SUPPLEMENTAL MATERIAL

Supplemental Material 1. Reconstitution of acetylcholine for endothelial function testing in the cardiac catheterization lab

- Drug used for testing - acetylcholine chloride intraocular solution (generic name) or Miochol-E (brand name)
- Drug comes packaged with a powder (20 mg) and a dilutant (2 ml)
- The powder is combined with dilutant
- 0.5 ml of this combination is mixed with 250 ml of normal saline to create a concentration of 20 mcg/ml acetylcholine

Supplemental Table 1. Clinical Characteristics by Sex

Variable	Total (n=288)	Women (n=215)	Men (n=73)
Age, years	53.5±12.7	54.1±12.2	51.8±14.0
Race			
American Indian/Alaska Native	8 (2.8)	5 (2.3)	3 (4.1)
Asian or East Indian	28 (9.7)	23 (10.7)	5 (6.8)
Black/African American	15 (5.2)	9 (4.2)	6 (8.2)
Hispanic or Latino	30 (10.4)	28 (13.0)	2 (2.7)
Native Hawaiian or Other Pacific Islander	2 (0.7)	1 (0.5)	1 (1.4)
Caucasian	181 (62.8)	130 (60.5)	51 (69.9)
Other	23 (8.0)	18 (8.4)	5 (6.8)
Missing	1 (0.3)	1 (0.5)	0 (0.0)
Hypertension	136 (47.2)	111 (51.6)	25 (34.2)
Diabetes mellitus	55 (19.1)	44 (20.5)	11 (15.1)
Dyslipidemia	163 (56.6)	120 (55.8)	43 (58.9)
Smoking			
Former	43 (14.9)	28 (13.0)	15 (20.5)
Current	42 (14.6)	26 (12.1)	16 (21.9)
Missing	1 (0.3)	1 (0.5)	0 (0.0)

Family history of CAD	79 (27.4)	66 (30.7)	13 (17.8)
BMI, kg/m2	28.7±6.5	28.8±7.0	28.2±4.8
BSA, m2	1.89±0.24	1.82±0.21	2.08±0.23
Medications			
Aspirin	188 (65.3)	133 (62.1)	55 (75.3)
Plavix	18 (6.2)	12 (5.6)	6 (8.2)
Beta blockers	115 (39.9)	88 (41.1)	27 (37.0)
Calcium channel			
blocker	56 (19.4)	37 (17.3)	19 (26.0)
ACEI/ARB	48 (16.7)	36 (16.8)	12 (16.4)
Diuretics	34 (11.8)	29 (13.6)	5 (6.8)
Statins	146 (50.7)	104 (48.6)	42 (57.5)
Nitrates	107 (37.2)	78 (36.4)	29 (39.7)
Missing	1 (0.3)	1 (0.3)	0 (0)
Total cholesterol, mg/dL	167.6±41.4	173.5±42.2	150.2±33.4
Lipoprotein(a), mg/dL	14.6 [6.1, 33.0]	14.4 [5.7, 32.9]	16.3 [8., 33.6]
Triglycerides, mg/dL	75.0 [48.0, 110.0]	75.5 [49.2, 110.0]	65.0 [44.0, 107.0]
LDL, mg/dL	95.75±34.76	99.14±35.72	85.88±29.92
HDL, mg/dL	54.0±16.9	55.6±16.9	49.3±16.1
Fasting glucose, mg/dL	100.5±22.8	100.0±23.4	101.9±20.9
HbA1c, %	5.6±0.8	5.7±0.8	5.6±0.6
Insulin, μU/mL	8.0 [5.0, 13.0]	8.0 [5.0, 13.5]	8.0 [5.0, 13.0]
Homocysteine, μmol/L	8.3±3.1	7.9±2.9	9.5±3.5

HOMA index*	1.9 [1.1, 3.4]	1.9 [1.1, 3.4]	1.9 [1.2, 3.4]
hs-CRP, mg/dL,	1.4 [0.6, 3.9]	1.5 [0.8, 4.1]	0.8 [0.4, 2.2]
SAQ scores†			
Physical limitation	68.6±14.2	68.4±14.5	68.9±13.7
Anginal stability	39.4±24.6	38.4±25.3	42.6±22.5
Anginal frequency	59.5±23.1	57.7±23.2	65.1±22.2
Treatment satisfaction	85.9±17.2	85.3±17.5	86.3±16.3
Quality of life	50 [33.3, 58.3]	41.7 [33.3, 58.3]	50 [33.3, 58.3]

* HOMA index=insulin (μU/m)×[glucose (mg/dl)/405]; † Lower SAQ score represents worse symptoms. BMI=body mass index; BSA=body surface area; CAD=coronary artery disease; HbA1c=hemoglobin A1c; HDL=high-density lipoprotein; HOMA=homeostasis model assessment; hs-CRP=high sensitivity C-reactive protein; LDL=low-density lipoprotein; SAQ=Seattle angina questionnaire

Supplemental Table 2. Estimated change in MLD in μmeter for every 10μg change in Ach dosage adjusted for body surface area

Slopes	Women (estimates of MLD change/10μg Ach)		Men (estimates of MLD change/10μg Ach)	
Ach	No endothelial dysfunction	Endothelial dysfunction	No endothelial dysfunction	Endothelial dysfunction
doses >20μg	-7.0 [-35, 21]	-22 [-48, 3.6]	16 [-29, 32]	-38 [-65, -12]
doses >50μg	9.6 [-3.9, 23]	1.7 [-9.3, 13]	18 [1.9, 35]	-14 [-28, -1.2]
doses >100μg	-1.1[-11, 8.4]	-31 [-15, 8.9]	7.4 [-7.1, 22]	-19 [-33, -5.8]

The median change in MLD/10 μg of Ach when the curves were adjusted for BSA lies within the confidence interval of the change in MLD/10 μg of Ach with the model that was unadjusted for BSA, suggesting no significant difference in the results. Slope estimates presented in μmeter/10μg; Ach = acetylcholine

Supplemental Table 3. Estimated change in the MLD/10 µg of Ach when endothelial dysfunction is defined as %MLD constriction >20%, ≥1% and ≥50% after IC Ach

Endothelial dysfunction = constriction >20%				
Slopes	Women (estimates of MLD change/10µg Ach)		Men (estimates of MLD change/10µg Ach)	
	No endothelial dysfunction	Endothelial dysfunction	No endothelial dysfunction	Endothelial dysfunction
doses >20	-2.7 (-31.8 – 26.4)	-21.6 (-47.4 – 4.2)	3.9 (-27.3 – 35.1)	-37.4 (-63.8 - -11.0)
doses >50	9.5 (-4.4 – 23.3)	1.8 (-9.3 – 12.8)	16.1 (-0.67 – 32.8)	-14.0 (-27.3 - -0.76)
doses >100	-2.4 (-12.2 – 7.3)	-2.9 (-14.9 – 9.1)	4.2 (-10.8 – 19.1)	-18.7 (-32.3 - -5.1)
Endothelial dysfunction = constriction ≥1%				
doses >20	21 (-24 – 66)	-23 (-45 - -2.0)	38 (-10 – 87)	-38 (-60 - -15)
doses >50	11 (-13 – 34)	5.7 (-3.8 – 15)	28 (0.79 – 55)	-8.1 -20 - 3.3)
doses >100	-6.1 (-21 - 9.1)	5.5 (-3.3 – 14)	11 (-12 – 33)	-8.4 (-19 - 2.7)
Endothelial dysfunction = constriction ≥50%				
doses >20	-9.2 (-31 – 13)	-76 (-120 - -36)	-7.1 (-30 – 16)	-75 (-120 - -35)
doses >50	3.8 (-5.8 – 13)	-16 (-36 - 3.2)	5.8 (-6.5 – 18)	-16 (-37 - 5.1)
doses >100	3.1 (-5 – 11)	2.1 (-20 – 24)	5.1 (-6.8 – 17)	2.8 (-17 – 23)

The median change in the estimates of MLD/10 µg of Ach when endothelial dysfunction is defined as %MLD constriction ≥1% and ≥ 50% after IC Ach lie within the confidence interval

for the definition used in the current manuscript (constriction >20%), suggesting no significant difference in the results with a different threshold for the definition of endothelial dysfunction.

MLD = minimal lumen diameter