



Inflammatory response to mental stress and mental stress induced myocardial ischemia

Muhammad Hammadah, *Emory University*
Samaah Sullivan, *Emory University*
[Bradley Pearce](#), *Emory University*
Ibhar Al Mheid, *Emory University*
Kobina Wilmot, *Emory University*
Ronnie Ramadan, *Emory University*
Ayman Samman Tahhan, *Emory University*
Wesley T. O'Neal, *Emory University*
Malik Obideen, *Emory University*
Ayman Alkhoder, *Emory University*

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

Journal Title: Brain, Behavior, and Immunity

Volume: Volume 68

Publisher: Elsevier: 12 months | 2018-02-01, Pages 90-97

Type of Work: Article | Post-print: After Peer Review

Publisher DOI: 10.1016/j.bbi.2017.10.004

Permanent URL: <https://pid.emory.edu/ark:/25593/tng44>

Final published version: <http://dx.doi.org/10.1016/j.bbi.2017.10.004>

Copyright information:

© 2017

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 22, 2019 4:46 AM EST



Published in final edited form as:

Brain Behav Immun. 2018 February ; 68: 90–97. doi:10.1016/j.bbi.2017.10.004.

Inflammatory response to mental stress and mental stress induced myocardial ischemia

Muhammad Hammadah^{a,1}, Samaah Sullivan^{b,1}, Brad Pearce^b, Ibhar Al Mheid^a, Kobina Wilmot^a, Ronnie Ramadan^a, Ayman Samman Tahhan^a, Wesley T. O'Neal^a, Malik Obideen^a, Ayman Alkhoder^a, Naser Abdelhadi^a, Heval Mohamed Kelli^a, Mohamad Mazen Ghafeer^a, Pratik Pimple^b, Pratik Sandesara^a, Amit J. Shah^{a,b,c}, Kareem Mohammed Hosny^a, Laura Ward^b, Yi-An Ko^b, Yan V. Sun^b, Lei Weng^b, Michael Kutner^e, J. Douglas Bremner^{c,f}, David S. Sheps^g, Fabio Esteves^d, Paolo Raggi^{b,d,h}, Viola Vaccarino^{a,b,*2}, and Arshed A. Quyyumi^{a,*2}

^aDepartment of Medicine, Division of Cardiology, Emory University School of Medicine, Atlanta, GA, United States

^bDepartment of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, United States

^cAtlanta VA Medical Center, Decatur, GA, United States

^dDepartment of Radiology, Emory University School of Medicine, Atlanta, GA, United States

^eDepartment of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA, United States

^fDepartment of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, United States

^gUniversity of Florida Health Science Center, Department of Medicine, Division of Cardiovascular Medicine, Gainesville, FL, United States

^hMazankowski Alberta Heart Institute, University of Alberta, Edmonton, Alberta, Canada

Abstract

Background—Mental stress-induced myocardial ischemia (MSIMI) is associated with increased risk of adverse cardiovascular outcomes, yet the underlying mechanisms are not well understood. We measured the inflammatory response to acute laboratory mental stress in patients with coronary artery disease (CAD) and its association with MSIMI. We hypothesized that patients with MSIMI would have a higher inflammatory response to mental stress in comparison to those without ischemia.

*Corresponding authors at: Emory University, Department of Cardiology, Emory University School of medicine, 1462 Clifton Road N.E. Suite 507, Atlanta GA 30322. lvaccar@emory.edu (V. Vaccarino), aquyyum@emory.edu (A.A. Quyyumi).

¹These authors contributed equally to this work and should be both considered first authors.

²These authors contributed equally to this project and thus both should be considered as corresponding authors.

Conflicts of interest

None of the other authors report conflict of interest relevant to this article.

Methods—Patients with stable CAD underwent 99mTc sestamibi myocardial perfusion imaging during mental stress testing using a public speaking stressor. MSIMI was determined as impaired myocardial perfusion using a 17-segment model. Inflammatory markers including interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), matrix metalloproteinase 9 (MMP-9) and high-sensitivity C reactive protein (hsCRP) were measured at rest and 90 min after mental stress. Results were validated in an independent sample of 228 post-myocardial infarction patients.

Results—Of 607 patients analyzed in this study, (mean age 63 ± 9 years, 76% male), 99 (16.3%) developed MSIMI. Mental stress resulted in a significant increase in IL-6, MCP-1, and MMP-9 (all $p < 0.0001$), but not hsCRP. However, the changes in these markers were similar in those with and without MSIMI. Neither resting levels of these biomarkers, nor their changes with mental stress were significantly associated with MSIMI. Results in the replication sample were similar.

Conclusion—Mental stress is associated with acute increases in several inflammatory markers. However, neither the baseline inflammatory status nor the magnitude of the inflammatory response to mental stress over 90 min were significantly associated with MSIMI.

Keywords

Inflammation; Mental stress; Myocardial ischemia; C reactive protein; Interleukin 6; Matrix metalloproteinase 9; Monocyte chemoattractant protein-1

1. Introduction

Mental stress can provoke myocardial ischemia, and trigger acute coronary syndromes or sudden cardiac death (Vaccarino, 2016; Burg and Soufer, 2014). In the laboratory; acute exposure to mental stress using a standardized stress challenge can induce myocardial ischemia in patients with coronary artery disease (CAD) (Vaccarino, 2016; Burg and Soufer, 2014; Wei et al., 2014). Mental stress-induced myocardial ischemia (MSIMI) is usually silent, occurs at a lower workload than exercise-induced ischemia, and is known to be associated with adverse cardiovascular outcomes, like cardiovascular death and myocardial infarction (MI) (Vaccarino, 2016; Wei et al., 2014; Ersbøll et al., 2014; Pepine et al., 2014; Ramadan et al., 2013).

It has been suggested that recurring increases in inflammatory markers in response to exogenous challenges, including psychologic distress, may contribute to a heightened inflammatory state and hence to progression of CAD and clinical CAD events (Endrighi et al., 2016; Hackett et al., 2012; Goldman-Mellor et al., 2010; Miller et al., 2002; Ackerman et al., 1998; Maes et al., 1998; Goebel et al., 2000; Lu et al., 2013; Kop et al., 2002; Marsland et al., 2017). Of several circulating markers of acute inflammation, interleukin-6 (IL-6), matrix metalloproteinase-9 (MMP9), monocyte chemoattractant protein-1 (MCP-1), and high sensitivity C reactive protein (hsCRP) have shown a significant association with atherosclerosis and/or adverse cardiac outcomes (Lu et al., 2013; Ridker, 1998; Ridker et al., 2000; Eapen et al., 2013; Tunon et al., 2014; Yabluchanskiy et al., 2013; Fisman et al., 2006). IL-6 is a pleiotropic cytokine with pro- and anti-inflammatory properties. IL-6 *classic signaling* (via activation of the membrane bound IL-6 receptor) mediates the activation of anti-inflammatory pathways on target cells, and is involved in regenerative processes,

metabolism regulation, bone homeostasis, and in many neural functions (Pedersen and Febbraio, 2008; Scheller et al., 2011). In contrast, IL-6 *trans-signaling* (via activation of soluble IL-6 receptors) leads to activation of the immune system through recruitment of monocytes to the inflamed area. IL-6 is found in large quantities in atherosclerotic plaques (Fisman et al., 2006). MCP-1 recruits monocytes into atherosclerotic plaques (Tunon et al., 2014). MMP-9 is a proteolytic enzyme that leads to degradation of the extracellular matrix and remodeling in cardiomyocytes and atherosclerotic plaques; (Phatharajaree et al., 2007) while hsCRP is at least partly synthesized in the liver in response to increases in IL-6 and reflects vascular inflammation (Eapen et al., 2013). Ischemia of the myocardium during unstable angina or during dobutamine stress testing is associated with significant increases in inflammatory markers including IL-6 (Biasucci et al., 1996; Ikonomidis et al., 2005).

Although inflammation has been implicated in MSIMI, this mechanism has not been thoroughly examined to date. Only one study in 83 patients with CAD has shown an association between higher post-mental stress levels of hsCRP and a higher risk of MSIMI (Shah et al., 2006). In a large sample of patients with stable CAD; we sought to examine whether MSIMI is associated with higher levels of resting inflammation, and/or a higher inflammatory response to mental stress. We also validated our results in an independent cohort of post-MI patients younger than 61 years old who underwent an identical protocol. The answer to this question may help clarify whether stress-induced inflammation plays a role in MSIMI, and may provide clues for future studies examining mechanisms linking MSIMI to adverse cardiovascular outcomes. Our hypothesis was that mental stress will be associated with a significant inflammatory response and that patients with MSIMI will have a higher inflammatory response in comparison to those without ischemia.

2. Methods

2.1. Study sample

The study design has been published elsewhere. Briefly, (Hammadah et al., 2017) patients were enrolled into the Mental Stress Ischemia Prognosis Study (MIPS), a prospective study that recruited patients with stable CAD between June 2011 and August 2014 at Emory University affiliated hospitals. The presence of CAD was defined by an abnormal coronary angiogram demonstrating evidence of atherosclerosis with at least luminal irregularities, documented previous percutaneous or surgical coronary revascularization, documented MI, or a positive nuclear stress test. Patients were excluded if they had an acute coronary syndrome or decompensated heart failure in the prior week, severe psychiatric conditions other than major depression, pregnancy (women of childbearing age were screened by pregnancy test), uncontrolled high blood pressure ($\geq 180/110$ mmHg), or with contraindications for regadenoson administration. All patients in MIPS underwent both mental stress test, and conventional stress test (using treadmill exercise or regadenoson). Baseline studies were performed during two visits within a week. At the initial visit (visit 1), patients were consented and underwent a medical history and psychosocial/psychiatric assessments, and blood draw. This was followed by a stress SPECT study after either a mental stress test or a conventional (exercise or chemical) stress test. During visit 2, they had the other stress test performed. The sequence of the two stressors was randomly assigned.

The median time between the two stressors was 4.0 days (IQR: 3.0, 7.0). The research protocol was approved by the Institutional Review Board and all participants provided informed consent. Patients were tested in the morning after a 12-h fast. Anti-anginal medications (beta-blockers, calcium-channel blockers, and long-acting nitrates), xanthine derivatives and caffeine-containing products were withheld for 24 h prior to stress testing. Depression was assessed using the Beck depression inventory score (Beck et al., 1996; Frasure-Smith et al., 1995; Carney et al., 1987). Only data about mental stress testing will be presented in this manuscript.

2.2. Mental stress procedure

In a quiet, dimly lit, temperature controlled (21–23 °C) room, after a 30-min rest period, vital signs were measured and mental stress was induced by a standardized public speaking task (Ramadan et al., 2013; Hassan et al., 2009; Sheps et al., 2002). Briefly; patients were asked to imagine a situation in which a close relative had been mistreated in a nursing home. Patients were given two minutes to prepare and three minutes to deliver a speech in front of an evaluative audience. Blood pressure and heart rate were recorded throughout. Mental stress testing was performed by trained and experienced staff to standardize the psychophysiological stress-provoking elements of the test. At 60 s into the mental stress task, a regular dose of ^{99m}Tc-sestamibi (30–40 mCi based on weight) was injected intravenously.

2.3. Myocardial perfusion imaging and SPECT images interpretation

Myocardial perfusion imaging with ^{99m}Tc-sestamibi SPECT was performed at rest and 30–60 min after mental stress according to standard protocols (Ramadan et al., 2013). For resting myocardial blood flow assessment, patients were injected with a low dose ^{99m}Tc-sestamibi (10–14 mCi based on weight). Studies were interpreted by two experienced readers, who are co-investigators in the study (PR and FE), and who were blinded to any other subject data, including severity of CAD, medical history and outcomes. Discrepancies in the interpretation of SPECT images were resolved by consensus. Rest and stress images were visually compared for number and severity of perfusion defects using a 17-segment model. Each segment was scored from 0 to 4, with 0 being normal uptake, and 4 no uptake. Ischemia was defined as a new impairment with a score of ≥ 2 in any segment, or as worsening of a pre-existing impairment by at least 2 points if in a single segment, or by at least 1 point if in two or more contiguous segments (Holly et al., 2010).

2.4. Hemodynamic monitoring

Hemodynamic parameters, including the systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were recorded every 5 min during the resting period, every 1 min during the mental stress, and every 5 min during the recovery period. Hemodynamic responses to mental stress were calculated as the difference between the maximum value of each hemodynamic parameter during the speech minus the minimum resting value during the rest period.

2.5. Inflammatory biomarkers measurement

Previous studies have shown a delayed release of IL-6 between 60–120 min after acute mental stress (Steptoe et al., 1979; Song et al., 2015; Koelsch et al., 2016). According to our knowledge, this is the first study to assess MCP-1 and MMP-9 levels after acute mental stress. Giving these, in a pilot sample of 48 randomly selected patients, we have assessed inflammatory biomarkers, at rest, 45-min and 90-min after mental stress. Based on the results of our pilot analysis (see results section), we decided to assess inflammatory biomarkers only at rest ($n = 607$), and 90-min ($n = 554$) after mental stress for the rest of the cohort. Venous blood was collected into ice-cooled citrate tubes and immediately centrifuged at 4 °C; obtained plasma was snap-frozen at -70 °C until further processing. We employed high sensitivity assays using the MesoScale system (Meso Scale Diagnostics Rockville, Maryland) using the SECTOR Imager 2400 to quantitate IL-6, MCP-1, MMP-9 and hsCRP according to the protocols supplied by the manufacturer. The Mesoscale multiplex assay system uses electrochemiluminescence for high sensitivity and broad dynamic range. Lower limits of detection for our experiment were: IL-6: 0.06 pg/mL, MCP-1: 0.09 pg/mL, MMP-9: 0.011 ng/mL, and hsCRP: 1.33×10^{-6} mg/L. The inter assay coefficient of variations for midpoint standards were 5.78% for IL-6, 4.99% for MCP-1, 9.38% for MMP-9, and 3.06% for hsCRP. The intra-assay coefficients of variations were 3.29% for IL-6, 3.45% for MCP-1, 5.95% for MMP-9, 2.33% for hsCRP.

2.6. Validation cohort

We have validated our result in a separate group of patients who were enrolled into the Myocardial Infarction and Mental Stress 2 (MIMS2) study. Both studies shared testing protocols and data collection methods, study staff, investigators, facilities and equipment, but there were some differences in the inclusion criteria. The MIMS2 patients all had a documented history of MI within the previous 8 months and were younger than 61 years of age at the time of screening. The diagnosis of MI was verified by medical record review based on standard criteria of troponin level increase and ECG changes (Thygesen et al., 2007). The exclusion criteria were consistent with MIPS. Similar to MIPS, subjects underwent mental stress testing, SPECT imaging and inflammatory biomarkers assessment using the same methods described above (Hammadah et al., 2017).

2.7. Statistical and power analyses

To examine differences between groups (MSIMI positive vs. negative), we used Student's *t*-test for continuous variables and the chi-square test for categorical variables. We summarize normally distributed data as mean \pm SD and non-normally distributed data as median (25th and 75th percentiles). Given their skewed distribution, we used log base 2 transformations for inflammatory biomarkers. Spearman rank correlation was used to examine the factors associated with resting inflammation. To examine changes in inflammatory concentrations before and after mental stress testing, we used linear mixed models for repeated measures. To examine inflammatory response to mental stress (differences between 90-min post mental stress values and resting values) between groups (MSIMI positive vs negative), we used mixed linear models with random effects for plate in multivariable analyses. These models adjusted for factors that were associated with resting inflammation, such as, age, sex,

African American race, smoking, body mass index, beck depression inventory score, and diabetes, or known clinically to be associated with higher inflammation (previous MI) (Rooks et al., 2016). Mixed linear models for repeated measures were also used to investigate hemodynamic changes during mental stress. We also adjusted for the sequence of the stressor (whether mental/physical stress test was conducted first) in a final set of models.

There is only one previous study that assessed the relationship between inflammation (hsCRP) and MSIMI. According to this study, patients with MSIMI had 1.45 mg/dL higher hsCRP in comparison to those without (Shah et al., 2006). Assuming 15% of the patients would develop MSIMI, the power analysis indicated that a total of 600 patients ($n = 100$ MSIMI positives) will achieve 92% power to detect a difference of 1.45 mg/L in CRP (Shah et al., 2006) (Shah et al., 2006) between two groups. To the best of our knowledge, there are no other published studies of an association between other biomarkers and the provocation of MSIMI in the laboratory among patients with stable CAD.

3. Results

Of 695 CAD patients enrolled in MIPS, 15 patients had missing or poor quality rest/mental stress SPECT scans. Additional patients had missing plasma samples, because of technical difficulties in sample drawing or processing, or the patient refused. A total of 607 patients had inflammatory biomarkers measured and completed mental stress testing, and were thus included in this analysis. There were no statistical differences between those with and without missing samples, except for more females and Black participants in those with missing data.

A total of 99 (16.3%) patients developed MSIMI. Differences across baseline demographics by MSIMI status including, CAD risk factors, history of MI and medication use are summarized in Table 1. As expected, mental stress increased systolic blood pressure [26 ± 16 mmHg; $19 \pm 12\%$ increase, $\beta = 25.61$; 95% CI: 24.36, 26.85, $p = <0.0001$], diastolic blood pressure [13 ± 9 mmHg; $17 \pm 11\%$ increase, $\beta = 12.91$; 95% CI: 12.23, 13.59, $p = <0.0001$], and heart rate [11 ± 9 beat/min; $18\% \pm 15\%$ increase, $\beta = 11.10$; 95% CI: 10.35, 11.85, $p = <0.0001$], and plasma hemoglobin [0.3 ± 0.7 g/d L; $2.3 \pm 5.3\%$, $\beta = 0.29$; 95% CI: 0.23, 0.36) $p = <0.0001$].

Female sex, race, higher body mass index, smoking, depression, hypertension, and diabetes were all associated with higher baseline biomarker levels (Online Table 1).

3.1. Biomarker changes in response to mental stress

To determine optimal timing for biomarker assessment, we randomly selected 48 patients and assessed their biomarker levels at rest, 45-min and 90-min after mental stress. Concordant with previously reported literature, (Steptoe et al., 1979; Song et al., 2015; Koelsch et al., 2016) acute mental stress resulted in a delayed release of IL-6 and MCP-1 that can be better detected at 90 min (Online Fig. 1). We did not observe any significant changes in any biomarker at 45 min.

For the whole study population, and given the results of our pilot analyses, we assessed biomarker levels only at rest and 90 min after mental stress. In the whole cohort and in unadjusted models (Table 2), mental stress resulted in a significant increase in levels of IL-6 [33.4%, $p < 0.0001$], MCP-1 [7.2%, $p < 0.0001$] MMP-9 [13.0%, $p < 0.0001$], but a significant decrease in hsCRP [-3.5%, $p = 0.03$]. Results were similar after adjustment for factors that correlate with resting biomarker levels, or known to be associated with higher inflammation (a history of MI). After accounting for batch effect in a random effects model, mental stress resulted in a significant increase in levels of IL-6 [34.0%, $p < 0.0001$], MCP-1 [7.3%, $p < 0.0001$], MMP-9 [13.3%, $p < 0.0001$], but no significant change in hsCRP [-2.4%, $p = 0.11$]. Adjustment for the sequence of stress test (mental vs conventional) did not change the results.

There were significant correlations between the mental stress-induced changes in IL-6, MMP-9 and MCP-1, but not with hsCRP (Table 3).

3.2. Relationship between biomarker levels at resting and after mental stress and MSIMI

The resting levels of biomarkers were not significantly different between patients with and without MSIMI (Table 4). Adjustment for the factors that correlate with resting inflammation (Online Table 1), including age, sex, Black race, body mass index, smoking, diabetes, Beck depression inventory score, and hypertension, or known to be associated with higher inflammation (previous MI) did not change the results. Furthermore, the changes in biomarker levels with mental stress were also not significantly different in patients with and those without MSIMI (Table 4).

3.3. Replication analysis

We validated these findings in 228 patients enrolled in the MIMS2 study. Their mean age (\pm SD) was age 50.5 ± 6.8 years and 49% were males (Online Table 2). Out of 228 patients who completed the mental stress test and biomarker measurements, 37 (16.2%) patients developed MSIMI. Similar to MIPS, mental stress resulted in significant increases in IL-6, MMP-9 and MCP-1 but not hsCRP (Online Table 3). After adjustment for the aforementioned covariables, and consistent with the results in MIPS, there were no significant differences in the resting or mental stress-induced changes in these biomarkers between those with MSIMI and those without (Table 5). Results remained similar when the two cohorts were pooled together (Online Table 4).

4. Discussion

In the largest study to date that assesses the multiple inflammatory responses to acute mental stress in patients with CAD, and the first to assess its relationship with MSIMI, we found that acute mental stress was associated with an early and significant increase in circulating IL-6, MCP-1 and MMP-9, but not hsCRP levels. However, and in contrary to our hypothesis, neither the resting levels nor mental stress-induced increases in these inflammatory biomarkers were associated with MSIMI. These results were consistent in a validation cohort. These findings argue against inflammation as a mechanism involved in MSIMI.

Consistent with our findings, several studies have shown a significant activation of the systemic inflammatory cascade with mental stress (Endrighi et al., 2016; Hackett et al., 2012; Marsland et al., 2017; Adams et al., 2004; Ellins et al., 2008; Endrighi et al., 2016). For example, Miller et al. (2005) showed a significant increase in IL-6, 30-min after an acute mental stress episode in depressed and healthy subjects (Miller et al., 2005). In a recently published *meta-analysis* that have included 2253 healthy subjects and 314 diseased patients, enrolled from 24 studies and 9 studies, respectively, mental stress was found to be associated with significant increases in circulating IL-6, IL-1, IL-10 and tumor necrosis factor-alpha and a stimulated IL-1 β , IL-4 and interferon-gamma. However, only one study included in the *meta-analysis* enrolled patients with CAD; Kop et al. observed a significant increase in IL-6 after an anger recall test in 34 patients with CAD without evidence of ischemia (Kop et al., 2008). In comparison, our study is larger, more comprehensive, used a well described population of stable CAD, validated the results in an independent population who survived a recent MI, and we have also assessed MSIMI.

Mental stress is associated with significant activation of the sympathetic nervous system and with parasympathetic nervous system withdrawal (Adams et al., 2004; Grebe et al., 1950; Cole et al., 2010; Slavich and Irwin, 2014). These changes up-regulate the transcription of pro-inflammatory immune response genes (e.g., those encoding IL-1, IL-6 and tumor necrosis factor), and increase degranulation of inflammatory factors from leukocytes, resulting in increased inflammation (Adams et al., 2004; Grebe et al., 1950; Cole et al., 2010; Slavich and Irwin, 2014).

Despite the body of evidence linking acute psychological stress with immune activation and inflammation, the role of inflammation in MSIMI remains understudied. According to our knowledge, only one study examined inflammation in relation to MSIMI. Shah et al. measured hsCRP 24 h after mental stress in 83 patients (36% with MSIMI), and showed higher levels in patients with MSIMI (Shah et al., 2006). However, they did not assess resting hsCRP levels, making it difficult to conclude if higher hsCRP in those with MSIMI is due to baseline differences, up-regulation due to mental stress, or delayed recovery. There continues to be controversy regarding the change in hsCRP with mental stress (Shah et al., 2006; Steptoe et al., 1979; Beattie et al., 2003; Liuzzo et al., 1996). While some studies have found levels of hsCRP to be stable over time and not responsive to either acute stress or ischemia, (Beattie et al., 2003; Liuzzo et al., 1996; Gaspardone et al., 2000) others have shown a statistically significant, but minimal change with acute mental stress (Adams et al., 2004; Miller et al., 2005). It has been shown before that the response time of hepatic CRP release is about 6–24 h, (Myers and Fleck, 1988) thus a slight increase in CRP in previous studies could be due hemoconcentration rather than due to inflammatory response (Kop et al., 2002). We measured hsCRP levels 90 min after mental stress and may have missed the later rise. However, we also found no relationship between the rise in IL-6, a known stimulator of hsCRP, MCP-1 or MMP-9 and MSIMI. Its unlikely that we have missed an earlier or later inflammatory response in these biomarkers given the results of our pilot sample, validation cohort, and previously reported findings of delayed release of IL-6 (Steptoe et al., 1979; Song et al., 2015; Koelsch et al., 2016). One reason for not observing an increase in inflammatory markers with MSIMI may be because, laboratory tested MSIMI is generally brief and mild in intensity, thus it may not result in a significant myocardial

injury that can cause a higher inflammatory response (Ramadan et al., 2013; White, 2011). This is supported by our findings of no increase in high sensitivity cardiac troponin, a marker of myocardial injury, after mental stress in those with MSIMI, but a significant increase in those with ischemia provoked during exercise stress test (Hammadah et al., 2017). Based on the results by Shah et al. (2006) our study had power to observe a meaningful difference in inflammatory markers. Moreover, our findings were confirmed in an independent validation cohort, but it is possible that very small effect sizes may have been missed.

In previous studies, the reported incidence of MSIMI varied between 18% and 67%, a variation that is largely due to different methodologies and patient populations studied, mostly several decades ago (Wei et al., 2014; Hassan et al., 2009; Sheps et al., 2002; Babyak et al., 2010). Although the incidence of MSIMI in both of our study cohorts was ~16%, our investigations are not comparable with most previous studies because of the differences in the methodology for ischemia assessment, mental stress protocols, definition of ischemia, and characteristics of the study population (Hammadah et al., 2017). In addition, most previous investigations studied MSIMI only in patients with a documented positive exercise stress test, a subgroup that we have shown before to be at higher risk of MSIMI (Hammadah et al., 2017). In the present study, we have chosen to enroll a broadly defined patient population with stable CAD in order to make our sample more representative of a contemporary CAD population.

Although we did not find an association between MSIMI and the inflammatory response to mental stress, our findings confirm that mental stress induces a pro-inflammatory state in CAD patients, which then can contribute to a number of other adverse outcomes, including increased arterial stiffness, (Lipman et al., 2002; Vlachopoulos et al., 2009; Vlachopoulos et al., 2006) endothelial dysfunction; (Ghiadoni et al., 2000; Xue, 2015) atherosclerosis; (Harrison et al., 2003; Das and O'Keefe, 2006) cardiac arrhythmia; (Qintar et al., 2015) acute coronary syndromes; (Vaccarino, 2016; Burg and Soufer, 2014) and cardiovascular events (Das and O'Keefe, 2006; Rosengren et al., 2004). Importantly, heightened pro-inflammatory activation due to stress may herald more rapid progression of vascular disease over time (Ellins et al., 2008). Specifically, Ellins and colleagues measured carotid arterial stiffness 3 years after evaluating mental stress-induced cytokine (fibrinogen, tumor necrosis factor-alpha and IL-6) release. The magnitude of increase in plasma fibrinogen, tumor necrosis factor alpha and IL-6 levels, but not the resting levels, correlated with worse arterial compliance at 3 years, indicating that post-stress measurements may provide a more accurate measure of future risk than resting measurements. While these reports suggest that mental stress-induced systemic inflammation is a predictor of greater cardiovascular risk, based on our results, inflammation is not a major mechanism for MSIMI. Furthermore, whether mental stress-induced inflammatory responses can identify patients with heightened risk of adverse cardiovascular outcomes, or whether targeting patients with heightened inflammatory response with behavioral or pharmacological interventions could reduce their inflammatory reactivity needs to be further investigated.

4.1. Strengths and limitations

Strengths of this study are its large size, the diversity of the population studied and the use of state-of-the-art myocardial perfusion imaging for the detection of ischemia. Limitations include the relatively modest incidence of MSIMI in our population and a lack of long-term follow-up data although the collection of these data is currently underway. Although ours is the most comprehensive study of inflammation and MSIMI in CAD patients, we used a limited battery of inflammatory biomarkers. Future studies should examine whether other stress-induced biomarkers, or oxidative stress markers, are associated with MSIMI.

5. Conclusion

Among patients with CAD, mental stress is associated with a significant increase in several inflammatory markers. However, neither the baseline inflammatory status nor the magnitude of the acute inflammatory response to mental stress were associated with MSIMI. Other mechanisms of MSIMI must be at a play.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of funding

Dr. Vaccarino and Dr. Quyyumi report research support from NIH. Ernest V. Garcia receives royalties from the sale of the Emory Cardiac Toolbox, used for some analyses in this study.

This work was supported by the NIH (P01 HL101398, P20HL113451-01, P01HL086773-06A1, R56HL126558-01, R01 HL109413, R01HL109413-02S1, R01 HL125246, UL1TR000454, KL2TR000455, K24HL077506, K24 MH076955, and THL130025A). The sponsors of this study had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.bbi.2017.10.004>.

Abbreviations

MSIMI	mental stress induced myocardial ischemia
CAD	coronary artery disease
IL-6	interleukin-6 (IL-6)
MCP-1	monocyte chemoattractant protein-1
MMP-9	matrix metalloproteinase 9
HsCRP	high-sensitivity C reactive protein

MI	myocardial infarction
MIPS	Mental stress ischemia prognosis study
MIMS-2	Myocardial infarction and mental stress-2

References

- Ackerman KD, Martino M, Heyman R, Moyna NM, Rabin BS. Stressor-induced alteration of cytokine production in multiple sclerosis patients and controls. *Psychosom Med*. 1998; 60:484–491. [PubMed: 9710295]
- Adams V, Lenk K, Linke A, Lenz D, Erbs S, Sandri M, Tarnok A, Gielen S, Emmrich F, Schuler G, Hambrecht R. Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise-induced ischemia. *Arterioscler Thromb Vasc Biol*. 2004; 24:684–690. [PubMed: 14988094]
- Babyak MA, Blumenthal JA, Hinderliter A, Hoffman B, Waugh RA, Coleman RE, Sherwood A. Prognosis after change in left ventricular ejection fraction during mental stress testing in patients with stable coronary artery disease. *Am J Cardiol*. 2010; 105:25–28. [PubMed: 20102885]
- Beattie MS, Shlipak MG, Liu H, Browner WS, Schiller NB, Whooley MA. C-Reactive protein and ischemia in users and nonusers of β -blockers and statins: data from the heart and soul study. *Circulation*. 2003; 107:245–250. [PubMed: 12538423]
- Beck, AT., Steer, RA., Brown, GK. BDI-II Beck Depression Inventory: Second Edition. The Psychological Corporation; San Antonio, TX: 1996.
- Biasucci LM, Vitelli A, Liuzzo G, Altamura S, Caligiuri G, Monaco C, Rebuzzi AG, Ciliberto G, Maseri A. Elevated Levels of Interleukin-6 in Unstable Angina. *Circulation*. 1996; 94:874–877. [PubMed: 8790019]
- Burg MM, Soufer R. Psychological Stress and Induced Ischemic Syndromes. *Curr Cardiovasc Risk Rep*. 2014; 8:1–6.
- Carney RM, Rich MW, Tevelde A, Saini J, Clark K, Jaffe AS. Major depressive disorder in coronary artery disease. *Am J Cardiol*. 1987; 60:1273–1275. [PubMed: 3687779]
- Cole SW, Arevalo JM, Takahashi R, Sloan EK, Lutgendorf SK, Sood AK, Sheridan JF, Seeman TE. Computational identification of gene-social environment interaction at the human IL6 locus. *Proc Natl Acad Sci USA*. 2010; 107:5681–5686. [PubMed: 20176930]
- Das S, O'Keefe JH. Behavioral cardiology: Recognizing and addressing the profound impact of psychosocial stress on cardiovascular health. *Curr Atherosclerosis Rep*. 2006; 8:111–118.
- Eapen DJ, Manocha P, Patel RS, Hammadah M, Veledar E, Wassel C, Nanjundappa RA, Sikora S, Malayter D, Wilson PW, Sperling L, Quyyumi AA, Epstein SE. Aggregate risk score based on markers of inflammation, cell stress, and coagulation is an independent predictor of adverse cardiovascular outcomes. *J Am Coll Cardiol*. 2013; 62:329–337. [PubMed: 23665099]
- Ellins E, Halcox J, Donald A, Field B, Brydon L, Deanfield J, Steptoe A. Arterial stiffness and inflammatory response to psychophysiological stress. *Brain Behav Immun*. 2008; 22:941–948. [PubMed: 18316176]
- Endrighi R, Steptoe A, Hamer M. The effect of experimentally induced sedentariness on mood and psychobiological responses to mental stress. *Br J Psychiatry*. 2016; 208:245–251. [PubMed: 26294364]
- Endrighi R, Hamer M, Steptoe A. Post-menopausal women exhibit greater interleukin-6 responses to mental stress than older men. *Ann Behav Med*. 2016; 50:564–571. [PubMed: 26943141]
- Ersbøll M, Al Enezi F, Samad Z, Sedberry B, Boyle SH, O'Connor C, Jiang W, Velazquez EJ. Impaired resting myocardial annular velocities are independently associated with mental stress-induced ischemia in coronary heart disease. *JACC*. 2014; 7:351–361. [PubMed: 24631512]
- Fisman EZ, Benderly M, Esper RJ, Behar S, Boyko V, Adler Y, Tanne D, Matas Z, Tenenbaum A. Interleukin-6 and the risk of future cardiovascular events in patients with angina pectoris and/or healed myocardial infarction. *Am J Cardiol*. 2006; 98:14–18. [PubMed: 16784912]

- Frasure-Smith N, Lesperance F, Talajic M. Depression and 18-month prognosis after myocardial infarction. *Circulation*. 1995; 91:999–1005. [PubMed: 7531624]
- Gaspardone A, Perino M, Ghini AS, Tomai F, Versaci F, Proietti I, Crea F. Exercise induced myocardial ischaemia does not cause increase in C-reactive protein concentration. *Heart*. 2000; 84:668–669. [PubMed: 11083751]
- Ghiadoni L, Donald AE, Cropley M, Mullen MJ, Oakley G, Taylor M, O'Connor G, Betteridge J, Klein N, Steptoe A, Deanfield JE. Mental stress induces transient endothelial dysfunction in humans. *Circulation*. 2000; 102:2473–2478. [PubMed: 11076819]
- Goebel MU, Mills PJ, Irwin MR, Ziegler MG. Interleukin-6 and tumor necrosis factor-alpha production after acute psychological stress, exercise, and infused isoproterenol: differential effects and pathways. *Psychosom Med*. 2000; 62:591–598. [PubMed: 10949106]
- Goldman-Mellor S, Brydon L, Steptoe A. Psychological distress and circulating inflammatory markers in healthy young adults. *Psychol Med*. 2010; 40:2079–2087. [PubMed: 20219150]
- Grebe KM, Takeda K, Hickman HD, Bailey AL, Embry AC, Bennink JR, Yewdell JW. Cutting edge: Sympathetic nervous system increases proinflammatory cytokines and exacerbates influenza A virus pathogenesis. *J Immunol*. 1950; 184:540–544.
- Hackett RA, Hamer M, Endrighi R, Brydon L, Steptoe A. Loneliness and stress-related inflammatory and neuroendocrine responses in older men and women. *Psychoneuroendocrinology*. 2012; 37:1801–1809. [PubMed: 22503139]
- Hammadah M, Al Mheid I, Wilmot K, Ramadan R, Shah AJ, Sun Y, Pearce B, Garcia EV, Kutner M, Bremner JD, Esteves F, Raggi P, Sheps DS, Vaccarino V, Quyyumi AA. The mental stress ischemia prognosis study: objectives, study design, and prevalence of inducible ischemia. *Psychosom Med*. 2017; 79:311–317. [PubMed: 28002382]
- Hammadah M, Al Mheid I, Wilmot K, Ramadan R, Alkhoder A, Obideen M, Abdelhadi N, Fang S, Ibeanu I, Pimple P, Mohamed Kelli H, Shah AJ, Pearce B, Sun Y, Garcia EV, Kutner M, Long Q, Ward L, Bremner JD, Esteves F, Raggi P, Sheps D, Vaccarino V, Quyyumi AA. Association between high-sensitivity cardiac troponin levels and myocardial ischemia during mental stress and conventional stress. *JACC*. 2017 in press.
- Hammadah M, Alkhoder A, Al Mheid I, Wilmot K, Isakadze N, Abdulhadi N, Chou D, Obideen M, O'Neal WT, Sullivan S, Tahhan AS, Kelli HM, Ramadan R, Pimple P, Sandesara P, Shah AJ, Ward L, Ko YA, Sun Y, Uphoff I, Pearce B, Garcia EV, Kutner M, Bremner JD, Esteves F, Sheps DS, Raggi P, Vaccarino V, Quyyumi AA. Hemodynamic, catecholamine, vasomotor and vascular responses: Determinants of myocardial ischemia during mental stress. *Int J Cardiol*. 2017
- Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. *Am J Cardiol*. 2003; 91:7a–11a.
- Hassan M, York KM, Li H, Li Q, Lucey DG, Fillingim RB, Sheps DS. Usefulness of peripheral arterial tonometry in the detection of mental stress-induced myocardial ischemia. *Clin Cardiol*. 2009; 32:E1–E6.
- Holly TA, Abbott BG, Al-Mallah M, Calnon DA, Cohen MC, DiFilippo FP, Ficaro EP, Freeman MR, Hendel RC, Jain D, Leonard SM, Nichols KJ, Polk DM, Soman P. Single photon-emission computed tomography. *J Nucl Cardiol*. 2010; 17:941–973. [PubMed: 20552312]
- Ikonomidis I, Athanassopoulos G, Lekakis J, Venetsanou K, Marinou M, Stamatiopoulos K, Cokkinos DV, Nihoyannopoulos P. Myocardial ischemia induces interleukin-6 and tissue factor production in patients with coronary artery disease. *Dobutamine Stress Echocardiography Study*. 2005; 112:3272–3279.
- Koelsch S, Boehlig A, Hohenadel M, Nitsche I, Bauer K, Sack U. The impact of acute stress on hormones and cytokines, and how their recovery is affected by music-evoked positive mood. *Sci Rep*. 2016; 6:23008. [PubMed: 27020850]
- Kop WJ, Gottdiener JS, Tangen CM, Fried LP, McBurnie MA, Walston J, Newman A, Hirsch C, Tracy RP. Inflammation and coagulation factors in persons > 65 years of age with symptoms of depression but without evidence of myocardial ischemia. *Am J Cardiol*. 2002; 89:419–424. [PubMed: 11835923]
- Kop WJ, Weissman NJ, Zhu J, Bonsall RW, Doyle M, Stretch MR, Glaes SB, Krantz DS, Gottdiener JS, Tracy RP. Effects of acute mental stress and exercise on inflammatory markers in patients with

- coronary artery disease and healthy controls. *Am J Cardiol.* 2008; 101:767–773. [PubMed: 18328837]
- Lipman RD, Grossman P, Bridges SE, Hamner JW, Taylor JA. Mental stress response, arterial stiffness, and baroreflex sensitivity in healthy aging. *J Gerontol Series A.* 2002; 57:B279–B284.
- Liuzzo G, Biasucci LM, Rebuffi AG, Gallimore JR, Caligiuri G, Lanza GA, Quaranta G, Monaco C, Pepys MB, Maseri A. Plasma protein acute-phase response in unstable angina is not induced by ischemic injury. *Circulation.* 1996; 94:2373–2380. [PubMed: 8921776]
- Lu XT, Zhao YX, Zhang Y, Jiang F. Psychological stress, vascular inflammation, and atherogenesis: potential roles of circulating cytokines. *J Cardiovasc Pharmacol.* 2013; 62:6–12. [PubMed: 23318990]
- Maes M, Song C, Lin A, De Jongh R, Van Gastel A, Kenis G, Bosmans E, De Meester I, Benoy I, Neels H, Demedts P, Janca A, Scharpe S, Smith RS. The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. *Cytokine.* 1998; 10:313–318. [PubMed: 9617578]
- Marsland AL, Walsh C, Lockwood K, John-Henderson NA. The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. *Brain Behav Immun.* 2017; 64:208–219. [PubMed: 28089638]
- Miller GE, Cohen S, Ritchey AK. Chronic psychological stress and the regulation of pro-inflammatory cytokines: a glucocorticoid-resistance model. *Health Psychology.* 2002; 21:531–541. [PubMed: 12433005]
- Miller GE, Rohleder N, Stetler C, Kirschbaum C. Clinical depression and regulation of the inflammatory response during acute stress. *Psychosom Med.* 2005; 67:679–687. [PubMed: 16204423]
- Myers MA, Fleck A. Observations on the delay in onset of the acute phase protein response. *Br J Exp Pathol.* 1988; 69:169–176. [PubMed: 3132197]
- Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev.* 2008; 88:1379–1406. [PubMed: 18923185]
- Pepine CJ, Petersen JW, Bairey Merz CN. A, microvascular-myocardial diastolic dysfunctional state and risk for mental stress ischemia: A revised concept of ischemia during daily life. *JACC.* 2014; 7:362–365.
- Phatharajaree W, Phrommintikul A, Chattipakorn N. Matrix metalloproteinases and myocardial infarction. *Can J Cardiol.* 2007; 23:727–733. [PubMed: 17622396]
- Qintar M, George JJ, Panko M, Bea S, Broer KA, St John J, Blissett KA, Ching E, Sears SF, Pedersen SS, Pozuelo L, Chung MK. A prospective study of anxiety in ICD patients with a pilot randomized controlled trial of cognitive behavioral therapy for patients with moderate to severe anxiety. *J Interventional Cardiac Electrophysiol.* 2015; 43:65–75.
- Ramadan R, Sheps D, Esteves F, Maziar Zafari A, Douglas Bremner J, Vaccarino V, Quyyumi AA. Myocardial ischemia during mental stress: role of coronary artery disease burden and vasomotion. *J Am Heart Assoc.* 2013; 2
- Ridker PM. Inflammation, infection, and cardiovascular risk. How good is the evidence? *Circulation.* 1998; 97:1671–1674. [PubMed: 9591759]
- Ridker PM, Hennekens CH, Buring JE, Rifai N. C-Reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med.* 2000; 342:836–843. [PubMed: 10733371]
- Rooks CR, Ibeanu I, Shah A, Pimple P, Murrah N, Shallenberger L, Pace T, Douglas Bremner J, Raggi P, Vaccarino V. Young women post-MI have higher plasma concentrations of interleukin-6 before and after stress testing. *Brain Behav Immun.* 2016; 51:92–98. [PubMed: 26263552]
- Rosengren A, Hawken S, Ounpuu S, Sliwa K, Zubaid M, Almahmeed WA, Blackett KN, Sitthi-amorn C, Sato H, Yusuf S. Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet.* 2004; 364:953–962. [PubMed: 15364186]
- Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Res.* 2011; 1813:878–888.

- Shah R, Burg MM, Vashist A, Collins D, Liu J, Jadbabaie F, Graeber B, Earley C, Lampert R, Soufer R. C-reactive protein and vulnerability to mental stress-induced myocardial ischemia. *Mol Med*. 2006; 12:269–274. [PubMed: 17380191]
- Sheps DS, McMahon RP, Becker L, Carney RM, Freedland KE, Cohen JD, Sheffield D, Goldberg AD, Ketterer MW, Pepine CJ, Raczynski JM, Light K, Krantz DS, Stone PH, Knatterud GL, Kaufmann PG. Mental stress-induced ischemia and all-cause mortality in patients with coronary artery disease: Results from the Psychophysiological Investigations of Myocardial Ischemia study. *Circulation*. 2002; 105:1780–1784. [PubMed: 11956119]
- Slavich GM, Irwin MR. From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychol Bull*. 2014; 140:774–815. [PubMed: 24417575]
- Song S, Graham-Engeland JE, Corwin EJ, Ceballos RM, Taylor SE, Seeman T, Klein LC. The role of multiple negative social relationships in inflammatory cytokine responses to a laboratory stressor. *PeerJ*. 2015; 3:e959. [PubMed: 26056615]
- Steptoe A, Willemsen G, Owen N, Flower L, Mohamed-Ali V. Acute mental stress elicits delayed increases in circulating inflammatory cytokine levels. *Clin sci*. 1979; 101:185–192.
- Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. *J Am Coll Cardiol*. 2007; 50:2173–2195. [PubMed: 18036459]
- Tunon J, Blanco-Colio L, Cristobal C, Tarin N, Higuera J, Huelmos A, Alonso J, Egido J, Asensio D, Lorenzo O, Mahillo-Fernandez I, Rodriguez-Artalejo F, Farre J, Martin-Ventura JL, Lopez-Bescos L. Usefulness of a combination of monocyte chemoattractant protein-1, galectin-3, and N-terminal probrain natriuretic peptide to predict cardiovascular events in patients with coronary artery disease. *Am J Cardiol*. 2014; 113:434–440. [PubMed: 24295549]
- Vaccarino, V. Mental Stress-Induced Myocardial Ischemia. In: Baune, TB., Tully, JP., editors. *Cardiovascular Diseases and Depression: Treatment and Prevention in Psychocardiology* Cham. Springer; International Publishing: 2016. p. 105-121.
- Vlachopoulos C, Kosmopoulou F, Alexopoulos N, Ioakeimidis N, Siasos G, Stefanadis C. Acute mental stress has a prolonged unfavorable effect on arterial stiffness and wave reflections. *Psychosom Med*. 2006; 68:231–237. [PubMed: 16554388]
- Vlachopoulos C, Xaplanteris P, Alexopoulos N, Aznaouridis K, Vasiliadou C, Baou K, Stefanadi E, Stefanadis C. Divergent effects of laughter and mental stress on arterial stiffness and central hemodynamics. *Psychosom Med*. 2009; 71:446–453. [PubMed: 19251872]
- Wei J, Rooks C, Ramadan R, Shah AJ, Bremner JD, Quyyumi AA, Kutner M, Vaccarino V. Meta-analysis of mental stress-induced myocardial ischemia and subsequent cardiac events in patients with coronary artery disease. *Am J Cardiol*. 2014; 114:187–192. [PubMed: 24856319]
- White HD. Pathobiology of troponin elevations: do elevations occur with myocardial ischemia as well as necrosis? *J Am Coll Cardiol*. 2011; 57:2406–2408. [PubMed: 21658560]
- Xue YT, Tan QW, Li P, Mou SF, Liu SJ, Bao Y, Jiao HC, Su WG. Investigating the role of acute mental stress on endothelial dysfunction: a systematic review and meta-analysis. *Clin Res Cardiol*. 2015; 104:310–319. [PubMed: 25391292]
- Yabluchanskiy A, Ma Y, Iyer RP, Hall ME, Lindsey ML. Matrix metalloproteinase-9: Many shades of function in cardiovascular disease. *Physiology*. 2013; 28:391–403. [PubMed: 24186934]

Table 1

Characteristics of the study population (MIPS Study).

Clinical variable	MSIMI- %, Mean \pm SD	MSIMI+ %, Mean \pm SD	P-value
Number	508	99	
Age, years	63.1 \pm 9.2	62.5 \pm 9.2	0.57
Male, %	75	78	0.61
Body mass index, kg/m ²	29.6 \pm 5.2	29.9 \pm 5.4	0.57
African American, %	26	33	0.14
Diabetes, %	32	38	0.18
Hypertension, %	75	77	0.71
Current Smoking, %	14	14	0.98
Myocardial infarction, %	36	39	0.48
Beck Depression Inventory	8.3 \pm 8.2	8.0 \pm 8.6	0.74
<i>Medications</i>			
ACE inhibitor, %	45	55	0.071
ARB, %	17	12	0.21
Aspirin, %	86	86	0.93
Beta blockers, %	74	77	0.53
Clopidogrel, %	34	40	0.19
Statin, %	86	85	0.85
<i>Resting hemodynamics</i>			
Systolic blood pressure, mmHg	135.1 \pm 18.1	136.2 \pm 16.5	0.56
Diastolic Blood pressure, beat/min	78.6 \pm 10.4	78.7 \pm 9.7	0.98
Heart rate, beat/min	62.9 \pm 10.6	66.2 \pm 13.0	0.016

Abbreviations: MSIMI, Mental stress induced myocardial ischemia; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker.

Table 2

Resting and post-mental stress values of inflammatory biomarkers (MIPS Study).

	Rest		Post stress		Unadjusted		Adjusted Model 1**		Adjusted Model 2†		Adjusted Model 3‡	
	Median (IQR)	Median (IQR)	β (95% CI)	P value*	β (95% CI)	P value*	β (95% CI)	P value*	β (95% CI)	P value*	β (95% CI)	P value*
IL-6, pg/mL	1.4 (1.0–2.1)	1.7 (1.2–2.6)	0.33 (0.29, 0.38)	<0.0001	0.34 (0.29, 0.39)	<0.0001	0.34 (0.29, 0.39)	<0.0001	0.34 (0.29, 0.39)	<0.0001	0.32 (0.28, 0.37)	<0.0001
MCP-1, pg/mL	123.1 (102.1–147.5)	128.3 (106.7–155.3)	0.07 (0.05, 0.09)	<0.0001	0.07 (0.05, 0.09)	<0.0001	0.07 (0.05, 0.09)	<0.0001	0.07 (0.05, 0.09)	<0.0001	0.07 (0.05, 0.09)	<0.0001
MMP-9, ng/mL	61.5 (41.5–96.7)	68.6 (46.2–98.7)	0.13 (0.09, 0.17)	<0.0001	0.13 (0.09, 0.17)	<0.0001	0.13 (0.09, 0.17)	<0.0001	0.13 (0.09, 0.18)	<0.0001	0.13 (0.09, 0.18)	<0.0001
hsCRP, mg/L	1.6 (0.6–3.8)	1.4 (0.6–3.6)	-0.03 (-0.06, 0.002)	0.03	-0.03 (-0.06, 0.0003)	0.05	-0.02 (-0.05, 0.01)	0.11	-0.02 (-0.05, 0.01)	0.22	-0.02 (-0.05, 0.01)	0.22

Abbreviations: IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MMP-9, matrix metalloproteinase 9; hsCRP, high-sensitivity C reactive protein. A log 2 transformation was used for biomarker values in the analysis.

* p values were obtained from repeated measures model.

** Adjusted model 1: Adjusted for age, sex, African American race, body mass index, smoking, diabetes, hypertension, beck depression inventory score, and previous myocardial infarction.

† Adjusted model 2: Adjusted model 1 + accounting for biomarker plate as a random effect.

‡ Adjusted model 3: Adjusted model 2 + adjusting for sequence of mental/physical stress.

Table 3

Spearman correlations of mental stress-induced changes in inflammatory biomarkers (MIPS Study).

	IL-6 response	MMP-9 response	MCP-1 response	HsCRP response
IL-6 response	–	–	–	–
MMP-9 response	0.15 ^{**}	–	–	–
MCP-1 response	0.27 ^{**}	0.09 [*]	–	–
Hs-CRP response	0.07 [*]	0.03	–0.02	–

Abbreviations: IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MMP-9, matrix metalloproteinase 9; HsCRP, high-sensitivity C reactive protein.

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Table 4

Resting values of inflammatory biomarkers and their changes with mental stress according to MSIMI status (MIPS Study).

	MSIMI		Unadjusted		Adjusted Model 1**		Adjusted Model 2†		Adjusted Model 3‡	
	Negative (n = 508) Median (IQR)	Positive (n = 99) Median (IQR)	β (95 % CI)	P value*						
A) Resting inflammatory biomarkers										
IL-6, pg/mL	1.4 (1.0–2.1)	1.3 (1.0–2.1)	0.01 (-0.19, 0.21)	0.91	0.01 (-0.18, 0.20)	0.93	0.03 (-0.14, 0.20)	0.74	-0.02 (-0.20, 0.16)	0.87
MCP-1, pg/mL	122.9 (101.3–147.1)	123.5 (107.0–147.9)	0.05 (-0.04, 0.14)	0.27	0.05 (-0.03, 0.14)	0.20	0.06 (-0.02, 0.13)	0.16	0.05 (-0.03, 0.13)	0.22
MMP-9, ng/mL	61.6 (40.6–97.1)	61.0 (44.9–93.1)	0.08 (-0.12, 0.28)	0.42	0.09 (-0.11, 0.28)	0.38	0.08 (-0.11, 0.28)	0.39	0.08 (-0.12, 0.29)	0.43
Hs-CRP, mg/L	1.6 (0.6–3.7)	1.4 (0.7–5.1)	0.19 (-0.22, 0.61)	0.36	0.19 (-0.20, 0.57)	0.35	0.14 (-0.27, 0.56)	0.50	0.10 (-0.38, 0.55)	0.67
B) Mental stress induced inflammatory responses§										
IL-6, pg/mL	0.3 (0.02–0.7)	0.2 (-0.04 to 0.8)	-0.06 (-0.19, 0.07)	0.37	-0.05 (-0.17, 0.08)	0.47	-0.02 (-0.15, 0.10)	0.74	-0.02 (-0.15, 0.10)	0.75
MCP-1, pg/mL	4.9 (-4.1 to 15.4)	7.4 (-2.7 to 16.1)	0.01 (-0.05, 0.06)	0.81	0.01 (-0.05, 0.06)	0.84	0.01 (-0.04, 0.07)	0.65	0.03 (-0.03, 0.09)	0.27
MMP-9, ng/mL	5.0 (-8.3 to 20.7)	7.3 (-5.4 to 24.7)	0.02 (-0.09, 0.14)	0.68	0.02 (-0.10, 0.13)	0.76	0.01 (-0.10, 0.13)	0.81	0.002 (-0.12, 0.12)	0.98
Hs-CRP, mg/L	-0.01 (-0.1 to 0.08)	-0.01 (-0.2 to 0.04)	-0.06 (-0.14, 0.02)	0.13	-0.07 (-0.15, 0.01)	0.08	-0.04 (-0.12, 0.05)	0.39	-0.02 (-0.10, 0.07)	0.70

Abbreviations: MSIMI, mental stress-induced myocardial ischemia; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MMP-9, matrix metalloproteinase 9; HsCRP, high-sensitivity C reactive protein.

Data was presented as median (25th and 75th percentiles). A log 2 transformation was used for biomarker values.

* p values were obtained from mixed linear models.

** Adjusted model 1: Adjusted for age, sex, African American race, body mass index, smoking, diabetes, hypertension, beck depression inventory score, and previous myocardial infarction.

† Adjusted model 2: Adjusted model 1 + accounting for biomarker plate as a random effect.

‡ Adjusted model 3: Adjusted model 2 + adjusting for sequence of mental/physical stress.

§ Responses are calculated as the differences between 90-min post mental stress values and resting values. Inflammatory response was modeled as the outcome adjusted for resting levels.

Table 5

Resting values of inflammatory biomarkers and their changes with mental stress according to MSIMI status in the validation cohort (MIMS2 Study).

	MSIMI		Unadjusted		Adjusted Model 1**		Adjusted Model 2†		Adjusted Model 3‡	
	Negative (n = 191) Median (IQR)	P value*	Positive (n = 37) Median (IQR)	P value*	β (95 % CI)	P value*	β (95 % CI)	P value*	β (95 % CI)	P value*
A) Resting inflammatory biomarkers										
IL-6, pg/mL	1.5 (1.0–2.4)	1.7 (1.2–2.8)	0.18 (–0.13, 0.50)	0.25	0.18 (–0.12, 0.47)	0.23	0.18 (–0.11, 0.47)	0.23	0.14 (–0.15, 0.44)	0.35
MCP-1, pg/mL	120.0 (101.3–139.7)	140.6 (117.6–153.0)	0.16 (0.02, 0.31)	0.03	0.10 (–0.04, 0.25)	0.16	0.09 (–0.05, 0.24)	0.20	0.05 (–0.09, 0.19)	0.48
MMP-9, ng/mL	56.3 (39.4–92.0)	51.1 (38.9–85.3)	–0.09 (–0.40, 0.23)	0.59	–0.08 (–0.39, 0.22)	0.59	–0.07 (–0.38, 0.23)	0.65	–0.05 (–0.35, 0.26)	0.77
Hs-CRP, mg/L	3.1 (1.3–6.9)	3.7 (0.8–5.4)	–0.05 (–0.76, 0.67)	0.90	–0.09 (–0.76, 0.57)	0.78	–0.07 (–0.73, 0.60)	0.84	–0.10 (–0.78, 0.58)	0.34
B) Mental stress induced inflammatory responses§										
IL-6, pg/mL	0.8 (0.4–1.8)	1.2 (0.7–2.1)	0.22 (–0.05, 0.49)	0.11	0.21 (–0.06, 0.48)	0.12	0.21 (–0.06, 0.48)	0.12	0.19 (–0.08, 0.46)	0.17
MCP-1, pg/mL	6.7 (–7.8 to 23.3)	12.1 (–2.5 to 30.8)	0.07 (–0.02, 0.17)	0.14	0.08 (–0.01, 0.17)	0.10	0.08 (–0.01, 0.17)	0.10	0.07 (–0.02, 0.17)	0.14
MMP-9, ng/mL	4.5 (–3.9 to 14.5)	4.6 (–5.7 to 23.2)	0.03 (–0.17, 0.24)	0.75	0.05 (–0.16, 0.27)	0.62	0.05 (–0.16, 0.26)	0.64	0.09 (–0.12, 0.31)	0.39
Hs-CRP, mg/L	–0.01 (–0.3 to 0.16)	0.003 (–0.4 to 0.3)	0.06 (–0.12, 0.24)	0.50	0.09 (–0.09, 0.27)	0.34	0.09 (–0.09, 0.27)	0.34	0.07 (–0.11, 0.25)	0.46

Abbreviations: MSIMI, mental stress-induced myocardial ischemia; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MMP-9, matrix metalloproteinase 9; HsCRP, high-sensitivity C reactive protein.

Data was presented as median (25th and 75th percentiles). A log 2 transformation was used for biomarker values.

* p values were obtained from mixed linear models.

** Adjusted model 1: Adjusted for age, sex, African American race, body mass index, smoking, diabetes, hypertension, beck depression inventory score, and previous myocardial infarction.

† Adjusted model 2: Adjusted model 1 + accounting for biomarker plate as a random effect.

‡ Adjusted model 3: Adjusted model 2 + adjusting for sequence of mental/physical stress.

§ Responses are calculated as the differences between 90-min post mental stress values and resting values. Inflammatory response was modeled as the outcome adjusted for resting levels.