

Online Supplementary Data

**Relationship Between Flow-mediated Vasodilation and Cardiovascular Risk
Factors in a Large Community-based Study**

Running title: Confounding factor of FMD

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Methods

Measurement of FMD

A high-resolution linear artery transducer (10-MHz) was coupled to computer-assisted analysis software (UNEXEF18G, UNEX Co, Nagoya, Japan) that used an automated edge detection system for measurement of brachial artery diameter. A blood pressure cuff was placed around the forearm. The brachial artery was scanned longitudinally 5 to 10 cm above the elbow. When the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained, the transducer was held at the same point throughout the scan by a special probe holder (UNEX Co) to ensure consistency of the image. Depth and gain setting were set to optimize the images of the arterial lumen wall interface. When the tracking gate was placed on the intima, the artery diameter was automatically tracked, and the waveform of diameter changes over the cardiac cycle was displayed in real time using the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 seconds after cuff deflation. Blood flow velocity was calculated from the color Doppler data and was displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 seconds, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 minutes. The longitudinal image of the artery was recorded continuously until 5 minutes after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 seconds at baseline and for 10 seconds immediately after cuff deflation. Changes in

brachial artery diameter were immediately expressed as percentage change relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage change in peak vessel diameter from the baseline value. Percentage of FMD $(\text{Peak diameter} - \text{Baseline diameter} / \text{Baseline diameter})$ was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (πr^2). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow. The observers were blind to the form of examination.

Table I. Univariate Analysis of the Relation Between Flow-Mediated Vasodilation and Variables

Variables	r	Total P value
Age, y	-0.27	<0.001
Body mass index, kg/m ²	-0.14	<0.001
Systolic blood pressure, mm Hg	-0.18	<0.001
Diastolic blood pressure, mm Hg	-0.13	<0.001
Heart rate, bpm	0.02	0.20
Total cholesterol, mmol/L	-0.07	<0.001
Triglycerides, mmol/L	-0.10	<0.001
HDL cholesterol, mmol/L	0.06	<0.001
LDL cholesterol, mmol/L	-0.04	0.01
Glucose, mmol/L	-0.14	<0.001
HbA1c, %	-0.14	<0.001
Framingham risk score	-0.29	<0.001
Baseline brachial artery diameter, mm	-0.43	<0.001

HDL indicates high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Table II. Clinical Characteristics of the Decade of Age

Variables	Age, y						P value
	20-29 (n=763)	30-39 (n=620)	40-49 (n=1511)	50-59 (n=1759)	60-69 (n=448)	70≤ (n=196)	
Age, y	22.7±2.7	35.4±3.0	45.2±3.3	53.8±3.2	62.8±2.7	75.0±4.6	<0.001
Body mass index, kg/m ²	21.2±2.44	22.9±3.66	23.5±3.38	23.4±3.11	23.2±2.87	22.6±3.39	<0.001
Systolic blood pressure, mm Hg	112.8±11.9	119.0±14.5	124.4±15.5	127.8±17.2	133.8±18.6	140.2±18.9	<0.001
Diastolic blood pressure, mm Hg	63.4±9.0	72.7±11.4	78.4±12.1	79.7±12.2	80.3±12.8	77.3±10.8	<0.001
Heart rate, bpm	64.7±10.8	63.8±9.5	63.5±10.2	63.3±10.3	65.6±11.6	68.9±11.8	<0.001
Total cholesterol, mmol/L	4.50±0.64	4.98±0.84	5.36±0.86	5.44±0.84	5.35±0.90	5.01±0.86	<0.001
Triglycerides, mmol/L	1.00±1.11	1.28±1.04	1.53±1.12	1.50±0.91	1.50±1.31	1.31±0.76	<0.001
HDL cholesterol, mmol/L	1.66±0.33	1.52±0.38	1.55±0.41	1.59±0.44	1.54±0.43	1.52±0.44	<0.001
LDL cholesterol, mmol/L	2.49±0.66	3.01±0.75	3.30±0.78	3.30±0.77	3.20±0.92	2.91±0.75	<0.001
Glucose, mmol/L	4.90±0.44	5.12±0.74	5.51±0.98	5.69±1.15	5.92±1.99	6.16±1.80	<0.001
HbA1c, %	4.82±0.22	4.94±0.40	5.09±0.54	5.23±0.61	5.42±0.75	5.65±0.76	<0.001
Framingham risk score	-6.60±6.39	-2.68±5.82	2.31±3.09	5.05±2.53	7.51±2.70	8.94±2.41	<0.001
Baseline brachial artery diameter, mm	3.65 ±0.52	3.84±0.58	4.08±0.60	4.12±0.55	4.10±0.60	4.10±0.65	<0.001
FMD, %	7.33±3.51	7.13±3.22	6.45±3.20	5.61±3.00	4.88±2.90	4.10±2.45	<0.001
Hypertension, n (%)	4 (0.5)	25 (4.1)	142 (9.4)	281 (16.0)	170 (37.9)	115 (60.2)	<0.001
Dyslipidemia, n (%)	42 (5.5)	205 (33.2)	755 (50.1)	901 (51.3)	267 (59.6)	93 (48.4)	<0.001
Diabetes mellitus, n (%)	0 (0)	4 (0.6)	77 (5.1)	134 (7.6)	78 (17.5)	43 (22.4)	<0.001
Smoking, n (%)	61 (8.0)	163 (26.5)	482 (32.7)	606 (34.6)	120 (27.1)	51 (27.1)	<0.001
Coronary heart disease, n (%)	0 (0)	3 (0.5)	14 (0.9)	44 (2.5)	32 (7.2)	24 (12.7)	<0.001
Cerebrovascular disease, n (%)	1 (0.13)	1 (0.16)	4 (0.35)	7 (0.55)	18 (4.0)	15 (8.1)	<0.001

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; FMD, flow-mediated vasodilation.

Table III. Clinical Characteristics of Male and Female Subjects

Variables	Male (n=4131)	Female (n=1183)	P value
Age, y	47±13	45±16	<0.001
Body mass index, kg/m ²	23.4±3.1	21.6±3.4	<0.001
Systolic blood pressure, mm Hg	126.5±16.1	118.2±19.5	<0.001
Diastolic blood pressure, mm Hg	77.5±12.7	71.4±12.8	<0.001
Heart rate, bpm	63.6±10.4	65.4±10.6	<0.001
Total cholesterol, mmol/L	5.28±0.87	5.19±0.91	0.01
Triglycerides, mmol/L	1.55±1.11	1.01±0.66	<0.001
HDL cholesterol, mmol/L	1.51±0.40	1.78±0.42	<0.001
LDL cholesterol, mmol/L	3.24±0.80	2.98±0.81	<0.001
Glucose, mmol/L	5.60±1.09	5.28±1.45	<0.001
HbA1c, %	5.2±0.9	5.1±0.6	<0.001
Hypertension, n (%)	577 (14.0)	161 (13.8)	0.82
Dyslipidemia, n (%)	1933 (46.9)	331 (28.3)	<0.001
Diabetes mellitus, n (%)	266 (6.5)	70 (6.0)	0.56
Smoking, n (%)	1417 (34.5)	78 (6.7)	<0.001
Coronary heart disease, n (%)	98 (2.4)	19 (1.7)	0.13
Cerebrovascular disease, n (%)	36 (1.1)	10 (0.9)	0.50
Flow-mediated vasodilation, %	5.92±3.05	6.99±3.77	<0.001
Baseline brachial artery diameter, mm	4.19±0.50	3.38±0.48	<0.001

HDL indicates high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Table IV. Univariate Analysis of the Relationship Between Flow-Mediated Vasodilation and Variables in Male and Female Subjects

Variables	Male		Female	
	r	P value	r	P value
Age, y	-0.20	<0.001	-0.41	<0.001
Body mass index, kg/m ²	-0.10	<0.001	-0.15	<0.001
Systolic blood pressure, mm Hg	-0.12	<0.001	-0.26	<0.001
Diastolic blood pressure, mm Hg	-0.08	<0.001	-0.19	0.002
Heart rate, bpm	0.013	0.39	-0.007	0.82
Total cholesterol, mmol/L	-0.01	0.63	-0.21	<0.001
Triglycerides, mmol/L	0.07	<0.001	-0.17	<0.001
HDL cholesterol, mmol/L	0.01	0.62	0.06	0.04
LDL cholesterol, mmol/L	0.03	0.09	-0.21	<0.001
Glucose, mmol/L	-0.10	<0.001	-0.19	<0.001
HbA1c, %	-0.10	<0.001	-0.25	<0.001
Framingham risk score	-0.18	<0.001	-0.39	<0.001
Baseline brachial artery diameter, mm	-0.43	<0.001	-0.43	<0.001

HDL indicates high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Table V. Clinical Characteristics of Blood Pressure Categories

Variables	Blood pressure categories				P value
	Normal (n=2097)	Prehypertension (n=1984)	Stage 1 (n=876)	Stage 2 (n=312)	
Age, y	41.2±13.5	47.5±12.2	52.6±10.8	55.4±10.3	<0.001
Body mass index, kg/m ²	21.8±2.9	23.4±2.99	24.5±2.6	24.4±3.66	<0.001
Systolic blood pressure, mm Hg	108.9±7.5	127.4±6.2	142.8±7.9	162.2±13.0	<0.001
Diastolic blood pressure, mm Hg	65.5±7.4	77.7±7.4	89.2±6.8	101.3±10.1	<0.001
Heart rate, bpm	61.6±9.6	64.4±10.4	67.2±11.0	69.3±10.9	<0.001
Total cholesterol, mmol/L	5.10±0.87	5.31±0.89	5.40±0.83	5.47±0.90	<0.001
Triglycerides, mmol/L	1.21±0.82	1.52±1.10	1.61±1.23	1.72±1.20	<0.001
HDL cholesterol, mmol/L	1.65±0.41	1.53±0.41	1.52±0.40	1.51±0.42	<0.001
LDL cholesterol, mmol/L	3.02±0.80	3.26±0.79	3.30±0.78	3.35±0.87	<0.001
Glucose, mmol/L	5.24±0.82	5.61±1.15	5.80±1.38	6.11±2.00	<0.001
HbA1c, %	5.02±0.48	5.15±0.57	5.27±0.69	5.34±0.76	<0.001
Framingham risk score	-0.46±6.32	3.36±3.74	5.92±3.09	7.60±2.97	<0.001
Baseline brachial artery diameter, mm	3.82±0.60	4.11±0.56	4.16±0.57	4.18±0.59	<0.001
FMD, %	6.75±3.33	5.96±3.15	5.56±3.07	5.07±3.07	<0.001
Hypertension, n (%)	72 (3.4)	250 (12.6)	284 (32.5)	131 (42.0)	<0.001
Dyslipidemia, n (%)	604 (28.8)	975 (49.2)	482 (55.1)	197 (63.1)	<0.001
Diabetes mellitus, n (%)	67 (3.2)	133 (6.7)	90 (10.3)	46 (14.7)	<0.001
Smoking, n (%)	537 (25.7)	592 (30.0)	268 (30.6)	93 (30.0)	0.005
Coronary heart disease, n (%)	38 (1.9)	45 (2.3)	19 (2.2)	15 (4.8)	0.01
Cerebrovascular disease, n (%)	10 (0.6)	20 (1.2)	11 (1.4)	5 (1.8)	0.08

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; FMD, flow-mediated vasodilation.

Table VI. Clinical Characteristics of Brachial Artery Diameter Quartiles

Variables	Brachial artery diameter Quartiles				P value
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
	≤3.60 (n=1328)	3.61-4.03 (n=1328)	4.04-4.41 (n=1328)	≥4.42 (n=1328)	
Age, y	41.9±15.0	45.4±13.7	47.1±12.1	50.6±10.6	<0.001
Body mass index, kg/m ²	21.2±2.89	22.7±2.98	23.6±2.84	24.5±3.38	<0.001
Systolic blood pressure, mm Hg	117.4±17.6	124.6±16.4	126.9±16.0	129.7±16.5	<0.001
Diastolic blood pressure, mm Hg	71.1±12.3	75.5±12.9	77.8±12.5	80.1±12.4	<0.001
Heart rate, bpm	64.6±10.4	63.9±10.7	63.4±9.8	64.2±10.9	0.02
Total cholesterol, mmol/L	5.13±0.89	5.22±0.88	5.33±0.87	5.33±0.87	<0.001
Triglycerides, mmol/L	1.08±0.76	1.44±1.20	1.54±1.01	1.63±1.10	<0.001
HDL cholesterol, mmol/L	1.72±0.42	1.54±0.40	1.52±0.40	1.51±0.42	<0.001
LDL cholesterol, mmol/L	2.99±0.78	3.18±0.79	3.30±0.78	3.26±0.83	<0.001
Glucose, mmol/L	5.26±1.28	5.49±0.99	5.60±1.19	5.76±1.20	<0.001
HbA1c, %	5.04±0.55	5.13±0.56	5.15±0.54	5.22±0.66	<0.001
Framingham risk score	-1.22±7.99	3.15±4.25	3.73±3.45	4.49±3.17	<0.001
FMD, %	7.90±3.67	6.64±2.99	5.72±2.71	4.39±2.47	<0.001
Hypertension, n (%)	102 (7.7)	169 (12.8)	178 (13.4)	289 (22.0)	<0.001
Dyslipidemia, n (%)	358 (27.1)	538 (40.6)	638 (48.2)	730 (55.4)	<0.001
Diabetes mellitus, n (%)	55 (4.2)	79 (6.0)	90 (6.8)	112 (8.5)	<0.001
Smoking, n (%)	172 (13.1)	376 (28.4)	446 (33.8)	500 (38.1)	<0.001
Coronary heart disease, n (%)	16 (1.3)	32 (2.5)	24 (1.8)	45 (3.4)	<0.001
Cerebrovascular disease, n (%)	8 (0.64)	12 (1.06)	4 (0.39)	22 (2.20)	0.01

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; FMD, flow-mediated vasodilation.

Table VII Flow-Mediated Vasodilation in Each Baseline Brachial Artery Diameter Quartile in Age Decade

Age	Baseline brachial artery diameter quartiles				P value
	Quartile 1 ≤3.60	Quartile 2 3.61-4.03	Quartile 3 4.04-4.40	Quartile 4 4.41≤	
20-29 (n=763)	8.65±3.58	6.55±3.33	6.13±2.81	4.82±2.61	<0.001
30-39 (n=620)	8.82±3.32	7.08±2.90	6.21±2.56	4.87±2.72	<0.001
40-49 (n=1511)	8.28±3.82	7.12±2.95	6.00±2.73	4.71±2.57	<0.001
50-59 (n=1759)	6.99±3.59	6.56±2.90	5.47±2.61	4.26±2.39	<0.001
≥60 (n=644)	5.44±2.83	5.13±2.85	4.96±2.87	3.45±2.36	<0.001

Table VIII. Univariate Analysis of the Relation Between Flow-Mediated Vasodilation and Baseline Brachial Artery Diameter in Each Decade in Men and Women

Age	Total		Men		Women	
	r	P value	r	P value	r	P value
20-29 (n=763)	-0.41	<0.001	-0.30	<0.001	-0.37	<0.001
30-39 (n=620)	-0.46	<0.001	-0.43	<0.001	-0.33	<0.001
40-49 (n=1511)	-0.43	<0.001	-0.47	<0.001	-0.32	<0.001
50-59 (n=1759)	-0.37	<0.001	-0.44	<0.001	-0.32	<0.001
≥60 (n=644)	-0.29	<0.001	-0.34	<0.001	-0.33	<0.001

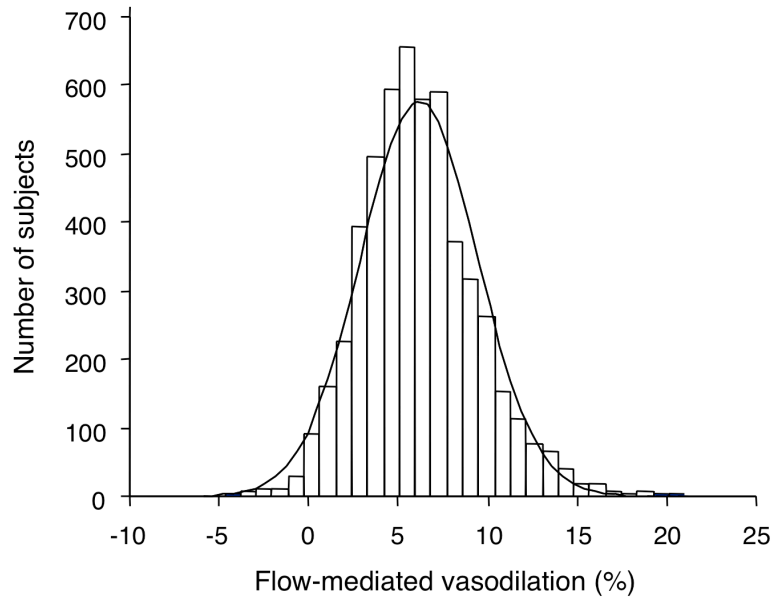
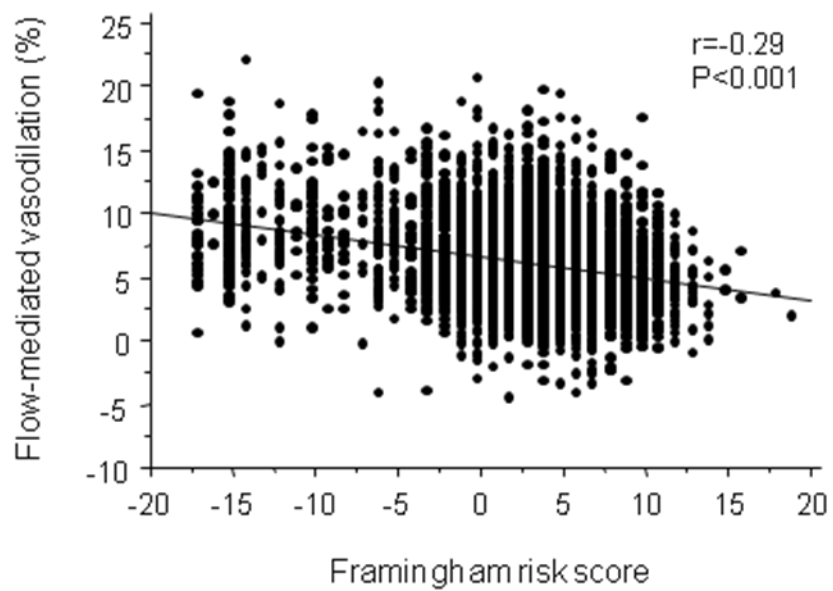
Figure S1

Figure S2

A



B

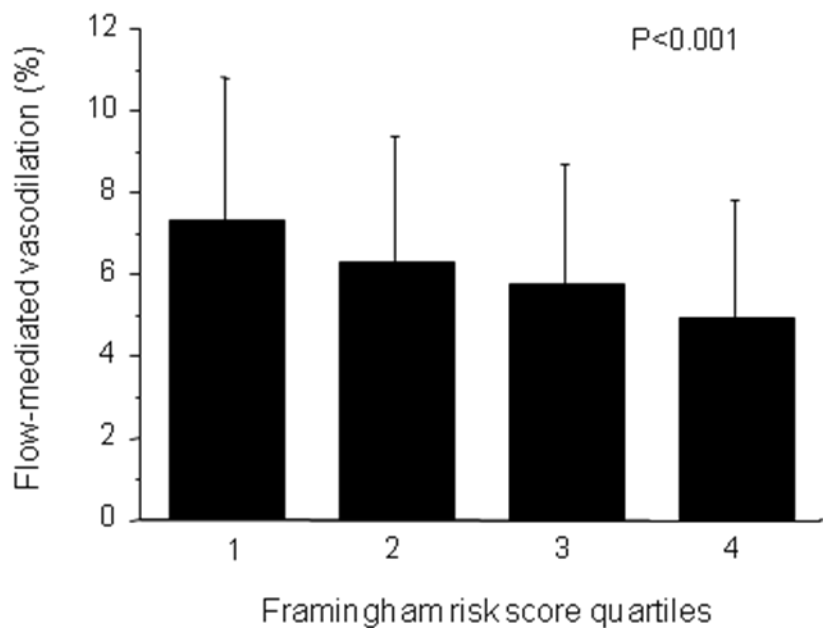


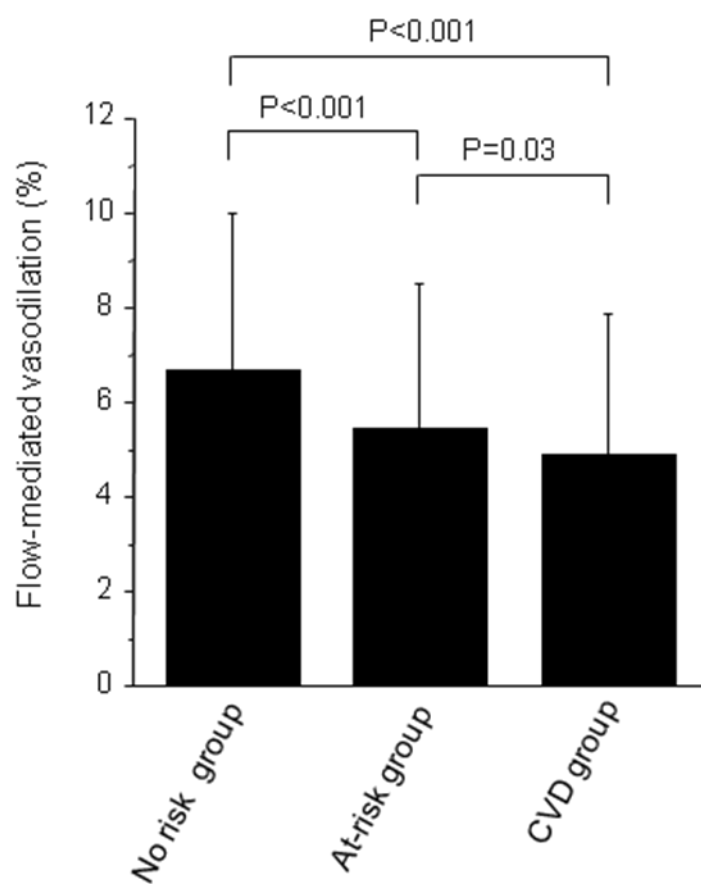
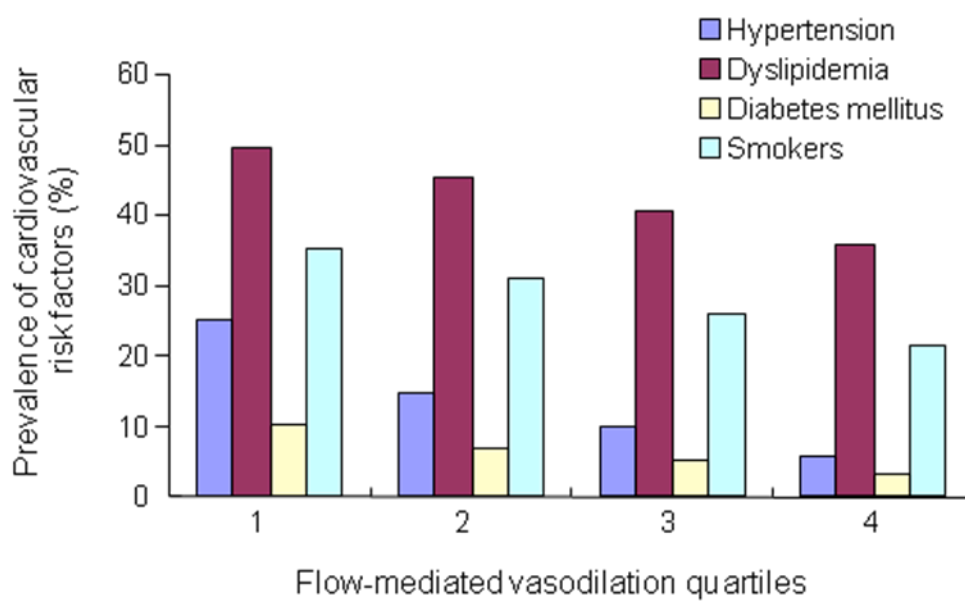
Figure S3

Figure S4

A



B

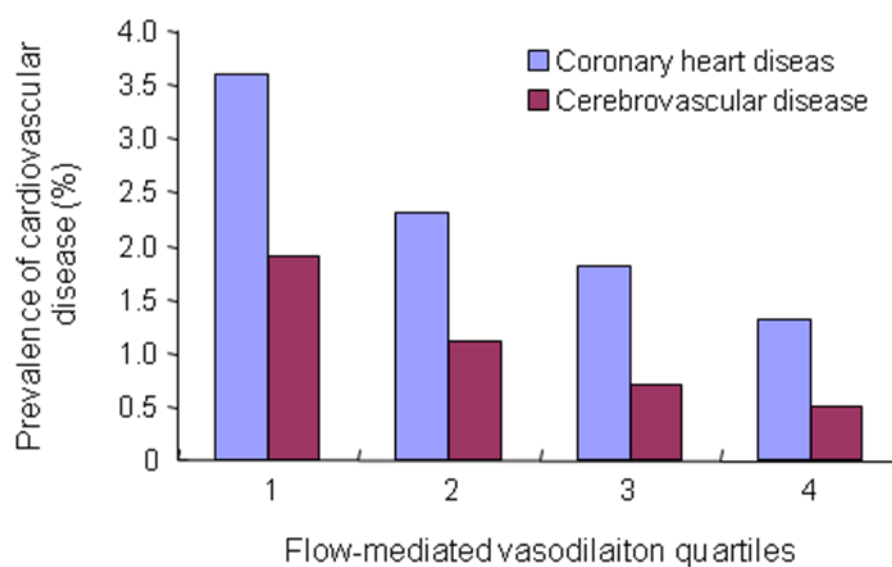


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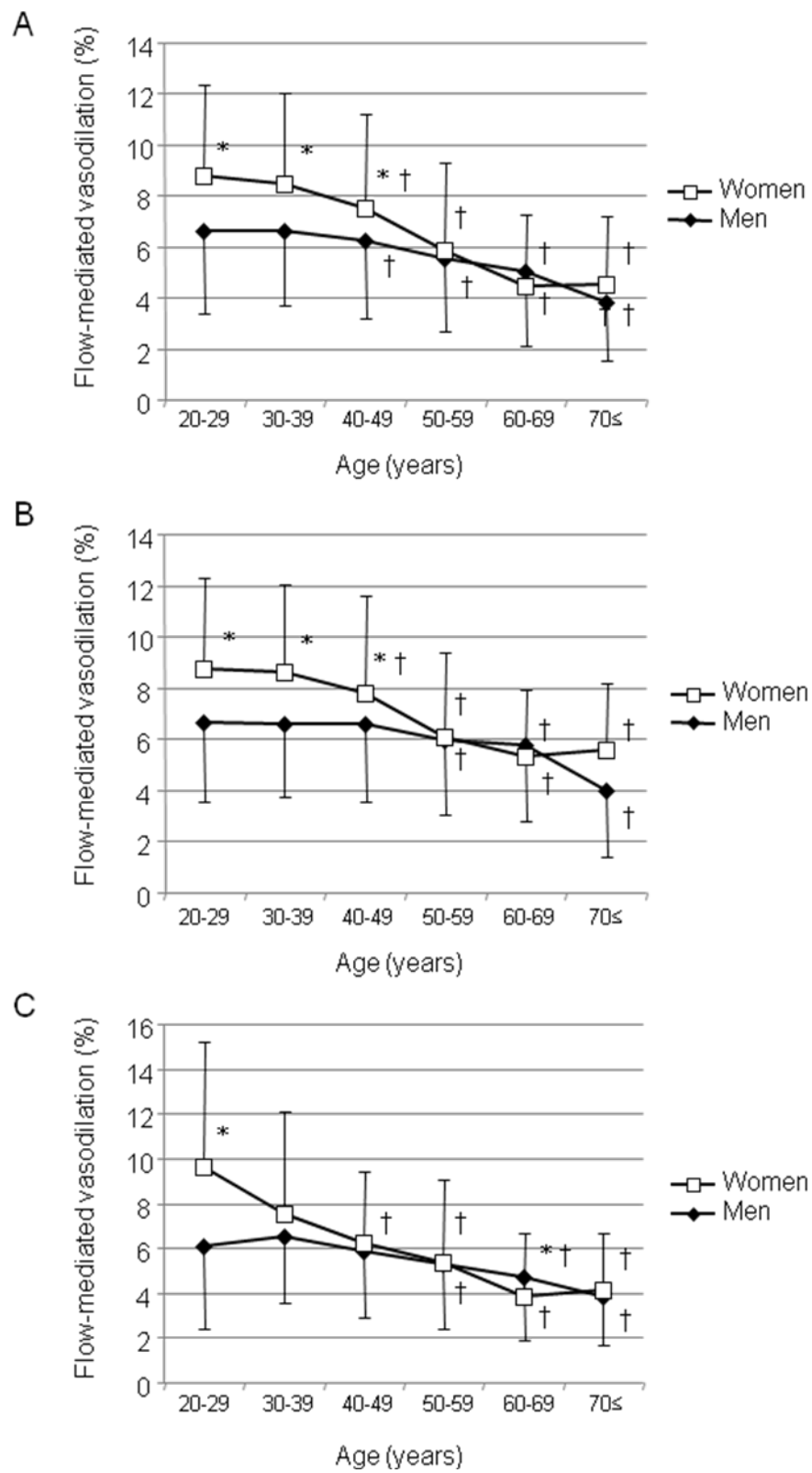


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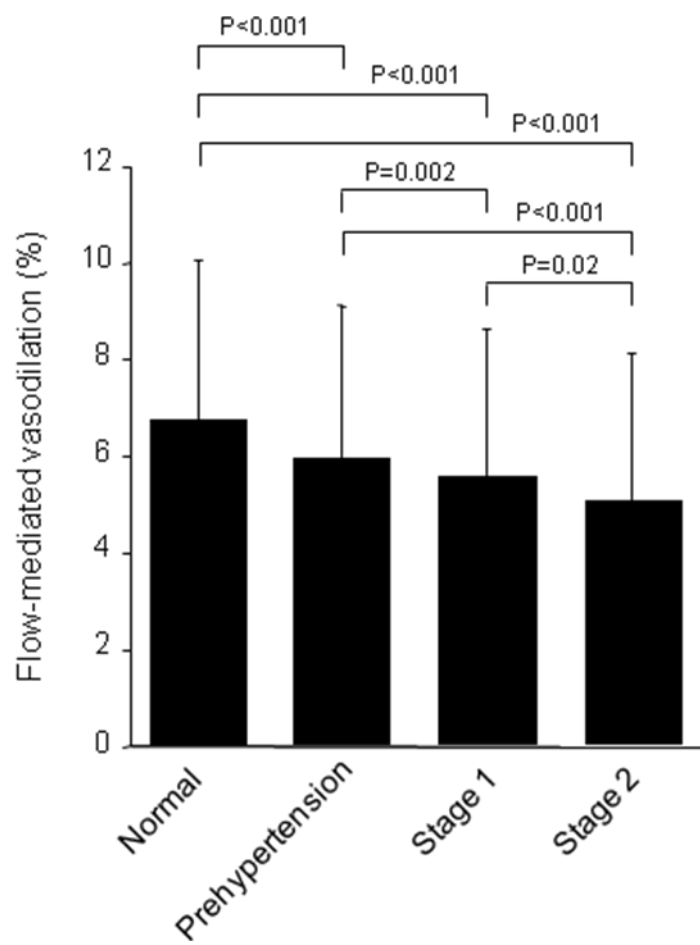


Figure legends

Figure S1. Histogram of flow-mediated vasodilation in all subjects.

Figure S2. A, Scatterplots show the relationship between flow-mediated vasodilation and Framingham risk score. B, Bar graphs show flow-mediated vasodilation of subjects classified into 4 groups based on Framingham risk score.

Figure S3. Bar graphs show flow-mediated vasodilation in the no risk group, at-risk group, and cardiovascular disease (CVD) group.

Figure S4. A, Bar graphs show the prevalence of cardiovascular risk factors in subjects classified into 4 groups based on flow-mediated vasodilation. B, Bar graphs show the prevalence of cardiovascular disease in subjects classified into 4 groups based on flow-mediated vasodilation.

Figure S5. A, Line graphs show flow-mediated vasodilation in all subjects classified into 6 groups based on decades of age. B, Line graphs show flow-mediated vasodilation in men and women classified into 6 groups based on decades of age in the no risk group. C, Line graphs show flow-mediated vasodilation in men and women classified into 6 groups based on decades of age in the at-risk and cardiovascular disease groups. * $P < 0.05$ vs. men at the same decade of age, † $P < 0.05$ vs. decade of the 20s in the same sex.

Figure S6. Bar graphs show flow-mediated vasodilation in subjects classified into 4 groups according to Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure VII (normal, prehypertension, stage 1 and stage 2).