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Sex Differences in the Neural Correlates of Mental Stress in Men and Women with Coronary Artery Disease

By

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Master of Public Health

Epidemiology

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By

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BS, University of California Los Angeles, 2012

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An abstract of
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Abstract

Sex Differences in the Neural Correlates of Mental Stress in Men and Women with Coronary Artery Disease

By Nicole Kasher

Chronic exposure to psychosocial stress has been linked to changes in structural and functional activation of brain regions involved in the control of cardiovascular regulation. Psychosocial stress is a risk factor for coronary artery disease (CAD) and has been associated with poorer prognosis and greater mortality in women than men. We investigated sex differences in the neural correlates of mental stress in a cross-sectional study of 53 women and 112 men with stable CAD. We used $[^{15}O]H_2O$ positron emission tomography (PET) to identify brain regions with cerebral blood flow changes with mental stress using mental arithmetic and public speaking tasks in women and men. Compared to men, women had significantly greater activation in the left superior temporal gyrus (Area 42) and greater deactivation in the anterior cingulate gyrus, bilaterally (Area 24, 32), right medial frontal gyrus (Area 8, 9), and right middle temporal gyrus (Area 21). Of interest, among those with mental stress ischemia (MSI), women had greater activation than men in the right and left anterior cingulate gyrus (Area 24, 32). In contrast, among those without MSI, there was no statistically significant increase in cerebral flow to any brain region outside of the cerebellum among women compared with men; however, there was greater hypoactivation to mental stress in women than men in the cingulate gyrus (Area 24, 32) and right middle temporal gyrus (Area 21). Among female participants, women with MSI had comparable sex differences in deactivation to mental stress as women without MSI, in addition to deactivation in the right and left prefrontal cortex (Areas 8, 11, 47). The results of our study are consistent with the hypothesis that stress is differentially associated with hyper- and hypoactivation of brain regions in men and women, particularly in the limbic system. Furthermore, our results demonstrate exaggerated stress responsivity in the prefrontal cortex, particularly the cingulate gyrus, in women with CAD and MSI.

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Table of Contents

Background

A. Introductions

Exposure to stressors triggers a series of adaptive physiological and behavioral responses that function together to maintain homeostasis. According to Selye's concept of general adaptation syndrome, exposure to chronic and uncontrolled stress may result in dysregulation of adaptational responses and leave individuals more vulnerable to the development of chronic diseases(1). Recent evidence suggests that psychosocial or mental health disturbances such as depression are risk factors for the development of cardiovascular disease (CVD) and for poorer health outcomes after myocardial infarction (MI) among men and women (2-8) . The prevalence of stress and stress-related disorders and their association with coronary artery disease (CAD) is more pronounced in women than men in several studies(8-11) suggesting potential sex differences in stress perception and/or processing in the brain.

B. Cardiovascular disease: magnitude of the problem and sex differences

CVD is the leading cause of mortality among men and women in the United States, accounting for approximately 1 of every 3 deaths the US in 2009 (12, 13) There are considerable differences in the burden and distribution of CVD and CVD types among men and women. The prevalence of CVD is greater in men than women in all age groups beginning at 20 years of age and continuing until 80 years of age where women surpass men (14). Among both men and women, coronary artery disease is the largest contributor to CVD-related mortality statistics (13). While the prevalence of CAD is greater in men than women across all age groups, the incidence of CAD in women exceeds that of men at 85 years of age and older (14).

Men and women are distinct with regards to the presentation and prognosis of CAD. Compared to men, women have a ten-year delay in the age at onset of increased CAD risk suggesting that younger women are protected from CAD with respect to men of similar age (2, 15, 16). Additionally, women

below 65 years of age, particularly younger women, are more likely to present with a greater number of cardiovascular risk factors and have a larger number of comorbidities and a longer length of stay when hospitalized for CAD than men, suggesting that young women who present with CAD are sicker relative to men in the same age group (17-20). Between 1979 and 2011, age-adjusted CAD mortality rates were greater in American men than women, in all age groups 25 years of age and older(21) While there has been a substantial decline in CAD-related mortality rates over the past several decades, the rate of change was lower among individuals less than 55 years of age and lowest among young women less than 55 years of age compared to men in the same age groups(21). Furthermore, multiple studies examining sex differences in prognosis after MI have identified a greater risk of in-hospital mortality and 30-day mortality post-hospitalization, in women younger than 75 years of age than men(17, 18, 22, 23). These differences in the presentation and prognosis of disease among men and women may indicate potential sex-based differences in the etiology, pathophysiology, or clinical course of cardiovascular disease.

C. Psychosocial stressors as risk factors for CVD and sex differences

Psychosocial stressors may be directly associated with cardiovascular disease risk or may act indirectly by impacting CVD risk factors. For example, in a large individual-participant data (IPD) meta-analysis of studies conducted between 1984 and 2003, job strain was associated with greater cardiovascular disease risk and several CVD-related risk factors such as smoking and obesity (Nyberg et al). Furthermore, in a case control study investigating cardiovascular risk factors for acute MI and spanning 52 countries, psychosocial stressors (defined as depression, locus of control, perceived stress, and life events) was found to independently account for 33% of the risk of myocardial infarction in the entire cohort(2, 7). Depression, in particular, has been recognized as a predictor of worse prognosis after CAD among men and women in the last decade (24).

Sex differences in the effect of psychosocial stressors on health suggest that stress-related pathways may differ in men and women or they may perceive and process stress differently. For example, depressive symptoms have been found to be stronger risk factors for worsening of CAD and CAD-related adverse events such as mortality in women below 55 years of age than men of similar age and older women (25). Interestingly, young women are more susceptible to developing depression and depressive symptoms (26); moreover, sex differences in the association between depression and survival after myocardial infarction have not been found in larger studies that do not consider the interaction of sex and age suggesting that young women in particular may be more prone to the ill-effects of depression on cardiovascular health (27, 28).

Finally, women have a greater burden of stress-related disorders than men. For example, Takotsubo syndrome, or stress cardiomyopathy, occurs primarily among post-menopausal women and onset of the disease usually follows an acute mental or emotional stressor. (11, 29). Additionally, mental stress ischemia (MSI, a phenomenon characterized by insufficient perfusion of blood to the heart during mental stress, affects anywhere from approximately 1/3 to 1/2 of all individuals with CAD (30). MSI is associated with poorer prognosis and higher mortality among individuals with CVD and disproportionately affects younger women (10, 31, 32). Additionally, the prevalence of depression is approximately two-fold higher in women compared to men, and these sex differences begin at puberty and decline after menopause (26). Furthermore, among individuals with a history of CVD, women are more likely to report higher levels of stress, depression and anxiety than men (10, 33).

D. The physiological stress response

The physiological stress response is mediated in part by changes in activity of a series of neural circuits including the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis (34-37). Immediately following exposure to an acute stressor, the autonomic nervous system releases

epinephrine and norepinephrine, which coordinate a series of physiological and behavioral responses to meet the demands of organ systems involved in the fight and flight response, such as redirecting blood flow and increasing blood pressure and respiration rates (37). A secondary slower response to stress is mediated by cortisol, a glucocorticoid released by the adrenal medulla following activation of the HPA axis by corticotropin releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus(37).

In addition to these circuits, there is a network of cortico-limbic regions which is involved in perceiving and processing external stressors and internal emotions as well as regulating an appropriate stress response (34). Neuroimaging studies in the literature have identified networks of brain regions that are activated or deactivated during stressful tasks and are correlated with changes in cardiovascular markers of stress. Heart rate, systolic blood pressure, mean arterial pressure, and cortisol reactivity during mental stress have all been positively correlated with changes in activation in multiple cortico-limbic-striatal structures that include members of the limbic system or share connections with the limbic system (such as the amygdala, hippocampus, parahippocampus, thalamus, hypothalamus, dorsal and anterior cingulate cortex, posterior cingulate cortex, bilateral insula, medial prefrontal cortex, and right orbitofrontal cortex), brain regions involved in generating voluntary movement (left premotor and supplementary cortex), regions involved in working memory, sensation, and sensory and motor coordination (dorsolateral prefrontal cortex and somatosensory cortex), the right superior temporal gyrus, the right caudate body, precuneus, and the cerebellum (38-46). Additionally, after exposure to mental stress tests that used emotional stressors, cardiovascular markers of stress were also correlated with greater activation in brain regions involved in memory and in the processing of visual information such as faces (fusiform, parahippocampal, occipital, and lingual gyri) (47-49). Among studies that measured increases as well as decreases in cerebral blood flow during mental stress testing through brain imaging, systolic blood pressure and heart rate have also been correlated with deactivation in

multiple cortico-limbic-striatal structures and their projections (38, 40-42, 49). These studies suggest that regulation of the autonomic nervous system depends on an organized network of brain structures within the limbic system and their projections and is not localized to a single or a few brain circuits.

E. Stress reactivity in CAD patients

Patients with CAD exhibit differences in brain reactivity to mental stress compared to healthy controls in multiple structures including the limbic system and prefrontal cortex, which are implicated in emotion and autonomic regulation as well as executive control. In a study by Soufer et al, 16 right handed male patients without a history of psychiatric illness, 10 of whom had a diagnosis of CAD, were exposed to a mental stress test involving mental arithmetic. Compared to healthy controls, CAD patients had greater activation in the left parietal cortex, left anterior cingulate, right visual association cortex, left fusiform gyrus and cerebellum (50). Furthermore, CAD patients had greater deactivation than healthy subjects in the right thalamus, right frontal gyrus, and right middle temporal gyrus (50). Differences in brain activity during stress among CAD patients and healthy controls provide evidence of potential alterations in the neurobiological pathways that mediate the stress response among individuals with CAD. Furthermore, in the same study, CAD patients with and without mental stress ischemia (MSI) were compared. Compared to CAD patients without MSI, CAD patients with MSI had significantly greater activation and deactivation in multiple limbic structures (left hippocampus and bilateral anterior cingulate) as well as the left superior and middle frontal and temporal gyri, and bilateral visual association cortex (50). MSI is associated with poorer prognosis of CAD (31, 32). Differences in brain reactivity of the limbic structures to mental stress among those with and without MSI suggest that functional alterations of structures within the limbic systems may be linked to dysregulation of the autonomic nervous system and worsening of CAD among those with MSI.

F. Stress reactivity and sex differences in healthy subjects

Mental stress tasks are used to reproduce acute psychosocial stressors under controlled settings and elicit measurable changes in activation of the stress response system. Standardized mental stress testing paradigms such as the Montreal Imaging Stress Task have been shown to reliably increase biomarkers of acute stress, such as cortisol, in subjects in lab settings (51). Mentally stressful tasks may be classified into two domains: cognitive stressors, such as mental arithmetic, public speaking, and word-color conflict, and emotive stressors, such as emotional face viewing (49). Changes in peripheral cardiovascular markers, such as blood pressure and cortisol levels, in response to these stressful tasks are useful indicators of the stress response mounted by the autonomic nervous system and the HPA axis, respectively, and may be used to show sex differences in stress reactivity.

Numerous epidemiological studies have shown that men have statistically significantly greater systolic and diastolic blood pressure reactivity, and cortisol reactivity (markers of sympathetic reactivity) than women after exposure to acute psychological stressors (52-58). However, several investigations failed to show sex differences in these cardiovascular markers of sympathetic reactivity after psychosocial stress (56, 59). Additionally, heart rate reactivity to mental stress was not significantly different between men and women in a majority of studies reviewed. However, investigations that failed to find sex differences in these markers had smaller sample sizes (56, 59), a unique combination of stress tests (56) or the cohort included subjects with a wider range in age (59). Sex differences in cardiovascular markers of stress have been shown to vary over stress task and participants age (58, 60). For example, in a meta-analysis of 186 studies that measured cardiovascular stress reactivity to a variety of psychological stressors, systolic blood pressure, diastolic blood pressure, heart rate, and overall sympathetic and parasympathetic reactivity varied significantly with either stress task or age (60). Systolic blood pressure reactivity was greater during speech tasks than all other stress tasks compared (60). Additionally, neuroendocrine and cardiovascular reactivity to stress may be affected by menstrual

cycle phase, oral contraceptive use, and menopause status among women, further supporting the complexity of sex differences in the neurobiological pathways of stress reactivity and the pathophysiology of stress-related disorders (61). For example, Goldstein et al used a cross-sectional study of 25 men and women between 35 and 46 years of age to show that sex differences in brain reactivity to mental stress (negative and neutral affective pictures) varied depending on the menstrual cycle phase that female participants were tested (62). When women were tested during their early follicular phase, men had greater activation than women in brain regions involved in emotional perception and autonomic regulation (amygdala and anterior cingulate). In contrast, when these same women were tested 2 weeks later during their midcycle phase, men had greater activation more broadly throughout subsets of the limbic system, frontal lobe, and periaqueductal gray, suggesting that sex differences in acute mental stress response may be affected by circulating sex hormones.

A limited number of studies assessed sex differences in the neural correlates of stress reactivity in healthy individuals. Interestingly, men and women exhibited different changes in brain activation upon exposure to mentally stressful tasks supporting sex differences in the effect of stress on the brain. Among studies that exposed subjects to emotionally stressful and achievement-related cognitive tasks, several suggested that functional connectivity and activation in the amygdala, a brain region implicated in fear and anxiety processing, may be greater in women than men (63-67), although this has been debated (62). Sex differences in stress reactivity of brain structures implicated in emotional processing, like the amygdala, may help explain differences in stress perception among men and women. Additionally, in a meta-analysis of studies published between 1990 and 2010 that analyzed sex differences in brain activation to positive and negative valence stimuli (emotional stress tests), men and women engaged different cortical and subcortical regions after exposure to emotional stressors (65). For example, women had greater activation than men in a number of brain regions, including components of the limbic system (the amygdala, hippocampus, mammillary body, the medial, frontal, and anterior

cingulate gyrus, and medial dorsal nucleus of the left thalamus) the right middle occipital gyrus, and the middle and inferior temporal gyrus (65). Men were more likely to have brain activation in different brain regions of the limbic system (the insula, posterior cingulate gyrus) and in the prefrontal cortex (inferior frontal gyrus), the right precentral gyrus, the right superior temporal gyrus, the right putamen, and the left middle temporal gyrus and fusiform gyrus (65). Moreover, in a cross-sectional study of 16 men and 16 women with a mean age of 22.8 who were exposed to a mentally stressful arithmetic task, the neural correlates of mental stress differed among men and women, particularly among structures in the limbic system and prefrontal cortex (68). Men had greater activation than women in thalamus and greater deactivation than women in the left orbitofrontal cortex and inferior frontal cortex; women had greater activation in the left orbitofrontal cortex, dorsal anterior cingulate cortex (dACC), left insula, and left parietal cortex and supramarginal gyrus (68). Differences in the mental stress tasks applied across these studies may contribute to the variability in brain regions that were affected across these studies. For example, in a cross-sectional study of 14 women and 12 men between 25 and 50 years of age who were exposed to recordings of themselves and others performing a social rejection ask, female participants reported experiencing greater stress than male participants, but did not exhibit greater activation in any brain regions than men, while male participants exhibited greater activation than female participants in 16 different brain regions (69).

Notably, similar brain regions that regulate the stress response may have different effects in men and women. For example, investigators Buchanan et al demonstrated that cortisol reactivity to stress among participants with medial prefrontal cortex (mPFC) damage is affected by the participants' sex. In women, those with mPFC damage had significantly greater cortisol release to stress (using the Trier Social Stress Test), than normal female controls (70). In contrast, men with mPFC damage had slightly less cortisol release with stress than healthy male controls (70). These studies emphasize the

importance of not only identifying differences in the neural correlates of stress among men and women, but understanding differences in their role in the stress response.

G. Stress reactivity and sex differences in animal studies

Several experimental studies have also shown sex differences in functional and structural changes of brain regions tied to executive function, emotional processing, and autonomic regulation (the limbic system and prefrontal cortex) during mental stress. For example, chronic stress differentially affects hippocampal cell neurogenesis and survival in male and female rats (71). Furthermore, gene expression in the hippocampus in response to mental stress has been shown to differ among male and female mice. Male and female mice (N = 177) were exposed to a traumatizing cold swim stress test and expression of 3 different genes sensitive to stress was measured using polymerized chain reaction (PCR.) These genes were Fos (a protein marker of immediate neuronal activity), Per1 (a regulatory protein involved in generating a circadian rhythm), and Sgk1 (a regulatory protein involved in regulating a wide array of cellular functions, such as ion transport, and stress response, such as apoptosis.)Female mice had greater Fos and Per1 expression in hippocampal cells than male mice after stress, suggesting that the activation of gene transcription in response to stress is greater in females than males in the hippocampus, which is a component of the HPA axis and an important mediator of the stress response system (72). Additionally, male and female rats have shown sex-dependent alterations in structural connectivity of the medial prefrontal cortex in response to chronic stress, which may be mediated by circulating estrogen levels in female rats (73, 74). After receiving chronic stress restraint treatment 3 hours daily for one week, changes in the dendritic morphology of pyramidal cells in layers II – III of the medial prefrontal cortex were measured in male and female rats. Dendritic apical branch number and length decreased in male rats and apical branch length increased in female rats. Furthermore, among ovariectomized female rats, alterations in dendritic branching was dependent on estrogen treatment;

only ovariectomized rats with estrogen treatment, and not those without estrogen treatment, demonstrated a statistically significant difference in dendritic morphology after chronic stress relative to their unstressed counterparts. These studies underscore the importance of considering sex-dependent differences in the effect of acute and chronic stress on the brain.

H. Conclusion

The effect of stress on the brain is supported by studies of both acute and chronic stressors. Acute stressors are associated with greater activation and deactivation of different brain regions, including structures implicated in autonomic and emotional regulation, and this brain reactivity is correlated with changes in cardiovascular markers of stress. Furthermore, a number of studies have demonstrated hyperactivation in structures associated with the limbic system during mental stress testing among individuals with CVD compared to healthy controls. Individuals with CVD experience a significantly higher prevalence of depression in addition to significant differences in activation in brain regions that are part of or communicate with the limbic system, including the hippocampus, parahippocampus, thalamus, and cingulate gyrus, compared to healthy controls. Structural changes to the limbic circuitry and other brain regions involved in regulation of the autonomic nervous system following chronic exposure to mental stressors, such as depression, may affect the ability of the autonomic nervous system to perform its function. If women are more susceptible to the effects of mental stress than men, then sex based differences in brain activation upon exposure to mental stress may help with identifying markers for the risk of developing CVD or for poor prognosis of CVD, and with identifying subsets of the population who are more susceptible to the effects of mental stress.

Introduction

Cardiovascular Disease (CVD) is the leading cause of mortality among men and women in the United States (12, 13). Recent evidence suggests that psychosocial stressors and mental health disturbances such as depression are risk factors for the development of cardiovascular disease (CVD) and for poorer health outcomes after myocardial infarction (MI) (2, 4-7, 24). The prevalence of stress and stress-related disorders and their association with coronary artery disease (CAD) tends to be more pronounced in women than men, suggesting potential sex differences in stress perception and processing in the brain (8, 9, 11).

Differences in characteristics and factors related to disease development, presentation, and prognosis of disease among men and women support sex differences in the pathophysiology of CVD. Compared to men, women have a ten year delay in the age at onset of CAD, suggesting that younger women have a sex-related advantage and protection from CAD compared with men of the same age (2, 15, 16). Women below 65 years of age diagnosed with CAD, particularly younger women, are more likely to present with a greater number of cardiovascular risk factors and comorbidities, and have a longer length of hospital stay for CAD and a greater risk of in-hospital death and 30 day mortality compared to men, even though they have less severe coronary atherosclerosis (17-20, 22, 23).

Women have a greater burden of stress and stress-related disorders than men. The prevalence of depression is approximately two-fold higher in women compared to men, and these sex differences begin at puberty and decline after menopause (26). Furthermore, among individuals with CAD, women report higher levels of perceived stress, depression and anxiety than men (10, 33). Additionally, mental stress ischemia (MSI, a phenomenon characterized by insufficient perfusion of blood to the heart during mental stress, affects anywhere from approximately 33% to 50% of all individuals with CVD (30). MSI is associated with poorer prognosis and mortality among individuals with CVD and disproportionately affects younger women (10, 31, 32).

Sex differences in the prevalence of psychosocial stress may contribute to observed differences in CAD development and outcome, and susceptibility to MSI, in women compared with men. Another possibility is that women and men differ in their physiological responses to emotional stress. Chronic stress is associated with activation and volumetric changes in brain regions implicated in cognition, executive function and emotion, especially areas within the limbic system and prefrontal cortex (75). Compared to healthy controls, individuals with CVD exhibit different activation patterns in brain regions that are part of, or communicate with, the limbic system (50, 76-79), suggesting that these brain areas are involved in CVD pathophysiology. The limbic system has direct and indirect outputs to brain areas involved in control of the autonomic nervous system, which in turn regulates the cardiovascular system (80). Thus, structural or functional changes to the limbic circuitry following acute or chronic exposure to emotional stress and/or stress-related disorders, such as depression, may disrupt regulatory functions of the autonomic nervous system on cardiac physiology. This may represent a pathway linking emotional stress to CVD. Therefore, sex-based differences in brain activation upon exposure to mental stress in patients with CAD may help identify risk pathways potentially involved in sex differences in vulnerability to MSI and poor prognosis of CVD.

Prior neuroimaging studies in healthy individuals and participants with CAD have demonstrated sex differences in brain reactivity to mental stress, but they tended to be small and included a limited number of women. Furthermore, to our knowledge, previous studies that conducted neuroimaging with mental stress in CAD patients and examined sex differences have not considered MSI. In the current, larger study, we addressed whether there are sex-related differences in brain activation and deactivation associated with mental stress in participants with CAD, and whether these differences are modified by MSI.

Methods

Study Design

We studied 60 female and 126 male subjects with confirmed CAD (N = 186) who were participants in the Mental Stress Ischemia Mechanisms and Prognosis Study (MIPS). Recruitment methods for MIPS cohort were described elsewhere (81). Briefly, 695 patients between 30 and 80 years of age with confirmed stable coronary artery disease (CAD) were prospectively enrolled from Emory-affiliated hospitals, Emory clinics, and external clinics between 2011 and 2014. All patients underwent mental stress testing using a standardized public speaking task, and ischemia was detected by Tc-99m sestamibi myocardial perfusion imaging. Patients also underwent conventional stress testing for myocardial ischemia (CSIMI) using exercise or pharmacological stress testing with regadenoson.

Using a cross-sectional study design, the objective of the present study was to investigate sex differences in neural correlates of mental stress in patients with a history of CAD. All subjects were participant in MIPS and satisfied the inclusion criteria of a documented clinical diagnosis of CAD by meeting at least one of the following: (1) angiographic evidence of CAD with at least 1 major vessel affected; (2) myocardial infarction at least 1 month before baseline visit; (3) atherosclerosis of at least 1 coronary vessel as demonstrated by an abnormal coronary intravascular ultrasound exam; (4) a history of coronary bypass surgery or angioplasty; and/or (5) a positive nuclear scan or exercise stress test. Patients were excluded if they had unstable angina, myocardial infarction, or decompensated congestive heart failure within one week of their baseline visit; if they had any severe comorbidities expected to decrease their life expectancy to the next 5 years; or if they had systolic or diastolic blood pressure above 190 and 115 mmHg, respectively, on the day of testing. Patients were also not eligible for participation if they had a history of alcohol or substance abuse in the past year, severe psychiatric disorders other than depression, inflammatory diseases, malignancies, organ transplants, permanent atrial fibrillation, severe neurological disorders such as dementia or Parkinson's disease, or

contraindications to regadenoson. Pregnant women, patients with chronic steroid use above 1500 mcg per day, and patients unable to complete testing were also excluded from study participation. Given that depression is common in CAD patients and exerts profound effects on brain activity, patients were selected into the current study so that approximately half of the sample would have depression (either current depressive episode or an elevated level of depressive symptoms). All study subjects provided informed consent and the study was approved by the Emory University Institutional Review Board.

Mental Stress Testing

Subjects underwent 8 PET brain imaging scans in conjunction with mental stress and control tasks in a single day. Participants were asked to hold beta-adrenergic antagonists and nitrate and calcium channels blockers for a minimum of 24 and 12 hours, respectively, prior to mental stress testing. After resting in a quiet room for 30 minutes, subjects were asked to perform two control and then two mentally stressful conditions, each lasting approximately 2 minutes. During each control condition, participants were scanned twice while counting out loud and twice while discussing a neutral event. The remaining four scans were collected during stress conditions in which subjects completed arithmetic (2 scans) and public speaking (2 scans) tasks, and the order of mentally stressful tasks was randomized. For the arithmetic task, participants were asked to solve a series of increasingly complicated math equations, including addition, subtraction, division, and multiplication, under time constraints and were given negative feedback regarding their performance by a staff member administering the test and wearing a white coat. For the public speaking task, participants were provided two scripted scenarios of stressful interpersonal situations and asked to develop a speech regarding these events. They were given 2 minutes to prepare each speech and 3 minutes to present it in front of an audience and camera. Subjects were told that their speeches would later be evaluated.

Brain Imaging Methods and Analysis

We conducted PET brain imaging scans using High Resolution Research Tomography (HRRT) (Siemens, Inc), with a spatial resolution of 2 mm. There was a total of eight brain scans, two scans during each of the 2 control (counting aloud and recalling a neutral event) and 2 stress (arithmetic and public speaking) conditions, and the duration of each task was approximately two minutes. Subjects were injected with 20mCi of O-15 water 10 seconds after the beginning of each tasks to assess cerebral blood flow. Analysis of PET images was automated using statistical parametric mapping (SPM8) software, following methods previously described (82, 83). Prior to analysis, all scans were realigned to the first image in the scanning session, smoothed, and normalized onto a standard brain template from the Montreal Neurological Institute (MNI). Subjects were compared based on sex and MSI status and sex to identify significant differences in brain activation and deactivation between groups. Significance thresholds were set at $p < 0.005$ and 11 voxels. We then converted MNI brain coordinates provided by SPM8 to Talairach coordinates using standardized piecewise affine transformation equations. MNI and Talairach atlases were used to identify the name and Brodmann area of regions with significant activity as highlighted by SPM8, and all results were reviewed by a neuroscientist.

Myocardial Perfusion Imaging Methods and Analysis

On a separate day, subjects completed single photon emission computed tomography (SPECT) cardiac imaging to measure myocardial perfusion at rest, as part of the MIPS protocol (81). Patients were injected with 10 - 14 mCi of [Tc-99m] sestambi at rest and SPECT imaging of the heart was collected 30 – 40 minutes later. To obtain cardiac imaging during stress, patients were injected with 30-40 mCi at the end of the speech stress condition and SPECT imaging of the heart was collected 30 - 40 minutes later. Cardiac data were acquired following a 17-myocardial segment model and scored separately by two experienced readers, blinded to the task condition and without prior knowledge of the subject's medical

history, on a scale of 0 (normal) to 4 (no perfusion). Disagreements were resolved by consensus or a third reader if needed. The summed stress score was calculated by dividing the summation of all scores collected during stress conditions by 68, the maximum possible score. Participants with a summed stress score of 3 or higher during mental stress testing were determined to be positive for MSI, while those with a score lower than 3 were negative for MSI.

Hemodynamic Reactivity

Hemodynamic reactivity measures were collected using an automatic oscillometric device and a 12 lead ECG. Measurements were recorded at baseline and during each control and mental stress tasks. The measurements obtained were averaged over the control and stress tasks to obtain mean control and mean mental stress measures. The mean rate pressure product during mental stress and control conditions for each subject was calculated as the product of the mean heart rate and the mean systolic blood pressure during control tasks and during mental stress tasks. Stress reactivity for systolic blood pressure, heart rate and rate pressure product was calculated as the difference between mean mental stress and mean control measures.

Other Measurements

The Structured Clinical Interview for DSM IV (SCID) was administered at the baseline visit to establish depression diagnosis. Sociodemographic characteristics, medical history, and medication history were collected by a research nurse using standard questionnaires, chart reviews and in-person interviews and were verified using medical records.

Data Analysis

Differences in demographic, clinical and behavioral variables between males and females were assessed using two sample t tests for continuous variables and chi square tests for categorical variables. The likelihood ratio test and 2-way ANVOA were used to calculate the interaction of sex with MSI for categorical and continuous variables, respectively. Linear regression was used to compare heart rate reactivity, systolic blood pressure reactivity, and rate pressure product reactivity between men and women, before and after adjusting for covariates. Variables adjusted for in the models included sociodemographic factors (age, income, education, and race), cardiovascular disease history and risk factors (BMI, diabetes, dyslipidemia, mental stress ischemia status, beta-blocker use, history of smoking, and history of myocardial infarction), and depression diagnosis, and were sequentially added to the model. Variables were selected for inclusion based on a priori considerations that they might confound the association, and if their inclusion caused at least a 10% change in the estimate for sex.

Results

Of the 186 individuals who were originally enrolled in our study, 165 completed the study with usable brain data and were included in the analysis (N = 53 female and 112 males). We excluded 7 female and 14 male participants because of poor scan quality or because participants were unable to complete their scans. Selected baseline characteristics of the sample can be found in Table 1. The 53 women and 112 men included in the final analysis had a mean age of 61.2±7.7 and 62.3±8.7, respectively. Women were more likely than men to meet DSM-IV criteria for lifetime major depression and to be on antidepressants. While there was no difference in history of myocardial infarction among men and women, the proportion of women with a history of heart failure was 2.4-fold greater than men. Additionally, women were more likely to be taking beta blockers and diuretics than men (all $p < 0.05$). All other measured socioeconomic, clinical, and lifestyle characteristics were evenly distributed among men and women in the dataset, even after stratifying by MSI (Tables 1 and 2).

Hemodynamic reactivity to psychosocial stress also differed between men and women (Table 3). Men had a significantly greater average increase in systolic blood pressure from control to mentally stressful tasks than women (10.4±7.8 vs 5.3±10.0, $p <0.001$). Additionally, the change in rate pressure product in response to mental stress was approximately 1.5-fold greater on average in men than women ($p = 0.04$). There were no statistically significant sex differences in heart rate reactivity to mental stress indicating that sex differences in RPP reactivity to psychosocial stress were driven by systolic blood pressure. To identify the factors that may contribute to the differences in hemodynamic reactivity among men and women, in a series of models we progressively adjusted for demographic factors, lifestyle characteristics and clinical risk factors associated with CAD (Table 4). Sex differences in rate pressure product and systolic blood pressure were no longer statistically significant after adjusting for demographic factors, and the association further weakened after adding to the model CVD risk factors, clinical history, MSI status, and depression (Table 4).

Next, we examined brain activation/deactivation patterns with mental stress relative to control conditions in women (Tables 5 and 6) and men (Tables 7 and 8). Among women, mental stress tasks, relative to control tasks, were associated with greater activation in the right middle frontal gyrus, bilateral cingulate gyrus, left inferior and superior frontal gyrus, bilateral inferior parietal lobe (including the supramarginal gyrus), and regions of the cerebellum (Table 5, Figures 1-2). Furthermore, in women, mental stress conditions relative to control conditions were associated with greater deactivation primarily in the left precentral gyrus, inferior frontal gyrus, and insula. Further regions with greater deactivation during mental stress than control in women include the right precentral gyrus, superior temporal gyrus, and insula as well as the bilateral medial frontal gyrus, bilateral cingulate gyrus, right medial occipital gyrus, right middle and superior temporal gyrus, and regions of the cerebellum (Table 6, Figures 3-5). Among men, mental stress tasks resulted in greater activation primarily in the left cingulate gyrus and bilateral medial frontal gyrus than control conditions. Additional regions with greater activation during psychosocial stress than control among men include the left middle and superior frontal gyrus, bilateral inferior parietal lobe, left middle temporal gyrus, and right superior temporal gyrus (Table 7, Figures 6-7). Furthermore, in men mental stress conditions relative to control conditions resulted in greater deactivation in the bilateral precentral and postcentral gyri, right insula, right superior and middle temporal gyrus, right medial occipital gyrus, left inferior parietal lobe, and regions of the cerebellum (Table 8, Figures 8-9).

Men and women were then compared to identify sex differences in regions with greater stress-related activation or deactivation. Compared to men, in women mental stress resulted in greater activation in the left superior temporal gyrus and cerebellum than men (Table 9). Additionally, women relative to men exhibited greater deactivation with mental stress compared to control conditions in the bilateral cingulate gyrus, right medial frontal gyrus, and right middle temporal gyrus (Table 9, Figures 10-11).

Sex differences in brain reactivity to stress among those with and without mental stress ischemia was also analyzed. Among participants with mental stress ischemia, the cingulate gyrus and cerebellum exhibited greater activation in women than men during mental stress compared to control (Table 10). In contrast, among participants without mental stress ischemia, only the cerebellum exhibited greater activation with stress in women than men (Table 10). Furthermore, women with mental stress ischemia had greater deactivation with stress, relative to men, in the right middle temporal gyrus, bilateral superior frontal gyrus, right inferior frontal gyrus, and the right cingulate gyrus (Table 10). In comparison, relative to men, women without mental stress ischemia had greater deactivation with stress compared to control conditions, in the bilateral cingulate gyrus and right middle frontal and temporal gyri (Table 10).

Discussion

The aim of this study was to explore sex differences in brain areas involved in mental stress among patients with CAD. While men and women had concurrent activation and deactivation in response to mental stress in many brain regions, our findings suggest that there are differences in the neural correlates of psychosocial stress among men and women with CAD especially involving the medial prefrontal cortex, and particularly the cingulate gyrus. Additionally, cardiovascular reactivity to mental stress was greater among men than women in two of the three markers we measured. Men had greater changes in systolic blood pressure and rate pressure product, and not heart rate, than women during mental stress compared to control. Interestingly, sex differences in systolic blood pressure were no longer statistically significant after controlling for differences in demographic and clinical risk factors associated with CAD, especially race and depression, underscoring potential differences in the pathophysiology of depression between men and women.

Men and women exhibited similar increases in activation and deactivation to psychosocial stress in brain regions that are implicated in mental arithmetic, phonological language processing, visual processing, working memory, spatial awareness, working memory and emotion regulation, all functions that potentially contribute to the execution of stressful mental arithmetic and public speaking tasks (84-91). Furthermore, in both men and women, mental stress was associated with greater deactivation of the insula than control conditions. The insula is a highly-interconnected region of the brain and a subset of the limbic system. The insula also receives sensory information regarding the internal physiological status of the body from multiple brain regions and houses efferent projections to the cardiovascular and respiratory system (92). Previous studies have shown that electrical stimulation of the insula is associated with cardiac irregularities, including changes in blood pressure and heart rate. Interestingly, in our study, women had deactivation in both the right and left insula while men only had deactivation

in the left insula (93). However, when we compared deactivation in the insula in women and men, there was no statistically significant sex difference in insular cortex deactivation.

While both men and women had greater activation with mental stress relative to control in subsets of the medial prefrontal cortex (mPFC), only women had greater deactivation with mental stress in the mPFC, specifically in the left and right cingulate gyrus and the right medial frontal gyrus. Greater deactivation in the cingulate cortex in women compared to men was preserved among those with and without MSI. However, women with MSI exhibited hyperactivation in the right and left cingulate cortex compared to men with MSI, a difference that was not observed among those without MSI.

Our results are consistent with a previous cross-sectional study that investigated the neural correlates of mental stress in 3 CAD patients with MSI and 7 CAD patients without MSI; compared to CAD patients without MSI, CAD patients with MSI had greater deactivation to mental stress in multiple brain regions including the bilateral anterior cingulate gyrus (area 24). (50). Furthermore, our results show that a general pattern of deactivation with stress in these regulatory brain areas is more marked among women than men irrespective of MSI status. Thus, alterations in functional activity of the anterior cingulate gyrus may be linked to dysregulation of the autonomic nervous system, particularly among women.

Women also had greater deactivation to mental stress than men in the right middle temporal gyrus and medial frontal gyrus (BA 21, BA 8 and 9, respectively), areas implicated in language and literacy, vision and eye movement, and social cognition (94-96). Among those with MSI, women also had greater deactivation to mental stress testing in BA 8 as well as areas related to the learning and processing of emotional information (right inferior frontal gyrus, BA 47, and the right superior frontal gyrus, BA 11) than men (97-99).

The effects of psychosocial stress on the limbic system are well documented. Previous studies have shown that chronic stress alters functional reactivity of the cingulate cortex and other subsets of

the limbic system to stress. In a meta-analysis of 40 studies published between 1998 and 2010 investigating brain regions with altered reactivity to mental stress, subjects with major depressive disorder (MDD)had greater hypoactivation in the anterior cingulate cortex and the insula than healthy controls (100). Furthermore, numerous cross-sectional studies investigating the neural correlates of mental stress in healthy individuals have consistently shown greater activation and deactivation of multiple corticolimbic structures, including the right and left cingulate cortex, to acute mental stress (38-43). In our study, women with CAD had significantly greater deactivation of the anterior cingulate cortex compared to men with CAD. Furthermore, among those with MSI, women with CAD had both hyperactivation and hypoactivation in the anterior cingulate cortex compared to men with CAD. Additionally, sex differences in hypoactivation of the anterior cingulate cortex was unique to those with MSI in our cohort. Our results suggest that women with CAD, particularly those with abnormal responses to psychosocial stress resulting in MSI, may have greater dysregulation of the anterior cingulate gyrus than men with CAD.

The cingulate cortex has been previously implicated in autonomic regulation of the heart. In a case series study of 3 patients with anterior cingulate lesions, patients had statistically significant dysregulation of systolic blood pressure and heart rate, when compared with a normative population, to different tests that challenged the autonomic nervous system, including mental arithmetic, orthostatic standing, isometric exercise, cutaneous cold, and hyperventilation tests (101). Additionally, altered stress responsivity in the anterior cingulate cortex was also found in subjects who were more susceptible to mental stress (42, 50). For examples, in a cross-sectional study of 40 young and healthy adults that were exposed to the Montreal Imaging Stress Test (MIST) to induce mental stress, subjects that were identified as cortisol responders (those with increasing cortisol release over the duration of the study) had greater deactivation than non-responders in the left anterior cingulate gyrus, the right orbitofrontal/inferior frontal gyrus and the left dorsolateral prefrontal cortex (DLPFC) than cortisol non-

responders (42). Furthermore, experimental studies in the literature also support direct and indirect regulation of the cardiovascular system by the anterior cingulate cortex (102). Finally, the cingulate gyrus is a component of the limbic system and a densely connected region of the brain. The anterior cingulate cortex shares projections with cortical and subcortical structures in the limbic system, parietal and frontal cortices, and sensory, motor, and reward related regions which include structures implicated in cardiovascular control, such as the insular cortex and amygdala (103).

Our study provides a foundation for the identification of neural biomarkers of mental stress in men and women with CAD. Due to the exploratory nature of the study, we were limited to group-level analyses and were unable to define individual level differences within participants. However, this is the first study that we are aware of that compares the neural correlates of mental stress in men and women with CAD and by mental stress ischemia status. Another limitation is that each activated and deactivated clusters were identified by a single coordinate in the brain which does not inform the reader of all the regions in each cluster with changes in brain activity. Additionally, transformation of the coordinates from the 3-D MNI space to the two dimensional Talairach space may introduce non-differential misclassification for the location of the highlighted region due to differences in the shape of the brain templates that were used to construct both atlases. However, final scan results were reviewed by a psychiatrist. Furthermore, we used PET imaging, a gold standard neuroimaging technique, to capture changes in brain activation as well as 2 different standardized and validated stress tasks to elicit mental stress.

In conclusion, our results suggest that neural correlates of mental stress differ between men and women with CAD, particularly in corticolimbic structures implicated in emotion and autonomic regulation. These results may have implications for further understanding of the complex brain pathways involved in regulation of the cardiovascular system and the effect of mental health on cardiovascular health. Future studies should focus on measuring changes in activation of components of

the prefrontal cortex, particularly the cingulate gyrus, in relation to mental health conditions such as depression and regulation of the cardiovascular system and identifying subgroups that may be more susceptible to the effects of mental health on cardiovascular disease.

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Tables and Figures

Table 1. Demographic and Clinical Characteristics of Study Participants by Sex (N = 165)

	Females (n = 53)	**Males (n = 112)**	***P***
Demography			
Age, y ± SD	61.2±7.7	62.3±8.7	0.42
African American, %	29(55)	32(29)	**0.001**
Income (below $20,000)	15(28)	19(17)	0.1
Education (high school)	12(23)	33(29)	0.36
Clinical Characteristics and Lifestyle Factors			
BMI, ±SD	31.2±7.4	29.5±5.2	0.09
Current major depressive episode, SCID, %	17(32)	24(22)	0.16
Lifetime major depression, SCID, %	31(58)	37(33)	**0.002**
Abnormal angiogram	37(76)	66(60)	0.06
Abnormal nuclear test	7(14)	14(13)	0.79
Abnormal exercise test, %	9(18)	13(12)	0.27
Diabetes, %	19(36)	38(34)	0.81
Dyslipidemia, %	40(75)	96(86)	0.11
Heart Failure, %	14(26)	12(11)	**0.01**
Hypertension, %	40(75)	85(76)	0.95
MI History, %	16(30)	47(42)	0.15
MSI, %	9(17)	35(31)	**0.05**
Smoking History			
Current Smoker, %	7(14)	16(15)	0.31
Former Smoker, %	21(43)	60(54)	
Never Smoker, %	21(38)	34(31)	
Medication Use			
ACE Inhibitors, %	21(40)	52(47)	0.38
Antidepressants, %	26(50)	29(26)	**0.003**
Aspirin, %	45(85)	96(86)	0.78
Beta Blockers, %	45(85)	77(69)	**0.03**
Diuretics, %	24(45)	26(23)	**0.004**
Statins, %	41(84)	99(91)	0.19
Vasodilators, %	5(9)	12(11)	0.79

Abbreviations: SPECT: single photon emission computed tomography, MSI: mental stress ischemia, BMI: body mass index, SCID: Structured Clinical Interview for DSM IV, MI: myocardial infarction.

Table 2. Demographic and Clinical Characteristics of Study Participants by Mental Stress Ischemia (MSI) Status (N = 165).

	Females (n= 53)[b]		Males (n = 112)[b]		*P for interaction*
	Females, MSI - (n =44)	**Females, MSI +** (n =9)	**Males, MSI -** (n =77)	**Males, MSI +** (n =35)	
Demography					
Age, y ± SD	61.5±7.6	59.4±8.6	61.7±8.9	63.5±8.4	0.28
African American, %	22(50)	7(78)	24(31)	8(23)	0.08
Income (below $20,000)	11(25)	4(44)	15(20)	4(11)	0.12
Education (high school)	10(23)	2(22)	26(34)	7(20)	0.51
Clinical Characteristics and Lifestyle Factors					
BMI ±SD	31.0±7.2	32.1±8.6	29.6±5.3	29.4±4.9	0.62
Current major depressive episode SCID, %	14(32)	3(33)	17(23)	7(20)	0.81
Lifetime major depression, SCID, %	27(61)	4(44)	27(36)	10(29)	0.67
Diabetes, %	14(32)	5(56)	27(35)	11(31)	0.18
Dyslipidemia, %	33(75)	7(78)	65(84)	31(89)	0.85
Heart Failure, %	12(27)	2(22)	9(12)	3(9)	0.95
Hypertension, %	32(73)	8(89)	60(78)	25(71)	0.18
MI History [a], %	14(32)	2(22)	34(44)	13(37)	0.83
Smoking History					
Current Smoker, %	6(14)	1(14)	13(17)	3(9)	0.35
Former Smoker, %	17(40)	4(57)	41(53)	19(58)	
Never Smoker, %	19(45)	2(29)	23(30)	11(35)	
Medication Use					
ACE Inhibitors, %	17(39)	4(44)	35(46)	17(49)	0.87
Antidepressants, %	23(52)	3(38)	23(30)	6(17)	0.88
Aspirin, %	40(91)	5(56)	66(87)	30(86)	0.54
Beta Blockers, %	36(82)	9(100)	54(71)	23(66)	0.06
Diuretics, %	19(43)	5(56)	17(22)	9(26)	0.72
Statins, %	36(86)	5(71)	70(92)	29(88)	0.73

Abbreviations: SPECT: single photon emission computed tomography, MSI: mental stress ischemia, BMI: body mass index, SCID: Structured Clinical Interview for DSM IV, MI: myocardial infarction.
Categorical and continuous variables are reported as n (%) and mean± standard deviation, respectively
[a] Test for interaction of sex with MSI status. P value obtained using likelihood ratio tests and 2-way ANOVA for categorical and continuous variables, respectively

Table 3. Sex Differences in Hemodynamic Reactivity Parameters in Response to Mental Stress, Unadjusted (N = 165)

	Females (n=53)	**Males (n=112)**	***95% CI***
Systolic Blood Pressure (mmHg)[a]			
Control	143.7±22.9	143.9±19.2	*(-6.5, 7.0)*
Stress	149.2±22.3	154.3±20.2	*(-1.8, 11.9)*
Systolic Blood Pressure Reactivity	5.5±10.0	10.4±7.8	*(2.0, 7.7)*
Heart Rate (beats/min)[a]			
Control	67.5±9.6	65.1±11.2	*(-5.8, 1.2)*
Stress	70.0±10.2	67.8±12.0	*(-6.0, 1.5)*
Heart Rate reactivity	2.6±1.7	2.6±1.8	*(-1.2, 1.4)*
Rate Pressure Product (mmHg*beats/min)[a]			
Control	9700.8±2152.6	9377.4±2087.3	*(-1017.6, 370.6)*
Stress	10446.6±2180.3	10478.6±2476.4	*(-753.4, 817.5)*
Rate Pressure Product Difference	745.7±850.3	1101.3±1088.3	*(20.3, 690.8)*

[a] Continuous variables are reported as mean± standard deviation. Average values during control and stress conditions were calculated for men and women. Values for systolic blood pressure reactivity, heart rate reactivity, and rate pressure product difference were calculated by taking the difference between the average stress and control values for each parameter

Table 4. Sex differences in Hemodynamic Reactivity Parameters in Response to Mental Stress, Adjusted (N = 165)

	Male-to-Female Difference (n = 165)	*Lower 95% CI*	*Upper 95% CI*	R^2
Systolic Blood Pressure (mmHg)				
model 1[a]	4.8±1.5	1.87	7.75	0.11
model 2[b]	4.3±1.7	1.02	7.56	0.12
model 3[c]	3.2±1.7	-0.22	6.58	0.16
Heart Rate (beats/min)				
model 1	0.05±0.67	-1.28	1.37	0.07
model 2	0.18±0.71	-1.23	1.58	0.19
model 3	0.05±0.74	-1.42	1.53	0.22
Rate Pressure Product (mmHg*beats/min)				
model 1	331.7±173.8	-11.57	674.89	0.06
model 2	325.7±188.4	-46.61	698.08	0.19
model 3	217.2±194.8	-167.94	602.25	0.23

[a] Adjusts for age at enrollment, high school education, income status, and African American race.

[b] In addition to the variables in model 1, model 2 also adjusted for BMI, diabetes, dyslipidemia, mental stress ischemia status, beta-blocker use, history of smoking, and history of myocardial infarction

[c] In addition to the variables in model 2, model 3 also adjusted for diagnosis of current depression based on the SCID

Table 5. Stress Activation in Female Participants with CAD (N = 53)

Voxel number*	Brain Regions	Brodmann's Area	TALAIRACH X	TALAIRACH Y	TALAIRACH Z	Z score**
4877	Right Middle Frontal Gyrus	9	48	27	26	7.00
	Left Cingulate Gyrus	32	-4	16	42	6.97
	Right Cingulate Gyrus	32	8	23	41	6.88
1189	Left Inferior Frontal Gyrus	47	-36	19	-6	6.64
	Left Inferior Frontal Gyrus	44	-46	11	33	5.68
	Left Inferior Frontal Gyrus	44	-42	2	31	5.54
42	Left Superior Frontal Gyrus	11	-24	46	-12	4.29
220	Right Inferior Parietal Lobe	40	50	-41	43	6.50
	Right Inferior Parietal Lobe	40	42	-52	43	5.76
	Right Inferior Parietal Lobe and Supramarginal Gyrus	40	61	-49	26	4.35
42	Left Inferior Parietal Lobe	40	-40	-48	41	4.68
	Left Inferior Parietal Lobe and Supramarginal Gyrus	40	-50	-47	36	3.59
	Left Inferior Parietal Lobe	40	-42	-37	44	2.73
126	Left Cerebellum		-24	-69	-25	3.97
	Left Cerebellum		-32	-63	-25	3.71

Changes in cerebral blood flow with mentally stressful public speaking and arithmetic tasks and control counting and recall tasks were measured with [15O]H2O positron emission tomography in female participants. Brain regions with increased cerebral blood flow during mental stress tasks compared to control are shown.

*Number of voxels in the highlighted cluster. Only clusters with a minimum of 11 contiguous voxels were considered an activation.

**one tailed Z-score > 2.75, $p<0.005$

Table 6. Stress Deactivation in Female Participants with CAD (N = 53)

Voxel number*	Brain Regions	Brodmann's Area	TALAIRACH X	TALAIRACH Y	TALAIRACH Z	Z score**
230	Left Gyrus Precentralis Pars Opercularis**	6	-55	0	6	6.54
	Left Inferior Frontal Gyrus	44	-51	-11	17	4.99
	Left Precentral Gyrus	6	-53	-7	10	4.52
	Left Insula‡		-43	4	-5	
155	Right Precentral Gyrus	6	51	-9	13	5.63
	Right Superior Temporal Gyrus	22	48	2	4	4.94
	Right Gyrus Precentralis Pars Opercularis**	6	57	0	7	4.06
	Right Insula					
11	Right Precentral Gyrus	6, 43	65	-6	26	3.98
48	Bilateral Medial Frontal Gyrus	11	0	46	-12	3.60
	Right Medial Frontal Gyrus	10	8	48	-9	3.55
69	Left Medial Frontal Gyrus	6, 8	-6	-23	44	3.46
	Left Cingulate Gyrus, paracentral lobule	24	-2	-11	45	3.21
11	Right Cingulate Gyrus	24	8	0	41	3.18
16	Right Cingulate Gyrus	24	2	7	31	3.57
28	Right Medial Occipital Gyrus and Middle Temporal Gyrus	19	48	-73	11	6.28
	Right Medial Occipital Gyrus and Middle Temporal Gyrus	19	40	-79	15	4.06
	Right Medial Occipital Gyrus and Middle Temporal Gyrus	37, 39	53	-68	7	3.63
39	Right Superior Temporal Gyrus	22	46	-12	-6	4.61
	Right Superior Temporal Gyrus	22	50	-17	3	3.29
17	Left Cerebellum		-18	-61	-19	3.42
51	Right Cerebellum		22	-65	-12	4.59

Changes in cerebral blood flow with mentally stressful public speaking and arithmetic tasks and control counting and recall tasks were measured with [15O]H2O positron emission tomography in female participants. Brain regions with increased cerebral blood flow during control tasks compared to mentally stressful tasks are shown.

*Number of voxels in the highlighted cluster. Only clusters with a minimum of 11 contiguous voxels were considered an activation.

**one tailed Z-score > 2.75, $p<0.005$

‡ Identified by psychiatrist viewing scan results and no associated Z score available.

Table 7. Stress Activation in Male Participants with CAD (N = 112)

Voxel number*	Brain Regions	Brodmann's Area	TALAIRACH X	TALAIRACH Y	TALAIRACH Z	Z score**
8316	Left Cingulate Gyrus	32	-6	29	26	65535.00
	Left Cingulate Gyrus, Medial Frontal Gyrus	6, 32	-4	18	43	65535.00
	Right Medial Frontal Gyrus	6, 8	14	20	47	65535.00
83	Left Superior Frontal Gyrus	11	-24	46	-12	5.39
	Left Middle Frontal Gyrus	11	-34	38	-14	4.55
365	Right Inferior Parietal Lobe	40	46	-46	43	7.10
	Right Inferior Parietal Lobe and Supramarginal Gyrus	40	63	-47	26	5.69
70	Left Middle Temporal Gyrus	21	-57	-35	-3	5.39
	Left Middle Temporal Gyrus	21	-61	-30	-9	3.80
87	Left Middle Temporal Gyrus	21	57	-20	-4	4.58
24	Right Superior Temporal Gyrus	22	67	-37	7	3.60
66	Left Inferior Parietal Lobe	40	-46	-41	39	5.88
	Left Inferior Parietal Lobe	40	-40	-48	41	3.47

Changes in cerebral blood flow with mentally stressful public speaking and arithmetic tasks and control counting and recall tasks were measured with [15O]H2O positron emission tomography in male participants. Brain regions with increased cerebral blood flow during mentally stressful tasks compared to control tasks are shown.

*Number of voxels in the highlighted cluster. Only clusters with a minimum of 11 contiguous voxels were considered an activation.

**one tailed Z-score > 2.75, $p<0.005$

Table 8. Stress Deactivation in Male Participants with CAD (N = 112)

Voxel number*	Brain Regions	Brodmann's Area	TALAIRACH X	TALAIRACH Y	TALAIRACH Z	Z score**
475	Left Gyrus Precentralis Pars Opercularis**	6	-53	-5	9	65535.00
	Left Precentral Gyrus	6, 4, 3	-50	-7	22	6.35
	Left Postcentral Gyrus	40	-51	-20	18	6.18
47	Right Postcentral Gyrus	6, 4, 3, 1,2	63	-18	27	4.10
	Right Precentral Gyrus	6	65	-6	26	4.03
162	Right Precentral Gyrus	6	51	-5	11	6.62
	Right Insula and Superior Temporal Gyrus	22	40	-4	-1	6.27
	Right Superior Temporal Gyrus	22	50	2	5	4.76
24	Right Insula and Middle Temporal Gyrus	21	44	-10	-5	4.69
17	Right Medial Occipital Gyrus and Middle Temporal Gyrus	19	40	-79	15	4.62
	Right Medial Occipital Gyrus and Middle Temporal Gyrus	19, 39	50	-73	9	3.58
12	Left Inferior Parietal Lobe	40	-57	-36	28	3.28
143	Right Cerebellum		22	-65	-12	65535.00
	Right Cerebellum		16	-63	-19	6.99
172	Left Cerebellum		-20	-67	-17	5.73
	Left Cerebellum		-12	-61	-24	4.81
	Left Cerebellum		-12	-65	-17	4.53

Changes in cerebral blood flow with mentally stressful public speaking and arithmetic tasks and control counting and recall tasks were measured with [15O]H2O positron emission tomography in male participants. Brain regions with increased cerebral blood flow during mentally stressful tasks compared to control tasks are shown.

*Number of voxels in the highlighted cluster. Only clusters with a minimum of 11 contiguous voxels were considered an activation.

**one tailed Z-score > 2.75, $p<0.005$

Table 9. Brain Regions with Greater Stress-Induced Activation or Deactivation in Female (N = 53) Relative to Male (N = 112) Participants with CAD

			TALAIRACH			
Voxel number*	Brain Regions	Brodmann's Area	X	Y	Z	Z score**
	Greater Stress Activation in Women than Men					
15	Left Superior Temporal Gyrus	42	-53	-27	9	3.18
41	Left Cerebellum		-18	-69	-17	3.52
	Left Cerebellum		-22	-67	-25	2.96
11	Left Cerebellum		-32	-61	-24	3.17
33	Right Cerebellum		24	-67	-19	3.41
	Greater Stress Deactivation in Women than Men					
55	Left Cingulate Gyrus	32	-8	27	28	4.31
17	Left Cingulate Gyrus	24, 32	-6	30	19	2.89
44	Right Cingulate Gyrus	32	16	25	39	3.41
	Right Medial Frontal Gyrus	8	14	22	47	3.05
25	Right Cingulate Gyrus	24, 32	16	36	22	3.02
	Right Medial Frontal Gyrus	9	20	42	26	2.94
71	Right Middle Temporal Gyrus	21	57	-14	-1	4.25

Changes in cerebral blood flow with mentally stressful public speaking and arithmetic tasks and control counting and recall tasks were measured with [15O]H2O positron emission tomography. Brain regions with larger increases and decreases in cerebral blood flow in women than men with mental stress compared to control are shown.

*Number of voxels in the highlighted cluster. Only clusters with a minimum of 11 contiguous voxels were considered an activation.

**one tailed Z-score > 2.75, $p<0.005$

Table 10. Brain Regions with Greater Stress-Induced Activation or Deactivation in Female Relative to Male Participants with CAD, by Mental Stress Ischemia (MSI) Status

			TALAIRACH			
Voxel number*	**Brain Regions**	**Brodmann's Area**	**X**	**Y**	**Z**	**Z score****
	Stress Activation in Women (N = 9)>Men (N = 35), among MSI +					
	Right Anterior Cingulate Gyrus[ǂ]	24, 32	4	42	-5	
17	Right Cingulate Gyrus	24,32	4	14	40	3.38
11	Left Cingulate Gyrus	24	-2	-6	39	3.34
17	Bilateral Cerebellum		0	41	-4	3.30
23	Left Cerebellum		-18	-69	-17	3.69
	Stress Activation in Women (N = 44)>Men (N = 77), among MSI -					
14	Right Cerebellum		24	-67	-19	3.36
11	Left Cerebellum		-22	-67	-25	3.06
	Stress Deactivation in Women (N = 9)>Men (N = 35), among MSI +					
37	Right Middle Temporal Gyrus	21	53	-16	-3	3.74
17	Left Superior Frontal Gyrus	8	-4	51	44	3.54
15	Right Superior Frontal Gyrus	11	24	54	-14	3.29
12	Right Inferior Frontal Gyrus	47	44	27	-6	3.05
33	Right Cingulate Gyrus	24, 32	14	26	23	3.33
	Stress Deactivation in Women (N = 44) > Men (N = 77), among MSI -					
80	Left Cingulate Gyrus	24, 32	-8	27	28	4.10
	Left Cingulate Gyrus	24, 32	-8	34	20	2.98
12	Right Middle Frontal Gyrus	8	22	29	39	2.80
	Right Cingulate Gyrus	32	16	23	39	2.79
24	Right Middle Temporal Gyrus	21	57	-14	-1	3.71

Changes in cerebral blood flow with mentally stressful public speaking and arithmetic tasks and control counting and recall tasks were measured with [15O]H2O positron emission tomography. Brain regions with larger increases and decreases in cerebral blood flow in women than men, stratified by MSI status, with mental stress compared to control were identified.

*Number of voxels in the highlighted cluster. Only clusters with a minimum of 11 contiguous voxels were considered an activation.

**one tailed Z-score > 2.75, p<0.005

ǂ Identified by psychiatrist viewing scan results and no associated Z score available.

Figure 1. Stress Activation in Female Participants with CAD (N = 53) Across Multiple Brain Regions

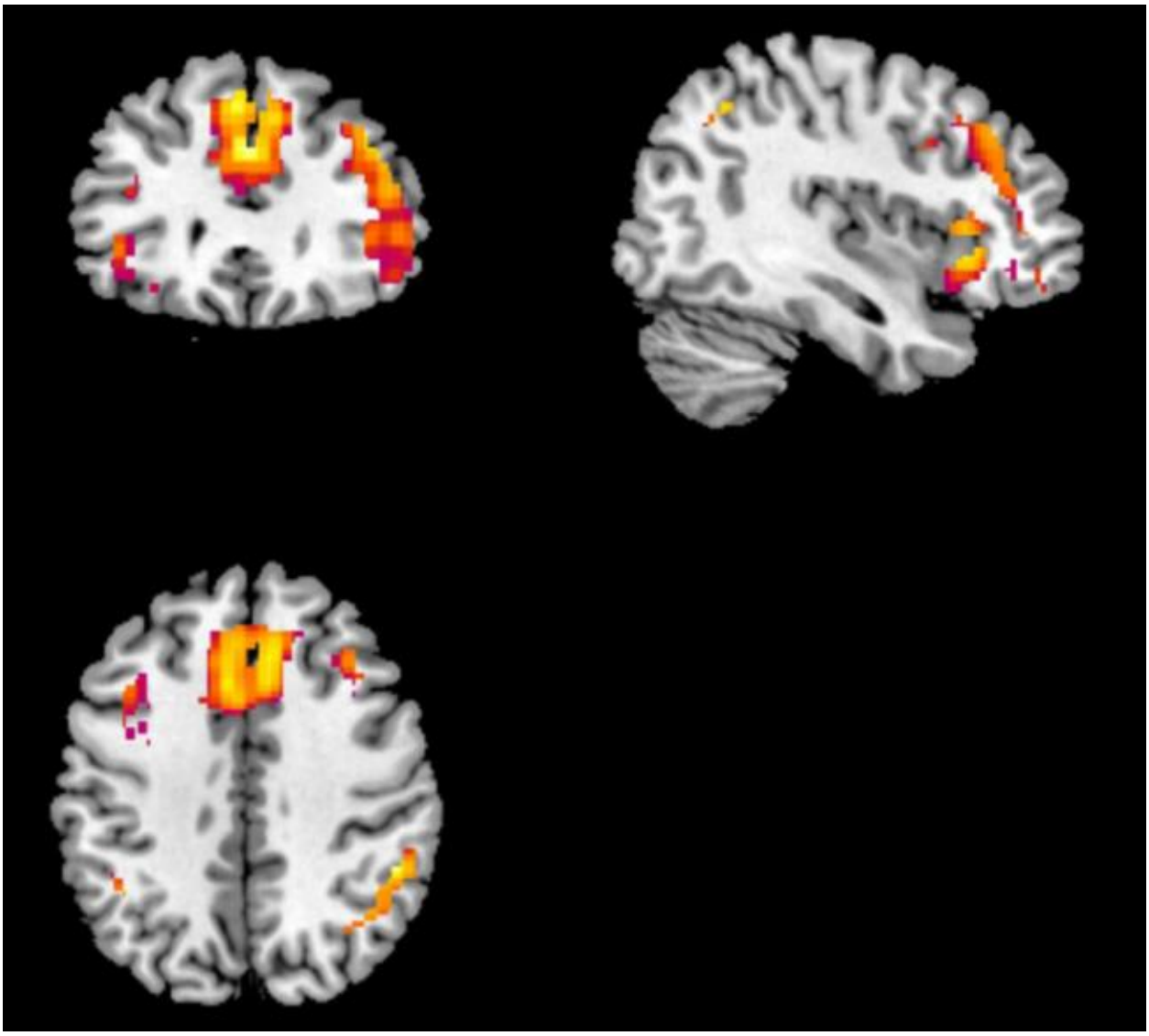

Figure 2. Stress Activation in Female Participants with CAD (N = 53) in the Inferior Frontal Gyrus (IFG)

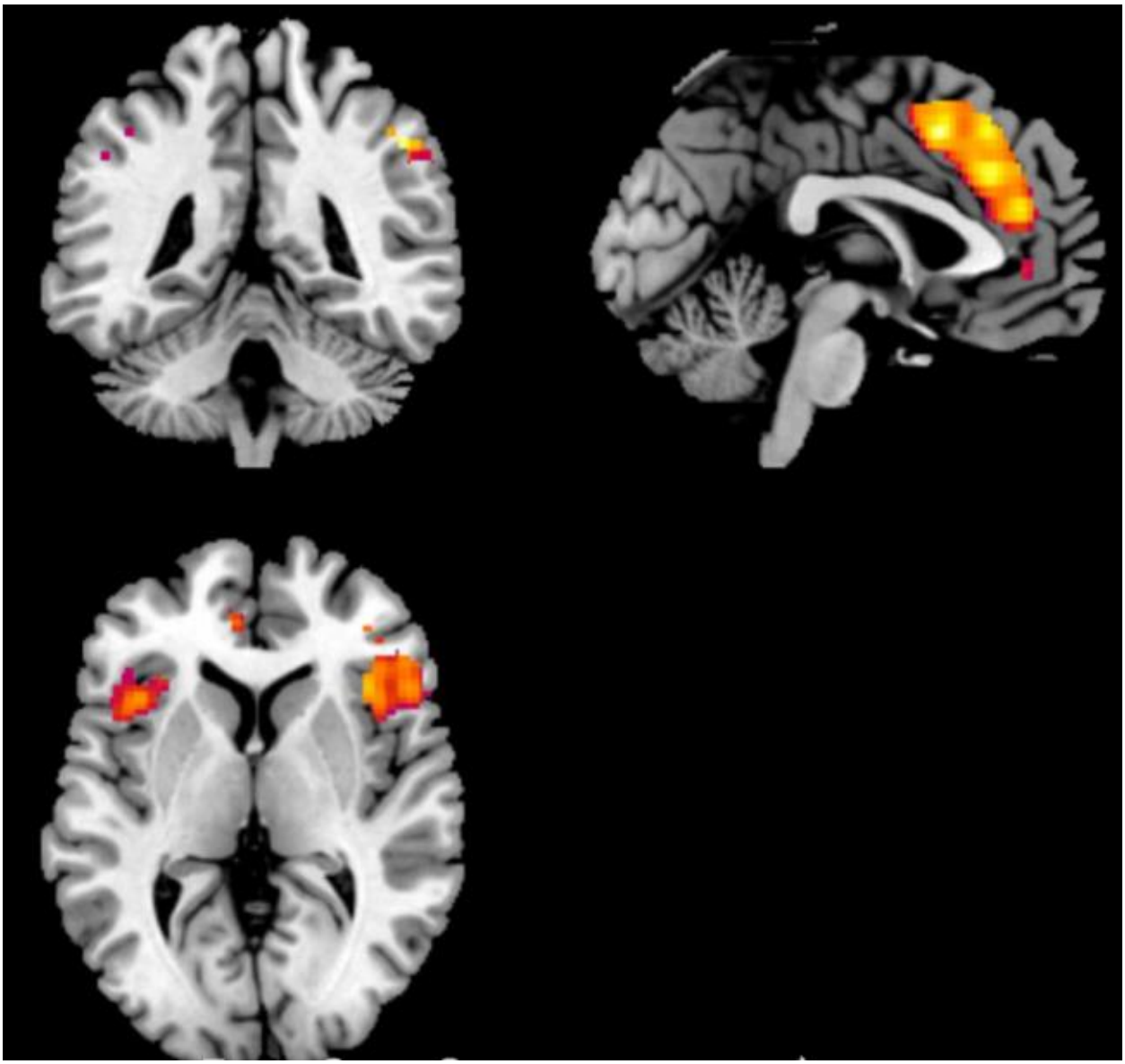

Figure 3. Stress Deactivation in Female Participants with CAD (N = 53) in the Anterior Cingulate

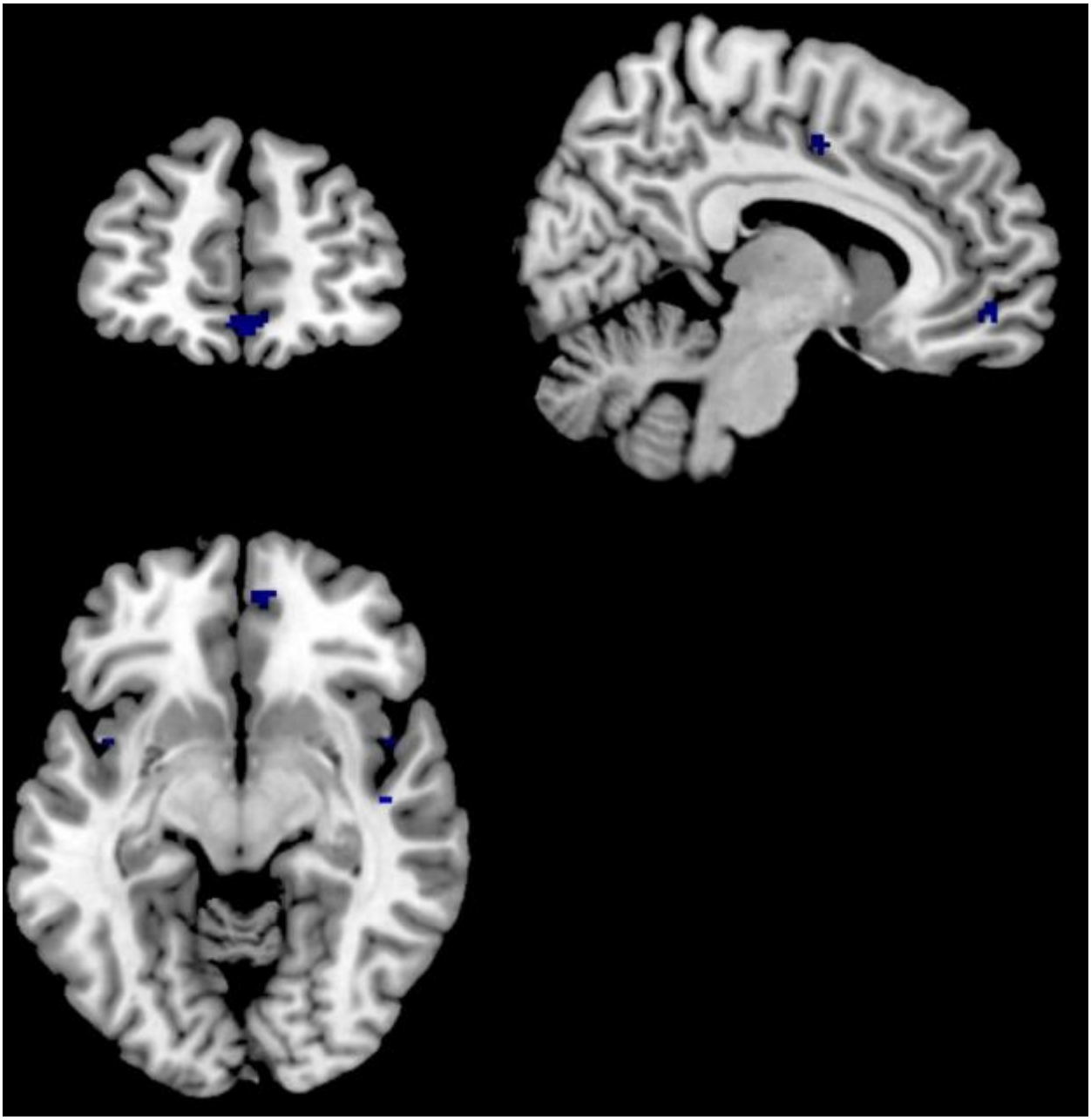

Figure 4. Stress Deactivation in Female Participants with CAD (N = 53) in the Insula

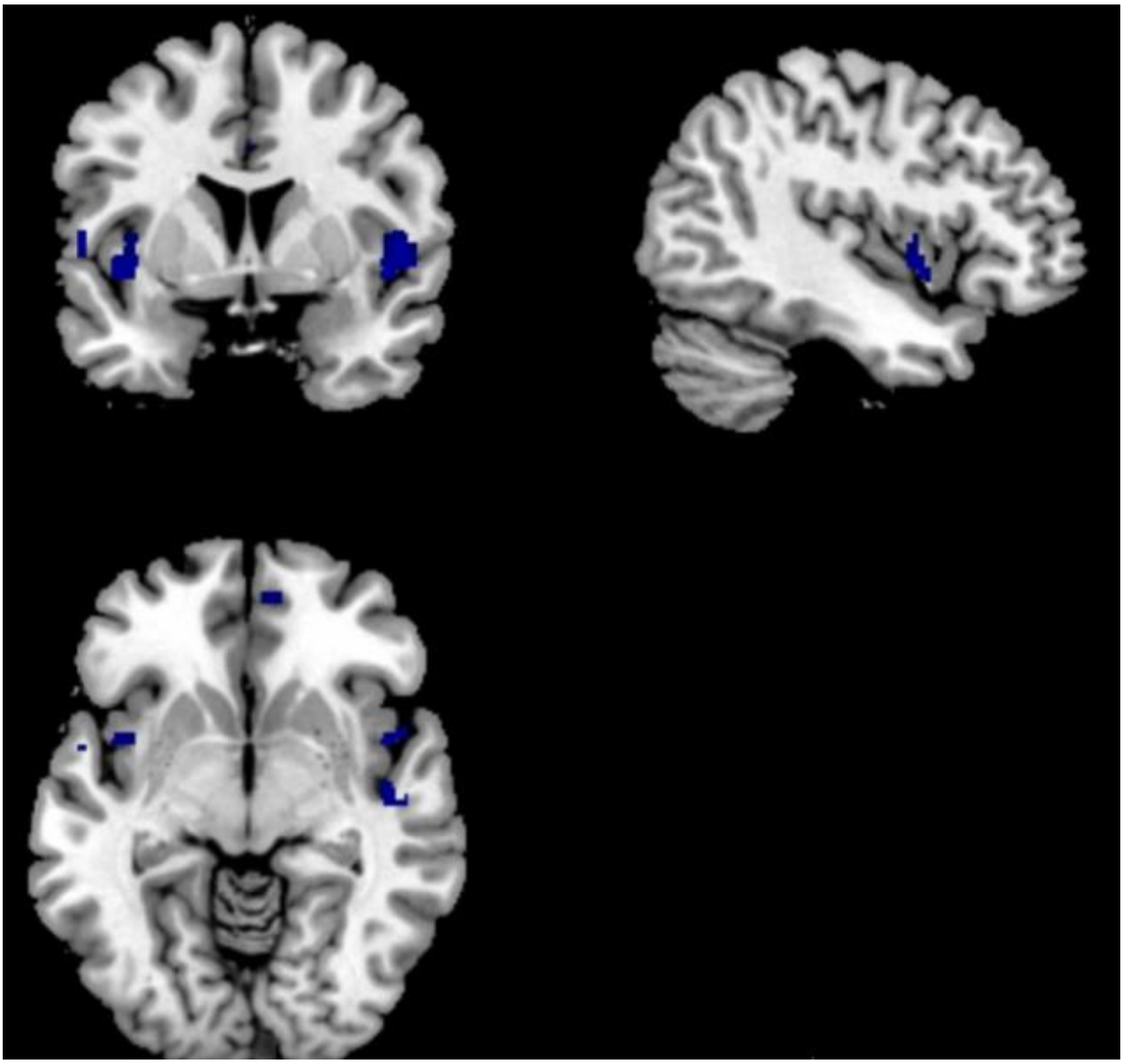

Figure 5. Stress Deactivation in Female Participants with CAD (N = 53) in the Middle Temporal Gyrus

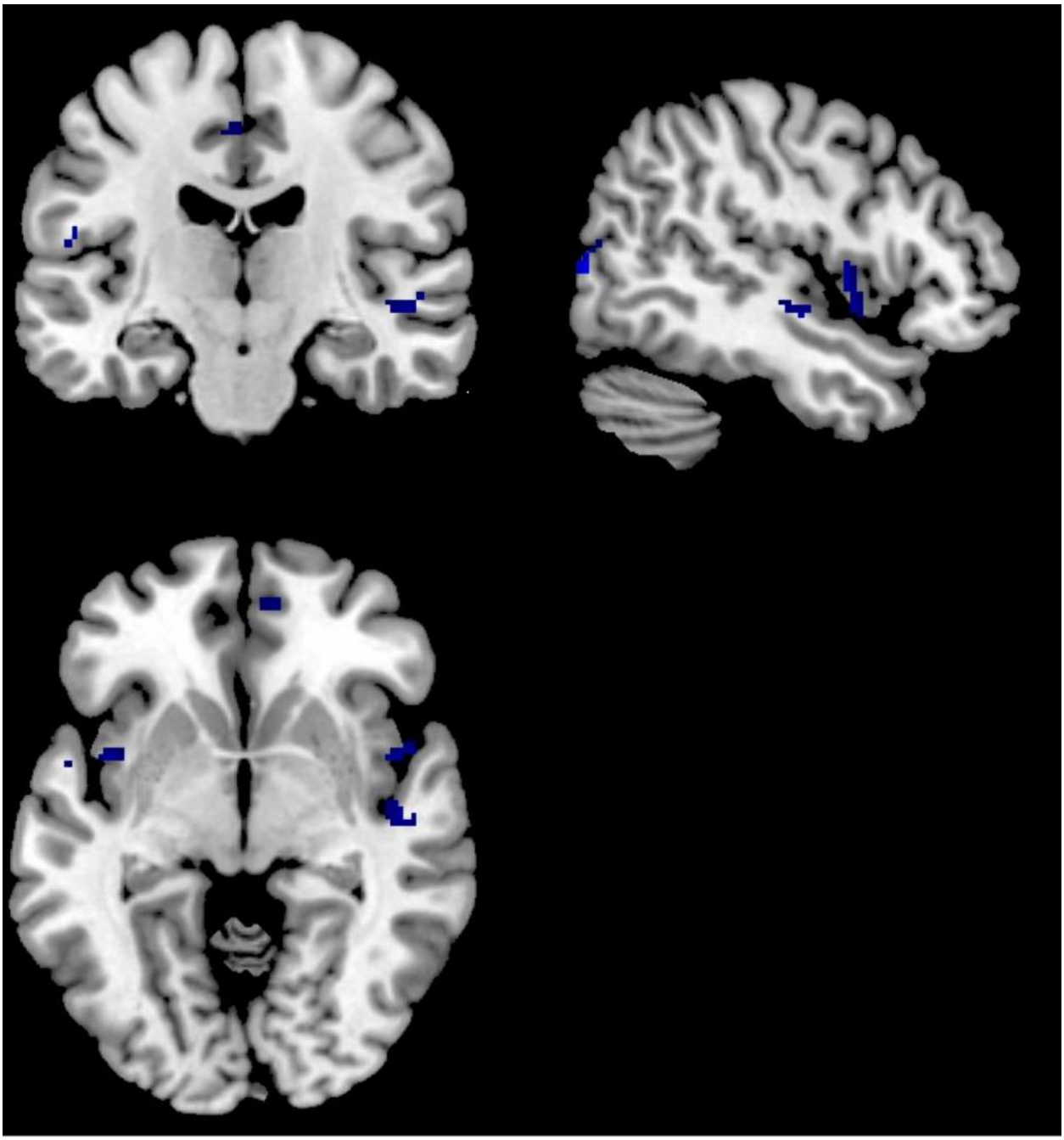

Figure 6. Stress Activation in Male Participants with CAD (N = 112) in the Anterior Cingulate Gyrus

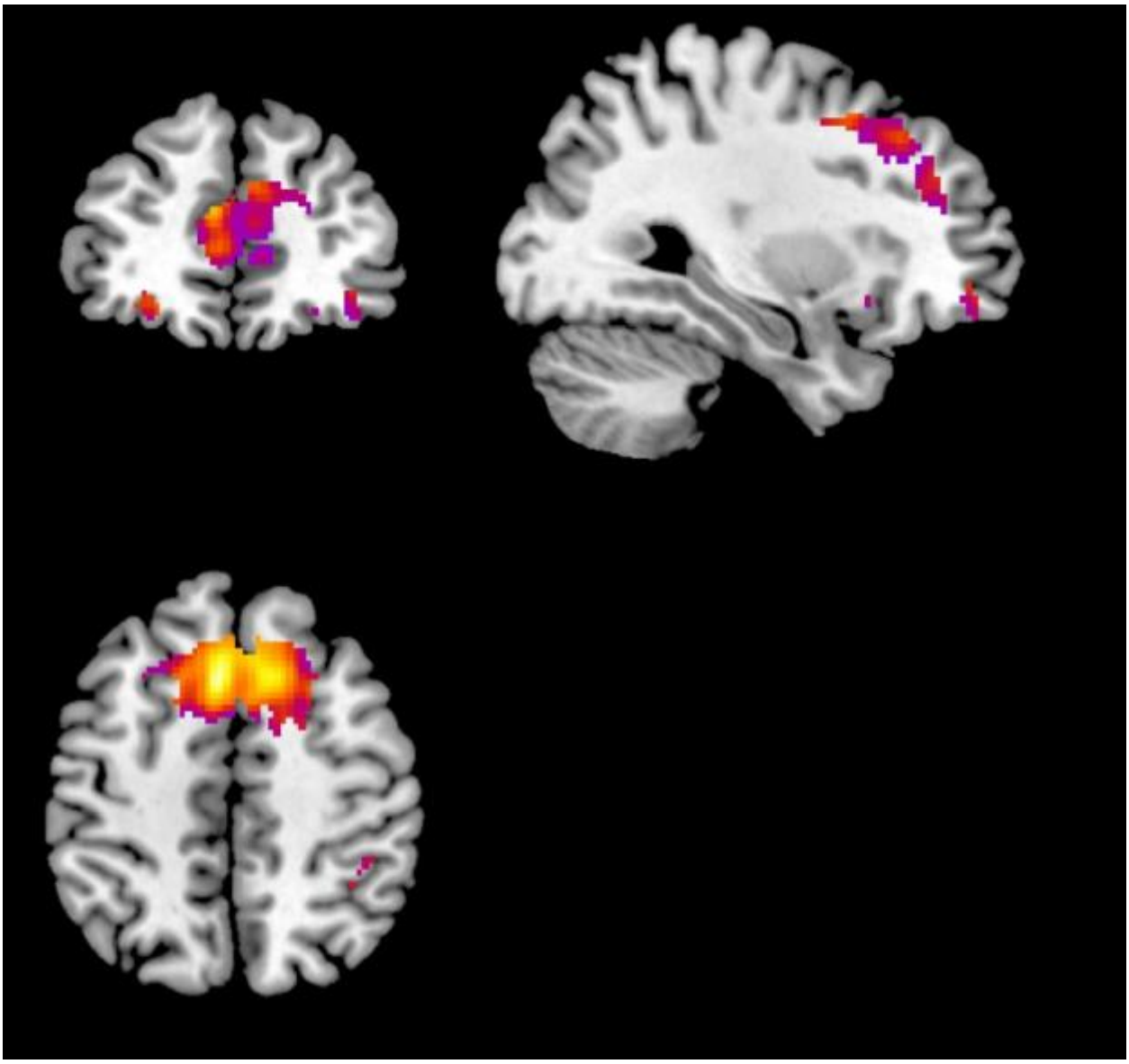

Figure 7. Stress Activation in Male Participants with CAD (N = 112) in the Insula

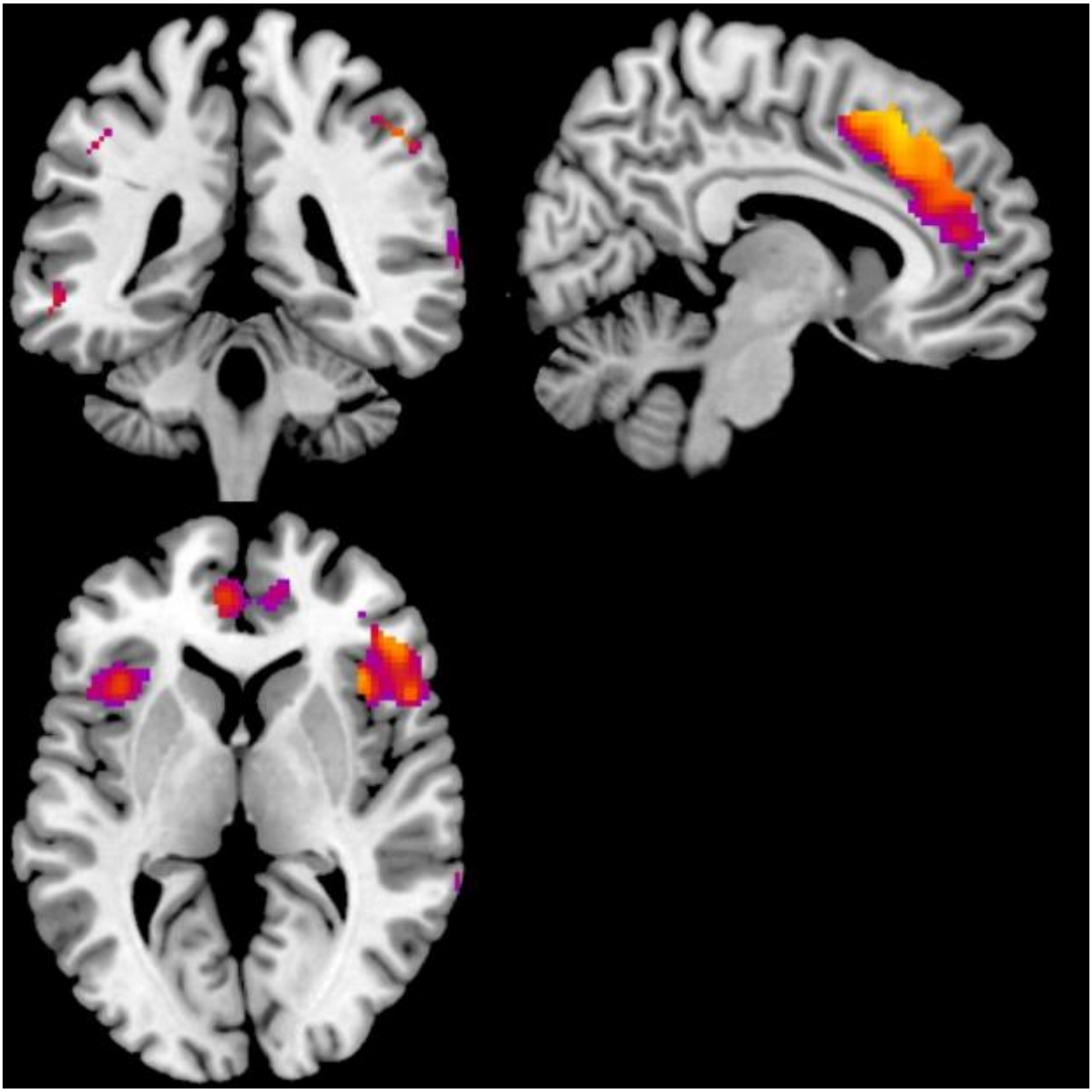

Figure 8. Stress Deactivation in Male Participants with CAD (N = 112) in the Middle Temporal Gyrus

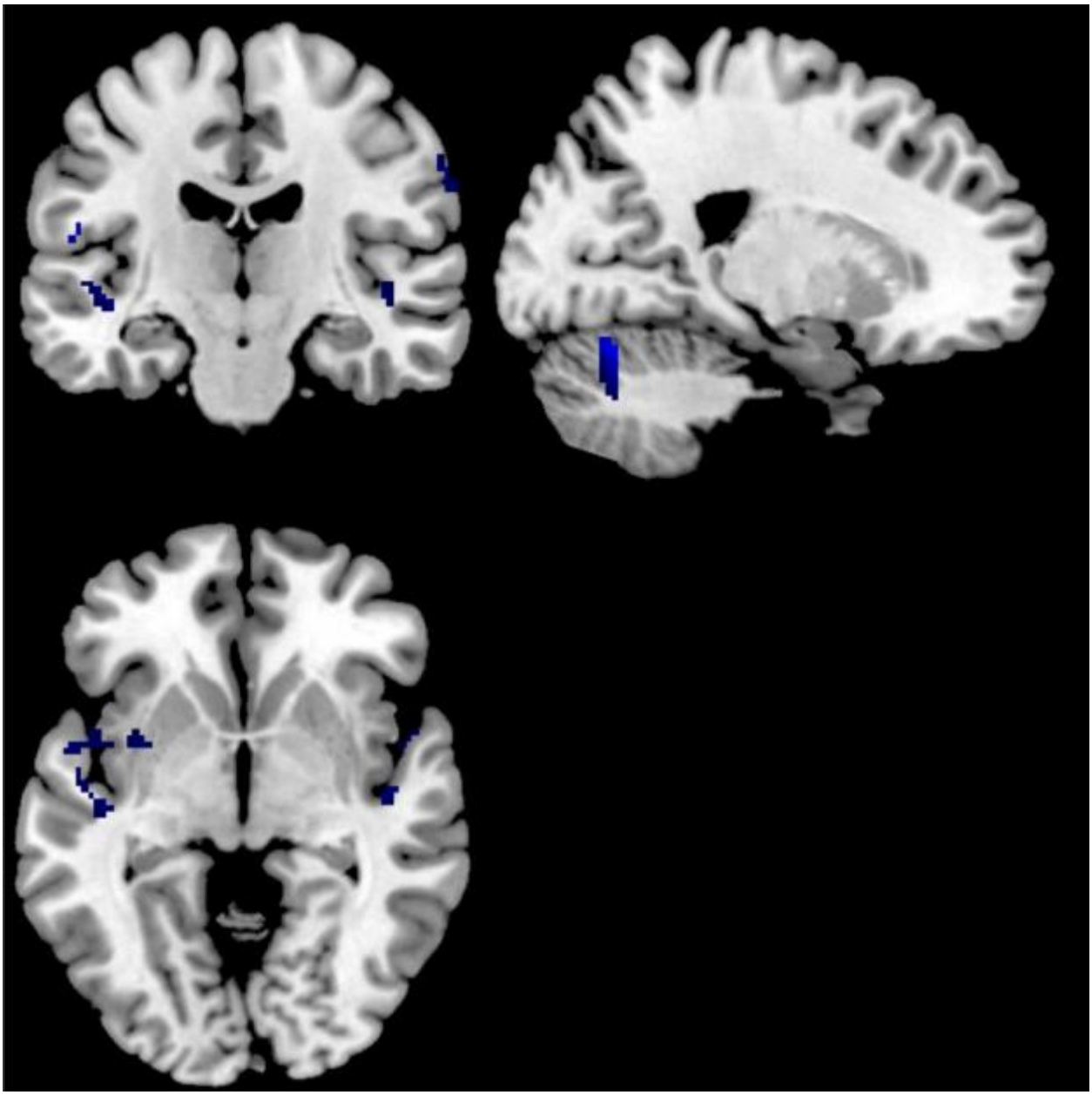

Figure 9. No Stress Deactivation in Male Participants with CAD (N = 112) in the Anterior Cingulate Gyrus

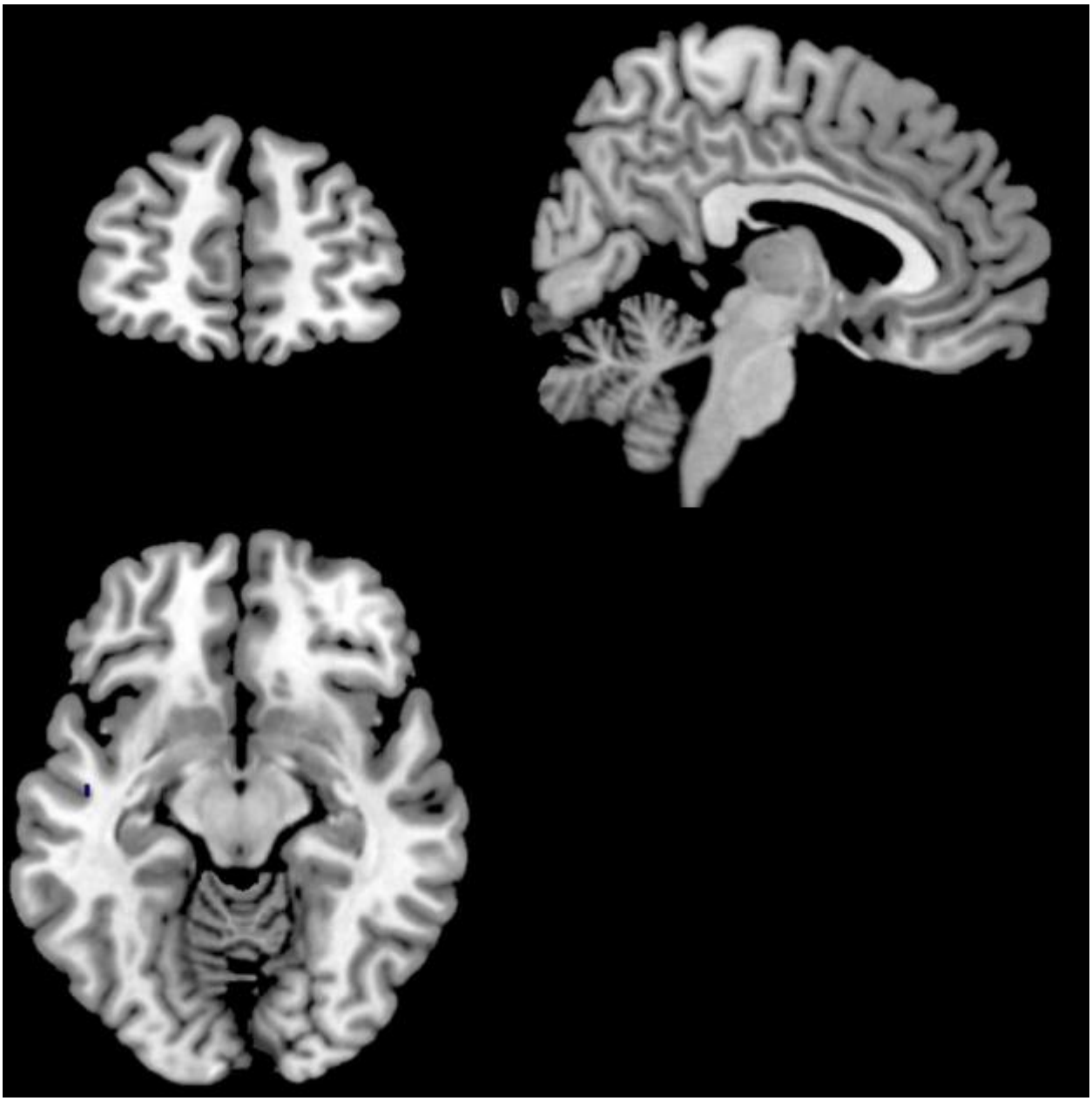

Figure 10. Greater Stress-Induced Deactivation in Female (N = 53) Relative to Male (N = 112) Participants with CAD in the Anterior Cingulate Gyrus

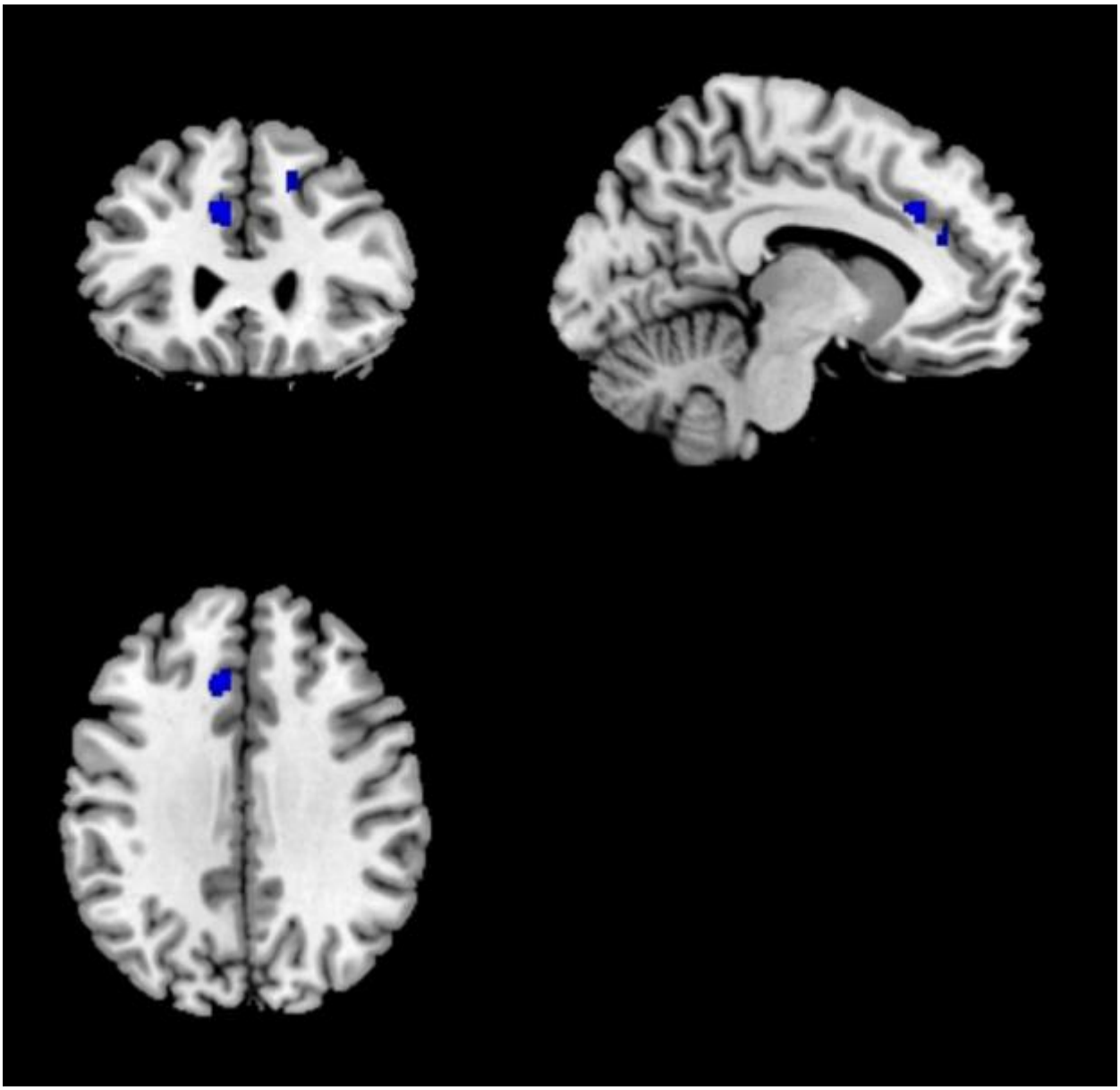

Figure 11. Greater Stress-Induced Deactivation in Female (N = 53) Relative to Male (N = 112) Participants with CAD in the Middle Temporal Gyrus