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Effects of smoking cessation on endothelial function as assessed by flow-mediated total dilation

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Abstract

Background In assessing the effects of smoking cessation on endothelial function, low-flow-mediated constriction (L-FMC) may provide complementary information to flow-mediated dilation (FMD). However, the value of flow-mediated total dilation (FMTD), an index that incorporates L-FMC into FMD, remains underreported. We aimed to evaluate the effect of smoking cessation on endothelial function, as assessed by FMD and FMTD, and clarify its associated clinical factors.

Methods We enrolled 118 consecutive current smokers without previous coronary artery disease (72.9% were men; age: 59 ± 11 years) who underwent smoking cessation treatment. The clinical variables %FMD, %L-FMC, and %FMTD were examined before and 20 weeks after treatment initiation. A multivariate linear regression model was used to investigate the effects of smoking cessation on %FMD and %FMTD and the interaction between smoking cessation and baseline clinical variables.

Results After 20 weeks, 85 smokers (69.4% were men; age: 59 ± 12 years) ceased smoking (abstainers), whereas 33 smokers (81.8% were men; age: 58 ± 11 years) did not (continued smokers). The estimated group differences (abstainers - continued smokers) in changes in the %FMD and %FMTD were 0.77% (95% confidence interval [CI], -0.22–1.77%; $p = 0.129$) and 1.17% (95% CI, 0.16–2.18%; $p = 0.024$), respectively. Smoking cessation-associated improvement in %FMTD was greater in women than in men (5.41% [95% CI, 3.15–7.67%] versus 0.24% [95% CI, -0.81–1.28%]; *p*-value for interaction, < 0.001). Additionally, a greater %FMTD improvement was observed in patients who smoked fewer cigarettes per day (*p*-value for interaction, 0.042) and those who had a smaller resting baseline lumen diameter (D_{base}) (*p*-value for interaction, 0.023).

Conclusions Smoking cessation was associated with an improvement in %FMTD. Sex, cigarettes smoked per day, and D_{base} significantly affected this improvement. The FMTD may help in risk stratification after smoking cessation.

Keywords Smoking cessation, Endothelial function, Flow-mediated dilation, Low-flow mediated constriction

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Background

Smoking predisposes individuals to endothelial dysfunction and eventually to arteriosclerosis; it is regarded as a major risk factor for cardiovascular disease (CVD) [1–3]. Therefore, smoking cessation remains the most crucial intervention in preventive and pre-emptive medicine [4, 5].

Endothelial dysfunction is an important factor in the development of atherosclerosis, which precedes the asymptomatic structural vascular alterations and clinical manifestations of CVD. Flow-mediated dilation (FMD) of the brachial artery is an endothelium-dependent, largely nitric oxide (NO)-mediated, dilatation of the conduit arteries in response to an imposed increase in blood flow and shear stress [6]. Thus, FMD is used to assess endothelial function [6, 7]. Smoking intensity is independently associated with endothelial function, as evaluated by measuring FMD [8], which improves after smoking cessation [9–11]. However, the effects of smoking cessation on endothelial function are not always consistent and may differ according to an individual's sex, age, past smoking levels, duration of smoking cessation, and background [12].

Vasoconstriction during the low-flow state, also known as low-flow-mediated constriction (L-FMC), has recently been introduced and proven useful in patients with cardiovascular risk [13, 14]. L-FMC is an endothelial response to reduced blood flow due to forearm compression (resting shear stress) and is induced by several factors such as the release of endothelin-1 and inhibition of the release of cyclooxygenase-dependent products [15, 16]. L-FMC and FMD complement each other, and flow-mediated total dilation (FMTD), an index that incorporates L-FMC into FMD, has been proposed to assess endothelial function. Furthermore, the association between FMTD and cardiovascular risk has been closely examined [17, 18]; we demonstrated that FMTD may be a good alternative to FMD for estimating cardiovascular risk in smokers [18].

When assessing the effects of smoking cessation on endothelial function, L-FMC may provide complementary information to that of FMD. However, no previous study has evaluated the incorporation of the L-FMC index before and after smoking cessation. Thus, we aimed to evaluate the effect of smoking cessation on endothelial function, as assessed by FMD and FMTD, and clarify the associated clinical factors affecting endothelial function response.

Methods

Study population

Consecutive current smokers without a history of coronary artery disease (CAD), who visited our smoking cessation outpatient department and received smoking cessation treatment between April 2010 and July 2017, were enrolled. The inclusion criteria were (1) aged ≥ 20 years, (2) a Brinkman index (number of cigarettes per day × years of smoking) ≥ 200, (3) a nicotine-dependence score ≥ 5 (Tobacco Dependence Screener), and (4) motivation to quit smoking. The criteria complied with the Japanese drug use system for nicotine-dependent outpatients. Smokers with insulin-treated diabetes mellitus and previous CAD were excluded. We used varenicline (a partial agonist of alpha-4 beta-2 nicotinic receptors) to aid in smoking cessation.

Study protocol

For all enrolled participants, FMD and FMTD were measured once at baseline, before initiating the smoking cessation treatment. After vascular tests and venous blood collection at baseline, varenicline was administered and then titrated up as follows: 0.5 mg once daily for 3 days followed by 0.5 mg twice daily for 4 days and finally 1.0 mg twice daily for 11 weeks. Varenicline was discontinued at week 12. All patients were advised to visit our smoking cessation clinic at weeks 2, 4, 8, 12, and 20. Self-reported smoking status, adverse event information, body weight, and exhaled carbon monoxide (CO) concentrations were assessed at each visit. Smoking cessation was confirmed by the participants' self-reported smoking status and an exhaled CO level ≤ 3 ppm at week 20 [19, 20]. Venous blood was collected and the FMD and FMTD were measured again at week 20. Smoking cessation at week 12 but resumption at week 20 was considered unsuccessful smoking cessation in this study. Patients who were followed up at week 20 were analysed.

Measurement of FMD, L-FMC, and FMTD

FMD was measured according to the guidelines for FMD assessment at baseline and week 20 [21]. All participants were required to fast for at least 12 h; avoid heavy exercise for at least 24 h; avoid consuming caffeine-containing products, alcohol, and antioxidant vitamins for at least 6 h; withhold all drugs for at least 12 h; and sleep soundly for at least 6 h the night before the measurement. For premenopausal female participants, examinations were performed during the menstrual period. All participants rested in a sitting position in a quiet, dimmed, temperature-controlled room (22–25 °C) for 15 min and were subsequently placed in a supine position for 15 min. Brachial artery measurements were performed between 7:00 and 11:59 a.m.

We used a 10-MHz H-type probe (UNEXEF; UNEX, Nagoya, Japan) equipped with a semiautomatic vessel wall tracking software that provided one longitudinal image, two short-axis B-mode images, and one processed A-mode line image of the brachial artery [22]. Through continuous and simultaneous monitoring, two B-mode

short-axis vessel images were obtained using the edge-detection method, which was designed to maintain the same brachial artery position by adjusting the deviation of the probe position before and after forearm compression. The centre of the brachial artery and the positional stability of the A-mode lines were preserved throughout the FMD examination. A-mode lines were used to automatically measure vessel lumens on longitudinal images. A total of 41 A-mode lines, including 20 bilateral points surrounding the designated point, were measured at 0.15-mm intervals to precisely measure the vessel lumen. The measured values were averaged and presented in the images. Electrocardiogram gating was used during image acquisition, in which the onset of the R-wave was used to identify end diastole. A B-mode edge detection method was designed to automatically maintain the same position in the brachial artery by adjusting the deviation in the probe position before and after forearm compression to precisely measure the vessel lumen [21]. After determining the probe position at which the clearest baseline image could be obtained, an occlusive cuff was wrapped around the forearm with the proximal edge of the cuff at the elbow. The forearm cuff was inflated to a systolic blood pressure (SBP) of at least 50 mm Hg for 5 min. Longitudinal images of the brachial artery were automatically and continuously recorded from 0 s after cuff inflation to 5 min after cuff release.

L-FMC was defined as vasoconstriction during the 30 s before cuff release (Fig. 1) [12]. The presence of L-FMC was defined as L-FMC of 0.05 mm based on our previous study, which demonstrated that the intra-observer mean difference for lumen diameter measurements was 0.021 ± 0.016 mm using the same ultrasound system [20]. We calculated the %L-FMC as the change from the resting baseline lumen diameter (D_{base}) to the minimum lumen diameter during the 30 s before cuff release (D_{min}) divided by the D_{base}.

$$\%\mathrm{L-FMC} = \frac{D_{min} - D_{base}}{D_{base}} \times 100$$

The percentage of maximum change from D_{base} to the hyperaemic state (D_{max}) divided by D_{base} was defined as %FMD (Fig. 1). The sum of the %FMD and absolute %L-FMC values was defined as %FMTD, which was calculated as the percentage of the maximum change from D_{min} to D_{max} divided by D_{base} (Fig. 1) [14]. These indices were calculated using the following formulas:

$$\%\mathrm{FMD} = \frac{D_{max} - D_{base}}{D_{base}} \times 100$$

$$\%\mathrm{FMTD} = \frac{D_{max} - D_{min}}{D_{base}} \times 100$$

Clinical data and definition

Laboratory data were obtained using fasting blood samples collected on the same day as the FMD measurements. Hypertension was defined as a SBP ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, and/or antihypertensive medication use. Diabetes mellitus was defined as a fasting blood glucose level ≥ 126 mg/dL, glycosylated haemoglobin ≥ 6.5%, and/or use of an oral hypoglycaemic agent. Dyslipidaemia was defined as high-density lipoprotein cholesterol (HDL-C) levels < 40 mg/dL, low-density lipoprotein cholesterol levels ≥ 140 mg/dL, triglyceride levels ≥ 150 mg/dL, and/or use of a lipid-lowering medication. The change was defined as the value at week 20 minus the value at baseline, and the difference

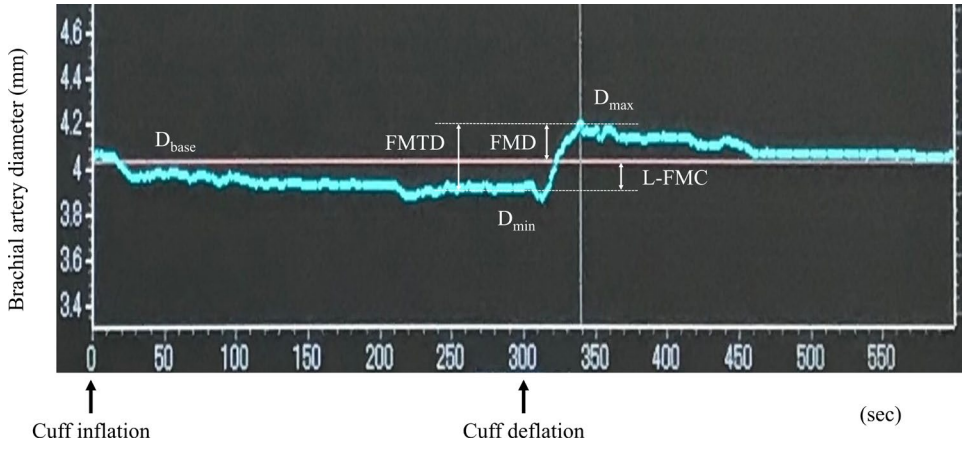

Fig. 1 A representative case with the presence of L-FMC. This patient demonstrated vasoconstriction of no less than 0.05 mm during the last 30 s before cuff release. %FMD and %FMTD were calculated as 3.23% and 6.45%, respectively. D_{base} = resting baseline lumen diameter; D_{max} = the lumen diameter of the hyperemic state; D_{min} = minimum lumen diameter during the last 30 s before cuff release; L-FMC = low-flow-mediated constriction; FMD = flow-mediated dilation; FMTD = flow-mediated total dilation

was defined as the value in abstainers minus the value in continued smokers.

Statistical analysis

The participants' clinical characteristics are presented as mean ± standard deviation for continuous variables and as percentage (number) for categorical variables. Student's *t*-test was used to compare continuous variables, and the χ^2 test was used to compare categorical variables (men vs. women) at baseline. The change in each clinical parameter from baseline to week 20 was compared between smokers who successfully ceased smoking and those who did not using the Student's *t*-test. A linear regression model was used to investigate the effect of smoking cessation on endothelial function as assessed by %FMD and %FMTD. In the linear regression model, we included changes in %FMD and %FMTD as dependent variables and success in smoking cessation (yes or no) and adjustment variables (sex, age, body mass index [BMI], cigarettes smoked per day, hypertension, and Dbase at baseline) as explanatory variables. Another model was developed to examine the interactions between successful smoking cessation and each factor. Statistical analyses were performed using SPSS software (version 28.0; SPSS, IBM Corp., Inc., Chicago, IL, USA) and R version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria). A p-value < 0.05 was defined as statistically significant.

Table 1 Baseline characteristics

	Overall (*n* = 118)	Females (*n* = 32)	Males (*n* = 86)	*p* value
Age, years	59 ± 11	59 ± 12	59 ± 11	0.868
BMI, kg/m^2	23.7 ± 3.7	22.1 ± 2.8	24.3 ± 3.8	0.003**
Brinkman index	815 ± 400	700 ± 308	858 ± 422	0.055
Cigarettes per day	23 ± 10	21 ± 6	24 ± 10	0.088
CO concentration, ppm	15.4 ± 10.3	14.1 ± 10.6	15.9 ± 10.3	0.414
Hypertension	37.3% (*n* = 44)	31.3% (*n* = 10)	39.5% (*n* = 34)	0.522
SBP, mmHg	119.0 ± 17.8	117.5 ± 19.9	119.6 ± 17.0	0.558
DBP, mmHg	75.9 ± 10.1	74.3 ± 11.1	76.5 ± 9.7	0.294
HR, beats/min	66.0 ± 11.8	66.6 ± 10.8	65.8 ± 12.2	0.769
Diabetes mellitus	23.7% (*n* = 28)	28.1% (*n* = 9)	22.1% (*n* = 19)	0.659
HbA1c, %	6.0 ± 0.9	6.0 ± 0.8	6.0 ± 0.9	0.870
Fasting glucose, mg/dl	100.7 ± 20.5	99.2 ± 18.7	101.2 ± 21.3	0.628
Dyslipidemia	68.6% (*n* = 81)	68.8% (*n* = 22)	68.6% (*n* = 59)	1.000
HDL-C, mg/dl	50.4 ± 14.5	60.5 ± 15.7	46.6 ± 12.1	< 0.001**
LDL-C, mg/dl	114.6 ± 33.0	123.7 ± 33.3	111.2 ± 32.4	0.067
Triglycerides, mg/dl	151.4 ± 99.2	127.6 ± 70.6	160.2 ± 106.9	0.119
eGFR, ml/min/1.73 m^2	75.9 ± 20.0	74.7 ± 26.4	76.4 ± 17.1	0.694
Medication				
Antihypertensive	30.2% (*n* = 35)	28.1% (*n* = 9)	31.0% (*n* = 26)	0.767
Oral hypoglycemic	15.7% (*n* = 18)	21.9% (*n* = 7)	13.3% (*n* = 11)	0.254
Lipid lowering	24.3% (*n* = 28)	31.3% (*n* = 10)	21.7% (*n* = 18)	0.284
D_{base}, mm	4.11 ± 0.67	3.45 ± 0.54	4.35 ± 0.54	< 0.001**
L-FMC	33.9% (*n* = 40)	21.9% (*n* = 7)	38.4% (*n* = 33)	0.143
%L-FMC, %	-1.16 ± 1.99	-1.08 ± 2.37	-1.19 ± 1.85	0.788
%FMD, %	4.05 ± 2.13	3.95 ± 2.26	4.09 ± 2.09	0.759
%FMTD, %	5.21 ± 2.54	5.03 ± 2.54	5.28 ± 2.55	0.641
baIMT, mm	0.31 ± 0.07	0.28 ± 0.06	0.32 ± 0.08	0.029 *

BMI = body mass index; CO = carbon monoxide; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; HbA1c = glycosylated hemoglobin; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; eGFR = estimated glomerular giltration rate; D_{base} = resting baseline lumen diameter; L-FMC = low-flow-mediated constriction; FMD = flow-mediated dilation; FMTD = flow-mediated total dilation; baIMT = brachial artery intima-media thickness

*p < 0.05, **p < 0.01

Results

Patient characteristics

We included 118 participants who were followed-up for 20 weeks after the initiation of the smoking cessation treatment. Their baseline characteristics (72.9% were men; age: 59 ± 11 years) are shown in Table 1. Regarding antihypertensive medications, 27 smokers (23%) used calcium channel blockers, 22 (19%) used renin-angiotensin system inhibitors, and 2 (2%) used beta-blockers. At baseline, L-FMC was demonstrated in 33.9% of the smokers; %FMD was 4.05% ± 2.13%, and %FMTD was 5.21% ± 2.54%. No significant differences were observed between men and women except for BMI, HDL-C levels, D_{base}, and brachial artery intima-media thickness.

Changes in clinical parameters

At week 20, 85 smokers (male: 69.4% were men; age: 59 ± 12 years) successfully ceased smoking (abstainers), whereas 33 smokers (81.8% were men; age: 58 ± 11 years) did not (continued smokers). The clinical parameters of abstainers and continued smokers at baseline and at week 20 are presented in Table 2. No significant differences were observed between abstainers and continued smokers, except for changes in SBP and HDL-C levels.

Effects of smoking cessation on endothelial function

At week 20, 27.1% of the abstainers demonstrated L-FMC, with %FMD and %FMTD values of 4.89% ± 2.22% and 5.77% ± 2.91%, respectively. In continued smokers, L-FMC was demonstrated in 24.2%, with %FMD and %FMTD values of 4.38% ± 2.91% and 4.86% ± 3.11%, respectively (Table 2). After adjusting for sex, age, BMI, cigarettes smoked per day, hypertension, and D_{base} at baseline, the estimated group differences (abstainers

Table 2 Comparison of changes in clinical parameters between abstainers and continued smokers

	Abstainers ($n=85$)			Continued smokers ($n=33$)			*p*-value[a]
	Baseline	Week 20	Change	Baseline	Week 20	Change	
Sex, % males	69.4% ($n=59$)			81.8% ($n=27$)			
Age, years	59 ± 12			58 ± 11			
BMI, kg/m^2	23.7 ± 3.7	24.4 ± 3.8	0.7 ± 0.9	23.8 ± 3.8	24.3 ± 4.1	0.5 ± 0.8	0.157
Brinkman index	817 ± 410			808 ± 378			
Cigarettes per day	22 ± 10			24 ± 10			
CO concentration, ppm	14.3 ± 9.7	1.9 ± 0.8	-12.5 ± 9.8	18.2 ± 11.7	7.6 ± 5.0	-10.8 ± 11.8	0.520
Hypertension	34.1% ($n=29$)			45.4% ($n=15$)			
SBP, mmHg	117.7 ± 19.1	121.7 ± 17.1	4.0 ± 11.5	122.5 ± 13.7	121.8 ± 15.8	-0.7 ± 11.2	0.048*
DBP, mmHg	74.6 ± 10.6	77.0 ± 9.7	2.4 ± 7.6	79.1 ± 7.8	79.2 ± 9.2	0.2 ± 7.7	0.161
HR, beats/min	65.2 ± 10.9	66.5 ± 10.3	0.7 ± 7.6	68.2 ± 13.8	67.2 ± 13.5	-0.3 ± 10.5	0.576
Diabetes mellitus	23.5% ($n=20$)			24.2% ($n=8$)			
HbA1c, %	6.0 ± 0.9	6.2 ± 1.1	0.1 ± 0.6	6.0 ± 0.9	6.0 ± 1.3	0.0 ± 0.5	0.139
Fasting glucose, mg/dl	100.7 ± 22.9	108.7 ± 33.5	7.9 ± 19.6	100.8 ± 12.5	103.3 ± 24.0	2.5 ± 17.8	0.180
Dyslipidemia	71.8% ($n=61$)			60.6% ($n=20$)			
HDL-C, mg/dl	50.9 ± 15.7	54.0 ± 17.8	3.0 ± 9.6	49.1 ± 10.8	46.8 ± 11.3	-2.3 ± 9.1	0.007**
LDL-C, mg/dl	116.0 ± 34.5	118.5 ± 38.5	2.3 ± 27.7	110.7 ± 28.6	111.1 ± 34.9	0.4 ± 27.1	0.741
Triglycerides, mg/dl	153.0 ± 98.0	170.7 ± 206.9	17.6 ± 171.0	147.1 ± 103.7	167.1 ± 94.1	20.0 ± 119.1	0.941
eGFR, ml/min/1.73 m^2	75.5 ± 21.1	74.6 ± 16.4	-1.0 ± 16.1	77.1 ± 17.0	75.0 ± 25.8	-2.1 ± 14.8	0.721
D_{base}, mm	4.04 ± 0.67	4.12 ± 0.65	0.08 ± 0.36	4.27 ± 0.66	4.29 ± 0.62	0.02 ± 0.43	0.459
L-FMC	35.3% ($n=30$)	27.1% ($n=23$)		30.3% ($n=10$)	24.2% ($n=8$)		
%L-FMC, %	-1.15 ± 1.90	-0.88 ± 1.71	0.27 ± 2.19	-1.20 ± 2.24	-0.48 ± 1.11	0.76 ± 2.31	0.292
%FMD, %	3.93 ± 1.91	4.89 ± 2.22	0.95 ± 2.28	4.35 ± 2.62	4.38 ± 2.91	0.06 ± 2.81	0.079
%FMTD, %	5.08 ± 2.36	5.77 ± 2.91	0.69 ± 2.59	5.55 ± 2.96	4.86 ± 3.11	-0.70 ± 2.03	0.007**
baIMT, mm	0.30 ± 0.08	0.31 ± 0.08	0.00 ± 0.06	0.32 ± 0.06	0.32 ± 0.06	0.00 ± 0.06	0.824

BMI = body mass index; CO = carbon monoxide; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; HbA1c = glycosylated hemoglobin; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; eGFR = estimated glomerular giltration rate; D_{base} = resting baseline lumen diameter; L-FMC = low-flow-mediated constriction; FMD = flow-mediated dilation; FMTD = flow-mediated total dilation; baIMT = brachial artery intima-media thickness

*$p<0.05$, **$p<0.01$

[a]Change, abstainers vs. continued smokers

- continued smokers) in changes in the %FMD and %FMTD were 0.77% (95% confidence interval [CI], -0.22–1.77%; $p=0.129$) and 1.17% (95% CI, 0.16–2.18%; $p=0.024$), respectively (Fig. 2; Table 3).

Interaction between smoking cessation and each factor

The interaction between smoking cessation and each factor after adjusting for sex, age, BMI, cigarettes smoked per day, hypertension, and D_{base} at baseline is presented in Table 3; Fig. 3. No significant interaction was observed between smoking cessation and any of the factors affecting the %FMD. Conversely, smoking cessation-associated improvement in %FMTD was greater in women than in men (5.41% [95% CI, 3.15–7.67%] versus 0.24% [95% CI, -0.81–1.28%]; *p*-value for interaction, <0.001). Additionally, a greater %FMTD improvement was observed in patients who smoked fewer cigarettes per day (*p*-value for interaction, 0.042) and had a smaller D_{base} (*p*-value for interaction, 0.023). No significant interactions were observed between smoking cessation and other clinical factors, including age, BMI, or hypertension at baseline.

Discussion

This study demonstrated that smoking cessation was associated with improvement in %FMTD; however, %FMD was not significantly different at 20 weeks after treatment initiation. In addition, smoking cessation-associated improvement in %FMTD was greater in women than in men, as well as in patients who smoked fewer cigarettes per day and those with a smaller D_{base}. To the best of our knowledge, this is the first study to assess the effects of smoking cessation on endothelial function using the FMTD.

A previous study showed that smoking cessation for 1 year improved endothelial function as assessed by FMD [9]. Another study also supported the notion of partial reversibility of smoking-induced endothelial dysfunction, showing that endothelial function in former smokers, as assessed by FMD, was impaired compared to that in normal non-smoking controls [8]. Moreover, the maximum improvement in vascular function after environmental cigarette smoke withdrawal was observed more than 2 years after smoking cessation [23], and years since quitting significantly improved atherosclerotic CVD risk

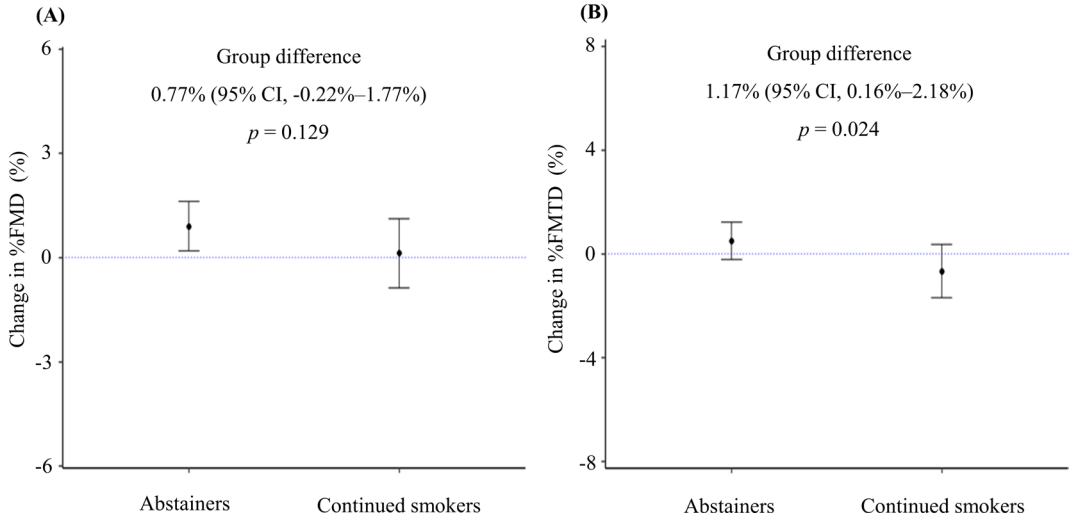

Fig. 2 The estimated changes in %FMD and %FMTD in abstainers and continued smokers. A linear regression model included changes in %FMD (**A**) and %FMTD (**B**) as dependent variables and success in smoking cessation (yes or no) and adjustment variables (sex, age, body mass index, cigarettes per day, hypertension, and Dbase at baseline) as explanatory variables. D_{base} = resting baseline lumen diameter; FMD = flow-mediated dilation; FMTD = flow-mediated total dilation

prediction [7]. These findings suggest that a longer smoking cessation period results in more evident improvements in endothelial function after smoking cessation. In short-term evaluations, some studies have reported increased %FMD in abstainers [10, 11, 24, 25]; however, the results are not always consistent and may differ according to sex, age, past smoking levels, duration of cessation, and individual background [12]. In short-term evaluations, the effects of smoking cessation analysed using multivariate analysis with continued smokers as the control group have not yet been fully elucidated. The lack of a significant association between the %FMD and successful smoking cessation in our study may be partly attributable to the short evaluation period.

Smoking-induced endothelial dysfunction is associated with multiple factors, including decreased plasma NO concentration, enhanced oxidative damage, and impaired prostacyclin production [26–29]. Several previous studies have investigated whether FMD measurement alone can adequately reflect endothelial responsiveness to altered haemodynamic stimuli [30], as it mainly measures the NO-dependent part of endothelial function [21]. Simultaneous measurement of L-FMC, which appears to be NO-independent, has been proposed [31, 32]. L-FMC is induced by several factors such as the release of endothelin-1 and inhibition of the release of cyclooxygenase-dependent products [15]. FMTD has also been proposed and shown to be superior to FMD in estimating the cardiovascular risk in both smokers and non-smokers [17, 18]. In our study, the L-FMC responses tended to be attenuated after treatment in both abstainers and smokers. As the presence of L-FMC has been reported to be associated with a lower BMI in smokers [18], L-FMC attenuation might be, in part, due to increased BMI after treatment. Another study showed a reduced L-FMC response in smokers [14]. In abstainers, smoking cessation was expected to have a positive effect on L-FMC responses. Therefore, the complementary value of L-FMC in assessing the effect of smoking cessation achievement on endothelial function may explain our finding that changes in %FMTD, rather than in %FMD, were significantly associated with smoking cessation.

Smoking approximately one cigarette per day increases the risk of developing coronary heart disease [33]. However, smoking cessation, but not smoking reduction, is associated with reduced CVD risk [34], further emphasising the importance of successful smoking cessation to reduce the risk of CVD. After smoking cessation, pack-years smoked may play an important role in estimating CVD risk [35], and smoking cessation benefits may depend on past smoking levels [36]. Our study showed greater smoking cessation-associated %FMTD improvement in patients who smoked fewer cigarettes per day. These findings suggest that smoking-induced endothelial dysfunction reversibility is dependent on past smoking levels; thus, patients who smoked more cigarettes per day had less endothelial function improvement after smoking cessation.

A recent study demonstrated that excess CVD risk among former heavy smokers compared with never smokers can persist for up to 16 years after cessation [37]. However, data on how sex may influence the risk of CVD in former smokers compared with current smokers are limited because most large prospective studies with data on smoking cessation have focused heavily on men [38]. Smoking is a greater risk factor for CVD in women

Table 3 Interaction between smoking cessation and each clinical factor

	Change in %FMD								Change in %FMTD							
	Abstainers		Continued smokers		Difference		*p*-value[a]	*p*-value for interaction	Abstainers		Continued smokers		Difference		*p*-value[a]	*p*-value for interaction
	Estimate (95% CI)		Estimate (95% CI)		Estimate (95% CI)				Estimate (95% CI)		Estimate (95% CI)		Estimate (95% CI)			
Overall	0.89	(0.18 to 1.60)	0.12	(-0.87 to 1.11)	0.77	(-0.22 to 1.77)	0.129		0.49	(-0.23 to 1.21)	-0.67	(-1.69 to 0.34)	1.17	(0.16 to 2.18)	0.024*	
Sex								0.189								< 0.001**
Males	0.80	(0.07 to 1.52)	0.37	(-0.70 to 1.43)	0.43	(-0.69 to 1.55)	0.452		0.24	(-0.45 to 0.92)	0.00	(-1.00 to 1.01)	0.24	(-0.81 to 1.28)	0.658	
Females	1.75	(0.63 to 2.87)	-0.38	(-2.49 to 1.73)	2.13	(-0.14 to 4.40)	0.066		2.37	(1.33 to 3.42)	-3.03	(-5.20 to -0.87)	5.41	(3.15 to 7.67)	< 0.001**	
Age, years								0.966								0.065
40	0.89	(-0.23 to 2.00)	0.08	(-1.60 to 1.77)	0.81	(-1.11 to 2.73)	0.411		1.01	(-0.08 to 2.10)	-1.75	(-3.52 to 0.02)	2.76	(0.79 to 4.72)	0.006**	
50	0.89	(0.08 to 1.70)	0.10	(-1.03 to 1.24)	0.79	(-0.43 to 2.01)	0.207		0.71	(-0.09 to 1.50)	-1.14	(-2.32 to 0.03)	1.85	(0.61 to 3.09)	0.003**	
60	0.89	(0.17 to 1.61)	0.13	(-0.89 to 1.14)	0.77	(-0.26 to 1.79)	0.145		0.41	(-0.31 to 1.13)	-0.54	(-1.56 to 0.48)	0.95	(-0.08 to 1.97)	0.070	
70	0.89	(-0.03 to 1.81)	0.15	(-1.30 to 1.59)	0.74	(-0.80 to 2.29)	0.346		0.11	(-0.81 to 1.03)	0.06	(-1.39 to 1.52)	0.04	(-1.51 to 1.60)	0.955	
BMI (baseline), kg/m^2								0.876								0.284
20.0	1.17	(0.20 to 2.14)	0.50	(-0.84 to 1.83)	0.68	(-0.82 to 2.17)	0.371		0.99	(0.04 to 1.94)	-0.76	(-2.07 to 0.54)	1.75	(0.29 to 3.22)	0.019*	
22.0	0.98	(0.18 to 1.78)	0.26	(-0.84 to 1.36)	0.72	(-0.44 to 1.88)	0.220		0.72	(-0.07 to 1.50)	-0.74	(-1.82 to 0.34)	1.46	(0.32 to 2.59)	0.012*	
24.0	0.79	(0.06 to 1.53)	0.03	(-1.02 to 1.08)	0.76	(-0.28 to 1.80)	0.148		0.44	(-0.28 to 1.16)	-0.72	(-1.75 to 0.31)	1.16	(0.14 to 2.18)	0.026*	
26.0	0.60	(-0.19 to 1.39)	-0.20	(-1.41 to 1.00)	0.81	(-0.39 to 2.01)	0.185		0.16	(-0.61 to 0.94)	-0.70	(-1.88 to 0.49)	0.86	(-0.32 to 2.04)	0.150	
Cigarettes per day								0.206								0.042*
10	1.45	(0.46 to 2.45)	-0.25	(-1.93 to 1.42)	1.71	(-0.12 to 3.53)	0.067		0.93	(-0.04 to 1.90)	-1.78	(-3.41 to -0.15)	2.71	(0.93 to 4.49)	0.003**	
20	0.94	(0.21 to 1.66)	-0.07	(-1.15 to 1.00)	1.01	(-0.10 to 2.12)	0.073		0.55	(-0.16 to 1.25)	-1.07	(-2.11 to -0.02)	1.61	(0.54 to 2.69)	0.004**	
30	0.42	(-0.40 to 1.24)	0.11	(-1.01 to 1.22)	0.32	(-0.89 to 1.52)	0.606		0.16	(-0.64 to 0.96)	-0.36	(-1.44 to 0.72)	0.52	(-0.66 to 1.70)	0.383	
40	-0.09	(-1.29 to 1.10)	0.29	(-1.46 to 2.03)	-0.38	(-2.39 to 1.63)	0.709		-0.22	(-1.39 to 0.95)	0.35	(-1.35 to 2.05)	-0.57	(-2.53 to 1.39)	0.563	
Hypertension								0.577								0.577

Table 3 (continued)

	Change in %FMD								Change in %FMTD							
	Abstainers		Continued smokers		Difference		*p*-value[a]	*p*-value for interaction	Abstainers		Continued smokers		Difference		*p*-value[a]	*p*-value for interaction
	Estimate (95% CI)		Estimate (95% CI)		Estimate (95% CI)				Estimate (95% CI)		Estimate (95% CI)		Estimate (95% CI)			
Yes	0.79	(-0.25 to 1.83)	0.37	(-0.94 to 1.67)	0.42	(-1.15 to 2.00)	0.595		0.14	(-0.89 to 1.17)	-0.71	(-2.00 to 0.57)	0.85	(-0.70 to 2.41)	0.280	
No	0.93	(0.17 to 1.69)	-0.08	(-1.33 to 1.18)	1.01	(-0.35 to 2.36)	0.144		0.54	(-0.21 to 1.29)	-0.89	(-2.12 to 0.35)	1.42	(0.09 to 2.76)	0.037*	
D_{base} (baseline), mm								0.552								0.023*
3.00	0.80	(-0.58 to 2.18)	-0.59	(-2.87 to 1.69)	1.39	(-0.95 to 3.74)	0.241		0.95	(-0.38 to 2.28)	-2.58	(-4.78 to -0.39)	3.53	(1.28 to 5.79)	0.002**	
4.00	0.88	(0.12 to 1.64)	-0.01	(-1.14 to 1.12)	0.89	(-0.23 to 2.02)	0.119		0.56	(-0.17 to 1.30)	-1.12	(-2.20 to -0.03)	1.68	(0.59 to 2.76)	0.003**	
5.00	0.96	(-0.08 to 1.99)	0.56	(-0.89 to 2.02)	0.39	(-1.21 to 1.99)	0.627		0.17	(-0.83 to 1.17)	0.35	(-1.06 to 1.76)	-0.18	(-1.73 to 1.36)	0.816	

A linear regression model was used after adjusting for sex, age, BMI, cigarettes per day, hypertension, and D_{base} at baseline

BMI = body mass index; CI = confidence interval; D_{base} = resting baseline lumen diameter

*$p<0.05$, **$p<0.01$

[a] Abstainers vs. continued smokers

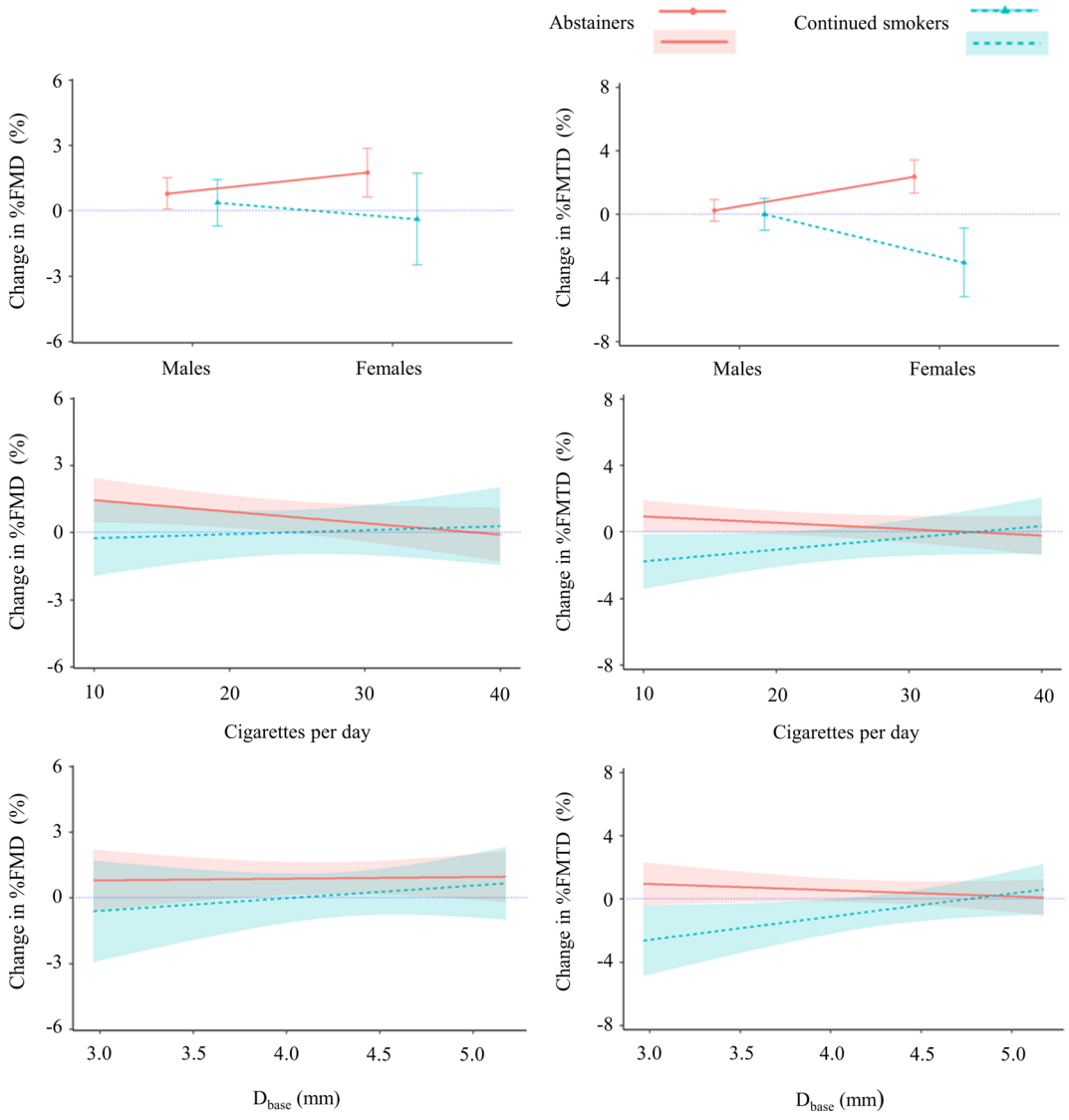

Fig. 3 Interaction between smoking cessation and each clinical factor. A linear regression model was used after adjusting for sex, age, body mass index, cigarettes smoked per day, hypertension, and D_{base}. D_{base} = resting baseline lumen diameter; FMD = flow-mediated dilation; FMTD = flow-mediated total dilation

than in men [39, 40], and the possible underlying mechanism may involve endothelial function [41]. The chemical constituents of cigarette smoke have high oxidant and inflammatory effects that can potentiate an inflammatory response, and women may extract a greater quantity of toxic agents from the same number of cigarettes than do men [42, 43]. However, our study showed greater smoking cessation-associated %FMTD improvement in women than in men, suggesting that women smokers may benefit more at 20 weeks after the initiation of smoking cessation treatment. Although the precise mechanism is unknown, cigarette smoke exposure increases oxidative stress, whereas smoking cessation reduces oxidative damage in endothelial cells [9, 44]. These characteristics of endothelial damage associated with smoking may partially account for the results of the present study.

Large lumen diameters are mostly accompanied by low basal tone and are prone to constriction under low-flow conditions, as indicated by L-FMC [30]. Moreover, restructuring the cellular and non-cellular components of the vessel wall in response to long-term changes in haemodynamic conditions and/or chronic atherogenic exposure may change the vessel luminal diameter [45]. An enlarged brachial artery diameter has been described as a significant predictor of cardiovascular events in a population-based cohort study [46]. Our study showed greater smoking cessation-associated %FMTD improvement in patients with smaller baseline brachial artery diameters. These findings suggest that patients with advanced

vascular morphological changes have less responsive endothelial function after smoking cessation.

Endothelial function measurements can be used to monitor responses to lifestyle changes, and patients whose endothelial function does not improve with interventions may be at considerable risk of further adverse events [6, 47]. These studies support the potential use of repeated endothelial function assessments, rather than a single measure, to predict future cardiovascular events [48]. In cross-sectional studies, the FMTD can better indicate the estimated CVD risk in both smokers and non-smokers in primary prevention settings [17, 18]. L-FMC may provide complementary and additive information to that of FMD on how individual endothelial function responds to smoking cessation treatment; however, no previous studies have investigated this possibility. Our study results suggest that FMTD can be a better indicator of the effects of smoking cessation on endothelial function and identify the clinical factors that interact with these effects, which may help in risk stratification after cessation.

The present study has certain limitations. First, although we enrolled consecutive smokers who visited the smoking cessation outpatient department, completed smoking cessation treatment, and were evaluated for endothelial function, the number of enrolled participants were few to adjust for all covariates to correctly estimate changes in the %FMD and %FMTD. Further prospective studies with a larger number of participants are necessary to clarify the effects of smoking cessation on the L-FMC index. Second, 27 smokers (23%) used calcium channel blockers, 22 (19%) used renin-angiotensin system inhibitors, and two (2%) used beta-blockers. Any of the patients were not using nebivolol. In this study, all participants were required to withhold all medications for at least 12 h before endothelial function measurements. However, the possibility that medications affected endothelial function cannot be completely eliminated. Additionally, we enrolled participants who used varenicline as a smoking cessation aid. To minimise the effect of varenicline on endothelial function, we set the time to determine the effect at week 20, which was 8 weeks after participants completed 12 weeks of the smoking cessation program. However, to accurately assess the effect of smoking cessation on endothelial function, it is necessary to evaluate participants who achieved smoking cessation without using any pharmacotherapies. Further examinations are warranted to clarify the impact of smoking cessation on the L-FMC-incorporated index over a longer period and the association between endothelial responsiveness to smoking cessation treatment and future cardiovascular events.

Conclusions

Smoking cessation was significantly associated with %FMTD but not with %FMD improvement at 20 weeks after treatment initiation. In addition, greater smoking cessation-associated %FMTD improvements were noted in women, those who smoked fewer cigarettes per day, and those who had a smaller D_{base}. FMTD may be a better indicator to assess the effects of smoking cessation on endothelial function and identify the clinical factors that interact with its effects, which is important in risk stratification after cessation. Further prospective studies with larger sample sizes and longer follow-up periods are required to confirm our findings.

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Author contributions

Naoki Okuyama: Data curation, Formal analysis, Methodology, Writing-Original draft. Kazuo Fukumoto: Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing-Original draft, Writing-review & editing. Yasuhiko Takemoto: Conceptualization, Methodology, Supervision, Writing-review & editing. Takeshi Yamauchi: Data curation. Ayako Makuuchi: Data curation. Hiroki Namikawa: Data curation. Hiromitsu Toyoda: Data curation. Yoshihiro Tochino: Data curation. Yasuhiro Izumiya: Writing-review & editing. Daiju Fukuda: Writing-review & editing. Taichi Shuto: Conceptualization, Supervision, Writing-review & editing.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participant

The study protocol adhered to the Declaration of Helsinki and was approved by the Ethics Committee of Osaka City University (approval nos. 1744 and 1752).

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors declare no competing interests.

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