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ORIGINAL ARTICLE

Acute Effects of Smoking on the Arterial Function of Young Healthy Smokers

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ABSTRACT

BACKGROUND: Several studies have supported the idea that smoking induces changes with direct impact on endothelial function and arterial stiffness. The main purpose of this study was to assess the acute effects of smoking on vascular function in healthy young adults.

METHODS: We designed a quasi-experimental study, including ninety participants aged between 18 and 25 years. Participants were divided into three groups: Active exposure group (AG) - 30 usual smokers; passive exposure group (PG) and non-exposure control group (CG), each including 30 non-smokers matched for age, gender and overall lifestyle. Heart rate (HR), brachial blood pressure (bBP), flow-mediated dilation (FMD), aortic pulse wave velocity (PWV) and carotid pulse wave analysis (PWA) were evaluated in two moments: baseline and 30 minutes after smoking exposition (AG and PG) or 30 minutes after the first evaluation (CG).

RESULTS: Significant changes from baseline were observed only in the AG, with an increase in PWV, from 5.6 ± 0.7 m/s to 6.1 ± 0.2 m/s post-smoking (p = 0.040), and a decrease in FMD of about -5.7 ± 2.3% after smoking one cigarette (p < 0.001). A significant increase in brachial and central BP was also observed in the AG. A trend for increase in brachial and central BP, and decrease in the FMD was observed in the PG and no significant changes were depicted in the CG.

CONCLUSION: Just one cigarette produces significant detrimental acute effects on the vascular endothelium and hemodynamic profile of healthy young short-term smokers. Changes are also observed, although to a lesser extent, in passive smokers.

Key words: Smoking; Endothelial Dysfunction; Arterial Stiffness; Risk Factors

Key points: Smoking impairs vascular function in young healthy short-term smokers; Smoking one cigarette acutely and significantly depresses endothelial function; Smoking one cigarette increases immediately arterial stiffness; Endothelial-dependent vasodilation is acutely depressed in young smokers.

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INTRODUCTION

According to the World Health Organization (WHO), smoking accounts for over 8% of the deaths worldwide, being the leading cause of avoidable and preventable disease in the western world and a major risk factor for the development and progression of cardiovascular diseases[1]. Passive smoking has also been linked to an increased risk of dying from atherosclerotic heart disease[2].

Smoking has been linked to arterial stiffness and to accelerated sclerotic processes affecting both the larger and the smaller arteries[3-9]. Induced endothelial dysfunction, an important precursor event in the atherosclerotic continuum, has been proposed as the prevailing mechanism underlying the vascular injury associated with long-term smoking[5]. However, the majority of the studies supporting this pathophysiological link were performed in middle aged adults, with long-term exposition to smoking[3,4,6,8]. Fewer studies have addressed

the acute effects of smoking, particularly in young short-term smokers, thus, the real effects of acute exposure to tobacco may be underestimated due to possible long-term adaptations to continuous vascular aggression. Also, such acute effects over passive smokers are not well described, although evidence exists demonstrating adverse consequences of such indirect exposition[9]. In fact, cigarettes burn at a higher temperature during inhalation, which breaks down or filters some toxic components of tobacco. Considering that the majority of the environmental tobacco smoke consists of sidestream smoke (from the burning ends of cigarettes), with only 15% resulting from exhaled mainstream smoke, the sidestream smoke has higher concentrations of many toxic constituents to which passive smokers are exposed[3,4,5].

Even so, the endothelium-dependent vasodilation was shown to be impaired in apparently healthy young smokers in one study[7]. Also, according to Celermajer et al[10], endothelial dysfunction may occur in the systemic arteries of healthy teenagers and young adults as a result of passive smoking, which can be related with reduced nitric oxide (NO) bioavailability. A few studies addressing arterial stiffness have also shown an increase in pulse wave velocity (PWV) after acute smoking[2,4,8], and changes in aortic pressure waveform after acute exposure to second-hand smoke[9].

Most previous research has been focused on the long-term effects of smoking. Notwithstanding, the understanding of the impact of a single cigarette would add relevant information on the pathophysiological impact of smoking, mainly demonstrating the functional changes induced by one single cigarette, the duration of the observed deleterious effects and the existence of any cumulative effects. Thus, this work aims at evaluating the acute effects of smoking (one cigarette) on vascular function in young and healthy short-term smokers and passive smokers, focusing on the study of endothelial function and arterial stiffness.

METHODS

Population and Study design

Ninety participants of both genders (56 females), aged between 18 and 25 years old were enrolled in a quasi-experimental study. Participants were recruited from the students' community of the Polytechnic Institute in Coimbra, Portugal. Participants were all clinically healthy and free of any chronic medication. Thirty active smokers' were selected to participate in the study, all of them having a smoking habit of less than 2 years duration. Sixty non-smokers matched for age, gender and overall lifestyle were also selected, including physical activity, alcohol and coffee consumption and dietary pattern. All participants were sedentary, with recreational alcohol consumption habits and low coffee intake. The diet pattern was verified with the PREDIMED questionnaire[11,12], and an overall low adherence to the Mediterranean diet was found (mean score: 6.9 ± 1.2). Participants were divided into three groups: Active exposure group (AG), including active smokers; passive exposure group (PG) and non-exposure control group (CG), each with 30 participants.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Polytechnic Institute of Coimbra. Anonymity and confidentiality of the collected data were assured, since the study was developed for scientific purposes only, free of any financial or economic interests. All participants signed an informed consent prior to the study.

Heart Rate and Blood Pressure

Heart rate (HR) and brachial blood pressure (bBP) were obtained using an automatic and clinically validated device (Riester ri-champion® N; Riester, India). With the patient in supine position, we obtained both values through the right brachial artery, with the cuff at heart level and adjusted to the arm circumference about 2 to 3 cm above the antecubital fossa. Three measurements were performed at 1 minute intervals, and the arithmetic mean of the three measurements was considered for analysis. We measured brachial systolic and diastolic BP (bSBP and bDBP, respectively), and the HR. Brachial pulse pressure (bPP) was calculated as bSBP - bDBP, and brachial mean arterial pressure (bMAP) was estimated as bDBP + (bPP/3). As blood pressure consists of both a steady component (bMAP) and a pulsatile component (bPP), these BP components add relevant information to the hemodynamic characterization of the participants, with bMAP being mainly determined for the ventricle ejection and peripheral vascular resistance and bPP for the viscoelastic properties of the large arteries and the wave reflection[13,14].

Aortic pulse wave velocity and carotid pulse wave analysis Carotid-femoral pulse wave analysis (PWV), a measure of aortic stiffness, and carotid pulse wave analysis (PWA), were assessed simultaneously with the Complior® Analyse device (Alam Medical, Saint-Quentin-Fallavier, France) according to a previously described technique[13,15,16]. The measurements were made with the subjects in supine position with the neck in a slight hyperextension, and slightly rotated to the left, after a resting period of 10 minutes. Brachial blood pressure (bBP) was measured and entered on the Complior® Analyse software, and then signal acquisition was launched. When the operator observed pulse waveforms of adequate quality, simultaneous carotid and femoral pressure curves were recorded for 15 seconds. The distance travelled by the pulse waveforms was measured between the two recording sites directly on the body surface, and was automatically corrected according to the equation "0.8 x direct distance", subtracting the manubrium-to-carotid distance as previously recommended[13]. The aortic transit time was calculated according to the intersecting tangent algorithm, as previously recommended[13]. PWV was then calculated using measurements of transit time and corrected distance travelled by the pulse wave, between the two recording sites: $PWV\ (m/s) = \frac{corrected\ distance\ (meters)}{time\ (seconds)}$.

The averaged carotid waveform for pulse wave analysis was extracted from the 15 seconds recording window of carotid pulse waves acquired during the PWV procedure, and were calibrated with brachial diastolic pressure (bDBP) and mean arterial pressure (bMAP). The pressure curve was analysed, and its morphological and temporal components were extracted for analysis. Central systolic blood pressure (cSBP), central pulse pressure (cPP) and augmentation index (Aix) were assessed by analysing the morphology and timeline of the curves.

All measurements were performed by a highly experienced operator, with high reproducibility scores, as previously published[15], and a noteworthy concordance between invasive arterial parameters and the Complior-based pulse wave analysis method, which also been previously documented[16].

Flow-Mediated Dilation

The assessment of FMD was performed by ultrasonography using a Vivid *i*® ultrasound system, (GE Healthcare, USA) equipped with a 10L linear vascular transducer (frequency range: 7-12MHz), according to a previously described method[17,18].

With the participant in supine position, after a resting period of 10 minutes in a quiet environmental, the right brachial artery was identified with ultrasound imaging and color Doppler in its longitudinal section, on the medial aspect of the arm, about 2-3 cm above the antecubital fossa.

A basal measurement of the diameter of the right brachial artery in a linear plane was made, as the distance between the two hyperechogenic rows closest to the intima of the vessel. After determining the basal diameter (D1), a cuff was placed on the forearm, distal to the site of the ultrasound assessment, and was inflated into a suprasystolic pressure (about 50mmHg above the previously measured bSBP), keeping the ischemia for a 5 minute period, after which the cuff was deflated. About 1 minute after the complete deflation of the cuff, the diameter of the brachial artery was measured at the same site, to determine D2, that is, the brachial diameter post-reactive hyperemia. The FMD was calculated as the percent increase in brachial diameter after reactive hyperemia, FMD=[(D2 - D1) / D1] x 100, providing information on the endothelium-dependent vasodilation. All measurements were blindly performed by the same experienced operator, with high reproducibility scores (ICC = 0.95; unpublished data), and always in the in the right arm of the participants.

Procedure

Each participant was evaluated in an adequate quite laboratory, with controlled temperature and humidity, and always during the morning. The participants abstained from alcohol, caffeine, and tobacco, as well as food for 8 hours before the study. All participants initially filled a structured questionnaire aimed at retrieving information on clinical personal and family history. Height and weight were measured, and body mass index (BMI) was calculated as BMI=Weight (kg)/Height^2(m^2). Afterward, with the participants in supine position, all the baseline evaluations were performed (HR, bBP, FMD, PWV and PWA). The participants of the AG were then instructed to smoke one cigarette, of the same brand (10 mg tar, 0.8 mg nicotine and 10 mg carbon monoxide), in a smokers' room available at the Institution. For each participant of the AG, one participant of the PG followed to the smokers' room, to implement the passive exposition condition. The participants of the CG were instructed to wait in a quiet room near the research laboratory. The second evaluation occurred 30 minutes after the first moment, which, in the AG and the PG, corresponded to 30 minutes after being exposed to one cigarette, and the same measurements were obtained in all participants (HR, bBP, FMD, PWA and PWV). All procedures were implemented by the same operators of the research team.

Statistical Analysis

Data was gathered in Excel 2016 (Microsoft Office, Redmond, WA), and then imported to SPSS Statistics version 24 (IBM, Armonk, NY) for statistical analysis. A priori required sample was calculated with the GPower software version 3.1.9.2 (Universität Kiel, Germany) and a sample of 30 participants per group was identified as adequate for detecting a medium effect size for a power coefficient of 0.9.

Categorical variables were reported as frequencies and percentages, and χ^2 or Fisher *exact* tests were used when appropriate. The Shapiro-Wilks test was used to confirm normal distribution of all continuous variables, expressed as mean and standard deviation. Variables with a non-normal distribution were log-transformed (Log10). Baseline inter-group comparisons were made with the one-factor ANOVA. A 2-factor mixed-design ANOVA was used to evaluate modifications of variables between the baselines to the post-exposition evaluation in each and between groups. The Greenhouse-Geisser correction was used when sphericity was violated, and the Bonferroni adjustment was adopted for multiple comparisons designed to locate the significant effects of a factor. For between-groups comparison, an additional ANCOVA was performed over the post-intervention data, adjusting for the baseline data (entering as a covariate into the model). A 2-tailed $p<0.05$ was considered significant.

RESULTS

Population

Ninety healthy Caucasian participants were enrolled in this study. Ages ranged from 18 to 25 years and females were predominant (66.7%). The smokers consumed a mean 9.33 ± 5.18 cigarettes/day for up to two years. Participant's baseline characteristics are summarized in table 1. No significant differences were observed in the overall baseline clinical and demographic characteristics, with the exception for the baseline FMD, which was significantly lower in the AG (7.5 ± 2.8%) comparing with both the CG and the PG (12.3 ± 5.4% and 12.5 ± 4.3%, respectively; $p = 0.001$ for AG versus CG and PG; $p = 0.996$ for CG versus PG).

Heart Rate and Blood Pressure

All the values obtained in either evaluation moments were within normality range. The HR had a significant increase in the AG with an increase of 9.6 ± 2.0 bpm from the baseline moment to the post smoking moment ($p < 0.001$), explaining a significant inter-group difference in the HR between the AG and both the CG and the PG ($p = 0.001$). No differences in HR were found between the CG and the PG in the second evaluation. Regarding brachial BP, all values increased significantly in the AG 30 minutes after smoking a cigarette, as depicted in table 2. A trend for an increase in brachial BP was also observed in the PG group, reaching statistical significance for the bDBP, bMAP and the bPP. These increases in bBP in the AG and the PG resulted in a significantly higher bBP profile in comparison with the CG (Table 2).

Pulse Wave Velocity and Pulse Wave Analysis

The within-group analysis concerning PWV and PWA parameters, presented in table 3, depicted significant variations only in the AG participants, with the central pressures increasing from 106.8 ± 8.6 mmHg to 115.2 ± 7.1 mmHg (SBP; $p < 0.001$) and from 41.3 ± 9.2 mmHg to 45.1 ± 8.4 mmHg (PP; $p = 0.029$), and PWV increasing from 5.6 ± 0.7 m/s to 6.1 ± 0.2 m/s ($p = 0.023$). The differences maintained statistical significance after adjusting the analysis HR variation. No significant variations were observed within the CG and the PG, although an increase in the average values of cSBP, cPP and PWV was also identified after exposition in the PG participants.

Flow Mediated Dilatation

Regarding FMD, lower baseline values were identified in the AG as compared with the CG and the PG (Table 1 and figure 1). Considering the FMD changes after the exposition, a significant decrease was observed in the AG (Figure 1, right upper and lower panel) and no changes were observed in the CG and the PG. After the exposition, the FMD was significantly lower in the AG compared with the CG and the PG ($p < 0.001$), and a significant Time*Group interaction was observed for [$F(2,41)=4.7$; $p = 0.01$]. No significant differences were observed between the CG and the PG, even though the final average FMD was lower in the PG. Interestingly, there was a significant correlation between the number of cigarettes/day and the baseline values of FMD in the AG, with lower values of FMD for increasing number of cigarettes/day ($r = - 0.432$; $p < 0.05$).

DISCUSSION

The cardiovascular risk associated with smoking is a consequence

of its effects over the heart and circulation, such as increase cardiac output, deficient vasomotor function, cardiac remodelling, systemic and local inflammation, platelet activation and amongst other molecular important effects[1-5,19]. In the present study, smoking was associated with significant acute changes in several cardiovascular parameters, reflecting an important impact over vascular function. Indeed, smoking just one cigarette produced significant acute increases in HR, brachial and central blood pressure. This immediate hemodynamic response may be partially explained by the direct effect of nicotine, a major component of cigarettes[20], stimulating the sympathetic nervous system and causing the release of catecholamine by the adrenal medulla[20-22], and was similarly found in other studies[23-27]. Passive exposition to smoking did not produce significant hemodynamic changes, as in Giannini et al[28], although an increase in HR and BP was also observed, thus indicating the presence of a slighter immediate cardiovascular impact in the passive smokers. Of notice, Giannini et al documented significant depression of endothelial function (FMD) in passive smokers, in well controlled environmental conditions, measuring the amount of carbon monoxide in the control and polluted conditions, and identifying a strong correlation between the FMD reduction and the carboxyhemoglobin level[28].

Even though these hemodynamic adaptations may reflect sympathetic activation, the evidence regarding endothelial function depression points clearly to other causes rather than a mere autonomic modulation. Endothelial dysfunction is a pathophysiological state in which normal homeostatic properties of the vessels wall are damaged or lost[20], and consistently with other studies involving either short-term[20-25] or long-term smokers[29,30], our results clearly demonstrated changes in endothelial function as evaluated with the FMD method. It is noteworthy that baseline FMD was significantly lower in AG compared to both the CG and the PG, hence some degree of depression of endothelial function was already present in the smokers, as in Yufu et al[31], in which 26 young active smokers were compared with 31 young non-smokers, and FMD was shown to be significantly lower in the smoking group, even though no significant difference was found regarding brachial-ankle PWV. Of notice also is the identification of an immediate and severe depression of endothelial function in such young and short-term group of smokers, indicating a clear effect over the production of NO[22], to which the secondary secretion of vasoconstrictors, such as endothelin[32], together with increased oxidative stress that further reduces NO production[9], adds to the central direct effect of nicotine,

Table 1 Clinical and demographic characterization of the participants.

	CG (n = 30)	PG (n = 30)	AG (n = 30)	ANOVA ρ
Male, n(%)	10 (33.3)	10 (33.3)	10 (33.3)	1
Female, n(%)	20 (66.7)	20 (66.7)	20 (66.7)	
Age, years	20.7 ± 1.2	20.3 ± 1.3	20.3 ± 1.3	0.984
BMI, Kg/m2	23.7 ± 3.4	22.3 ± 2.3	22.3 ± 2.3	0.572
Basal HR, bpm	62.3 ± 10.5	61.2 ± 8.8	61.2 ± 8.8	0.623
Basal bSBP, mmHg	114.7 ± 12.7	113.7 ± 10.4	113.7 ± 10.4	0.426
Basal bDBP, mmHg	68.3 ± 7.2	66.1 ± 10.1	66.1 ± 10.1	0.799
Basal bMAP, mmHg	81.7 ± 8.6	82.0 ± 9.9	82.0 ± 9.9	0.567
Basal bPP, mmHg	47.3 ± 9.0	47.5 ± 7.2	47.5 ± 7.2	0.611
Basal cSBP, mmHg	105.1 ± 13.1	106.9 ± 13.1	106.9 ± 13.1	0.56
Basal cPP, mmHg	40.4 ± 9.8	40.4 ± 7.1	40.4 ± 7.1	0.711
Basal PWV, ms	5.7 ± 0.9	5.6 ± 0.8	5.6 ± 0.8	0.978
Basal AIx, %	-17.6 ± 13.1	-14.2 ± 12.7	-14.2 ± 12.7	0.89
Basal FMD, n	12.5 ± 4.3	12.3 ± 5.4	12.3 ± 5.4	0.001

CG: non-exposure control group; AG: active exposure group; PG: passive exposure group; HR: Heart rate; bSBP: systolic blood pressure; bDBP: diastolic blood pressure; bMAP: mean arterial pressure; bPP: pulse pressure; cSBP: central systolic blood pressure; cPP: central pulse pressure; PWV: pulse wave velocity; AIx: augmentation index; FMD: flow mediated dilation.

Table 2 Heart rate and brachial blood pressure variation from baseline to post 30 minutes in the three experimental groups.

		CG	PG	AG	ANOVA p	Interaction
HR, bpm	Basal	62.3±10.5	61.2±8.8	63.5±10.4	0.623	0.985
	Post	58.9±8.4	60.4±18.7	73.1±14.9	0.001	
	Difference	-3.4±2.1	-0.8±3.3	9.6±2.0		
	p	0.202	0.589	<0.001		
bSBP, mmHg	Basal	114.7±12.7	113.7±10.4	116.4±10.2	0.426	0.89
	Post	112.9±9.0	122.0±11.7	125.1±9.4	<0.001	
	Difference	-1.8±2.3	8.3±2.1	8.7±1.3		
	p	0.6	0.091	<0.001		
bDBP, mmHg	Basal	68.3±7.2	66.1±10.1	67.1±9.2	0.799	0.801
	Post	61.2±7.2	71.0±6.6	71.7±9.3	<0.001	
	Difference	-7.1±2.82	4.9±1.6	4.6±1.9		
	p	0.114	0.03	0.032		
bMBP, mmHg	Basal	81.7±8.6	82.0±9.9	81.6±7.2	0.567	0.876
	Post	78.6±7.0	88.6±7.4	87.7±7.6	<0.001	
	Difference	-3.1±2.4	6.6±1.6	6.1±1.7		
	p	0.169	0.001	0.003		
bPP, mmHg	Basal	47.3±9.0	47.5±7.2	48.9±9.9	0.611	0.193
	Post	51.6±7.4	53.0±9.0	53.0±8.0	0.73	
	Difference	4.3±2.4	5.5±1.8	4.1±1.7		
	p	0.164	0.028	0.034		

CG: non-exposure control group; AG: active exposure group; PG: passive exposure group; HR: Heart rate; bSBP: systolic blood pressure; bDBP: diastolic blood pressure; bMAP: mean arterial pressure; bPP: pulse pressure.

Table 3 Changes in pulse wave velocity, augmentation index and central blood pressures from baseline to post 30 minutes in the three experimental groups.

		CG	PG	AG	ANOVA p	Time x group Interaction
cSBP, mmHG	Basal	105.1 ± 13.3	106.9 ± 13.1	106.8 ± 8.6	0.56	0.446
	Post	105.4 ± 14.2	112.7 ± 12.0	115.2 ± 7.1	0.021	
	Difference	0.3 ± 3.9	5.8 ± 2.1	8.4 ± 1.5		
	p	0.954	0.17	<0.001		
cPP, mmHg	Basal	40.4 ± 9.8	40.4 ± 7.1	41.3 ± 9.2	0.711	0.122
	Post	40.7 ± 10.1	42.8 ± 12.3	45.1 ± 8.4	0.107	
	Difference	0.3 ± 1.7	2.4 ± 3.2	3.8 ± 1.5		
	p	0.894	0.421	0.029		
PWV, ms	Basal	5.7 ± 0.9	5.6 ± 0.8	5.6 ± 0.7	0.978	0.8
	Post	5.8 ± 0.7	5.9 ± 0.5	6.1 ± 0.2	0.6	
	Difference	0.1 ± 0.2	0.3 ± 0.2	0.5 ± 0.2		
	p	0.47	0.29	0.023		
AIx, %	Basal	-17.6 ± 13.1	-14.2 ± 12.7	-18.0 ± 17.1	0.89	0.38
	Post	-16.4 ± 15.1	-17.1 ± 28.4	-18.7 ± 18.1	0.987	
	Difference	1.2 ± 2.8	-2.9 ± 13.7	-0.7 ± 0.8		
	p	0.672	0.678	0.37		

CG: non-exposure control group; AG: active exposure group; PG: passive exposure group; HR: Heart rate; bSBP: systolic blood pressure; bDBP: diastolic blood pressure; bMAP: mean arterial pressure; bPP: pulse pressure.

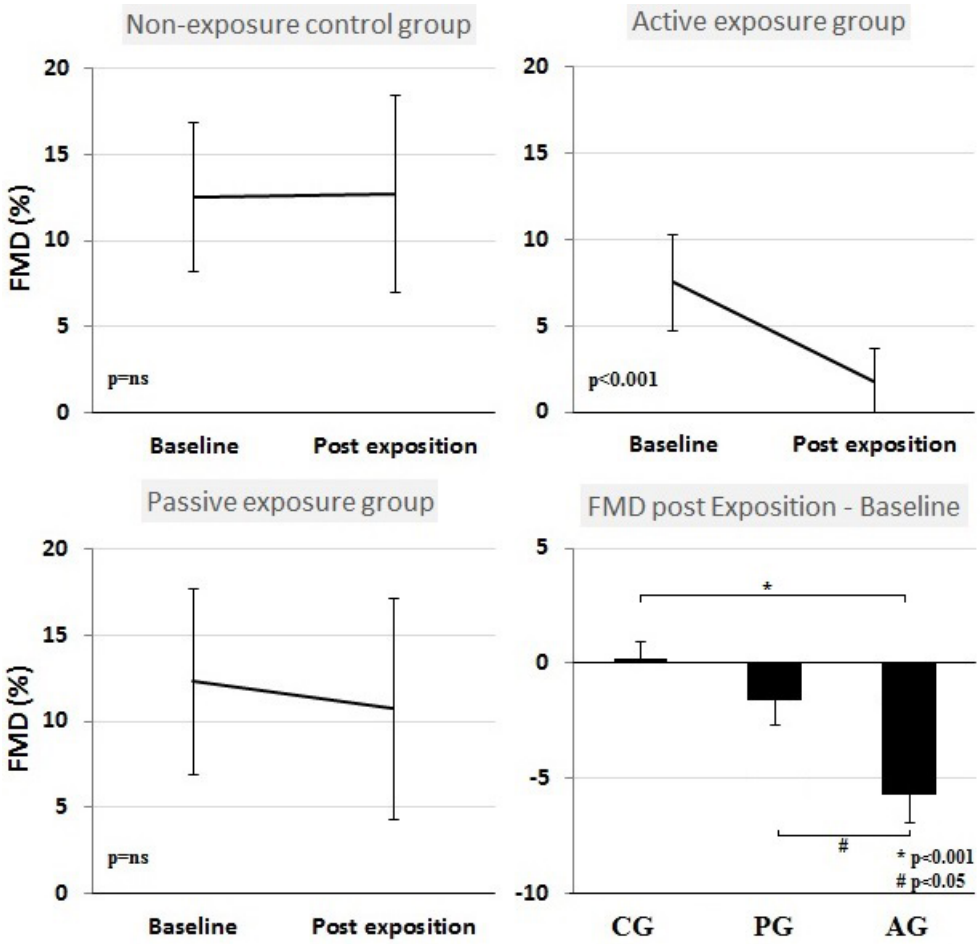

Figure 1 Changes in flow mediated dilation from baseline to post 30 minutes in the three experimental groups. CG: non-exposure control group; AG: active exposure group; PG: passive exposure group; FMD: flow mediated dilation.

increasing the sympathetic signaling, and contributing additively to the changes in the hemodynamic state of the smokers through a reactive vasoconstriction[27]. Curiously, a recent study[33] demonstrated the ability of red wine to reverse this effects in a group of healthy smokers, thus suggesting that smoking increases oxidative stress and/or decreases NO levels, and antioxidants in red wine may neutralize such effects and reverse its influence on FMD.

The relaxation impairment observed in response to the exposition provides the basis to understand the significant changes also observed in PWV, a reference and integrated marker of arterial stiffness[34]. As in previous studies[3,27,35], PWV increased after the cigarette smoking, indicating an acute increase of arterial stiffness, possibly due to the smoking-induced endothelial dysfunction and the sympathetic activation[3], which in turn may produce increased smooth muscle tonus and vasoconstriction. As PWV directly relates to the overall cardiac workload, consequences are also expected to occur in the heart, and thus, should be addressed in future research.

Regarding the passive exposition condition, although no significant changes were identified, again, a trend towards a reduction in FMD was also observed in the PG, in line with previous findings[7,9,28], so further research is needed to identify the mechanisms of vascular compromise associated with passive exposition to smoking, namely identifying dose-dependency relations in terms of the overall amount of exposition and its cumulative effect over vascular biology and function.

This study has several limitations that should be considered. The small number of participants is a significant aspect, even though the results were consistent in the most relevant outcomes considered in the study. Also, the study enrolled young healthy participants, with a good overall cardiovascular health, and a short-term history of smoking habits, so the detrimental effect of tobacco could be underestimated. Even so, the effect observed in the AG reveals a significant depression of endothelial in a particular favorable clinical context, and, to the best of our knowledge, is the first study of the acute effects of smoking in the vascular function of Portuguese young and healthy. Additionally, the measurements for the FMD estimations were performed manually, and even considering the experience of the operator and the blinding methodology, must be acknowledge as an important technical limitation[18].

In conclusion, our results combined with the available evidences, clearly demonstrated that smoking only one cigarette produces detrimental acute effects on vascular endothelium and arterial stiffness in healthy and short term smokers.

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