled with multivariable analyses conducted in 2 phases. First, single-predictor regression analysis was used to examine the relationships of background variables and cotinine levels or ultrasound measures. The Cochran-Mantel-Haenszel method for nonzero correlation was used for association of categorical background variables with categorical cotinine levels. All variables showing considerable effect ($P<0.10$) in these primary models were included as covariates in the further analyses of ultrasound measures, conducted with multivariable regression models with a backward stepwise selection method with exclusion criteria $P>0.10$. Cotinine levels were entered

in these regression models with consecutive values (nondetectable cotinine=0, low cotinine=1, top-decile cotinine=2). To assess the sensitivity of the results to the coding of the cotinine group variable, we also analyzed the cross-sectional data using geometric cotinine mean values of the groups (nondetectable cotinine=0, low cotinine=0.6, top-decile cotinine=2.8) in regression models with similar results. Repeated-measures ANOVA was used to test whether the magnitude of FMD responses measured between 40 and 180 seconds after cuff release differed between the cotinine groups. Results were considered significant at a value of $P<0.05$. Statistical analyses were performed with the SPSS 11.0 package for Windows (SPSS, Chicago, Ill) and SAS software, release 9.1.3 (SAS Institute, Cary, NC).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

At 11 years of age, 402 children (73% of the age cohort) had both cotinine values and ultrasound variables measured. Serum cotinine concentration was above the detection limit (0.16 ng/mL) in 43% of the children (Figure 1) and showed no difference between genders ($P=0.45$) or between the intervention and control groups of the project ($P=0.20$). Comparison of serum cotinine concentrations between children with and without ultrasound data revealed no significant difference ($P=0.70$). None of the children reported active smoking. Sixteen percent of the mothers and 25% of the fathers smoked either daily or occasionally. The smoking frequencies of intervention and control parents showed no significant difference ($P=0.14$ for mothers, $P=0.77$ for fathers).

Eleven-Year-Old Children in 3 Cotinine Groups

The characteristics of the 11-year-old children in the 3 cotinine groups are shown in Table 1. Cotinine values ranged from 1.7 to 6.8 ng/mL (geometric mean 2.8 ng/mL) in the

TABLE 1. Characteristics of 11-Year-Old Study Children in 3 Cotinine Groups

	Nondetectable Serum Cotinine Concentration (n=229)	Low Serum Cotinine Concentration (n=134)	Top-Decile Serum Cotinine Concentration (n=39)	P for Trend
Proportion of boys, %	53	57	38	0.38
In STRIP intervention group, %	51	44	44	0.19
Cotinine, ng/mL*	Nondetectable	0.6 (0.56–0.68)	2.8 (2.43–3.12)	...
BMI, kg/m ²	17.9±2.8	18.0±2.8	18.1±2.4	0.58
Systolic blood pressure, mm Hg	104±8	105±9	103±7	0.97
Diastolic blood pressure, mm Hg	63±5	64±6	63±5	0.98
Total cholesterol, mmol/L	4.44±0.74	4.54±0.76	4.61±0.64	0.10
LDL cholesterol, mmol/L	2.77±0.63	2.85±0.67	2.87±0.67	0.21
HDL cholesterol, mmol/L	1.30±0.27	1.34±0.29	1.25±0.21	0.95
Triglycerides, mmol/L*	0.77 (0.73–0.82)	0.73 (0.67–0.78)	0.80 (0.71–0.91)	0.79
Pubertal, %	59	54	59	0.56
hsCRP, mg/L*	0.35 (0.30–0.42)	0.31 (0.25–0.38)	0.34 (0.23–0.51)	0.55
Brachial baseline diameter, mm	2.9±0.3	3.0±0.3	3.0±0.4	0.11
Hyperemia, %	358±155	354±153	338±156	0.52
Maximal nitrate-mediated dilation, %	11.95±4.69	12.55±4.89	10.55±4.41	0.57

Hyperemia indicates percent increase in blood flow after cuff release. Values are mean±SD unless otherwise indicated. BMI indicates body mass index; HDL, high-density lipoprotein.

*Geometric mean (95% CI), logarithmic transformation used in statistical analyses.

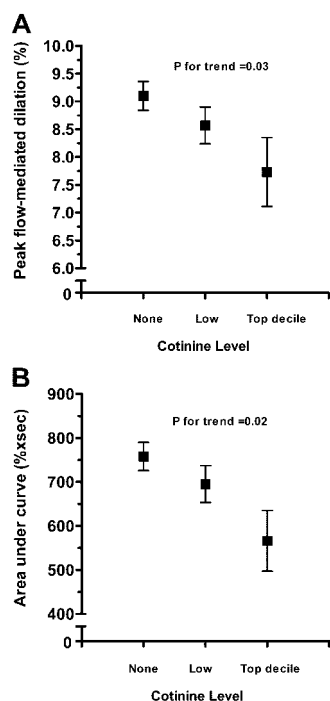


Figure 2. Peak FMD of brachial artery (A) and AUC (B) in 11-year-old children according to cotinine group. Values are mean \pm SEM.

top-decile cotinine group and from 0.2 to 1.6 ng/mL (geometric mean 0.6 ng/mL) in the low-cotinine group. The proportion of boys or the proportion of children belonging to the STRIP intervention group did not differ in the cotinine groups. No significant trend across cotinine groups was observed in BMI, blood pressure, serum lipids, the proportion of pubertal children, or hsCRP. In addition, brachial baseline diameter, increase in blood flow after cuff release, and endothelium-independent (maximal nitrate-mediated) dilation were similar across cotinine groups. Results shown in Table 1 remained similar when geometric cotinine means of the groups were used in the analyses (data not shown).

Peak endothelium-dependent (flow-mediated) dilation showed a significant trend across the 3 cotinine-level groups (nontcotinine group 9.10% [3.88%]; low-cotinine group 8.57% [3.78%]; and top-decile cotinine group 7.73% [3.85%]; $P=0.03$ for trend; Figure 2A). Similarly, AUC was affected by the cotinine level (nontcotinine group 759% \cdot s [491% \cdot s]; low-cotinine group 695% \cdot s [488% \cdot s]; and top-decile cotinine group 566% \cdot s [434% \cdot s]; $P=0.02$ for trend; Figure 2B). When the geometric cotinine means were entered in the regression models instead of groups represented by consecutive values, the results remained unchanged (FMD $P=0.03$ for trend; AUC $P=0.02$ for trend).

Figure 3 shows that the temporal development of the FMD responses measured between 40 and 180 seconds after cuff release was similar across the cotinine groups, but the magnitude of the response was marginally reduced in children with higher cotinine values (effect of group $P=0.054$).

LDL cholesterol was inversely associated with AUC ($\beta=-74.1$, $P=0.048$; Table 2). No other significant associations between measures of endothelial function and estab-

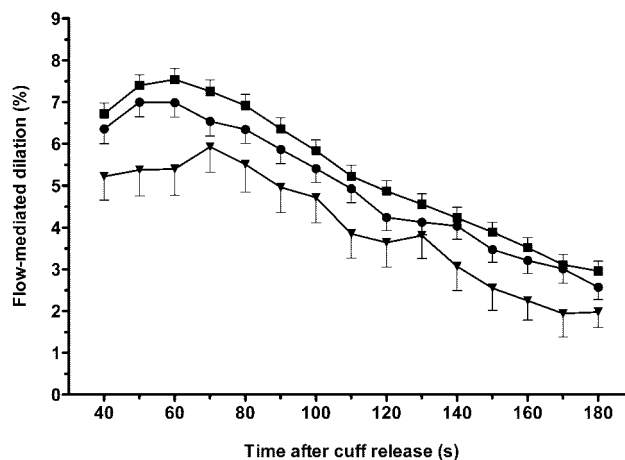


Figure 3. Temporal development of brachial artery FMD responses (mean \pm SEM) of 11-year-old children in the 3 cotinine groups. The top-decile group ($n=39$) is marked with triangles (\blacktriangle), the low-cotinine group ($n=134$) with circles (\bullet), and children belonging to the nontcotinine group ($n=229$) with squares (\blacksquare). The temporal development of the FMD responses was similar across cotinine groups, but the magnitude of the response was marginally reduced in children with higher cotinine values (effect of group $P=0.054$).

lished risk factors were found. In the multivariable regression model, controlling for LDL cholesterol had no effect on the association between an increase in cotinine concentration and impaired FMD (adjusted $P=0.03$ for trend) or decreased AUC (adjusted $P=0.03$ for trend). Respective probability values were 0.03 and 0.02 when geometric cotinine means were used in regression models.

Children With Longitudinal Cotinine Values

The characteristics of the children with all 4 cotinine measurements between the ages of 8 and 11 years did not differ across the cotinine groups with regard to gender, STRIP study group, puberty staging, BMI, blood pressure, serum lipid values, hsCRP, brachial baseline diameter, increase in blood flow after cuff release, or maximal nitrate-mediated dilation (Table 3). FMD and AUC were attenuated in children with several high cotinine concentrations during follow-up (FMD: persistently low cotinine values, 9.14% [4.00%]; remittent cotinine values 8.33% [3.61%]; and repeatedly high cotinine values 7.14% [3.20%]; $P=0.01$ for trend; Figure 4A; AUC: persistently low cotinine values 759% \cdot s [495% \cdot s]; remittent cotinine values 638% \cdot s [476% \cdot s]; and repeatedly high cotinine values 530% \cdot s [379% \cdot s]; $P=0.008$ for trend; Figure 4B). Figure 5 shows that the temporal development of the FMD responses measured between 40 and 180 seconds after cuff release differed in cotinine groups (time-by-group interaction $P<0.001$), and the magnitude of the responses was reduced in children with repeatedly high cotinine values (effect of group $P=0.02$). In the multivariable regression model, the association between decreased FMD and decreased AUC and several high cotinine values was unchanged after adjustment for LDL cholesterol (FMD adjusted $P=0.01$ for trend; AUC adjusted $P=0.007$ for trend).

Discussion

We have shown that endothelial function, a measure of arterial health, is attenuated in children who have been

TABLE 2. Single-Predictor Regression Analysis for Determinants of Peak FMD and Total Dilation Response (AUC)

Explanatory Variable	No.	FMD		AUC	
		Regression Coefficient- β	<i>P</i>	Regression Coefficient- β	<i>P</i>
Male gender	402	0.236	0.54	68.5	0.16
STRIP control group	402	-0.502	0.19	-18.5	0.70
Pubertal	402	0.064	0.87	14.3	0.77
BMI, kg/m ²	402	0.076	0.28	8.2	0.35
Systolic blood pressure, mm Hg	392	0.013	0.60	2.3	0.44
Diastolic blood pressure, mm Hg	392	0.025	0.50	2.2	0.64
Total cholesterol, mmol/L	400	0.054	0.84	8.8	0.79
LDL cholesterol, mmol/L	400	-0.447	0.13	-74.1	0.048
HDL cholesterol, mmol/L	400	-0.578	0.42	-74.9	0.41
Triglycerides, mmol/L*	400	0.617	0.16	61.0	0.27
hsCRP, mg/L*	402	0.061	0.68	-0.3	0.99

BMI indicates body mass index; HDL, high-density lipoprotein.

*Logarithmic transformation.

exposed to environmental tobacco smoke compared with unexposed children. The present data suggest that passive smoking alters endothelial function in childhood and thus has a role in the development of early atherosclerosis. The association between tobacco smoke exposure and decreased endothelium-dependent dilation was dose dependent, although the exposure to tobacco smoke even in the top-decile cotinine group was not very extensive.

Long-term tobacco smoke exposure impairs arterial health both in active smokers¹¹ and in passive smokers.^{12,26} Celer-

majer et al¹² showed that passive smoking is associated in a dose-dependent manner with significant endothelial dysfunction in teenagers and young adults. Exposure to tobacco smoke was determined by self-report, and the passive smoking group was exposed for at least 1 hour daily for at least 3 years. FMD was similarly impaired in passive and active smokers compared with control subjects. Woo et al²⁶ found that in young adults, heavy passive smoking (>8 hours daily for at least 2 years) in the workplace is associated with impaired endothelial function. The present study confirms

TABLE 3. Characteristics of 11-Year-Old Study Children With Serum Cotinine Measured 4 Times Between Ages of 8 and 11 Years

	Persistently Low Serum Cotinine Concentration (n=211)	Remittent Serum Cotinine Concentration (n=96)	Repeatedly High Serum Cotinine Concentration (n=20)	<i>P</i> for Trend
Proportion of boys	54	53	30	0.14
In STRIP intervention group, %	50	50	40	0.59
Cotinine, ng/mL*	Nondetectable	0.2 (0.16–0.24)†	0.7 (0.37–0.94)†	...
BMI, kg/m ²	17.9±2.8	18.0±2.8	17.7±2.5	0.98
Systolic blood pressure, mm Hg	104±8	104±9	104±10	0.99
Diastolic blood pressure, mm Hg	63±5	63±6	64±5	0.69
Total cholesterol, mmol/L	4.49±0.79	4.45±0.68	4.66±0.80	0.75
LDL cholesterol, mmol/L	2.82±0.69	2.74±0.60	2.99±0.67	0.92
HDL cholesterol, mmol/L	1.30±0.25	1.31±0.30	1.30±0.31	0.78
Triglycerides, mmol/L*	0.75 (0.71–0.80)	0.78 (0.71–0.86)	0.76 (0.66–1.12)	0.52
Pubertal, %	63	58	53	0.24
hsCRP, mg/L*	0.37 (0.31–0.44)	0.28 (0.21–0.36)	0.38 (0.22–0.64)	0.29
Brachial baseline diameter, mm	2.9±0.3	3.0±0.3	2.8±0.3	0.94
Hyperemia, %	368±150	343±164	378±145	0.51
Maximal nitrate-mediated dilation, %	12.58±4.97	11.35±4.90	12.79±4.23	0.29

Values are mean±SD, unless otherwise indicated. BMI indicates body mass index; HDL, high-density lipoprotein.

Persistently low serum cotinine concentration indicates cotinine concentration never in the top decile during follow-up; remittent serum cotinine concentration, cotinine concentration once in the top decile during follow-up; repeatedly high serum cotinine concentration, cotinine concentration in the top decile 2 to 4 times during follow-up; and hyperemia, percent increase in blood flow after cuff release.

*Geometric mean (95% CI), logarithmic transformation used in statistical analyses.

†Cotinine values are geometric means of the 4 cotinine concentrations measured between ages 8 and 11 years.

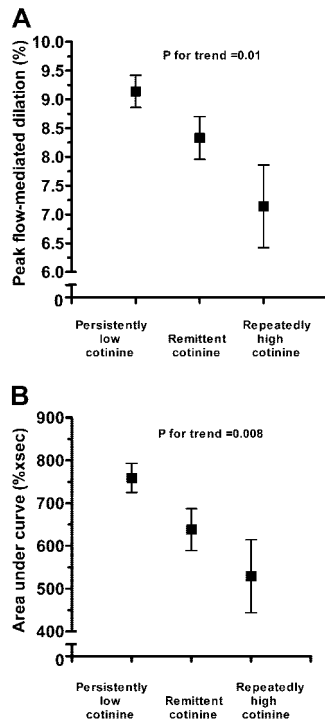


Figure 4. Peak FMD of brachial artery (A) and AUC (B) in children with serum cotinine measured 4 times between ages 8 and 11 years. Values are mean \pm SEM.

these findings in children using an objective biomarker to verify tobacco smoke exposure.

Short-term tobacco smoke exposure has also been associated with endothelial dysfunction in adults. Lekakis et al²⁷

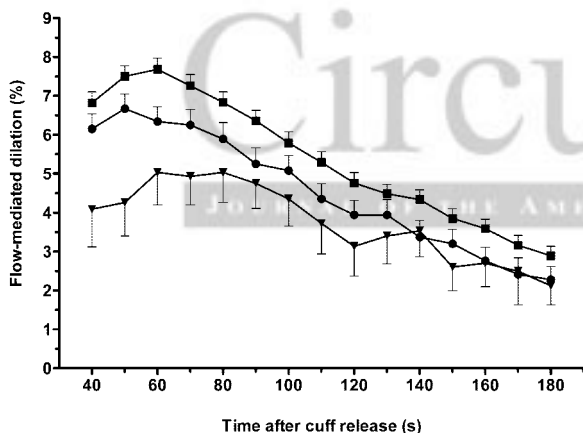


Figure 5. Temporal development of brachial artery FMD responses (mean \pm SEM) in 11-year-old children with longitudinal cotinine values between the ages of 8 and 11 years. The group of children with repeatedly high cotinine values (cotinine concentration in the highest decile at least 2 times, n=20) is marked with triangles (\blacktriangledown), children with remittent cotinine values (cotinine concentration once in the highest decile, n=96) are marked with circles (\bullet), and children with persistently low cotinine values (cotinine concentration never in the highest decile, n=211) are marked with squares (\blacksquare). The temporal development of FMD responses differed in cotinine groups (time-by-group interaction $P<0.001$), and the magnitude of the response was reduced in children with repeatedly high cotinine values (effect of group $P=0.02$).

showed that smoking of 1 cigarette was associated with significant impairment of flow-mediated arterial dilation, and the response persisted for at least 60 minutes. Otsuka and colleagues,²⁸ in examining the short-term effects of passive smoking on endothelial function in the coronary circulation, found that 30 minutes of passive smoke exposure in non-smokers led to impairment of coronary flow velocity reserve, similar to the effect seen in smokers.

Exposure to environmental tobacco smoke is associated with increased risk of coronary heart disease,^{29,30} and one mechanism among multiple cardiovascular effects of second-hand smoke is endothelial dysfunction.² The brachial artery dilation caused by the sudden increase in flow is endothelium dependent and largely mediated by NO secreted from endothelial cells.²¹ In reaction to tobacco smoke, production of NO is decreased.³¹ Animal studies have shown that passive smoking reduces endothelium-dependent vasorelaxation, and dietary supplementation of an NO donor such as L-arginine improves endothelium-dependent dilation.³² In line with these observed tobacco-related effects on NO biosynthesis, we found that exposure to tobacco smoke was associated with impaired flow-mediated vasodilation, which is considered an NO-dependent response, but not with a direct smooth muscle dilation response produced by administration of an exogenous NO donor. Reduced endothelial NO may precede development of atherosclerosis,³³ because NO inhibits several pathophysiological phenomena (ie, platelet aggregation, smooth muscle proliferation, and monocyte adhesion) that may participate in the development of vascular disease.³⁴

Environmental tobacco smoke contains more than 4000 components, many of which are toxic and carcinogenic.^{3,35} The majority of environmental tobacco smoke is sidestream smoke, which contains many toxic substances in higher concentrations than in mainstream smoke because of different combustion conditions.³⁶ Thus, many effects of passive smoking occur at very low exposure levels.²

Several factors influence the intensity of exposure to environmental tobacco smoke, such as the number of cigarettes smoked in the room, exposure time, proximity to the smoker, surface materials, ventilation, and the size of the room.¹² Parents smoking at home are mostly responsible for children's exposure to tobacco smoke^{37,38} but other people's smoking also affects children's exposure³⁹ and becomes increasingly important when children grow older.⁴⁰ We previously showed that although children's exposure to environmental tobacco smoke correlates with smoking by the parents, much variability is present in children's cotinine values both in smoking and in nonsmoking families.²⁵ Thus, we deliberately did not rely on parents' smoking as an indicator of their children's exposure to tobacco smoke but aimed to measure passive smoking with an objective method.

The strength of the present study is its prospective design in determining exposure to tobacco smoke. Passive smoking of children was defined by serum cotinine concentration, which reflects exposure to tobacco smoke during the previous 2 or 3 days, because the half-life of cotinine in serum is \approx 20 hours.⁴ Serum cotinine concentration is a suitable biomarker because it is a highly sensitive and specific reflector of the exposure.^{3,4} Children in the present study were not heavily

exposed to tobacco smoke. More than half had a nondetectable cotinine concentration, the majority had cotinine values near the detection limit, and even the highest cotinine values indicated only modest exposure. Exposure to tobacco smoke in these Finnish children appears to be less severe than previously reported in the United States and the United Kingdom.^{15,38}

The present study has limitations. Serum cotinine concentration characterizes only recent tobacco use and exposure to only 1 component (nicotine) of the smoke. Many of the deleterious effects of tobacco smoke on the cardiovascular system, including the endothelium, are caused by components other than nicotine.^{36,41} However, intake of nicotine reflects exposure to other constituents of environmental tobacco smoke reasonably well.⁴ We recognize that cotinine concentration is not a measure of past exposure, but so far, no methods are available to objectively quantify long-term exposure to tobacco smoke. However, the longitudinal design of the present study probably to some extent reflects children's long-term exposure to tobacco smoke.

One limitation is that the ultrasound study was performed only in a subgroup of the initial study cohort; however, serum cotinine concentrations did not differ in 11-year-old children with and without ultrasound measurements. Furthermore, we previously showed that those children who had complete ultrasound data did not differ significantly from the other children at baseline of the STRIP project or at the 11-year study with respect to anthropometry, serum lipoproteins, or blood pressure values.¹⁴ Multiple statistical tests were performed for brachial ultrasound data in the full study sample and in a subsample with repeated observations. This may increase type I error, and thus the results need to be interpreted cautiously.

As before,²⁰ we found an inverse association between LDL cholesterol and endothelial function in healthy children; however, the main results of the present study were unchanged when we controlled for LDL cholesterol. In addition, the other traditional cardiovascular risk factors presumably did not affect the main results, because no trends between risk factors and serum cotinine levels were found.

C-reactive protein may have a direct proatherogenic role by disturbing endothelial function,⁴² and cigarette smoking has been associated with CRP elevations.⁴³ In the present study, however, no significant trend was detected in hsCRP values across the cotinine groups, and arterial measures were not associated with hsCRP concentrations. Thus, mechanisms other than the induction of systemic inflammation probably contribute to the association between exposure to tobacco smoke and endothelial function in children.

Exposure to tobacco smoke has deleterious effects on endothelial function.^{12,28} Importantly, the present study suggests that this association may be seen already in 11-year-old children. In addition, according to the present data, even modest exposure to tobacco smoke alters endothelial function in children and may thus impact early development of atherosclerosis. Because endothelial dysfunction related to passive smoking may be only partially reversible after cessation of the exposure,⁴⁴ the present data strongly emphasize the

importance of implementing smoke-free environments for children at home and in public places.

Acknowledgments

We thank Mia Laakkonen and Marja-Leena Toukkari for skillful technical assistance.

Sources of Funding

This study was financially supported by Finnish Cardiac Research Foundation; Academy of Finland (grants 34316, 73583, 64/2003); Sigrid Juselius Foundation; Specified Government Transfer, Turku University Hospital; Yrjö Jahnsson Foundation; Juho Vainio Foundation; the Regional Fund of Southwestern Finland of the Finnish Cultural Foundation; Foundation for Pediatric Research; Turku University Foundation; Finnish Medical Foundation; and Research Foundation of Orion Corporation, Finland.

Disclosures

None.

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CLINICAL PERSPECTIVE

Atherosclerosis is known to begin in childhood, and arterial endothelial function might be involved in the early pathophysiology of atherosclerosis. Endothelial function, a marker of arterial health, can already be measured with a feasible noninvasive ultrasound method in children. Our results suggest that even modest exposure to tobacco smoke may affect the arteries of healthy children by disturbing endothelial function. The association between tobacco smoke exposure and decreased endothelium-dependent flow-mediated vasodilation was dose dependent, which suggests that even a slight amount of passive smoke exposure may be hazardous. However, the long-term effects and possible reversibility of endothelial dysfunction of children remain to be explored. In addition to showing the effects of passive smoking on endothelial function, exposure to tobacco smoke has numerous adverse effects on the cardiovascular system. Importantly, passive smoking is a preventable risk factor of cardiovascular diseases. Thus, clinicians should ask and advise about involuntary tobacco smoke exposure. The importance of creating smoke-free environments for children and adolescents both at their homes and in public places is supported by this study.