The effect of chronic tobacco smoking on arterial stiffness

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Aims

Cardiovascular disease caused by smoking is related to the pathophysiological burden placed on the vascular endothelium. We studied the effect of chronic cigarette smoking on arterial wave reflection (study 1) and smoking cessation on pulse wave analysis (study 2).

Methods

Fifty smokers and 50 age- and sex-matched nonsmokers participated in study 1. Study 2 recruited 20 volunteers from the stop smoking clinic at the Royal Hallamshire Hospital, Sheffield, UK. Systemic augmentation index (Alx) and carotid-femoral pulse wave velocity (PWV) were measured using the SphygmoCor system. Brachial blood pressure (BP) (Omron 705-CP-E), Alx and PWV were recorded at a single visit in study 1. Study 2 measured these variables on 'quit day' and 4 weeks later.

Results

In study 1, Alx was significantly higher in smokers than in nonsmokers (median 17.25 vs. 11.75%, $P = 0.004$). Multiple regression analysis showed a significant correlation between Alx and age, diastolic BP, smoking status ($P < 0.001$), blood glucose ($P = 0.045$) and weight ($P = 0.049$). In study 2, Alx significantly reduced after 4 weeks of abstinence in successful quitters ($n = 10$) compared with relapsed smokers ($n = 4$) (median 5.0 vs. -9.5 ; $P = 0.013$). PWV did not reach significance in either study.

Conclusions

Chronic tobacco smoking is associated with endothelial dysfunction and increased Alx in subjects of a wide age range free from additional cardiovascular risk factors, which is partially reversible after 4 weeks of smoking cessation.

Introduction

Smoking is one of the leading modifiable risk factors for cardiovascular disease [1]. Much of the increased cardiovascular risk caused by smoking may be due to adverse effects on the vascular endothelium, induction of coronary vasoconstriction and changes in basal nitric oxide (NO) or endothelial nitric oxide synthase (eNOS) protein production [1–3]. Mahmud and Feely

have demonstrated that smoking a single cigarette produces an acute increase in arterial stiffness in chronic smokers and nonsmokers [4]. Vlachopoulos *et al.* have shown similar findings where a significant undesirable combined effect of smoking and caffeine was found on arterial stiffness [5]. Increased carotid arterial stiffness and systemic augmentation index (AIx) have also been previously reported following

exposure to environmental tobacco smoke in non-smokers [6, 7].

The risk of myocardial infarction (a major endpoint of cardiovascular disease) is known to return to normal levels within a few years of quitting smoking, suggesting that the cardiovascular damage caused by smoking may be reversible [8]. It may therefore be hypothesized that reduced basal NO secretion associated with smoking is also reversible and that endothelial function returns to normal after quitting smoking. Measurement of endothelial dysfunction may be a potential target for cardiovascular risk factor modification [9]. Arterial stiffness is emerging as an important cardiovascular risk factor predominately due to new non-invasive technologies which enable measurements to be taken in large-scale clinical trials. The shape of the arterial pressure waveform provides a measure of systemic arterial stiffness and can be assessed using the technique of pulse wave analysis (PWA) [10]. It has been demonstrated as a reproducible method for determining AIx and pulse wave velocity (PWV) [10, 11]. Arterial stiffness can be used as a surrogate measure of endothelial function since it is partially dependent on vascular smooth muscle tone [12, 13]. PWV measures large artery stiffness; carotid-femoral PWV is considered to be the most clinically relevant because the aorta and its first branches are responsible for regulating blood pressure in the periphery and maintaining diastolic coronary artery flow. PWA has previously been used to demonstrate increased arterial wave reflection in a cohort of healthy young smokers compared with nonsmokers [4].

We have performed two studies to investigate the effect of smoking on arterial stiffness. Study 1 investigated the effect of chronic cigarette smoking in healthy volunteers aged 18–60 years on systemic AIx and PWV in 50 smokers vs. 50 age- and sex-matched nonsmoking controls. Study 2 collected preliminary data describing the effect of smoking cessation on arterial stiffness and endothelial function using PWA at baseline and 4 weeks post quit day.

Methods

Subject population

Study 1 One hundred volunteers aged between 18 and 60 years (mean $\pm$ SD 37.9 ± 11.4) with a body mass index (BMI) of 19.2–39.2 (25.8 ± 3.9) participated in the study. Fifty were smokers who had smoked 10 cigarettes or more (16.2 ± 5.1) per day for at least 1 year prior to recruitment. The remaining 50 volunteers were age- (within 5 years) and sex-matched nonsmokers who had not smoked at all over the past year. Thirteen had

been smokers, with 10 stopping between 5 and 27 years and three between 1.5 and 5 years before recruitment. All participants were screened by medical history and blood tests [serum total cholesterol, high-density lipoprotein (HDL)-cholesterol, glucose, creatinine, C-reactive protein (CRP)] to exclude those with pre-existing disease that may have confounded the PWA results [10]. Serum nicotine and cotinine levels were determined by mass spectrometry based on the method described by Stolker *et al.* [14]. Smokers had their smoking status confirmed by expired carbon monoxide (CO) testing (Bedfont Scientific Ltd, Rochester, UK). A subset of volunteers ($n = 26$ matched pairs) provided blood samples for high-sensitivity CRP analysis (hsCRP; Biocheck, Inc., Burlingame, CA, USA). The study protocol had local ethics committee approval and all subjects provided written informed consent.

Study 2 Twenty patients who were enrolled into the stop smoking clinic at the Royal Hallamshire Hospital, Sheffield, UK took part in the study. Subjects were aged 57.2 ± 10.4 years and had a BMI of 27.9 ± 5.7 . Eight (40%) subjects had established cardiovascular disease, 12 (60%) had respiratory disease and were taking 6.1 ± 5.6 concomitantly prescribed drugs. At baseline, subjects smoked 22.2 ± 9.4 cigarettes per day for 37.1 ± 17.3 years, had a mean heart rate of 66 ± 11 beats per minute and brachial blood pressure of $127/76 \pm 15/9$ mmHg. All subjects receiving nitrate medication were excluded, but other cardiovascular drugs were allowed as patients were acting as their own control (drug treatment was kept constant throughout the study). Successful quitters were defined as patients who were not smoking by 4 weeks after quit date and had used less than 10 cigarettes during this time. The study protocol had local ethics committee approval and all subjects provided written informed consent.

Experimental protocol

Study 1 To avoid measuring the acute effects of smoking and dietary nitrates, subjects fasted for 12 h from food, smoking, alcohol and caffeine-containing beverages. Subjects were studied supine in a quiet room after 5 min rest. PWA was performed at the radial pulse to determine AIx and the carotid and femoral pulses to determine PWV. All measurements were repeated, with AIx measurements standardized for heart rate at 75 beats per minute [10].

Study 2 Patients were asked to attend study visits on quit day and 4 weeks later. A detailed dietary history of

the previous 48 h was obtained at baseline in order to give an indication of dietary nitrate consumption. All volunteers were asked to eat an equivalent nitrate diet during the 48 h before their next study visit, and to follow a similar procedure for caffeine and alcohol consumption. Smoking status was assessed and documented at each visit.

Blood pressure measurements Brachial blood pressure (BP) was recorded supine in a quiet room at room temperature after 5 min rest using an automated digital oscillometric BP monitor (Omron 705-CP-E; Omron Corp., Tokyo, Japan). Three readings separated by 1-min intervals were taken and the mean of the last two readings were recorded. The estimated arterial BP was determined following analysis of the pressure waveform by the SphygmoCor software version 6.31 (AtCor Medical, Sydney, Australia).

Analysis of the arterial pressure waveform AIx and PWV were determined by applanation tonometry following BP measurements. A high-fidelity micro pressure probe (SPC-301; Millar Instruments, Houston, TX, USA) was used to record systemic arterial stiffness at the radial artery and aortic pulse wave velocity by analysing the arterial pressure wave at the carotid and femoral arteries. The SphygmoCor software automatically captures the pressure waveforms and has a built-in quality control [10], and has been used to record smoking-related changes in arterial stiffness [4, 5, 7].

Statistical analyses

In Study 1, AIx and PWV were studied between matched pairs using the Wilcoxon signed-rank nonparametric test. Multiple regression analysis was used to identify factors associated with changes in AIx and PWV. We were unable to obtain AIx readings from $n = 2$ subjects, PWV readings from $n = 12$ subjects and were missing data for one or more of the regression model predictors from $n = 10$ subjects, which is consistent with other PWA studies [15]. Two matched pairs were excluded from the Wilcoxon AIx analysis ($n = 96$) and 10 matched pairs were excluded from the PWV analysis ($n = 80$). A sample size of $n = 88$ was employed in the AIx model and $n = 78$ subjects were included in the PWV model. High-sensitivity CRP levels were compared between a subset of smokers and nonsmokers using the Wilcoxon signed-rank test ($n = 52$). In study 2, subjects were divided into successful quitters and relapsed smokers. Changes in AIx and PWV between baseline and 4 weeks post quit day were analysed using the Mann–Whitney nonparametric test. Six patients did not attend their 4-week study visits and were assumed

to have gone back to regular tobacco smoking. Five successful quitters and two relapsed smokers were excluded from the PWV analyses ($n = 7$) due to difficulties palpating the arterial study sites. Minitab™ software version 14.12.0 (Minitab Inc., State College, PA, USA) was used to carry out all statistical tests. $\alpha = 0.05$ was used in all cases.

Results

Study 1

Baseline characteristics of the volunteers are shown in Table 1. There was no significant difference in age, gender, height, weight, BMI, creatinine, glucose, hsCRP or total cholesterol between the matched pairs, although the smokers did have a significantly lower HDL-cholesterol, higher total : HDL-cholesterol ratio and a higher alcohol intake than the nonsmokers. AIx in smokers was significantly higher than that in nonsmokers [median 17.25 vs. 11.75%, median difference 5.5, 95% confidence interval (CI) 2.0, 8.75; $P = 0.004$]. Figure 1 demonstrates the increasing proportion of smokers with higher AIx readings compared with nonsmokers. However, PWV was not significantly different between the two groups (median nonsmokers 5.6 vs. smokers 5.9 m s⁻¹, median difference 0.225, 95% CI -0.55, 0.075; $P = 0.15$).

Multiple regression analysis demonstrated a significant positive association between AIx and age, diastolic BP and smoking status, and a negative association with glucose and weight (Table 2). The model explained 78.3% of the variability in AIx observed in the study ($P < 0.001$). Multiple regression analysis for PWV investigating the same predictors as for AIx and including height [16] showed that age was the only significant predictor ($P < 0.001$).

Matched-pair analysis in the 52 subjects with serum hsCRP levels did not show a significant difference between nonsmokers and smokers (median nonsmokers 1.2 vs. smokers 1.5 mg l⁻¹, median difference 0.10, 95% CI -0.79, 0.42, $P = 0.48$). Univariate regression analysis indicated that hsCRP levels independently predicted aortic PWV ($P = 0.028$) and approached significance for AIx ($P = 0.059$).

Study 2

At 4 weeks post quit day, 10 patients had successfully reduced their smoking to <10 cigarettes over the 4-week period (0 cigarettes, $n = 6$; 1–3 cigarettes, $n = 2$; 4–6 cigarettes, $n = 2$). Four patients had smoked 10 or more cigarettes during this time (10–20 cigarettes, $n = 2$; 20+ cigarettes, $n = 2$). Six patients did not attend the 4-week follow-up study visit and were withdrawn from the study.

Table 1

Study 1: subjects' baseline characteristics

	Smokers (n = 50)	Nonsmokers (n = 50)	P-value
Gender (M:F)	15 : 35	15 : 35	
Age (years)	37.8 ± 11.8	38.1 ± 11.1	
Height (cm)	1.7 ± 0.1	1.7 ± 0.1	
Weight (kg)	74.6 ± 15.8	71.7 ± 12.6	
Body mass index	26.3 ± 4.1	25.3 ± 3.5	
Creatinine (mmol l ⁻¹)	74.1 ± 13.4	77.9 ± 15.8	
Glucose (mmol l ⁻¹)	5.3 ± 1.1	5.0 ± 0.8	
Cholesterol			
Total cholesterol (mmol l ⁻¹)	5.1 ± 1.1	4.8 ± 1.1	
HDL-cholesterol (mmol l ⁻¹)	1.3 ± 0.4	1.4 ± 0.4	0.012
Total:HDL ratio	4.3 ± 1.6	3.5 ± 1.0	0.001
hsCRP (mg l ⁻¹)	1.42 ± 1.15	1.66 ± 1.31	
Alcohol consumption (units week ⁻¹)	9.8 ± 8.0	6.9 ± 6.3	0.037
Smoking status			
Daily cigarette consumption	16.2 ± 5.1	0 ± 0	<0.001
Pack years smoked	16.4 ± 12.7	1.3 ± 3.8	<0.001
Haemodynamics			
Heart rate (bpm)	61.0 ± 9.4	61.5 ± 9.7	
Brachial SBP (mmHg)	112.9 ± 14.9	112.2 ± 14.0	
Brachial DBP (mmHg)	70.0 ± 8.3	69.2 ± 9.2	
Alx* (%)	17.25 (–18.5 to 40)	11.75 (–22 to 35.5)	0.004
PWV* (m s ⁻¹)	5.9 (4.1–8.4)	5.6 (4.1–13.4)	

Data are mean ± SD. There were no significant differences between the groups unless P-value is stated. *Median (range) cited. HDL, High-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; Alx, augmentation index; PWV, pulse wave velocity.

Table 2

Study 1: results of the multiple regression analysis using augmentation index as the dependent variable (n = 88)

Predictor	B	β	P-values
Age	0.723	0.602	<0.001
Diastolic blood pressure	0.653	0.415	<0.001
Smoking status	6.106	0.223	<0.001
Blood glucose	–1.866	–0.126	0.045
Weight	–0.146	–0.158	0.049
Systolic blood pressure	–0.170	–0.181	0.091
Sex	4.523	0.154	0.168
Total : HDL-cholesterol ratio	0.713	0.069	0.328
Creatinine	–0.029	–0.032	0.680
Height	–3.882	–0.028	0.787
Alcohol	–0.027	–0.014	0.812

B, Unstandardized regression coefficient; β, standardized regression coefficient. HDL, High-density lipoprotein.

Figure 1

Study 1: augmentation index (Alx) (%) of age- and sex-matched nonsmokers (■) vs. smokers (■)

Table 3 Study 2: individual patient data recorded at baseline and 4 weeks post quit day ($n = 14$)

Age	Sex	Baseline (quit day)		Cotinine (ng ml ⁻¹)	Alx (%)	4 weeks post quit day		
		Cigarettes per day	Expired CO (p.p.m.)			% change expired CO	% change cotinine	Alx (%)
<i>Successful quitters (n = 10)</i>								
51.5	M	20	12	276	22	-92	-14	13
52.4	F	23	7	555	29	-100	-86	19
61.0	F	20	7	192	30	-86	-50	34
65.9	F	5	5	99	30	-100	+47*	37
66.6	F	20	1	350	31	+400†	-47	27
67.4	F	20	5	130	40	-100	-34	32
46.8	F	15	6	282	40	-83	-70	34
76.4	F	5	12	276	26	-100	-63	23
54.2	M	35	11	462	37	-91	-96	35
50.2	M	20	2	430	32	-100	-40	26
<i>Relapsed smokers (n = 4)</i>								
65.1	M	25	4	141	18	-75	-48	36
53.7	F	20	12	331	35	-42	-36	35
59.7	F	20	10	361	11	-100	-42	22
63.8	M	35	28	282	40	-100	-17	48

*Cotinine concentration increased 47% from baseline due to nicotine replacement therapy; smoking status validated by CO monitor. †Anomalous increase in expired CO to 5 p.p.m. at 4 weeks post quit day (probably due to passive smoking and very low baseline reading).

The change in AIx from baseline to 4 weeks post quit day was significantly different between the quitters and relapsed smokers (Table 3), quitters showing a reduced AIx following 4 weeks of abstinence from smoking (median change 5.0 vs. -9.5; difference 14.0, 96% CI 4.0, 22.0; Mann-Whitney $P = 0.013$). The change in PWV did not reach significance between the two groups.

Discussion

Many of the traditional cardiovascular risk factors, including smoking, have been associated with endothelial dysfunction, which may be the pathological process responsible for the development of cardiovascular disease [17]. Basal NO production and other measures of endothelial dysfunction are very difficult to measure non-invasively. However, NO is a potent smooth muscle relaxant and is a principal regulator of arterial smooth muscle tone [18]. AIx and pulse wave velocity are both indices of arterial stiffness that can be measured non-invasively using pulse wave analysis.

Mahmud and Feely have demonstrated that smoking a single cigarette produces an acute increase in arterial stiffness in chronic smokers and nonsmokers [4]. In the same paper, they examined the chronic effects of

smoking on AIx in a young population (mean age 23 ± 5 years) by comparing 41 smokers vs. 116 non-smokers. AIx was significantly higher in smokers, indicating increased wave reflection, but PWV and therefore the contribution of aortic arterial stiffness was not measured. In the current study, we used a matched-pair analysis to demonstrate a significantly higher AIx, but not PWV, in smokers. The effects on vascular tone of impaired endothelium-dependent vasodilation, previously demonstrated in smokers, are greater in peripheral muscular arteries than in central elastic arteries, where elastin is the major determinant of arterial stiffness. Thus, the effects of smoking on smooth muscle tone would be expected to affect systemic, but not necessarily aortic arterial stiffness, as our results have shown.

The magnitude of the difference in AIx between smokers and nonsmokers was similar to that observed in the previous study, which used a younger subject population. In addition, our multiple regression model showed that smoking was the strongest predictor of AIx after age and diastolic BP. Our data did not show a dose-response relationship between difference in smoking and nonsmoking AIx and cigarette consumption, so that light smokers and heavy smokers both had a similar

elevation in AIx compared with nonsmoking counterparts. This may suggest that smoking 10 cigarettes per day (the cut-off for inclusion as a smoker) is enough to induce marked endothelial dysfunction. Indeed, recent work by Barua *et al.* [2] found that basal NO production and eNOS expression and activity were all impaired to the same extent in light and heavy smokers.

A number of other factors are known to influence AIx and although efforts were made to match the smoking and control groups as closely as possible, HDL-cholesterol, total:HDL ratio and alcohol consumption were still significantly higher among the smoking cohort. The relationship between smoking and these variables has been reported previously [16, 19]. However, in our multiple regression model, total:HDL ratio and alcohol intake showed no significant association with either AIx or PWV, whereas smoking status was still a significant independent determinant of AIx ($P < 0.001$, Table 2).

In study 1, increased systemic arterial stiffness was demonstrated in healthy smokers when compared with nonsmoking controls. The next logical question is whether this effect is reversible on quitting smoking. There is some evidence that an improvement in endothelial function follows smoking cessation. One study demonstrated significantly reduced plasma NO levels in current smokers but no difference in former smokers and never smokers [20]. The time since stopping smoking among the former smokers ranged from 6 months to 12 years and there was no association with basal plasma NO levels. This suggests that most of the improvement in endothelial function may occur in the first few months after quitting [20]. This corroborated previously published data demonstrating an improved flow-mediated dilation in former smokers compared with current smokers, which approached the levels of nonsmoking controls [21]. In keeping with the observations of Node *et al.* [20], flow-mediated dilation was not related to time since smoking cessation among the former smokers.

Contrary to previously reported findings, our data failed to show an association between hsCRP levels and smoking status [22, 23]. However, both studies involved more than 700 subjects whilst our data involved only a subset of the study 1 participants ($n = 52$). The significant finding of hsCRP levels predicting aortic PWV in healthy volunteers is consistent with a recent study which also employed the SphygmoCor system to perform PWA [24].

In study 2, we found that AIx decreased after 4 weeks of smoking cessation in successful quitters. Conversely, subjects who resumed regular smoking demonstrated an overall increase in AIx at 4 weeks and the difference in AIx response after 4 weeks was significantly different between the quitters and smokers ($P = 0.013$). An inter-

esting finding was that AIx increased, rather than remaining unchanged, at 4 weeks with continued smoking. This may be explained by an acute effect of smoking on AIx [4, 5], since all subjects who were recruited into study 2 were asked to refrain from smoking overnight before attending the clinical unit, but could smoke freely up to their study visit if they had relapsed at 4 weeks. These measurements could therefore reflect an acute effect of smoking on systemic arterial stiffness.

Our findings suggest an almost immediate recovery of endothelial function, although this is difficult to determine in such small numbers and without further follow-up. A study of similar design, but not measuring PWA, indicated an improvement in basal NO production after only 1 week of smoking cessation [25]. It monitored the change in exhaled NO levels among 25 smoking cessation clinic attendees over an 8-week timescale. At baseline smokers showed a reduced basal exhaled NO compared with matched nonsmoking controls. Ten of the 25 smokers were abstinent after 8 weeks and showed an increase towards normal exhaled NO levels at 1 week, followed by levels at 8 weeks that were not significantly different from those of the nonsmokers. It should be noted that the exhaled NO was probably the product of NOS in lung epithelial cells rather than the vascular endothelium; however, recovery of epithelial NOS function may indicate similar improvement in the endothelium. Further large-scale studies are required to elucidate the time-related effects of smoking cessation on arterial properties, including follow-up beyond 12 weeks. In addition, attempts should be made to quantify the improvement by comparison with that of a healthy nonsmoker.

In both the above studies, arterial stiffness measured by pulse wave analysis was used to demonstrate impaired basal arterial tone among smokers, an indicator of endothelial dysfunction. It may provide a predictive role in risk assessment, a surrogate endpoint in clinical trials and a target for therapeutic intervention [9]. However, one limitation of the SphygmoCor apparatus was that readings could not be obtained for 14 subjects, due to a combination of faint radial, carotid or femoral pulses, and rejection of the pulse wave trace by the software's quality control algorithms. Such measurement difficulties have been cited previously in the literature [15]. In addition, there is some debate among experts in the field regarding the use of a single general transfer function to estimate central pulse waveforms [26, 27].

In summary, these studies have shown that chronic smoking affects systemic arterial stiffness, as measured by AIx, in healthy subjects free from other cardiovascular risk factors. Chronic smoking does not seem to affect carotid-femoral PWV, which may indicate that the major-

ity of the effect on arterial stiffness is mediated through the effect of smoking on endothelial dysfunction. The effect of chronic smoking on AIx may be partly reversed after only 4 weeks of smoking cessation. Additional investigations are required to confirm the long-term effects of smoking cessation on PWA and endothelial function. If improvement in arterial function can be demonstrated, then use of AIx in the clinical setting may prove a useful motivational tool in smoking cessation.

Conflict of interest

None to declare.

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