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Associations of Tobacco Smoking with Impaired Endothelial Function: The Circulatory Risk in Communities Study (CIRCS)

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Aims: Smoking impairs endothelial function as an acute effect. However, few population-based studies have examined the association between smoking status and endothelial function or the dose–response and duration–response association of smoking with endothelial function. We examined whether smoking habits were associated with impaired endothelial function depending on smoking dose and duration.

Methods: We conducted a cross-sectional study of 910 men and women aged 30–79 years from 2013 to 2016. Statistical analyses of the data were conducted between 2016 and 2017. Endothelial function was assessed by brachial artery flow-mediated dilation (FMD) measurement. Low FMD was defined in two ways as the cutoff point based on the lowest quartile of %FMD (<5.1%) and median of %FMD (<6.8%), regarding as impaired endothelial function. We investigated the smoking status in terms of cigarettes consumed per day and the duration of smoking.

Results: Heavy and chronic smokers were associated with a high prevalence of impaired endothelial function. Those associations did not change substantially after adjustment for other cardiovascular risk factors. Among all participants, the multivariable-adjusted ORs (95% CIs) of low FMD (<5.1%) with reference to never smokers were 2.23 (1.00–5.14) for current heavy smokers of ≥ 30 cigarettes per day, 1.83 (1.04–3.20) for heavy smokers of ≥ 40 pack-years, and 2.16 (1.15–4.06) for chronic smokers of ≥ 40 years. For low FMD (<6.8%) those values was 2.17 (1.01–5.05), 1.70 (1.01–2.86), and 1.98 (1.07–3.69), respectively.

Conclusions: Similar associations were observed among only men. Heavy or long-term tobacco smoking may induce impaired endothelial function.

Key words: Flow-mediated dilation, Impaired endothelial function, Smoking

1. Introduction

Tobacco smoking is attributable to around six million deaths[1)]. Tobacco use is estimated to cause 12% of deaths among persons aged 30 and over worldwide[1)]. More than 7000 different chemicals[2)], mostly poisonous, are contained in tobacco and tobacco smoke. Despite strong epidemiological evidence[3-5)], the mechanisms about the cardiovascular effects of smoking are complex; they are the least understood among the risk factors of CVD[6)].

Impaired endothelial function is considered a key early disorder in the development of coronary atherosclerosis[7)], and its early detection may be useful in pre-

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venting CVD. Increasing evidence indicates that smoking may damage endothelial function through oxidative stress and inflammation[8-12]. Indications regarding endothelial function are most widely assessed using flow-mediated dilation (FMD). FMD is a noninvasive measurement of brachial artery diameter changes following an increase in shear stress induced by reactive hyperemia[13]. According to systematic reviews with meta-analysis[14, 15],brachial FMD showed a significant predictive value for cardiovascular events.

Smoking has an acute adverse effect on FMD[16, 17]. However, there is limited evidence about the association between active smoking status and FMD.

The aim of this study was to investigate the association among active smoking status, duration of smoking (pack-years), and impaired endothelial function in a general population.

2. Materials and Methods

2.1 Study Population Sample

We conducted FMD measurements in two communities covered by the Circulatory Risk in Communities Study (CIRCS): a mid-western urban suburban community (Yao, Osaka Prefecture) and a northeastern rural community (Ikawa, Akita Prefecture). The CIRCS is a dynamic community-based cohort study that covers five communities in Japan, including Yao and Ikawa[18]. From 2013 to 2016, we recruited 517 men and 393 women aged 30–79 years who underwent annual cardiovascular risk surveys. Statistical analyses of the data were conducted between October 2016 and April 2017. The study protocol was approved by the ethics committee of the Osaka Center for Cancer and Cardiovascular Disease Prevention and of Osaka University (No. 13483-4).

2.2 Measurement of FMD and Cardiovascular Risk Factors

FMD measurement was performed in a room with the participants in a resting, supine state during the measurement. Trained operators measured FMD using high-resolution ultrasonography and a forearm occlusive cuff according to current guidelines[13]. The right brachial artery diameter was measured by a high-resolution linear artery transducer (10 MHz), equipped with computer-assisted analysis software (UNEXEF18G, UNEX Co., Nagoya, Japan)[19]. The brachial artery was imaged at a location 5–10 cm above the elbow. When the clearest image of the anterior and posterior intimal interfaces between the lumen and vessel was obtained, the transducer was held at the same point throughout the scan. To standardize the position of the probe, we used a specially designed armrest and probe holder. To create a flow stimulus in the brachial artery, a sphygmomanometric cuff was placed on the forearm. After the brachial artery diameter was measured at baseline, the cuff was inflated to at least 50 mmHg above systolic pressure to occlude artery inflow for 5 min and then deflated. FMD was expressed as the percentage change in peak vessel diameter from that of baseline. The coefficient of inter-operator variability for FMD measurements in our laboratory was 5.7%; that of intra-operator variability was 11.1% after 2 months and 10.8% after 4 months[20].

Information about smoking status (number of cigarettes smoked per day and duration of smoking) was obtained by trained interviewers. Individuals who smoked ≥ 1 cigarettes per day were defined as current smokers; those who had quit smoking were defined as former smokers. Height was measured with the participants in their stocking feet, and weight was measured while wearing light clothing. We calculated body mass index (BMI) as weight (kg) divided by the square of height (m^2). After 5 min of rest and with the participants seated, blood pressure was measured by physicians and nurses using standard mercury sphygmomanometers on the right arm[18]. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg, or the use of antihypertensive medication. Serum total cholesterol and plasma glucose were measured by the enzymatic and hexokinase methods[21], respectively. Diabetes mellitus was defined as borderline or high plasma glucose (fasting glucose ≥ 110 mg/dL or non-fasting glucose ≥ 140 mg/dL) or being under medication for diabetes. An interview was conducted to ascertain alcohol drinking status, usual weekly physical activity, and medication use for hypertension. For alcohol drinking habits, the interviewer collected information about usual weekly intake of alcohol in units of go (a traditional Japanese unit of volume corresponding to 23 g ethanol). Participants who reported consuming ≥ 0.3 go per week were considered current drinkers.

2.3 Statistical Analysis

Low FMD was defined in two ways as the cutoff point of the lowest quartile[22, 23] of %FMD (<5.1% in the present study) and the median[24, 25] of %FMD (<6.8% in the present study). Pack-years was calculated as (number of cigarettes smoked per day/20 × number of years smoked). We present the characteristics of the study participants according to smoking status, pack-years, and years of smoking as age-adjusted and sex-adjusted means (standard errors) or proportions. We examined differences in the mean values of the confounding factors among smoking sta-

tus, pack-years, and years of smoking by analysis of covariance; we assessed the proportions of potential confounding factors using the chi-square test; we examined differences in the median values of FMD using the nonparametric test. We calculated age-adjusted, sex-adjusted, and multivariable-adjusted ORs and 95% CIs of low FMD (either $<5.1\%$ or $<6.8\%$) using logistic regression analysis according to smoking categories: never smokers, former smokers, current smokers of <30 cigarettes per day, and current smokers of ≥ 30 cigarettes per day; <20 pack-years, 20–40 pack-years, and ≥ 40 pack-years; duration of smoking of <20 years, 20–40 years, and ≥ 40 years. We adjusted for the potential confounding factors with the following: age (years); sex; community; BMI (kg/m^2); systolic blood pressure (mmHg); high-density lipoprotein (HDL)-cholesterol and non-HDL cholesterol levels (mmol/L); serum triglycerides (mmol/L); diabetes mellitus (yes or no); current drinking status (yes or no); physical activity (yes or no); use of antihypertensive medication (yes or no); medication use for diabetes (yes or no); medication use for dyslipidemia (yes or no); and baseline brachial artery diameter (mm). We tested the p for trend by using the median of each group as a continuous variable.

We performed all statistical analyses with SAS, version 9.4 software (SAS Institute Inc., Cary, NC, USA). All probability values for statistical tests were two tailed, and $p<0.05$ was regarded as statistically significant.

3. Results

The mean values of %FMD were 7.1% among all participants: 6.7% in men, 7.8% in women ($p<0.001$). The proportion of current smokers was 22% of all participants: 33% in men, but only 9% in women ($p<0.001$).

Table 1 shows age-adjusted and sex-adjusted mean values and proportions of cardiovascular risk factors according to smoking status, pack-years, and duration of smoking. The prevalence of low FMD (either $<5.1\%$ or $<6.8\%$), absence of diabetes mellitus, and mean value of systolic blood pressure were higher among current ≥ 30 cigarettes per day smokers, ≥ 40 pack-year smokers, and smokers who had smoked for ≥ 40 years than in the less smoking groups ($p<0.05$); those variables were higher than in the never smokers. The mean values of %FMD were lower for heavy and longer smokers than for never smokers ($p<0.05$).

As evident in **Table 2**, compared with never smokers, former and current smokers who smoked <30 cigarettes per day showed no significantly higher prevalence of low FMD (either $<5.1\%$ or $<6.8\%$); however, smokers who currently smoked ≥ 30 cigarettes per day did show a significantly higher prevalence among total subjects and men. Such an association was not observed among women because no women smoked ≥ 30 cigarettes per day. The multivariable-adjusted ORs (95% CIs) of low FMD ($<5.1\%$) with reference to never smokers were 1.18 (0.75–1.84) for former smokers, 1.26 (0.75–2.13) for currently smoking <30 cigarettes per day, and 2.23 (1.00–5.14) for currently smoking ≥ 30 cigarettes per day among total subjects; among men, those values were 0.92 (0.52–1.62), 1.25 (0.78–2.00), and 2.22 (1.01–4.95), respectively. The corresponding multivariable-adjusted ORs (95% CIs) of low FMD ($<6.8\%$) were 1.05 (0.73–1.49), 1.07 (0.70–1.64), and 2.17 (1.01–5.05) among total subjects; among men, those values were 0.75 (0.44–1.27), 1.08 (0.69–1.69), and 2.16 (1.00–5.00).

Age-adjusted, sex-adjusted and multivariable-adjusted ORs (95% CIs) of low FMD (either $<5.1\%$ or $<6.8\%$) according to pack-years and years of smoking appear in **Table 3**. Smokers who had smoked ≥ 40 pack-years had a higher prevalence of low FMD than never smokers among total subjects and men. Such an association was not observed among women because only two participants had smoked ≥ 40 pack-years; among those, none had low FMD. After further adjustment for other cardiovascular risk factors, those associations did not change substantially. Multivariable-adjusted ORs (95% CIs) of low FMD ($<5.1\%$) with reference to never smokers were 1.10 (0.70–1.70) for smokers <20 pack-years, 1.14 (0.69–1.90) for smokers 20–40 pack-years, and 1.83 (1.04–3.20) for smokers ≥ 40 pack-years among total subjects (p for the trend $=0.03$); among men those values were 0.85 (0.46–1.57), 1.04 (0.58–1.88), and 1.75 (1.00–3.28), respectively (p for the trend $=0.03$). The corresponding multivariable-adjusted ORs (95% CIs) of low FMD ($<6.8\%$) were 1.00 (0.70–1.42), 1.04 (0.67–1.61), and 1.70 (1.01–2.86) among total subjects; among men, those values were 0.74 (0.42–1.28), 0.96 (0.64–1.46), and 1.76 (1.13–3.09). With regard to smoking duration, the multivariable-adjusted ORs (95% CIs) of low FMD ($<5.1\%$) for participants who had smoked for ≥ 40 years versus those who never smoked were 2.16 (1.15–4.06) among total subjects (p for the trend $=0.03$), and 1.97 (1.00–3.89) (p for the trend $=0.01$) among men. The corresponding multivariable-adjusted ORs (95% CIs) of low FMD ($<6.8\%$) were 1.98 (1.07–3.69) and 2.05 (1.10–3.86).

Table 1. Age- and Sex-adjusted Mean Values and Proportion of Cardiovascular Risk Factors according to Smoking Status

	Never	Former	Current	
			< 30 cigarettes/day	≥ 30 cigarettes/day
Number	364	342	170	34
Age	50.7 (0.5)	54.3 (0.5)***	51.6 (0.7)	50.8 (1.6)
Men, %	25	76***	79***	100***
Mean of flow-mediated dilation, %	7.37 (0.17)	7.05 (0.16)	7.01 (0.23)	6.21 (0.51)*
Flow-mediated dilation < 5.1, %	18	26*	28*	42**
Median of flow-mediated dilation, %	7.20	6.60*	6.45*	6.00*
Flow-mediated dilation < 6.8, %	48	50	53	68*
Minimum and maximum of flow-mediated dilation, %	0.7, 20.3	0.7, 18.2	1.3, 17.4	1.9, 16.2
Baseline brachial artery diameter, mm	3.94 (0.03)	3.95 (0.03)	3.97 (0.04)	4.11 (0.09)
Current drinkers, %	45	65***	59**	82***
Physical activity, %	49	50	35**	21**
Body mass index, kg/m^2	23.5 (0.2)	23.4 (0.2)	23.2 (0.3)	23.9 (0.6)
Systolic blood pressure, mmHg	122 (1)	125 (1)*	125 (1)*	129 (3)*
Diastolic blood pressure, mmHg	80 (1)	80 (1)	80 (1)	81 (2)
Hypertension, %	20	30*	30*	33**
Use of antihypertensive medication, %	15	16	25**	23
Total cholesterol, mg/dL	211.2 (2.1)	208.1 (2.1)	205.7 (2.9)	207.8 (6.4)
HDL cholesterol, mg/dL	63.2 (0.9)	64.3 (0.8)	58.8 (1.2)**	59.8 (2.6)
Triglycerides, mg/dL	101.4 (7.2)	112.4 (6.9)	148.9 (9.7)***	167.0 (21.5)**
Diabetes mellitus, %	13	14	17	38***

	Never	Number of cigarettes smoked, pack-years		
		1-	20-	40-
Number	364	256	175	115
Age	50.7 (0.5)	48.8 (0.5)**	55.1 (0.6)***	60.4 (0.8)***
Men, %	25	59***	93***	98***
Mean of flow-mediated dilation, %	7.37 (0.17)	7.10 (0.19)	7.01 (0.24)	6.63 (0.30)*
Flow-mediated dilation < 5.1, %	18	23	24	33**
Median of flow-mediated dilation, %	7.20	6.80	6.40*	5.90***
Flow-mediated dilation < 6.8, %	48	50	51	60*
Maximum and minimum of flow-mediated dilation, %	0.7, 20.3	0.7, 17.4	1.3, 18.2	1.5, 17.8
Baseline brachial artery diameter, mm	3.94 (0.03)	3.91 (0.03)	4.03 (0.04)	4.01 (0.05)
Current drinkers, %	45	65***	60**	64***
Physical activity, %	49	48	42	36*
Body mass index, kg/m^2	23.5 (0.2)	23.4 (0.2)	23.5 (0.3)	23.0 (0.4)
Systolic blood pressure, mmHg	122 (1)	122 (1)	126 (1)*	127 (2)*
Diastolic blood pressure, mmHg	80 (1)	79 (1)	82 (1)	79 (1)
Hypertension, %	20	23	34*	28
Use of antihypertensive medication, %	15	15	26*	26*
Total cholesterol, mg/dL	211.2 (2.1)	207.9 (2.3)	207.5 (3.0)	205.6 (3.6)
HDL cholesterol, mg/dL	63.2 (0.9)	62.9 (0.9)	61.6 (1.2)	61.6 (1.5)
Triglycerides, mg/dL	101.4 (7.2)	126.6 (7.7)	125.4 (10.1)	128.7 (12.3)
Diabetes mellitus, %	12	12	17	30***

(Cont Table 1)

	Never	Years smoked, years		
		1-	20-	40-
Number	364	209	259	78
Age	50.7 (0.5)	48.6 (0.6)**	53.6 (0.5)***	64.6 (1.0)***
Men, %	25	67***	91***	100***
Mean of flow-mediated dilation, %	7.37 (0.17)	7.14 (0.21)	7.03 (0.19)	6.33 (0.35)**
Flow-mediated dilation <5.1, %	18	21	26	37**
Median of flow-mediated dilation, %	7.20	6.90	6.40*	5.50***
Flow-mediated dilation <6.8, %	48	48	53	65*
Maximum and minimum of flow-mediated dilation, %	0.7, 20.3	0.7, 18.2	1.3, 17.4	1.5, 17.8
Baseline brachial artery diameter, mm	3.94 (0.03)	3.92 (0.04)	4.00 (0.04)	3.99 (0.06)
Current drinkers, %	45	68***	61***	59*
Physical activity, %	49	52	38*	38*
Body mass index, kg/m^2	23.5 (0.2)	23.5 (0.2)	23.4 (0.2)	23.0 (0.4)
Systolic blood pressure, mmHg	122 (1)	121 (1)	125 (1)	127 (2)*
Diastolic blood pressure, mmHg	80 (1)	79 (1)	81 (1)	79 (1)
Hypertension, %	20	24	29	31
Use of antihypertensive medication, %	15	16	24*	22
Total cholesterol, mg/dL	211.2 (2.1)	210.3 (2.5)	204.7 (2.4)	207.6 (4.3)
HDL cholesterol, mg/dL	63.2 (0.9)	64.6 (1.0)	61.1 (1.0)	59.2 (1.8)
Triglycerides, mg/dL	101.4 (7.2)	113.4 (8.6)	119.1 (8.0)	140.3 (14.6)***
Diabetes mellitus, %	13	11	19*	24*

*$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with never smokers

4. Discussion

The core findings of the present population-based study are as follows: (1) The %FMD was lower in current smokers than in never smokers; (2) smokers who had smoked 30 or more cigarettes per day had a twofold higher prevalence of low FMD (either <5.1% or <6.8%) than never smokers; (3) heavy and chronic tobacco smoking was associated with a higher prevalence of low FMD (either <5.1% or <6.8%): heavy smokers of either 40 or more pack-years or chronic smokers who had smoked for 40 or more years had an approximately twofold higher prevalence of low FMD than never smokers.

The lower mean values of %FMD in smokers compared with never smokers was consistent with previous reports on a positive association between tobacco smoking and impaired endothelial function[26-28]. A UK case-reference study of 200 men and women (80 never smokers, 40 former smokers, and 80 current smokers) aged 15–57 years reported mean values (standard deviations) of %FMD of 10.0% (3.3%) for nonsmokers, 5.1% (3.8%) for former smokers, and 4.0% (3.9%) for current smokers[26]. A Japanese case-reference study of 57 men and women (31 nonsmokers and 26 smokers) aged 20–40 years found mean values (standard deviations) of %FMD of 16.1% (6.6%) for nonsmokers and 12.4% (5.8%) for smokers[27]. According to an Italian study of 206 men (68 never smokers, 53 light smokers, and 85 regular smokers) with a mean age of 51.5 years, the mean values (95% CIs) of %FMD were 9.00% (7.79%–10.21%) for never smokers, 6.09% (5.00%–7.18%) for light smokers, and 6.12% (4.99%–7.24%) for regular smokers[28]. To the best of our knowledge, the present investigation is the first to show an association between detailed smoking status (dose and duration) and impaired endothelial function in a large population-based study.

The mechanisms of tobacco smoking and functional changes in the endothelium are complicated and still unclear. A possible mechanism for lowering FMD induced by smoking is reduced production of nitric oxide (NO) and increased expression of adhesion molecules, leading to impaired endothelial function[29]. NO is known to be a key signaling molecule in vascular homeostasis; it is formed by endothelial NO synthase[30, 31]. Abnormalities in NO production accompany such diseases as angiogenesis-associated disorders because NO performs an important function in regulating blood vessel diameter and blood flow[29, 31, 32]. NO rapidly diffuses out of vascular endothelial cells and into the underlying smooth muscle cells, causing relaxation, and into nearby adhering

Table 2. Odds Ratios (95% CIs) of Low FMD according to Smoking Status

	Never	Former	Current	
			<30 cigarettes/day	≥ 30 cigarettes/day
Total subjects				
No. of subjects	364	342	170	34
No. of low FMD <5.1	62	93	46	14
Proportion of low FMD <5.1, %	17	27	27	41
Age- and sex-adjusted OR (95% CI)	1.00	1.17 (0.76-1.78)	1.26 (0.77-2.06)	2.24 (1.01-4.97)*
Multivariable OR (95% CI)[a]	1.00	1.18 (0.75-1.84)	1.26 (0.75-2.13)	2.23 (1.00-5.14)*
No. of low FMD <6.8	161	185	93	24
Proportion of low FMD <6.8, %	44	54	55	71
Age- and sex-adjusted OR (95% CI)	1.00	1.07 (0.76-1.52)	1.21 (0.80-1.83)	2.32 (1.03-5.24)*
Multivariable OR (95% CI)[a]	1.00	1.05 (0.73-1.49)	1.07 (0.70-1.64)	2.17 (1.01-5.05)*
Men				
No. of subjects	89	260	134	34
No. of low FMD <5.1	23	76	43	14
Proportion of low FMD <5.1, %	27	29	32	41
Age-adjusted OR (95% CI)	1.00	0.97 (0.56-1.68)	1.32 (0.85-2.06)	2.13 (1.02-4.46)*
Multivariable OR (95% CI)[a]	1.00	0.92 (0.52-1.62)	1.25 (0.78-2.00)	2.22 (1.01-4.95)*
No. of low FMD <6.8	50	146	79	24
Proportion of low FMD <6.8, %	56	56	59	71
Age-adjusted OR (95% CI)	1.00	0.85 (0.51-1.40)	1.26 (0.83-1.91)	2.27 (1.04-4.95)*
Multivariable OR (95% CI)[a]	1.00	0.75 (0.44-1.27)	1.08 (0.69-1.69)	2.16 (1.00-5.00)*
Women				
No. of subjects	275	82	36	0
No. of low FMD <5.1	38	17	3	0
Proportion of low FMD <5.1,%	14	21	8	0
Age-adjusted OR (95% CI)	1.00	1.73 (0.91-3.30)	0.51 (0.15-1.72)	-
Multivariable OR (95% CI)[a]	1.00	1.73 (0.85-3.53)	0.57 (0.16-2.06)	-
No. of low FMD <6.8	111	39	14	0
Proportion of low FMD <6.8, %	40	48	39	0
Age-adjusted OR (95% CI)	1.00	1.47 (0.89-2.44)	0.91 (0.45-1.84)	-
Multivariable OR (95% CI)[a]	1.00	1.52 (0.89-2.59)	0.99 (0.47-2.10)	-

*$p<0.05$ compared with never smokers
a. Multivariable variables included age, sex, community, baseline brachial artery diameter, body mass index, systolic blood pressure, use of antihypertensive medication, non-HDL cholesterol, HDL cholesterol, triglycerides, medication use for dyslipidemia, diabetes mellitus, medication use for diabetes, current drinking status and physical activity

platelets in the lumen of the blood vessel, inhibiting platelet adhesion and aggregation[30-32]. Numerous studies have reported that components involved in tobacco smoke induce oxidative stress and inflammation[8-10, 33]; thus, smoking increases oxidative damage to the endothelium and increases platelet adhesion to the endothelial membrane[34, 35]. Because of this damage, the activation and expression of endothelial NO synthase is inhibited and synthesis of NO is decreased[30, 31, 33, 34], resulting in impaired endothelial function.

One strength of the present investigation is that it is the first population-based study to allow generalization of the findings. Another strength is the use of a noninvasive technique for measuring %FMD and the standardized measurements for other cardiovascular risk factors, which assure the quality of this epidemiological study.

With respect to limitations, we were unable to define a causal relationship in the present study owing to its cross-sectional design. We were unable to reliably examine the association between smoking and low FMD in women: women had lower and shorter consumption of tobacco than men. Only two women had smoked 40 or more pack-years, and no woman smoked 30 or more cigarettes per day or had smoked

Table 3. Odds Ratios (95% CIs) of Low FMD according to Pack-years and Years of Smoking

	Never	Number of cigarettes smoked, pack-years			*P* for trend
		1-	20-	40-	
Total					
No. of subjects	364	256	175	115	
No. of low FMD <5.1	62	59	50	44	
Proportion of low FMD <5.1, %	17	23	29	38	<0.01
Age- and sex-adjusted OR (95% CI)	1.00	1.13 (0.73-1.73)	1.21 (0.73-1.99)	1.81 (1.06-3.11)*	0.02
Multivariable OR (95% CI)[a]	1.00	1.10 (0.70-1.70)	1.14 (0.69-1.90)	1.83 (1.04-3.20)*	0.03
No. of low FMD <6.8	161	129	97	76	
Proportion of low FMD <6.8, %	44	50	55	66	<0.01
Age- and sex-adjusted OR (95% CI)	1.00	1.07 (0.76-1.51)	1.11 (0.72-1.70)	1.70 (1.02-2.79)*	0.05
Multivariable OR (95% CI)[a]	1.00	1.00 (0.70-1.42)	1.04 (0.67-1.61)	1.70 (1.01-2.86)*	0.06
Men					
No. of subjects	89	152	163	113	
No. of low FMD <5.1	24	40	49	44	
Proportion of low FMD <5.1, %	27	26	30	39	0.04
Age-adjusted OR (95% CI)	1.00	0.97 (0.54-1.75)	1.16 (0.66-2.07)	1.73 (1.01-3.15)*	0.03
Multivariable OR (95% CI)[a]	1.00	0.85 (0.46-1.57)	1.04 (0.58-1.88)	1.75 (1.00-3.28)*	0.03
No. of low FMD <6.8	50	83	90	76	
Proportion of low FMD <6.8, %	56	55	55	67	0.05
Age-adjusted OR (95% CI)	1.00	0.94 (0.55-1.59)	0.96 (0.57-1.62)	1.67 (1.05-2.66)*	0.07
Multivariable OR (95% CI)[a]	1.00	0.74 (0.42-1.28)	0.96 (0.64-1.46)	1.76 (1.13-3.09)*	0.06
Women					
No. of subjects	275	104	12	2	
No. of low FMD <5.1	38	19	1	0	
Proportion of low FMD <5.1, %	14	18	8	0	0.76
Age-adjusted OR (95% CI)	1.00	1.39 (0.76-2.55)	0.57 (0.07-4.52)	-	0.98
Multivariable OR (95% CI)[a]	1.00	1.58 (0.83-3.00)	0.59 (0.07-4.93)	-	0.91
No. of low FMD <6.8	111	46	7	0	
Proportion of low FMD <6.8, %	40	44	58	0	0.46
Age-adjusted OR (95% CI)	1.00	1.17 (0.74-1.85)	2.07 (0.64-6.68)	-	0.53
Multivariable OR (95% CI)[a]	1.00	1.25 (0.78-2.01)	2.53 (0.74-8.63)	-	0.35

for 40 or more years. Further investigation is necessary to confirm whether heavy and chronic smoking induce impaired endothelial function in women.

We found that tobacco smoking—specifically heavy and chronic smoking—was associated with endothelial function in a general Japanese population.

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(Cont Table 3)

	Never	Years smoked, year			*P* for trend
		1-	20-	40-	
Total					
No. of subjects	364	209	259	78	
No. of low FMD <5.1	62	46	74	33	
Proportion of low FMD <5.1, %	17	22	29	42	<0.01
Age- and sex-adjusted OR (95% CI)	1.00	1.01 (0.64-1.61)	1.31 (0.85-2.04)	2.14 (1.18-3.86)*	<0.01
Multivariable OR (95% CI) [a]	1.00	1.07 (0.66-1.75)	1.20 (0.75-1.94)	2.16 (1.15-4.06)*	0.03
No. of low FMD <6.8	161	103	144	55	
Proportion of low FMD <6.8, %	44	49	56	71	<0.01
Age- and sex-adjusted OR (95% CI)	1.00	0.99 (0.69-1.44)	1.20 (0.83-1.73)	2.07 (1.16-3.71)*	0.02
Multivariable OR (95% CI) [a]	1.00	0.98 (0.66-1.45)	1.05 (0.71-1.57)	1.98 (1.07-3.69)*	0.08
Men					
No. of subjects	89	140	210	78	
No. of low FMD <5.1	24	32	68	33	
Proportion of low FMD <5.1, %	27	23	32	42	<0.01
Age-adjusted OR (95% CI)	1.00	0.80 (0.44-1.48)	1.30 (0.75-2.25)	1.99 (1.04-3.80)*	<0.01
Multivariable OR (95% CI) [a]	1.00	0.75 (0.40-1.41)	1.14 (0.64-2.02)	1.97 (1.00-3.89)*	0.01
No. of low FMD <6.8	50	72	122	55	
Proportion of low FMD <6.8, %	56	51	58	71	0.04
Age-adjusted OR (95% CI)	1.00	0.83 (0.48-1.41)	1.08 (0.66-1.78)	2.10 (1.21-3.64)*	0.02
Multivariable OR (95% CI) [a]	1.00	0.69 (0.39-1.21)	0.89 (0.52-1.50)	2.05 (1.10-3.86)*	0.05
Women					
No. of subjects	275	69	49	0	
No. of low FMD <5.1	38	14	6	0	
Proportion of low FMD <5.1, %	14	20	12	0	0.78
Age-adjusted OR (95% CI)	1.00	1.59 (0.81-3.13)	0.87 (0.35-2.18)	-	0.98
Multivariable OR (95% CI) [a]	1.00	1.87 (0.89-3.94)	0.76 (0.28-2.10)	-	0.90
No. of low FMD <6.8	111	31	22	0	
Proportion of low FMD <6.8, %	40	45	45	0	0.44
Age-adjusted OR (95% CI)	1.00	1.21 (0.71-2.05)	1.20 (0.65-2.22)	-	0.48
Multivariable OR (95% CI) [a]	1.00	1.30 (0.74-2.25)	1.27 (0.67-2.42)	-	0.39

*$p<0.05$ compared with never smokers
a. Multivariable variables included age, sex, community, baseline brachial artery diameter, body mass index, systolic blood pressure, use of antihypertensive medication, non-HDL cholesterol, HDL cholesterol, triglycerides, medication use for dyslipidemia, diabetes mellitus, medication use for diabetes, current drinking status and physical activity

Conflict of Interest

All the authors have no conflicts of interest to report.

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