SUPPLEMENTAL MATERIAL

Microvascular function contributes to the relation between aortic stiffness and cardiovascular events: the Framingham Heart Study

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Supplemental Content:

This supplementary material has been provided by the authors to give the readers additional information about their work.

Expanded Methods and Results

Brachial diameter and FMD assessed via Binning and LOESS methods

A binning method and locally weighted smoothing procedure (PROC LOESS) were used to obtain characteristics of the time course. Each bin was created by taking ten-second intervals and calculating the average diameter within each interval. Through PROC LOESS, we were able to fit a smoothed curve to the data instead of using a linear modeling approach. We used a smoothing parameter of 0.15 in PROC LOESS; this specified the percentage of data to use in each local weighted regression. Measurements in the first 15 seconds and measurements after 125 seconds were removed. Deflation ultrasound images were acquired for an additional 2 minutes, and Doppler flow was obtained during the first 15 seconds. Technicians measured the images using commercial software from Medical Imaging Applications. The average of all baseline diameters was used to calculate a baseline diameter for each individual. The 60 second diameter was calculated by taking the average of diameters measured in the 55-65 second post-deflation window: X=average baseline diameter and Y=average 55-65 second deflation diameter, then FMD%=100*[(Y-X)/X].

Binning and LOESS vascular measures as predictors of a major CVD event

The mean post-deflation maximum brachial diameter in the sample was 4.4±0.8 mm (for both Binning and LOESS). The mean FMD in the sample was 5.3±3.6 % (Binning) and 5.5±3.6 % (LOESS). We used Cox proportional hazards models for measures obtained via these two alternative methods as predictors of a major CVD event. After multivariable adjustment for clinical covariates (age, sex, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking, diabetes mellitus, hypertension treatment, cohort, BMI, and relatedness), the relations between Binning FMD and LOESS FMD and incident CVD events were HR=0.82; 95% CI: 0.66, 1.00; *P*=0.053 and (HR=0.81; 95% CI: 0.66, 1.00; *P*=0.051) respectively. These results are consistent with the observations using FMD assessed at 55-65 seconds after cuff release, which are presented in the main text.

Table S1: Comparison of baseline clinical and vascular characteristics of excluded and included participants.

Variable	Included (N=4547)	Excluded* (N varies)
Clinical Measures		
Age, y	51±11	59±11
Women, N (%)	2446 (54)	945 (52)
Height, cm	169±10	168±9
Weight, kg	77.9±17.1	83.7±20.2
Body mass index, kg/m^2	27.0±4.9	29.7±6.5
Systolic blood pressure, mm Hg	121±17	127±18
Diastolic blood pressure, mm Hg	75±10	75±10
Heart rate, bpm	63±10	65±11
Total cholesterol, mg/dL	197±36	197±37
High-density lipoprotein cholesterol, mg/dL	55±17	51±16
Triglycerides, mg/dL	123±90	144±95
Hypertension treatment, N (%)	826 (18)	642 (35)
Diabetes mellitus, N (%)	291 (6)	275 (15)
Smoker, N (%)	703 (15)	236 (13)
Generation 3 Exam 1, N (%)	2626 (58)	399 (22)
Vascular measures		
Baseline brachial diameter, mm	4.2±0.9	4.4±0.9
Flow-mediated vasodilation, %	4.5±3.6	3.3±3.2
Hyperemic mean flow velocity, cm/sec	58±20	50±21
Carotid-femoral pulse wave velocity, m/s	8.4±2.7	10.1±4.2
Tonometry primary pressure wave, mm Hg	42±11	43±12

All values are mean ± standard deviation except as noted. *Since younger individuals (<35 years) did not contribute to events, stratification of excluded participants is not based on age; note that N varies (611–1810) for excluded participants based on availability of data.

Table S2. Comparison of baseline clinical and vascular characteristics of participants with concordant and discordant phenotypes (N=4547).

Variable	High HFV and Low CFPWV (N=1368)	Low HFV and Low CFPWV (N=878)	High HFV and High CFPWV (N=905)	Low HFV and High CFPWV (N=1396)
Clinical Measures				
Age, y	44.2±7	45±8	53±9	59±11
Women, N (%)	939 (69)	468 (53)	451 (50)	588 (42)
Height, cm	169±9	170±10	170±10	169±10
Weight, kg	74.1±16.0	74.9±17.1	80.4±17.2	81.7±17.1
Body mass index, kg/m^2	26.0±4.8	25.8±4.4	27.8±4.8	28.4±4.8
Systolic blood pressure, mm Hg	113±12	113±12	124±14	133±18
Diastolic blood pressure, mm Hg	73±9	73±9	78±9	79±10
Heart rate, bpm	62±9	59±9	66±10	65±11
Total cholesterol, mg/dL	191±34	191±34	203±36	201±37
High-density lipoprotein cholesterol, mg/dL	58±16	56±17	54±18	52±17
Triglycerides, mg/dL	102±72	104±81	142±101	144±96
Hypertension treatment, N (%)	95 (7)	72 (8)	176 (19)	483 (35)
Diabetes mellitus, N (%)	16 (1)	12 (1)	75 (8)	188 (13)
Smoker, N (%)	264 (19)	124 (14)	149 (16)	166 (12)
Generation 3 Exam 1, N (%)	1085 (79)	696 (79)	437 (48)	408 (29)
Vascular measures				
Baseline brachial diameter, mm	3.9±0.7	4.1±0.9	4.2±0.8	4.5±0.9
Flow-mediated vasodilation, %	6.6±3.6	4.7±3.4	5.1±3.1	2.1±2.5
Hyperemic mean flow velocity, cm/sec	75±13	46±9	73±13	38±12
Carotid-femoral pulse wave velocity, m/s	6.6±0.6	6.7±0.6	9.1±1.6	10.7±3.3
Tonometry primary pressure wave, mm Hg	39±9	40±9	41±10	45±13
Number of events, N (%)	13 (1.0)	11 (1.3)	38 (4.2)	170 (12.2)
Hazard ratio (LCL, UCL)*	*Reference group*	1.10 (0.49, 2.45)	1.80 (0.94, 3.46)	2.95 (1.58, 5.52)

All values are mean ± standard deviation except as noted. *Model for phenotype group as predictor of a major cardiovascular event adjusted for age, sex, and cohort; *P*=0.0002. LCL, UCL, lower and upper limits of the 95% confidence intervals. Hazard ratios are derived from multivariable Cox-regression models adjusting for age, sex, and cohort.

Table S3. Individual measures of vascular function as predictors of a major CVD event with an expanded model further adjusted for pulse pressure (N=4547).

Vascular measure	Hazard Ratio (LCL, UCL)	*P*
Baseline brachial diameter	1.09 (0.91, 1.32)	0.36
Flow-mediated vasodilation	0.86 (0.70, 1.06)	0.16
Hyperemic mean flow velocity	0.85 (0.72, 1.00)	0.05
Carotid-femoral pulse wave velocity	1.30 (1.06, 1.61)	0.01
Tonometry primary pressure wave	1.06 (0.87, 1.30)	0.06

Models add covariates to the vascular variables one at a time. Models adjusted for age, sex, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, pulse pressure, smoking, diabetes mellitus, hypertension treatment, cohort, BMI, and relatedness. LCL, UCL, lower and upper limits of the 95% confidence intervals. HRs expressed per 1 SD higher value, 232 events (5%) with median of 8.6 years of follow-up.