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Association of Vitamin D Status With Mental Stress Induced Myocardial Ischemia in Patients With Coronary Artery Disease

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Abstract

Background—Mental stress-induced (MSIMI) or physical stress-induced (PSIMI) myocardial ischemia portends a worse prognosis in CAD patients. Vitamin D insufficiency is associated with adverse cardiovascular outcomes, but its relationship to myocardial ischemia remains unclear. We hypothesized that vitamin D insufficiency will be associated with a higher prevalence of myocardial ischemia in CAD patients.

Methods—In 255 patients with stable CAD, myocardial perfusion imaging was performed to assess ischemia in response to mental and physical stress protocols. Vitamin D insufficiency was defined as serum 25-hydroxyvitamin-D [25(OH)D] levels below 30 ng/ml, collected on the day of stress testing.

Results—Mean 25(OH)D level was 30.8±12.8 ng/ml, and 139 (55%) patients had vitamin D insufficiency. MSIMI occurred in 30 (12%) patients and PSIMI in 67 (27%). Individuals with MSIMI had significantly lower levels of 25(OH)D as compared to those without MSIMI (24.0±8.6 vs. 31.7±12.9, $p=0.002$). The prevalence of MSIMI was higher in those with as compared to those without vitamin D insufficiency (17% vs. 6%, $p=0.009$). Moreover, low 25(OH)D levels remained independently associated with MSIMI after adjusting for potential confounders. Conversely, 25(OH)D levels were similar between those with or without PSIMI (29.8±13.0 vs. 31.4±12.7; $p=0.37$), as was the prevalence of PSIMI in those with or without vitamin D insufficiency (29% vs. 24%, $p=0.42$).

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Conflict of Interest: none declared.

Conclusions—Vitamin D insufficiency is associated with a higher prevalence of MSIMI, but not PSIMI among stable CAD patients. Whether this association serves as a potential mechanism linking low vitamin D status to adverse cardiovascular outcomes warrants further investigation.

Keywords

Coronary; Inducible ischemia; Mental stress; Stress testing; Vitamin D

Introduction

Vitamin D insufficiency is relatively common in the general population and may be more prevalent in patients with cardiovascular risk factors¹ and in those with coronary artery disease (CAD)². Numerous epidemiological studies have demonstrated an association between 25-hydroxyvitamin-D [25(OH)D] insufficiency and increased prevalence of hypertension, left ventricular hypertrophy, heart failure, peripheral arterial disease, incident CAD, myocardial infarction and cardiovascular mortality³⁻⁸. Additionally, low vitamin D status has been linked to pathophysiological pathways underlying atherosclerosis such as inflammation⁹ and abnormal vascular function including increased arterial stiffness, abnormal vascular endothelial reactivity, and vascular calcification¹⁰⁻¹². Finally, we have previously shown that vitamin D insufficiency is associated with decreased coronary blood flow reserve in response to pharmacological stress in asymptomatic individuals, which may be explained by altered vasomotor and endothelial function¹³. All of the above may predispose to increased risk of ischemia in CAD patients with vitamin D insufficiency due to obstructive CAD and/or coronary vasomotor dysregulation.

Whether vitamin D insufficiency is also linked to a more frequent ischemic myocardial response to emotional stress remains unknown. Mental stress-induced myocardial ischemia (MSIMI) occurs in a significant proportion of patients with CAD and is associated with fatal and non-fatal cardiovascular events, irrespective of traditional risk factors and presence of physical stress-induced myocardial ischemia (PSIMI)¹⁴⁻¹⁸. While the mechanisms underlying MSIMI remain a subject of investigation, endothelial dysfunction and abnormal coronary microcirculatory vasomotion, conditions associated with low vitamin D status^{10, 13}, have been suggested as potential contributors¹⁹⁻²¹. Notably, vitamin D has also been linked to pathways that are central to the adaptive responses to stress including the metabolism of catecholamines and activation of the renin-angiotensin-aldosterone system (RAAS), both of which are pathways believed to be involved in the precipitation of MSIMI²²⁻²⁴. Therefore, the aim of this study was to assess whether 25(OH)D levels are associated with MSIMI and/or PSIMI in patients with stable CAD.

Methods

Patient Sample

We studied 255 patients with documented stable CAD, who were recruited from Emory Healthcare sites or by advertisement as part of the ongoing 'Mental Stress Ischemia: Mechanisms and Prognosis' study between June 2011 and November 2012. Presence of CAD was defined by abnormal coronary angiography demonstrating atherosclerosis with at

least luminal irregularities (20-30% stenosis), previous percutaneous or surgical coronary revascularization, or documented myocardial infarction. Patients with unstable angina, decompensated heart failure, or myocardial infarction in prior two months, end stage renal disease, or unstable psychiatric conditions were excluded. Diagnosis of cardiovascular risk factors, previous cardiovascular history, and current medications were assessed by using standardized questionnaires and chart reviews. We administered the Beck Depression Inventory-II (BDI-II), a standardized scale providing a continuous measure of depressive symptoms²⁵. Angiographic data from the most recent coronary angiogram was used to estimate CAD severity based on a semi-quantitative scoring system by Gensini²⁶. The research protocol was approved by the Institutional Review Board of Emory University and all participants provided informed consent.

Study Protocol

All patients were tested in the morning after an overnight fast while receiving their usual medications except for antianginal medications (beta-blockers, calcium-channel blockers, and long-acting nitrates), which were withheld for 24 to 48 hours, depending on the half-life of these medications, before stress testing. Patients underwent mental and physical stress testing on two separate days up to one week apart. The sequence of stressors (mental vs. physical) was evenly balanced. Five patients could not complete the physical stress test protocol due to either personal or medical reasons.

Mental Stress Procedure—Baseline vital signs were measured in a quiet dimly lit, temperature controlled (21-23°C) room after a 30-minute of rest. The mental stress was then administered utilizing a standardized public speaking task as previously described²⁷. Briefly, subjects were asked to imagine a situation in which a close relative had been mistreated in a nursing home. Subjects were given two minutes to prepare followed by a three-minute speech during which they were asked to deliver their story in front of an evaluative audience. They were told that their speech would be videotaped and evaluated later for content, quality, and duration. All personnel participating in administering the test were white coated and made sure not to give the patients any information about the specific contents of the test before they submitted to it. Subjective stress levels were evaluated by use of a visual analog scale (ranging from 0% signifying no stress to 100% signifying extreme stress) before and after the speech task. Subjects were monitored for ten minutes following the completion of the mental stress task. Blood pressure (BP) and heart rate were recorded at five-minute intervals during the resting phase and at one-minute intervals during and after the mental stress task by the use of an automatic oscillometric device (Dynamap Critikon Inc., Tampa, Florida).

Physical Stress Procedure—Exercise stress testing was performed using the Bruce Protocol; pharmacological stress testing with regadenoson was performed when exercise was contraindicated or if patients could not complete the exercise protocol. Patients were monitored for at least seven minutes following this type of stress test. Blood pressure, heart rate, and electrocardiographic (ECG) monitoring were performed per standard clinical protocol.

Myocardial Perfusion Imaging for Stress Testing—Myocardial perfusion imaging with 99m-Tc-sestamibi was performed at rest, during mental stress, and with exercise/pharmacologic stress according to standard protocols²⁹. Xanthine derivatives and caffeine-containing products were discontinued 48 and 12 hours before stress testing, respectively. During mental stress testing, the radioisotope injection was given at one minute into the speech based on previous reports demonstrating that the maximal hemodynamic, neurohormonal, and ischemic responses to mental stress usually occur at the near onset of the stressful task³⁰. The radioisotope injection was given at peak exertion during the exercise test, or immediately after the regadenoson injection. Exercise was continued for at least one minute after the injection. Stress images were acquired 30-60 minutes later using conventional methodology with single-photon emission computed tomography (SPECT)²⁹.

An experienced nuclear medicine physician who was blinded to the stressor and clinical data interpreted all studies. Rest images were used for estimation of the left ventricular ejection fraction (LVEF). Rest and stress images were visually compared for the number and severity of perfusion defects using a 17-segment model. Each segment was scored from zero to four, with zero being normal uptake and four no uptake, yielding a total score. A reversible defect score (summed difference score [SDS]) was calculated as the difference between summed stress and summed rest scores. Ischemia was defined as new or worsening perfusion defects during mental, exercise or pharmacologic stress as compared to the resting baseline images with a SDS of ≥ 4 . For the purpose of comparison in this study, the same ischemia definition was used for all testing protocols.

Vitamin D Assessment—Vitamin D insufficiency was defined as serum 25(OH)D level <30 ng/ml³¹. Venous blood samples were obtained after an overnight fast on the morning prior to stress testing. Serum 25(OH)D levels were determined by ELISA (IDS Inc., Fountain Hills, AZ) as per the manufacturer's product manual and tested in duplicate. The intra- and inter-assay coefficients of variation were less than 10% and 15%, respectively. Accuracy of the 25(OH)D measurements was ensured by the lab's participation in the vitamin D external quality assessment scheme as well as the National Institute of Standards and Technology/National Institutes of Health Vitamin D Metabolites Quality Assurance program.

Statistical Methods

Results for normally distributed continuous variables are summarized as means $\pm$ standard deviation or as proportions for categorical variables. Continuous variables with a non-normal distribution are shown as the median value with interquartile intervals. The two-tailed unpaired Student's *t* test was used for comparison of normally distributed continuous variables. The Mann-Whitney U test was used to compare the difference in non-normally distributed variables. The chi-square test was used for comparison of categorical variables. Correlations between continuous variables were assessed with two-way Pearson or Spearman correlation tests, as appropriate. Multivariate logistic regression models, with SPECT ischemia as a binary dependent variable were used to adjust for potential confounding factors. The Hosmer-Lemeshow test was used to test for model goodness of fit. Statistical significance was based on 2-tailed tests, and *p* values ≤ 0.05 were considered

significant. Analyses were performed with SPSS (version 20.0, SPSS, Inc., Chicago, Illinois).

Results

The mean serum 25(OH)D level was 30.8 ± 12.8 ng/ml (range: 5.9–81.9). Overall, 139 patients (55%) had vitamin D insufficiency. Among the demographic and other cardiovascular risk factors, mean serum 25(OH)D concentration was significantly higher in Caucasians, in non-obese patients, and in samples drawn during April–October compared to November–March (32.1 ± 13.5 vs. 27.5 ± 10.1 ; $p=0.004$) (Table 1). 25(OH)D levels correlated negatively with body mass index ($r=-0.15$, $p=0.024$) and positively with LVEF ($r=0.25$, $p<0.001$). However, 25(OH)D levels did not correlate with depressive symptoms assessed by the Beck Depression Inventory ($p=0.69$) or with the severity of CAD ($p=0.15$). Moreover, the median Gensini score was similar in those with and without vitamin D insufficiency (24; IQR = [3–54] vs. 33; IQR = [5–65]; $p=0.32$, respectively).

The mental stress task resulted in a significant increase in perceived stress levels in the entire cohort ($p<0.001$), including those with or without MSIMI and those with or without vitamin D insufficiency. Moreover, the percent change in stress levels was similar regardless of the ischemic response ($p=0.16$) or vitamin D insufficiency ($p=0.36$).

Vitamin D Status and Physical Stress Ischemia

The mean 25(OH)D level was higher in the 160 of 250 subjects (64%) who completed the exercise stress protocol, compared to the 90 (36%) who underwent pharmacological stress testing (32.6 ± 13.1 vs. 28.1 ± 11.6 , $p=0.007$, respectively). The mean metabolic equivalents achieved during exercise testing were similar in those with or without PSIMI (9.0 ± 2.7 vs. 8.9 ± 2.5 ; $p=0.86$) and in those with or without vitamin D insufficiency (8.8 ± 2.5 vs. 9.0 ± 2.5 ; $p=0.59$).

Patients who developed PSIMI ($n=67$, 27%) had a mean SDS of 8.2 ± 4.7 , were more frequently male with a history of coronary artery bypass graft surgery (CABG) and dyslipidemia, and had more severe CAD (Table 2). Patients with PSIMI had similar mean 25(OH)D levels as those without PSIMI (29.8 ± 13.0 vs. 31.4 ± 12.7 ; $p=0.37$). Furthermore, the prevalence of PSIMI was similar in those with or without vitamin D insufficiency (29% vs. 24%, $p=0.42$, respectively). This was true both among patients undergoing either exercise ($p=0.72$) or pharmacological stress testing ($p=0.10$). Vitamin D level was not a predictor of PSIMI in either univariate analysis (odds ratio = 0.99, 95% confidence interval = 0.97–1.012; $p=0.37$) or multivariate analysis adjusting for cardiovascular risk factors (age, sex, hypertension, diabetes mellitus, dyslipidemia), race, season of blood collection (November–March vs. April–October)³², previous history of myocardial infarction, angiographic severity of CAD, body mass index, LVEF, and type of stress test (exercise vs. pharmacologic) (odds ratio = 1.00, 95% confidence interval = 0.97–1.034; $p=0.94$). Similarly, Vitamin D insufficiency was not a predictor of PSIMI in either univariate (odds ratio = 1.26, 95% confidence interval = 0.72–2.22; $p=0.42$) or multivariate analysis adjusting for aforementioned confounders (odds ratio = 1.09, 95% confidence interval = 0.49–2.40; $p=0.83$).

Finally, the number of perfusion defects during rest, physical stress, and the SDS during physical stress did not significantly correlate with the 25(OH)D level in the entire cohort. Even in those with PSIMI, the 25(OH)D level did not correlate with the SDS ($r = -0.14$, $p = 0.27$), indicating that the severity of ischemia during physical stress was not related to the serum vitamin D level.

Vitamin D Status and Mental Stress Ischemia

MSIMI occurred in 30 patients (12%) with a mean SDS of 5.9 ± 1.9 . There were no significant differences in the clinical characteristics of those with or without MSIMI (Table 2). However, patients with MSIMI had significantly lower mean serum 25(OH)D levels than those without MSIMI (24.0 ± 8.6 vs. 31.7 ± 12.9 ; $p = 0.002$). This was true regardless of whether patients with MSIMI had concomitant PSIMI or not (Figure 1). Compared to subjects without vitamin D insufficiency, those with insufficiency had higher prevalence of MSIMI (6% vs. 17%; $p = 0.009$). Moreover, the 25(OH)D level correlated negatively with the summed stress score ($r = -0.167$, $p = 0.007$) and the SDS during mental stress ($r = -0.174$, $p = 0.005$) in the entire cohort. Importantly, in those with MSIMI, the 25(OH)D level correlated negatively with the SDS, indicating a greater magnitude of ischemia in those with lower vitamin D levels (Figure 2).

A lower serum 25(OH)D level was an independent predictor of MSIMI in univariate analysis (odds ratio = 0.94, 95% confidence interval = 0.90–0.97; $p = 0.002$), and remained an independent predictor for development of MSIMI after adjusting for cardiovascular risk factors (age, sex, hypertension, diabetes mellitus, dyslipidemia), race, season of blood collection (November–March vs. April–October)³², previous history of myocardial infarction, angiographic severity of CAD, body mass index, and LVEF (odds ratio = 0.93, 95% confidence interval = 0.89–0.98; $p = 0.010$). Similarly, Vitamin D insufficiency was a predictor of MSIMI in both univariate (odds ratio = 3.1, 95% confidence interval = 1.3–7.5; $p = 0.013$) and multivariate analysis adjusting for aforementioned confounders (odds ratio = 3.5, 95% confidence interval = 1.2–10.6; $p = 0.026$).

Hemodynamic Measurements and Ischemia

We examined the heart rate and BP responses during mental stress in order to investigate whether the difference in the prevalence of MSIMI was due to an exaggerated hemodynamic response to mental stress in patients with low vitamin D levels. Patients with MSIMI had a higher baseline systolic BP and tended to have a higher diastolic BP reactivity due to mental stress testing, but otherwise had similar systolic BP, heart rate, and rate pressure product (RPP) reactivity compared to those without MSIMI (Table 3).

Patients with vitamin D insufficiency had higher baseline systolic BP, but otherwise had similar hemodynamic reactivity during mental stress. Thus, although 25(OH)D level inversely correlated with baseline systolic BP ($r = -0.2$, $p = 0.004$), there was no association with either the systolic or diastolic BP, or RPP reactivity during mental stress.

Discussion

We found that patients with stable CAD and vitamin D insufficiency had a higher prevalence and severity of myocardial ischemia during mental stress, but not during physical stress. Importantly, vitamin D status was an independent predictor for the development of MSIMI even after adjustment for several confounders including cardiovascular risk factors and season of testing. However, vitamin D insufficiency was not associated with either a heightened perceived stress or hemodynamic reactivity in response to the mental stress task, or with a lower exercise capacity during exercise stress testing. Although the mechanisms underlying MSIMI remain unclear, evidence suggests that its pathophysiology may differ from PSIMI. For example, compared to PSIMI, MSIMI is more often painless¹⁸, occurs at lower levels of oxygen demand¹⁸, and may not be accompanied by PSIMI¹⁶. The differential effect of vitamin D status on ischemia during mental versus physical stress in our study supports differing mechanisms for these two types of ischemia and suggests that vitamin D plays a pathophysiological role specifically in MSIMI.

Evidence has linked low vitamin D status to adverse effects on the cardiovascular system and to a wide range of metabolic risk factors implicated in the development and progression of atherosclerosis^{1, 3-8, 33}. Vitamin D exerts its effects via nuclear and/or plasma membrane vitamin D receptors that are present on a broad range of cell types including vascular cells, cardiomyocytes, and brain cells, which are also capable of converting circulating 25(OH)D to its active form^{2, 33-35}. Vitamin D insufficiency may thus lead to adverse cardiovascular events by promoting endothelial dysfunction¹⁰, microvascular dysfunction¹³, insulin resistance³⁶, inflammation³⁷, and activation of the RAAS leading to smooth muscle and myocardial hypertrophy^{24, 37}.

The pleiotropic effects of vitamin D on neuronal cells may help explain the link between vitamin D and various psychological conditions including depression and anxiety^{38, 39}. While evidence linking vitamin D to stress is sparse, a recent study showed a higher prevalence of vitamin D insufficiency in stress-induced cardiomyopathy⁴⁰. In fact, low vitamin D status may play a key role in the stress adaptive response by stimulating both central and peripheral pathways including the RAAS, which in turn activates adrenocorticotrophic hormones, corticosterone, aldosterone, and catecholamines⁴¹. Cross-talk between vitamin D and glucocorticoid receptors in the brain may augment the deleterious physiologic response to mental stress with vitamin D insufficiency. Glucocorticoids may also lower levels of vitamin D by increasing its catabolism and decreasing its absorption that may further exacerbate its effects⁴²⁻⁴⁵. Whether vitamin D insufficiency leads to pathophysiological changes predisposing individuals to ischemia in response to stress, or whether vitamin D insufficiency is merely a marker of an exaggerated stress load needs further investigation.

Vitamin D insufficiency may ultimately contribute to the pathophysiology of MSIMI by its adverse effects on vascular function. We have shown that low vitamin D levels are associated with endothelial dysfunction, arterial stiffness, and microcirculatory coronary and peripheral vascular dysfunction^{10, 13}. Notably, these vascular mechanisms are also potential

contributors to MSIMI, which has been associated with endothelial dysfunction¹⁹, epicardial coronary vasospasm²⁰, and increased microvascular tone²¹.

Vitamin D regulates blood pressure by inhibiting renin gene expression²⁴. Lower vitamin D levels have been associated with hypertension and its' supplementation with lowering of blood pressure^{46, 47}. Indeed, patients with vitamin D insufficiency in our study also had higher systolic BP and those with MSIMI tended to have a higher diastolic BP response to mental stress, a finding that has been associated with MSIMI in previous studies^{17, 20}. However, the higher rate of MSIMI in patients with vitamin D insufficiency was not related to either a greater hemodynamic reactivity to mental stress, or to a greater increase in oxygen demand, estimated by the rise in RPP, suggesting that changes in myocardial blood flow as a result of greater vasoconstriction during mental stress is the likely mechanism of ischemia.

Finally, we found that vitamin D levels were higher in Caucasians, non-obese subjects, and in samples drawn during the spring and summer seasons, as previously reported^{10, 13}. Interestingly, patients deemed clinically unsuitable to undergo or complete treadmill exercise testing had lower 25(OH)D levels, supporting prior observations of higher vitamin D levels in more physically active subjects⁴⁸.

Study Limitations

The cross-sectional design of our study precludes definitive conclusions regarding the causal relationship between MSIMI and vitamin D status. In addition, the frequency of MSIMI in this study was lower than previously reported, partly because most previous studies only investigated patients with exercise-induced ischemia, and partly because of differing methodologies and sensitivity of criteria used for defining ischemia^{15, 49}.

Conclusions

We found a unique association between low vitamin D status and MSIMI among stable CAD patients, both of which have been shown to be associated with adverse cardiovascular outcomes. While this association does not prove causality, our findings are hypothesis generating and offer a potential mechanism linking vitamin D insufficiency to increased risk of fatal and non-fatal cardiovascular events. Whether vitamin D supplementation improves coronary microvascular function and flow resulting in reversal or prevention of ischemia during mental stress, and whether this will result in improved long-term outcomes in these patients warrants further investigation.

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References

1. Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, Felsenfeld A, Levine B, Mehrotra R, Norris K. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin d in the united states: Data from the third national health and nutrition examination survey. *Arch Intern Med.* 2007; 167:1159–1165. [PubMed: 17563024]

2. Lee JH, O'Keefe JH, Bell D, Hensrud DD, Holick MF. Vitamin d deficiency an important, common, and easily treatable cardiovascular risk factor? *Journal of the American College of Cardiology*. 2008; 52:1949–1956. [PubMed: 19055985]
3. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS. Vitamin d deficiency and risk of cardiovascular disease. *Circulation*. 2008; 117:503–511. [PubMed: 18180395]
4. Brondum-Jacobsen P, Benn M, Jensen GB, Nordestgaard BG. 25-hydroxyvitamin d levels and risk of ischemic heart disease, myocardial infarction, and early death: Population-based study and meta-analyses of 18 and 17 studies. *Arterioscler Thromb Vasc Biol*. 2012; 32:2794–2802. [PubMed: 22936341]
5. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin d and risk of myocardial infarction in men: A prospective study. *Arch Intern Med*. 2008; 168:1174–1180. [PubMed: 18541825]
6. Tomson J, Emberson J, Hill M, Gordon A, Armitage J, Shipley M, Collins R, Clarke R. Vitamin d and risk of death from vascular and non-vascular causes in the whitehall study and meta-analyses of 12 000 deaths. *European heart journal*. 2012
7. Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, Kinkeldei J, Boehm BO, Weihrauch G, Maerz W. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med*. 2008; 168:1340–1349. [PubMed: 18574092]
8. Wasson LT, Shimbo D, Rubin MR, Shaffer JA, Schwartz JE, Davidson KW. Is vitamin d deficiency a risk factor for ischemic heart disease in patients with established cardiovascular disease? 10-year follow-up of the nova scotia health survey. *International journal of cardiology*. 2011; 148:387–389. [PubMed: 21396725]
9. Laird E, McNulty H, Ward M, Hoey L, McSorley E, Wallace JMW, Carson E, Molloy AM, Healy M, Casey MC, Cunningham C, Strain JJ. Vitamin d deficiency is associated with inflammation in older irish adults. *The Journal of Clinical Endocrinology & Metabolism*. 0:jc.2013-3507.
10. Al Mheid I, Patel R, Murrow J, Morris A, Rahman A, Fike L, Kavtaradze N, Uphoff I, Hooper C, Tangpricha V, Alexander RW, Brigham K, Quyyumi AA. Vitamin d status is associated with arterial stiffness and vascular dysfunction in healthy humans. *Journal of the American College of Cardiology*. 2011; 58:186–192. [PubMed: 21718915]
11. Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-hydroxyvitamin d deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension*. 2011; 57:63–69. [PubMed: 21115878]
12. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation*. 2004; 109:III27–32. [PubMed: 15198963]
13. Karohl C, Vaccarino V, Veledar E, Goldberg J, Tangpricha V, Bellasi A, Raggi P. Vitamin D status and coronary flow reserve measured by positron emission tomography: A co-twin control study. *The Journal of clinical endocrinology and metabolism*. 2013; 98:389–397. [PubMed: 23144471]
14. Jiang W, Babyak M, Krantz DS, Waugh RA, Coleman RE, Hanson MM, Frid DJ, McNulty S, Morris JJ, O'Connor CM, Blumenthal JA. Mental stress--induced myocardial ischemia and cardiac events. *JAMA*. 1996; 275:1651–1656. [PubMed: 8637138]
15. 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]
16. Ramachandruni S, Fillingim RB, McGorray SP, Schmalfuss CM, Cooper GR, Schofield RS, Sheps DS. Mental stress provokes ischemia in coronary artery disease subjects without exercise- or adenosine-induced ischemia. *J Am Coll Cardiol*. 2006; 47:987–991. [PubMed: 16516082]
17. Blumenthal JA, Jiang W, Waugh RA, Frid DJ, Morris JJ, Coleman RE, Hanson M, Babyak M, Thyrum ET, Krantz DS, O'Connor C. Mental stress-induced ischemia in the laboratory and ambulatory ischemia during daily life. Association and hemodynamic features. *Circulation*. 1995; 92:2102–2108. [PubMed: 7554188]

18. Rozanski A, Bairey CN, Krantz DS, Friedman J, Resser KJ, Morell M, Hilton-Chalfen S, Hestrin L, Bietendorf J, Berman DS. Mental stress and the induction of silent myocardial ischemia in patients with coronary artery disease. *N Engl J Med*. 1988; 318:1005–1012. [PubMed: 3352695]
19. 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]
20. Kop WJ, Krantz DS, Howell RH, Ferguson MA, Papademetriou V, Lu D, Popma JJ, Quigley JF, Vernalis M, Gottdiener JS. Effects of mental stress on coronary epicardial vasomotion and flow velocity in coronary artery disease: Relationship with hemodynamic stress responses. *Journal of the American College of Cardiology*. 2001; 37:1359–1366. [PubMed: 11300447]
21. Schoder H, Silverman DH, Campisi R, Karpman H, Phelps ME, Schelbert HR, Czernin J. Effect of mental stress on myocardial blood flow and vasomotion in patients with coronary artery disease. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*. 2000; 41:11–16.
22. Baksi SN, Hughes MJ. Chronic vitamin d deficiency in the weanling rat alters catecholamine metabolism in the cortex. *Brain Res*. 1982; 242:387–390. [PubMed: 6288172]
23. Puchacz E, Stumpf WE, Stachowiak EK, Stachowiak MK. Vitamin d increases expression of the tyrosine hydroxylase gene in adrenal medullary cells. *Brain Res Mol Brain Res*. 1996; 36:193–196. [PubMed: 9011759]
24. Li YC. Vitamin d regulation of the renin-angiotensin system. *J Cell Biochem*. 2003; 88:327–331. [PubMed: 12520534]
25. Beck AT, Steer RA, Ball R, Ranieri W. Comparison of beck depression inventories -ia and -ii in psychiatric outpatients. *Journal of personality assessment*. 1996; 67:588–597. [PubMed: 8991972]
26. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol*. 1983; 51:606. [PubMed: 6823874]
27. Ramadan R, Quyyumi AA, Zafari AM, Binongo JN, Sheps DS. Myocardial ischemia and angiotensin-converting enzyme inhibition: Comparison of ischemia during mental and physical stress. *Psychosomatic medicine*. 2013
28. Kaufmann PG, McMahon RP, Becker LC, Bertolet B, Bonsall R, Chaitman B, Cohen JD, Forman S, Goldberg AD, Freedland K, Ketterer MW, Krantz DS, Pepine CJ, Raczynski J, Stone PH, Taylor H, Knatterud GL, Sheps DS. The psychophysiological investigations of myocardial ischemia (pimi) study: Objective, methods, and variability of measures. *Psychosomatic Medicine*. 1998; 60:56–63. [PubMed: 9492241]
29. Walkers, F.; Soufer, R.; Zaret, BL. Nuclear cardiology. In: Braunwald, E.; Zipes, D.; Libby, P., editors. *Heart disease: A text book of cardiovascular medicine*. Philadelphia, Pennsylvania: W.B. Saunders; 2000. p. 273-323.
30. Goldberg AD, Becker LC, Bonsall R, Cohen JD, Ketterer MW, Kaufman PG, Krantz DS, Light KC, McMahon RP, Noreuil T, Pepine CJ, Raczynski J, Stone PH, Strother D, Taylor H, Sheps DS. Ischemic, hemodynamic, and neurohormonal responses to mental and exercise stress. Experience from the psychophysiological investigations of myocardial ischemia study (pimi). *Circulation*. 1996; 94:2402–2409. [PubMed: 8921780]
31. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin d deficiency: An endocrine society clinical practice guideline. *The Journal of clinical endocrinology and metabolism*. 2011; 96:1911–1930. [PubMed: 21646368]
32. Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA. Serum 25-hydroxyvitamin d status of the us population: 1988-1994 compared with 2000-2004. *Am J Clin Nutr*. 2008; 88:1519–1527. [PubMed: 19064511]
33. Holick MF. Vitamin d deficiency. *N Engl J Med*. 2007; 357:266–281. [PubMed: 17634462]
34. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin d receptor and 1 alpha-hydroxylase in human brain. *J Chem Neuroanat*. 2005; 29:21–30. [PubMed: 15589699]
35. Garcion E, Wion-Barbot N, Montero-Menei CN, Berger F, Wion D. New clues about vitamin d functions in the nervous system. *Trends Endocrinol Metab*. 2002; 13:100–105. [PubMed: 11893522]

36. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis d is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr.* 2004; 79:820–825. [PubMed: 15113720]
37. Zittermann A. Vitamin d and disease prevention with special reference to cardiovascular disease. *Prog Biophys Mol Biol.* 2006; 92:39–48. [PubMed: 16600341]
38. Kalueff AV, Lou YR, Laaksi I, Tuohimaa P. Increased anxiety in mice lacking vitamin d receptor gene. *Neuroreport.* 2004; 15:1271–1274. [PubMed: 15167547]
39. May HT, Bair TL, Lappe DL, Anderson JL, Horne BD, Carlquist JF, Muhlestein JB. Association of vitamin d levels with incident depression among a general cardiovascular population. *Am Heart J.* 2010; 159:1037–1043. [PubMed: 20569717]
40. Dande AS, Sena SF, Wasserman HS, Warshofsky MK, Belsky JL. Prevalence and consequences of vitamin d insufficiency in women with takotsubo cardiomyopathy. *The Journal of clinical endocrinology and metabolism.* 2013
41. Saavedra JM, Benicky J. Brain and peripheral angiotensin ii play a major role in stress. *Stress.* 2007; 10:185–193. [PubMed: 17514587]
42. Davidson ZE, Walker KZ, Truby H. Clinical review: Do glucocorticosteroids alter vitamin d status? A systematic review with meta-analyses of observational studies. *The Journal of clinical endocrinology and metabolism.* 2012; 97:738–744. [PubMed: 22188740]
43. Skversky AL, Kumar J, Abramowitz MK, Kaskel FJ, Melamed ML. Association of glucocorticoid use and low 25-hydroxyvitamin d levels: Results from the national health and nutrition examination survey (nhanes): 2001-2006. *The Journal of clinical endocrinology and metabolism.* 2011; 96:3838–3845. [PubMed: 21956424]
44. Dhawan P, Christakos S. Novel regulation of 25-hydroxyvitamin d3 24-hydroxylase (24(oh)ase) transcription by glucocorticoids: Cooperative effects of the glucocorticoid receptor, c/ebp beta, and the vitamin d receptor in 24(oh)ase transcription. *J Cell Biochem.* 2010; 110:1314–1323. [PubMed: 20564225]
45. Obradovic D, Gronemeyer H, Lutz B, Rein T. Cross-talk of vitamin d and glucocorticoids in hippocampal cells. *J Neurochem.* 2006; 96:500–509. [PubMed: 16336217]
46. Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin d improves endothelial function in patients with type 2 diabetes mellitus and low vitamin d levels. *Diabet Med.* 2008; 25:320–325. [PubMed: 18279409]
47. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin d, ethnicity, and blood pressure in the third national health and nutrition examination survey. *Am J Hypertens.* 2007; 20:713–719. [PubMed: 17586404]
48. Giovannucci E, Liu Y, Rimm EB, Hollis BW, Fuchs CS, Stampfer MJ, Willett WC. Prospective study of predictors of vitamin d status and cancer incidence and mortality in men. *Journal of the National Cancer Institute.* 2006; 98:451–459. [PubMed: 16595781]
49. Jiang W, Samad Z, Boyle S, Becker RC, Williams R, Kuhn C, Ortel TL, Rogers J, Kuchibhatla M, O'Connor C, Velazquez EJ. Prevalence and clinical characteristics of mental stress-induced myocardial ischemia in patients with coronary heart disease. *Journal of the American College of Cardiology.* 2013; 61:714–722. [PubMed: 23410543]

List of Abbreviations

25(OH)D	25-hydroxyvitamin-D
BDI-II	Beck Depression Inventory-II
BP	Blood Pressure
CABG	Coronary Artery Bypass Graft Surgery
CAD	Coronary Artery Disease
LVEF	Left Ventricular Ejection Fraction

MSIMI	Mental Stress-Induced Myocardial Ischemia
PSIMI	Physical Stress-Induced Myocardial Ischemia
RAAS	Renin-Angiotensin-Aldosterone System
RPP	Rate Pressure Product
SDS	Summed Difference Score
SPECT	Single-Photon Emission Computed Tomography

Figure 1. Vitamin D Level According to Overall Ischemia Status

Patients with MSIMI had significantly lower mean 25(OH)D levels regardless of the presence or absence of concomitant PSIMI. P value compares the stated group to patients without ischemia to either physical or mental stress [PSIMI (-)/MSIMI (-)]. The two-tailed unpaired Student's t test was used for comparison.

Figure 2. Relationship between Vitamin D Level and the Severity of Mental Stress-Induced Myocardial Ischemia (MSIMI)

Vitamin D level correlated negatively with the severity of ischemia, measured as sum difference score, in patients with positive MSIMI.

Table 1
Vitamin D concentration Stratified by Risk Factors and Medication Intake

	25(OH)D Concentration (mean ± SD)	P value*
Gender		
Male (n= 193)	30.4±11.9	0.50
Female (n= 62)	31.9±15.2	
Race		
White (n= 175)	33.5±12.5	<0.001
Other (n= 80)	24.9±11.4	
Hypertension		
Yes (n= 180)	30.9±13.5	0.85
No (n= 75)	30.6±10.8	
Diabetes Mellitus		
Yes (n= 79)	29.5±14.2	0.28
No (n= 176)	31.4±12.1	
Hyperlipidemia		
Yes (n= 195)	30.8±13.1	0.97
No (n= 60)	30.7±11.6	
Obesity (BMI>30)		
Yes (n= 101)	28.9±12.7	0.026
No (n= 139)	32.6±12.6	
Depression		
Yes (n= 27)	27.4±9.5	0.15
No (n= 228)	31.2±13.1	
Myocardial Infarction		
Yes (n= 87)	30.3±13.2	0.67
No (n= 168)	31.0±12.6	

	25(OH)D Concentration (mean ± SD)	P value*
CABG		
Yes (n= 95)	31.7±13.1	0.36
No (n= 160)	30.2±12.6	
ACEI		
Yes (n= 99)	30.3±13.9	0.61
No (n= 156)	31.1±12.0	
Beta Blocker		
Yes (n= 177)	30.1±12.9	0.17
No (n= 78)	32.5±12.3	
Statin		
Yes (n= 213)	30.8±12.9	0.93
No (n= 42)	30.6±12.3	

ACEI = angiotensin-converting enzyme inhibitor; BMI = Body Mass Index; CABG = coronary artery bypass graft surgery

*The two-tailed unpaired Student's t test was used for comparison.

Table 2

Clinical Characteristics of Study Population

Total	Exercise/Pharmacologic Stress Ischemia		Mental Stress Ischemia	
	Negative	Positive	Negative	Positive
	N=183	N=67	N=225	N=30
Age (mean $\pm$ SD), (years)	64 $\pm$ 8	65 $\pm$ 7	65 $\pm$ 8	65 $\pm$ 7
		0.88		0.58
Male gender, n (%)	193 (76%)	133 (73%)	172 (76%)	21 (70%)
		0.042		0.44
Previous MI, n (%)	87 (34%)	19 (28%)	75 (33%)	12 (40%)
		0.22		0.47
Previous PTCA, n (%)	150 (59%)	105 (57%)	131 (58%)	19 (63%)
		0.45		0.59
Previous CABG, n (%)	95 (37%)	60 (33%)	81 (36%)	14 (47%)
		0.030		0.26
Angina in the past 4 weeks, n (%)	74 (29%)	49 (27%)	61 (27%)	13 (43%)
		0.39		0.073
LVEF (mean $\pm$ SD), (%)	59 $\pm$ 15	58 $\pm$ 13	60 $\pm$ 15	58 $\pm$ 11
		0.19		0.44
Cardiovascular risk factors				
Hypertension, n (%)	180 (71%)	125 (68%)	157 (70%)	23 (77%)
		0.23		0.44
Dyslipidemia, n (%)	195 (76%)	133 (73%)	171 (76%)	24 (80%)
		0.042		0.63
Diabetes, n (%)	79 (31%)	50 (27%)	66 (29%)	13 (43%)
		0.080		0.12
Depression, n (%)	27 (11%)	20 (11%)	23 (10%)	4 (13%)
		0.65		0.60
Current or ex-smokers, n (%)	150 (59%)	108 (59%)	131 (58%)	19 (63%)
		0.80		0.63
Treatment at study entry				
Beta Blocker, n (%)	177 (69%)	123 (67%)	160 (71%)	17 (57%)
		0.37		0.11
ACEI, n (%)	99 (39%)	69 (38%)	86 (38%)	13 (43%)
		0.87		0.59
Statin, n (%)	213 (84%)	154 (84%)	187 (83%)	26 (87%)
		0.70		0.62
CCB, n (%)	49 (19%)	38 (21%)	44 (20%)	5 (17%)
		0.44		0.71
Hemodynamic Measures				

Total	Exercise/Pharmacologic Stress Ischemia		Mental Stress Ischemia	
	Negative	Positive	Negative	Positive
	N=183	N=67	N=225	N=30
Peak RPP (mean $\pm$ SD), (mmHg*beats/min)	22745 $\pm$ 3880	22743 $\pm$ 4607	12900 $\pm$ 3277	13972 $\pm$ 3566
				0.10
Angiographic Severity				
Gensini Severity Score, median (IQR)	18 (2–50)	46 (11–91)	29 (4–57)	20 (4–54)
				0.76

ACEI = angiotensin-converting enzyme inhibitor; CABG = coronary artery bypass graft surgery; CCB = calcium channel blocker; IQR = Interquartile range; LVEF = left ventricular ejection fraction; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty; RPP = rate-pressure product; SD = standard deviation.

P value compares participants with and without ischemia. The two-tailed unpaired Student's t test was used for comparison of normally distributed continuous variables. The Mann-Whitney U test was used to compare the difference in non-normally distributed variables. The chi-square test was used for comparison of categorical variables.

Table 3

Hemodynamic changes in Response to Mental Stress

	Mental Stress Ischemia		Vitamin D Insufficiency	
	Negative N=225	Positive N=30	No N=116	Yes N=139
Systolic BP (mm Hg)				
Rest	126±16	136±16	124±16	130±17
Mental stress	166±21	179±25	164±21	170±22
Reactivity	40±15	43±22	40±16	40±17
Diastolic BP (mm Hg)				
Rest	73±10	76±10	72±10	74±11
Mental stress	97±13	105±15	97±13	99±14
Reactivity	24±11	28±13	24±10	25±12
Heart rate (beats/min)				
Rest	61±11	59±11	61±12	60±11
Mental stress	77±15	78±18	78±16	77±15
Reactivity	17±10	19±14	17±11	17±11
RPP (mmHg*beats/min)				
Mental stress	12900±3277	13972±3566	12895±3299	13136±3350

BP = blood pressure; RPP = rate pressure product (mmHg*beats per minute)

Reactivity = the magnitude of peak hemodynamic increase during mental stress testing from baseline

Values are shown as mean ± standard deviation. The two-tailed unpaired Student's t test was used for comparison.