Mental stress peripheral vascular reactivity is elevated in women with coronary vascular dysfunction: Results from the NHLBI-sponsored Cardiac Autonomic Nervous System (CANS) study

Puja Mehta, Emory University
Melody Hermel, Cedars Sinai Heart Institute
Michael D. Nelson, Cedars Sinai Heart Institute
Galen Cook-Wiens, Cedars Sinai Medical Center
Elizabeth A. Martin, Cedars Sinai Heart Institute
Ayman A. Alkhoder, Emory University
Janet Wei, Cedars Sinai Heart Institute
Margo Minissian, Cedars Sinai Heart Institute
Chrisandra Shufelt, Cedars Sinai Heart Institute
Sailaja Marpuri, Cedars Sinai Heart Institute

Only first 10 authors above; see publication for full author list.

Journal Title: International Journal of Cardiology
Volume: Volume 251
Publisher: Elsevier: 12 months | 2018-01-15, Pages 8-13
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1016/j.ijcard.2017.10.061
Permanent URL: https://pid.emory.edu/ark:/25593/tn4gg

Final published version: http://dx.doi.org/10.1016/j.ijcard.2017.10.061

Copyright information:
© 2017 Elsevier B.V.
This is an Open Access work distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Accessed November 8, 2019 11:36 AM EST
Mental Stress Peripheral Vascular Reactivity is Elevated in Women with Coronary Vascular Dysfunction: Results from the NHLBI-Sponsored Cardiac Autonomic Nervous System (CANS) Study

Puja K. Mehta, MD¹, Melody Hermel, MD², Michael D. Nelson, PhD², Galen Cook-Wiens, MS³, Elizabeth A. Martin, PhD², Ayman A. Alkhodar, MD¹, Janet Wei², Margo Minissian, PhDc, ACNP², Chrisandra Shufelt, MD², Sailaja Marpuri, MS², David Hermel, MD², Amit Shah, MD, MSc³, Michael R. Irwin, MD⁵, David S. Krantz, PhD⁶, Amir Lerman, MD⁷, and C. Noel Bairey Merz, MD²

¹Emory Clinical Cardiovascular Research Institute, Emory University School of Medicine, Atlanta, GA
²Barbra Streisand Women's Heart Center, Cedars-Sinai Heart Institute, Los Angeles, CA
³Department of Epidemiology, Rollins School of Public Health, Emory University
⁴Biostatistics and Bioinformatics Research Center, Cedars-Sinai Medical Center, Los Angeles, CA
⁵Cousins Center for Psychoneuroimmunology, Semel Institute for Neuroscience, David Geffen SOM at UCLA
⁶Department of Medical and Clinical Psychology, Uniformed Services University, Bethesda, MD
⁷Cardiovascular Diseases, Mayo Clinic, Rochester, MN

Abstract

**Background**—Women with chest pain, ischemia, and no obstructive coronary artery disease often have coronary vascular dysfunction (CVaD). Peripheral vascular reactivity to mental stress may contribute mechanistic understanding of stress-induced ischemia in women with CVaD.
Methods—62 women (41 CVaD and 21 controls) underwent mental stress testing (MST) with anger recall, mental arithmetic, and forehead cold pressor (COP) challenge. Emotional arousal was measured (Likert scale). Reactive hyperemia index (RHI) was calculated before and after MST by peripheral arterial tonometry (PAT). Stress PAT ratio (SPR) of pulse amplitude during stress to rest was obtained to measure vasoconstriction. Wilcoxon rank sum test was used for analysis.

Results—Mean age of CVaD and control groups was 58±9 and 55±10 years (p=0.73). Baseline RHI correlated with coronary endothelial function (r=0.36, p=0.03) and inversely with RHI change post-MST (r=−0.51, p <0.001). During MST, 10% controls reported chest pain vs. 41% CVaD subjects (p=0.01). RHI did not change significantly after MST in either group. CVaD subjects had lower SPR vs. controls during mental arithmetic (0.54 [0.15, 1.46] vs. 0.67 [0.36, 1.8], p=0.039), not evident in the other tasks. Vasoconstriction inversely correlated with anxiety (r=−3.4, p=0.03), frustration (r=−0.37, p=0.02), and feeling challenged (r=−0.37, p=0.02) in CVaD but not controls.

Conclusions—Mental stress peripheral vascular reactivity is elevated in women with CVaD compared to controls. Elevated vascular reactivity may be one contributor to stress-induced chest pain in CVaD. Interventions that modulate vasoconstrictive responses may be of benefit and should be tested in clinical trials in women with CVaD.

Keywords
vascular reactivity; mental stress; microvascular dysfunction; women heart disease

Background

Chest pain with evidence of myocardial ischemia and no obstructive coronary arteries occurs more commonly in women and is associated with an adverse prognosis.1–3 Approximately 50% of these women have coronary vascular dysfunction (CVaD). 2,4 CVaD encompasses endothelial and non-endothelial dependent macro- and microvascular dysfunction, and data from the Women’s Ischemia Syndrome Evaluation (WISE) and other studies indicate that CVaD is associated with adverse cardiovascular prognosis, including myocardial infarction, stroke, congestive heart failure, and sudden cardiac death.3,5–7 Invasive coronary reactivity testing (CRT) can be performed to clarify the diagnosis when CVaD is suspected.8

Women with CVaD also often present with non-exertional, emotional stress-induced chest pain; while anxiety/pain disorders and abnormal cardiac nociception are also relatively more common in women with chest pain,9,10 the mechanistic pathways of emotional stress-induced chest pain are not well understood. Acute mental stress has been associated with endothelial dysfunction and impaired vasoreactivity in patients with obstructive CAD.11,12 This may result in mental stress-induced myocardial ischemia (MSIMI), which is more common in women with CAD. Patients with MSIMI are also more likely to have exaggerated peripheral vasoconstriction during mental stress.12,13 Further evidence suggests that mental stress induces peripheral vasoreactivity, which may be useful in the detection of MSIMI in patients with CAD.14–18 However, it is not known whether women with CVaD and no obstructive CAD have abnormal peripheral vasoreactivity during acute mental stress. Hence, this study evaluated peripheral vascular reactivity to acute mental stress in women with CVaD as compared to reference control subjects.
Methods

CVaD Subjects

44 CVaD women enrolled in the NHLBI-sponsored Women’s Ischemia Syndrome Evaluation Coronary Vascular Dysfunction (WISE-CVD) study (PI: Bairey Merz) at a single site (Cedars-Sinai Medical Center) were recruited and enrolled in the NHLBI-sponsored Cardiac Autonomic Nervous System (CANS) substudy between October 2010 - June 2015. These women had persistent chest pain, evidence of myocardial ischemia by routine stress testing, and no obstructive CAD on invasive coronary angiography. Inclusion and exclusion criteria were as previously published for WISE-CVD\textsuperscript{19}. Three subjects who did not have coronary reactivity testing (CRT) to diagnose CVaD were excluded due to inadequate data. The study was IRB approved, and all subjects were provided informed consent.

Coronary Reactivity Testing (CRT)

Clinically-indicated invasive CRT was performed to diagnose CVaD as previously described\textsuperscript{8}. Briefly, after confirmation of no obstructive CAD by angiography, a doppler Flowire (Volcano®) was placed in the proximal left anterior descending artery and vasoactive agents (adenosine 18 and 36 mcg, acetylcholine 36.4 mcg, and nitroglycerin 200 mcg) were used to assess endothelial and non-endothelial dependent, macro- and microvascular function, using previously published methods.\textsuperscript{7,20} The peak coronary flow reserve (CFR) response to either dose of adenosine was used for analysis. Abnormal CRT responses were defined as the following four pathways: (a) an abnormal non-endothelial microvascular response was CFR <2.5 to intracoronary adenosine; (b) an abnormal endothelial microvascular response was change in coronary blood flow (CBF) ≤50% to high dose acetylcholine; (c) an abnormal endothelial macro-vascular response was <5% change in coronary artery diameter to high dose acetylcholine; and (d) an abnormal non-endothelial macrovascular response was change in coronary artery diameter <20% to nitroglycerin.\textsuperscript{8,21} Coronary endothelial dysfunction was defined as abnormal coronary diameter response or abnormal coronary blood flow to acetylcholine. Patients with at least one abnormal pathway were included in the study.

Mental Stress Testing (MST)

MST was performed in the morning after an overnight fast; caffeine was withheld for 24 hours and smoking was prohibited for at least 6 hours. Subjects were asked to withhold caffeine and medications for 24 hours (beta blocker, short acting calcium channel blocker, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, renin blockers, and ranolazine) or for 48 hours (long acting calcium channel blockers and nitrates) when medically able. After IV placement, subjects rested for 30 minutes in a quiet, dimly lit, temperature-controlled room. A mood survey with Likert scale\textsuperscript{22–25} questions was administered to assess subjective levels of psychological stress, including anxiety, frustration, anger, and irritation. Baseline blood pressure, heart rate, and reactive hyperemia index (RHI) by peripheral arterial tonometry (PAT) were measured. All subjects underwent MST in supine position via a standardized protocol (Appendix Figure 1).
Two mental stress tests were administered in counterbalanced order with a two minute rest period between each stress test task: (a) *4 minute anger recall speech task* modified from Ironson et al.\textsuperscript{26} requires the subject to recall a situation of extreme anger or frustration and (b) *4-minute mental arithmetic task*, which requires the subject to count backwards by 7’s from a randomly chosen number. These are standard MST tasks that have been used successfully by our group and others.\textsuperscript{22,24,27} During the two minute rest period in between tasks, blood pressure and heart rate were assessed to ensure a return to baseline levels, prior to proceeding with the next task. During the MST tasks, BP was monitored every minute by automated cuff, and heart rate and rhythm continuously monitored. For the first 26 subjects, one-lead ECG monitoring was used, but for better detection of ST segment changes, we changed to 12-lead ECG monitoring (Mortara Instruments\textregistered) for the later subjects. Likert scale was re-administered after each MST task. PAT pulse amplitude was continuously monitored during MST.

At the end of MST, 3 minutes of modified forehead cold pressor (COP) test was performed. A 1.5 liter bag filled with 800ml crushed ice and 200 ml of water (Temp 4°C) was placed on subject’s forehead for 3 minutes. Hand PAT probes precluded traditional COP testing with hand in ice bucket, thus forehead COP was used. HR, BP, and PAT pulse amplitude response were also measured during COP test.

**Peripheral Arterial Tonometry**

Peripheral endothelial function and vascular reactivity were measured by peripheral arterial tonometric (PAT) plethysmographic device (EndoPAT 2000, Itamar Medical\textregistered) as previously described and during supine position, under conditions for MST described above.\textsuperscript{28,29} The device consists of a finger probe with a transducer to assess digital volume changes accompanying pulse-waves. A pressure of 40–70mmHg is applied by the probe to the index fingers for venous occlusion of both hands and arterial pulse amplitude is recorded. A blood pressure cuff is placed on one arm (test arm) while amplitudes are recorded from the test arm and the contralateral arm. After 10 minutes of equilibration period, reactive hyperemia index (RHI) is obtained by the following: 5 minutes of baseline amplitude signal detection, 5-minutes of arterial occlusion by suprasystolic blood pressure cuff inflation, and then 6-minutes of post-occlusion amplitude signal detection. RHI is a unit-less measure and a marker of endothelial function, and was determined at baseline and post MST by EndoPAT\textregistered automated software which takes into account the baseline signal and adjusts it to the changes in the contralateral arm.\textsuperscript{15,16,28} RHI of less than 2.0 has been reported to correlate with coronary endothelial dysfunction and coronary artery disease, and is prognostic (sensitivity and specificity of ~80%).\textsuperscript{30–32} Peripheral vascular reactivity was determined by stress PAT ratio (SPR) which is a ratio of stress to rest pulse amplitude as previously described.\textsuperscript{15} The lowest pulse amplitude for 30 second segment during mental stress test task was used to compare to an average 3 minute rest amplitude to obtain SPR. SPR was obtained from the arm not wearing a BP cuff and determined for each MST task, as a measure of vasoconstriction, where less than 0.8 is considered abnormal.\textsuperscript{28,33}
Reference Control Subjects

22 women were recruited from the Reference Control Study in Women (PI: Bairey Merz) at Cedars-Sinai and enrolled in the CANS study. One subject was a screen failure and excluded. Women in this group were age- and BMI-matched to the CVaD group, had no cardiac risk factors, were not on cardiac medications, and had a normal maximal exercise treadmill testing (Bruce Protocol) to serve as reference controls.

Statistical Analysis

Summary data are expressed as means, standard deviations, medians, and ranges for continuous variables and frequencies (%) for categorical ones. Comparison of categorical variables between groups was done using Fisher’s Exact test due to low counts in the control group. Wilcoxon Rank Sum tests were used to compare continuous outcomes between groups due to the presence of outliers. Spearman correlation coefficients are reported due to outliers. A p-value < 0.05 was considered to indicate statistical significance. Multiple linear regression was performed with RHI as the outcome and adjusted for various cardiac medications. All statistical analysis was performed using SAS (The SAS Institute, Cary, NC; ver. 9.3).

Results

Baseline characteristics are shown in Table 1 and demonstrate no difference between mean age and BMI between the two groups. Mean time between CRT and MST was 2.0 ± 1.6 years. There were only 7 subjects with MST within 6 months of CRT, precluding further analysis given small numbers. CRT results demonstrated that 34% had abnormal CFR (< 2.5), 63% had abnormal acetylcholine diameter response, 44% had abnormal coronary blood flow change to acetylcholine, and 61% had an abnormal smooth muscle vasodilation to nitroglycerin. Only one subject had CVaD diagnosis because of abnormal nitroglycerin response with the other 3 pathways being normal. Excluding this subject did not change the overall results.

Cardiovascular Reactivity and Likert Scale

Hemodynamic changes to anger, mental arithmetic, and COP were similar in both groups (Appendix Table 1). There were no stress-induced arrhythmias detected on continuous rhythm monitoring during MST. During MST, 2/21 (10%) reference controls reported transient, self-limited chest pain, compared to 17/41 (41%) of CVaD subjects (p=0.010). Chest pain was not associated with rhythm or ST segment changes, and no one required treatment with SL NTG for chest pain resolution during MST.

At baseline, there were no significant differences in emotional arousal measured by Likert scale subjective measures of emotion between the two groups. While both MST tasks resulted in significant changes in emotion compared to baseline in both groups (Table 2), CVaD subjects were more emotionally aroused compared to reference controls. During recovery at the end of both MST tasks, CVaD subjects remained significantly more emotionally aroused compared to controls (Table 2).
Peripheral Endothelial Function (PAT-RHI)

Baseline peripheral endothelial function, measured by RHI, was normal in both CVaD and reference controls (2.49 [1.25, 3.74] vs 2.31 [1.49, 4.67], p=0.54, respectively). RHI within CVaD patients with an abnormal coronary acetylcholine response was lower compared to those with normal acetylcholine response (RHI: (2.15 [1.25, 3.74]) vs. 3.04 [1.81, 3.59], p=0.049). Likewise, median RHI was lower in those subjects with hypertension (n = 9) compared to those without hypertension (n=23) (RHI: 2.05 [1.61, 2.74] vs. 2.68 [1.25, 3.74], p = 0.016). Median RHI remained normal after acute MST in both CVaD and controls (2.57 [1.39, 4.21] vs. 2.40 [1.52, 5.03], p=0.76, respectively). Baseline RHI correlated with coronary endothelial function (r=0.36, p=0.03) and inversely with RHI change post-MST (r = −0.51, p = <0.001). In those with abnormal coronary endothelial function (abnormal acetylcholine diameter response or abnormal CBF), there was no significant difference in RHI between pre- and post-MST (p = 0.20). There were no differences in RHI in the CVaD group based on cardiac medications.

Peripheral Vascular Reactivity (stress PAT ratio)

A comparison of individual tasks showed that CVaD subjects had a significantly lower SPR (consistent with more vasoconstriction) to arithmetic compared to control subjects (median [range]: 0.54 [0.15, 1.46] vs. 0.67 [0.36, 1.8], p=0.039) (Figure 1). Similar results were obtained when outliers were excluded. Women with coronary endothelial dysfunction demonstrated more vasoconstriction with mental arithmetic compared to controls (median [range]: 0.57 [0.32, 0.71] vs. 0.67 [0.54, 0.92] (p = 0.04). Subgroup analysis of those who had an abnormal CFR (n=13) (<2.5, i.e. non-endothelial microvascular dysfunction) compared to reference controls demonstrated no difference in peripheral vasoreactivity with the stressors. There was no difference in proportion of CVaD subjects who had a low SPR (defined as <0.8) during arithmetic vs. anger compared to controls. There was also no difference in the proportion of CVaD subjects who had an SPR <0.8 to at least one of the stressors compared to control subjects. There were no differences in SPR on the basis of medications. During anger recall, some measures of emotional arousal [anxiety (r=−3.4, p=0.03), frustration (r=−0.37, p=0.02), and feeling challenged (r=−0.37, p=0.02)], inversely correlated with vasoconstriction (SPR) in CVaD subjects, but not in controls. There were no significant correlations between emotional arousal and SPR during mental arithmetic in either group.

Invasive coronary reactivity and PAT measures

There was a moderate correlation between coronary endothelial function and coronary blood flow by acetylcholine testing to RHI (Figure 2). There were no significant correlations between peripheral RHI and non-endothelial dependent pathways (CFR to adenosine, r = −0.05, p = 0.77; NTG response, r = 0.20, p = 0.26). SPR to anger, mental arithmetic, or cold pressor did not correlate with any of the CRT measures.

Discussion

The novel finding of this investigation is that women with CVaD demonstrate more peripheral vasoconstriction to mental stress compared to reference control subjects. Women
with CVaD also reported more emotional arousal during MST and recovery compared to reference controls, with peripheral vasoconstriction correlating with emotional arousal in women with CVaD compared to controls. Other vascular measures, including baseline RHI, were not different between the two groups. To our knowledge, this is the first study that compares women with CVaD diagnosed by invasive CRT to reference controls in a systematic mental stress protocol to evaluate digital peripheral arterial tonometric response. Taken together, these data add novel mechanistic insight into emotionally triggered symptoms in CVaD.

Autonomic dysregulation may contribute to this elevated vasoconstrictor response. Our study indicates that while peripheral vasoconstriction with MST occurs in both CVaD subjects and controls, it occurs significantly more in CVaD compared to controls during mental arithmetic, suggesting that mental arithmetic is a more potent stress test to detect group differences to understand mechanistic pathways of CVaD in women. A prior study comparing CAD subjects to those with normal coronary angiograms demonstrated during acute mental stress (by 10-min video game) greater coronary microvascular dysfunction in a non-obstructed coronary among the CAD subjects compared to those with normal angiograms. Demand ischemia due to mismatch from increased myocardial oxygen demand and failure of the microvasculature to dilate is often used to explain angina and ischemia in those with no obstructive CAD, however our data suggest that abnormal vascular constriction may be a mechanistic pathway for emotionally triggered angina in CVaD. In a report from the WISE study among women who underwent flow-mediated brachial artery reactivity testing, brachial artery constriction post-hyperemia was a marker associated with adverse cardiovascular outcomes compared to those without constriction, and the risk was not related to CAD or traditional risk factors. There were some differences in risk factors among the two groups. While both groups had similar ages, BMI, and no current smokers, a small percentage had diabetes and hyperlipidemia, while a third had hypertension. Thus, it cannot be established from this study whether the observed differences in peripheral vasoreactivity is typical of subjects with CVaD compared to those with a large presence of cardiovascular risk factors.

The mean baseline PAT-RHI in our study was similar between CVaD and reference control subjects. RHI is determined by hyperemic blood flow in the index fingers (skin microcirculation), which is a different than coronary reactivity testing which uses vasoactive agents to assess coronary circulation. We report an RHI of 2.5 [1.25, 3.74] in CVaD, which is consistent with prior literature. Median RHI in the iPOWER study from Denmark in women with microvascular dysfunction (diagnosed by dipyridamole stress echo coronary flow velocity reserve) was similar to ours (mean 2.1 [range 1.6, 2.6]). They also reported a lack of correlation between CVaD and resting RHI. In a study by Martin et al, RHI scores in post-menopausal controls were 2.35 ± 0.49 (similar to our reference controls), and there was no difference between controls and those with history of Takotsubo syndrome, which has been linked to CVaD. However, patients with a history of stress cardiomyopathy with apical ballooning demonstrated increased peripheral vasoconstriction to mental stress compared to those post-menopausal controls or patients with myocardial infarction. In contrast, a study in women from Japan reported an abnormal low RHI of 1.58 [1.4, 1.78] in the no obstructive CAD group compared to 2.15 [1.85, 2.48] in those with no ischemia.
These differences could be explained by ethnic variation; however, a more likely explanation would be the older age in the Japanese study compared to ours (age: 64±10 vs. 58±9), and higher burden of risk factors of hypertension, hyperlipidemia, and diabetes in the Japanese study, which would impact RHI.

As expected, we found that peripheral endothelial function in those with a history of hypertension was lower compared to non-hypertensive women. We also found that those women who had an abnormal response to acetylcholine did have a lower RHI and lower SPR compared to those with normal acetylcholine response. We therefore interpret our RHI data to reflect the vast heterogeneity of CVaD mechanistic pathways in women. The non-endothelial pathways of CVaD did not correlate with RHI, possibly due to small numbers. However, the endothelial pathways of CVaD did moderately correlate with RHI in this study, as would be expected, since previous reports have published that RHI correlates with coronary endothelial function in those with no obstructive CAD. 31,39 Studies have demonstrated that RHI has a sensitivity of 85% and specificity of 80% for detection of endothelial dysfunction in CAD patients.40,41

Ramadan et al. reported that in patients with CAD, SPR is not related to the angiographic severity of CAD.12 In another study in patients with stable CAD, those who had mental stress induced myocardial ischemia on nuclear imaging had greater peripheral vasoconstriction to mental stress compared to those who did not have mental stress myocardial ischemia.42 We find that SPR does not correlate with severity of coronary flow reserve abnormalities. However, SPR correlates with some measures of emotional arousal in women with CVaD during MST. During anger recall, emotional arousal (higher anxiety, frustration, and feeling challenged) inversely correlated with SPR in CVaD subjects, but not in controls. There were no significant correlations between emotions and SPR during mental arithmetic in either group. While more detailed measures of anxiety, depression, and stress were not collected in this study, these findings are thought-provoking and serve as pilot data for a larger study which we are planning to connect psychological measures to various CVaD presentations and mechanisms.

Women often have emotional stress/low heart rate-related angina that is not always exertional; ischemia from mental stress typically occurs at low cardiac workloads, 25,43, and likely involves the autonomic nervous system.44–46 A large body of evidence links mental stress to endothelial dysfunction, atheroma progression, ischemia, and CAD morbidity and mortality.23,27,43,47,48 During mental stress, the normal 26–44% dilation response of coronary microvasculature is blunted in patients with atherosclerosis compared to patients with normal angiograms.34 An exaggerated mental stress-induced rate pressure product may trigger acute ischemia in the setting of chronic coronary endothelial dysfunction. A high rate-pressure product is also predictive of outcomes in CAD patients, particularly in post-menopausal women.49 In this study, however, the CVaD group did not demonstrate an exaggerated hemodynamic response compared to reference control women when exposed to MST or COP testing. The increase in HR and BP that we achieved is consistent with hemodynamic change related to mental stress induction as reported in the literature.22.
Limitations

It must be noted that diagnoses of CVaD by CRT was determined within 2 years of PAT, and not concomitantly. It is possible that if PAT RHI was performed around the same time as CRT, our results may have been different as symptoms and potential underlying mechanisms can fluctuate over time. A true control group for the CVaD group would be women who have a normal CRT, however, our reference control group was asymptomatic and invasive testing is not appropriate. It is possible that we missed eliciting a stronger autonomic response to pain because forehead cold pressor testing may not be as strong a stimulus as traditional hand in ice bucket cold pressor testing. While PAT provides insight into sympathetic vasoconstriction, direct measures of muscle and skin sympathetic nerve activity were not assessed and warrant further investigation. Menstrual cycle may affect endothelial function and in this study, menstrual phase was not assessed. There were relatively low numbers of women with CFR less than 2.5 to adenosine in this study. The doses of vasoactive substances used in CRT may impact the diagnosis (e.g. the highest dose of intracoronary adenosine used was 36 mcg per WISE-CVD study protocol, which may differ from CFR by intravenous adenosine). By design, there were no men enrolled, so conclusions about sex differences in peripheral vasoconstrictive response cannot be made.

Conclusions

Peripheral vasoreactivity to mental stress is higher in patients with coronary vascular dysfunction compared to reference controls. Increased vasoconstriction may be one of the mechanisms that may explain stress induced chest pain and ischemia in women with CVaD. Interventions that modulate autonomic vasoconstrictive responses may be of benefit and should be tested in women with CVaD.

Acknowledgments

Support: This work was supported by contracts from the National Heart, Lung and Blood Institute K23HL105787, K23HL127251, R01 HL090957, T32HL69751, N01-HV-68161, N01-HV-68162, N01-HV-68163, N01-HV-68164, U0164829, U01 HL649141, U01 HL649241, 1R03AG032631 from the National Institute on Aging, GCRC grant MO1-RR00425 from the National Center for Research Resources, the National Center for Advancing Translational Sciences (NCATS) grant UL1TR000124, and grants from the Gustavus and Louis Pfeiffer Research Foundation, Danville, NJ, The Women’s Guild of Cedars-Sinai Medical Center, Los Angeles, CA, The Ladies Hospital Aid Society of Western Pennsylvania, Pittsburgh, PA, and QMED, Inc., Laurence Harbor, NJ, Society for Women’s Health Research (SWHR), Washington, D.C., the Edythe L. Broad and the Constance Austin Women’s Heart Research Fellowships, the Barbra Streissand Women’s Cardiovascular Research and Education Program, the Linda Joy Pollin Women’s Heart Health Program, and the Erika Glazer Women’s Heart Research Initiative and the Adelson Family Foundation, Cedars-Sinai Medical Center, Los Angeles, CA.

References


Figure 1.
Stress PAT Ratio (SPR) to Mental Stress in CVaD vs. Reference Control Subjects. CVaD subjects had abnormally low peripheral vasoreactivity to mental arithmetic compared to reference controls.
Figure 2.
Correlation of coronary diameter response to acetylcholine and peripheral endothelial function. PAT determined RHI mildly correlated with invasive coronary endothelial function by acetylcholine and coronary blood flow determine by acetylcholine. There was no correlation between RHI and invasive adenosine CFR or coronary artery diameter response to nitroglycerin.
Appendix Figure 1.
Mental Stress Testing Protocol. All subjects underwent standardized mental stress testing with anger recall and mental arithmetic, with blood pressure, heart rate, and peripheral arterial tonometric response. RHI: reactive hyperemia index; SPR: stress PAT ratio.
Table 1

Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>CVaD Subjects (n = 41)</th>
<th>Reference Control Subjects (n=21)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58±9</td>
<td>55±10</td>
<td>0.73</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.8±5</td>
<td>26.6±4</td>
<td>0.81</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 (32%)</td>
<td>0</td>
<td>0.0064</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (9%)</td>
<td>0</td>
<td>0.5686</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>5 (11%)</td>
<td>0</td>
<td>0.3093</td>
</tr>
<tr>
<td>Previous history of smoking</td>
<td>16 (36%)</td>
<td>0</td>
<td>0.0028</td>
</tr>
<tr>
<td>Currently smoking</td>
<td>0 (0%)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>32 (73%)</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hormone Therapy</td>
<td>11 (25%)</td>
<td>3 (18%)</td>
<td>0.74</td>
</tr>
<tr>
<td>Beta-Blockers</td>
<td>22 (50%)</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Calcium Channel Blockers</td>
<td>9 (20%)</td>
<td>0</td>
<td>0.05</td>
</tr>
<tr>
<td>Nitrates</td>
<td>23 (52%)</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ACE-Inhibitors/ARBs</td>
<td>21 (48%)</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Statins</td>
<td>29 (67%)</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peak CFR to IC adenosine (36 mcg)</td>
<td>2.83 ±0.74</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CBF to IC acetylcholine (36.4 mcg)</td>
<td>84.07 ±107.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coronary Diameter Change to IC acetylcholine (36.4 mcg)</td>
<td>-1.39 ± 17.36</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diameter change to IC nitroglycerin (200 mcg)</td>
<td>16.47 ± 12.94</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CBF: coronary blood flow; CFR: coronary flow reserve; CVaD: coronary vascular dysfunction; IC: intracoronary; LAD: left anterior descending coronary artery; QCA: quantitative coronary angiography
### Table 2

Emotion During Mental Stress Testing and Recovery in CVaD vs. Controls

<table>
<thead>
<tr>
<th></th>
<th>CVaD n=41 (mean ±SD)</th>
<th>p-Value (Compared to Baseline)</th>
<th>Reference Controls n=21 (mean ± SD)</th>
<th>p-Value (Compared to Baseline)</th>
<th>p-Value (CVaD vs. Reference Controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Anger Recall</td>
<td>3.24 ± 2.21</td>
<td>&lt;0.0001</td>
<td>2.48 ± 2.16</td>
<td>&lt;0.0001</td>
<td>0.16</td>
</tr>
<tr>
<td>Δ Anxious</td>
<td>3.39 ± 1.93</td>
<td>&lt;0.0001</td>
<td>3.10 ± 1.84</td>
<td>&lt;0.0001</td>
<td>0.55</td>
</tr>
<tr>
<td>Δ Tense</td>
<td>3.80 ± 1.55</td>
<td>&lt;0.0001</td>
<td>3.62 ± 2.18</td>
<td>&lt;0.0001</td>
<td>0.02</td>
</tr>
<tr>
<td>Δ Irritated</td>
<td>4.39 ± 1.93</td>
<td>&lt;0.0001</td>
<td>3.29 ± 1.79</td>
<td>&lt;0.0001</td>
<td>0.008</td>
</tr>
<tr>
<td>Δ Depressed</td>
<td>4.05 ± 1.95</td>
<td>&lt;0.0001</td>
<td>3.71 ± 2.28</td>
<td>&lt;0.0001</td>
<td>0.14</td>
</tr>
<tr>
<td>Δ Stressed</td>
<td>4.29 ± 1.66</td>
<td>&lt;0.0001</td>
<td>3.62 ± 1.77</td>
<td>&lt;0.0001</td>
<td>0.42</td>
</tr>
<tr>
<td>Δ Mental Arithmetic</td>
<td>3.04±2.6</td>
<td>&lt;0.0001</td>
<td>2.29 ±2</td>
<td>&lt;0.0001</td>
<td>0.42</td>
</tr>
<tr>
<td>Δ Anxious</td>
<td>4.30 ± 1.68</td>
<td>&lt;0.0001</td>
<td>3.30 ± 1.89</td>
<td>&lt;0.0001</td>
<td>0.04</td>
</tr>
<tr>
<td>Δ Tense</td>
<td>4.13 ± 1.68</td>
<td>&lt;0.0001</td>
<td>3.95 ± 2.01</td>
<td>&lt;0.0001</td>
<td>0.94</td>
</tr>
<tr>
<td>Δ Frustrated</td>
<td>4.83 ± 1.57</td>
<td>&lt;0.0001</td>
<td>4.30 ± 1.95</td>
<td>&lt;0.0001</td>
<td>0.34</td>
</tr>
<tr>
<td>Δ Irritated</td>
<td>3.85 ± 2.21</td>
<td>&lt;0.0001</td>
<td>3.90 ± 1.86</td>
<td>&lt;0.0001</td>
<td>0.92</td>
</tr>
<tr>
<td>Δ Depressed</td>
<td>1.48±2.33</td>
<td>0.0003</td>
<td>1.00 ± 1.62</td>
<td>0.012</td>
<td>0.61</td>
</tr>
<tr>
<td>Δ Anxious</td>
<td>3.60 ± 2.37</td>
<td>&lt;0.0001</td>
<td>2.40 ± 2.58</td>
<td>0.0005</td>
<td>0.07</td>
</tr>
<tr>
<td>Δ Stressed</td>
<td>4.43 ± 1.81</td>
<td>&lt;0.0001</td>
<td>4.00 ± 2.08</td>
<td>&lt;0.0001</td>
<td>0.45</td>
</tr>
<tr>
<td>Δ Mental Arithmetic</td>
<td>4.16±2.01</td>
<td>&lt;0.0001</td>
<td>4.81 ± 1.64</td>
<td>&lt;0.0001</td>
<td>0.25</td>
</tr>
</tbody>
</table>
### Appendix Table 1

**Hemodynamic Changes with Mental Stress Testing**

<table>
<thead>
<tr>
<th></th>
<th>CVaD Subjects</th>
<th>Reference Control Subjects</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median [min, max]</td>
<td>Median [min, max]</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>67 [47, 90]</td>
<td>61 [50, 83]</td>
<td>0.27</td>
</tr>
<tr>
<td>SBP</td>
<td>115 [90, 165]</td>
<td>116 [94, 145]</td>
<td>0.70</td>
</tr>
<tr>
<td>DBP</td>
<td>64 [44, 80]</td>
<td>67 [31, 85]</td>
<td>0.27</td>
</tr>
<tr>
<td>RPP</td>
<td>7725 [4794, 12330]</td>
<td>7192 [4700, 9570]</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>Anger</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ HR</td>
<td>12 [2, 42]</td>
<td>11 [6, 52]</td>
<td>0.49</td>
</tr>
<tr>
<td>Δ SBP</td>
<td>18 [2, 50]</td>
<td>22 [−4, 54]</td>
<td>0.88</td>
</tr>
<tr>
<td>Δ DBP</td>
<td>12 [−2, 27]</td>
<td>12 [1, 69]</td>
<td>0.49</td>
</tr>
<tr>
<td>Δ RPP</td>
<td>2994 [724, 10020]</td>
<td>2702 [715, 13514]</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>Arithmetic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ HR</td>
<td>10 [−1, 53]</td>
<td>17 [−6, 41]</td>
<td>0.08</td>
</tr>
<tr>
<td>Δ SBP</td>
<td>17 [−5, 62]</td>
<td>23 [−6, 39]</td>
<td>0.23</td>
</tr>
<tr>
<td>Δ DBP</td>
<td>8 [−8, 29]</td>
<td>11 [0, 16]</td>
<td>0.27</td>
</tr>
<tr>
<td>Δ RPP</td>
<td>2682 [−217, 13472]</td>
<td>3267 [−212, 8349]</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Cold Pressor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ HR</td>
<td>1 [−21, 30]</td>
<td>−1 [−14, 17]</td>
<td>0.90</td>
</tr>
<tr>
<td>Δ SBP</td>
<td>19 [−15, 60]</td>
<td>21 [−4, 40]</td>
<td>0.60</td>
</tr>
<tr>
<td>Δ DBP</td>
<td>11 [−8, 29]</td>
<td>11 [−7, 27]</td>
<td>0.90</td>
</tr>
<tr>
<td>Δ RPP</td>
<td>1206 [−1594, 8700]</td>
<td>1525 [−1480, 3900]</td>
<td>0.87</td>
</tr>
</tbody>
</table>

DBP = diastolic blood pressure, HR = heart rate, RPP = rate pressure product, SBP = systolic blood pressure