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Inflammatory response to mental stress and mental stress induced myocardial ischemia

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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.
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 metallopeptidase 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.

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 metallopeptidase 9; HsCRP, high-sensitivity C reactive protein; MI, myocardial infarction; MIPS, Mental stress ischemia prognosis study; MIMS-2, Myocardial infarction and mental stress-2.

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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 metalloprotease-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 99mTc-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 sub-

ject 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 $\geq$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 ± 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 ± 12% increase, β = 25.61; 95% CI: 24.36, 26.85, p = <0.0001], diastolic blood pressure [13 ± 9 mmHg; 17 ± 11% increase, β = 12.91; 95% CI: 12.23, 13.59, p = <0.0001], and heart rate [11 ± 9 beat/min; 18% ± 15% increase, β = 11.10; 95% CI: 10.35, 11.85, p = <0.0001], and plasma hemoglobin [0.3 ± 0.7 g/dL; 2.3 ± 5.3%, β = 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

Table 1
Characteristics of the study population (MIPS Study).

Clinical variable	MSIMI− %, Mean ± SD	MSIMI+ %, Mean ± SD	P-value
Number	508	99	
Age, years	63.1 ± 9.2	62.5 ± 9.2	0.57
Male, %	75	78	0.61
Body mass index, kg/m^2	29.6 ± 5.2	29.9 ± 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 ± 8.2	8.0 ± 8.6	0.74
Medications			
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
Resting hemodynamics			
Systolic blood pressure, mmHg	135.1 ± 18.1	136.2 ± 16.5	0.56
Diastolic Blood pressure, beat/min	78.6 ± 10.4	78.7 ± 9.7	0.98
Heart rate, beat/min	62.9 ± 10.6	66.2 ± 13.0	0.016

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

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 (±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

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*
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	0.32 (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
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, 0.18)	<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

Abbreviations: IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MMP-9, matrix metallopeptidase 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 metallopeptidase 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*	β (95 % CI)	P value*	β (95 % CI)	P value*	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 metallopeptidase 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.

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-1β, 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

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)	Positive (n = 37) Median (IQR)	β (95 % CI)	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 metallopeptidase 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.

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 of 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.

Conflicts of interest and sources of funding

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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.

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