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ORIGINAL RESEARCH

Oxidative Stress Is Associated With Diastolic Dysfunction in Women With Ischemia With No Obstructive Coronary Artery Disease

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BACKGROUND: Women with signs and symptoms of ischemia and no obstructive coronary artery disease often have evidence of diastolic dysfunction. Oxidative stress (OS) is associated with cardiovascular risk factors and adverse outcomes. The relationship between systemic OS and diastolic dysfunction is unknown.

METHODS AND RESULTS: A subgroup of women (n=75) with suspected ischemia and no obstructive coronary artery disease who had both cardiac magnetic resonance imaging and OS measurements were enrolled in the WISE-CVD (Women Ischemia Syndrome Evaluation—Coronary Vascular Dysfunction) study. Left ventricular end-diastolic pressure was measured invasively. Left ventricular end-diastolic volume and peak filling rate were assessed using cardiac magnetic resonance imaging. Aminothiol levels of plasma cystine and glutathione were measured as markers of OS. Spearman correlation and linear regression analyses were conducted. The group mean age was 54±11 years, and 61% had a resting left ventricular end-diastolic pressure >12 mm Hg. Cystine levels correlated negatively with the peak filling rate (r=−0.31, P=0.007) and positively with left ventricular end-diastolic pressure (r=0.25; P=0.038), indicating that increased OS was associated with diastolic dysfunction. After multivariate adjustment including multiple known risk factors for diastolic dysfunction and cardiovascular medications, cystine levels continued to be associated with peak filling rate (β=−0.27, P=0.049) and left ventricular end-diastolic pressure (β=0.25; P=0.035). Glutathione levels were not associated with indices of diastolic function.

CONCLUSIONS: OS, measured by elevated levels of cystine, is associated with diastolic dysfunction in women with evidence of ischemia and no obstructive coronary artery disease, indicating the role of OS in patients with ischemia and no obstructive coronary artery disease. Its role in the progression of heart failure with preserved ejection fraction should be explored further.

Key Words: cardiac MRI ■ diastolic dysfunction ■ INOCA ■ oxidative stress

Two thirds of women undergoing clinically indicated coronary angiography in the original WISE (Women Ischemia Syndrome Evaluation) study had signs and symptoms of ischemia in the setting of no obstructive coronary artery disease (INOCA).[1] These women often have coronary vascular dysfunction, which is associated with future adverse cardiovascular outcome.[2] We previously demonstrated that diastolic function is often impaired in women with evidence of INOCA as assessed by cardiac magnetic resonance imaging (CMRI).[3,4] Elevation of resting left-ventricular end-diastolic pressure (LVEDP) is also a marker of diastolic dysfunction and associated with a higher mortality in ischemic heart disease independent of left ventricular systolic function.[5,6] Furthermore, diastolic dysfunction can progress to heart failure

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CLINICAL PERSPECTIVE

What Is New?

- Elevated levels of serum cystine, a marker of oxidative stress, are associated with diastolic dysfunction in women with ischemia and no obstructive coronary artery disease.
- This is the first human study to demonstrate that oxidative stress is associated with diastolic dysfunction using cardiac magnetic resonance imaging and resting left-ventricular end-diastolic pressure.

What Are the Clinical Implications?

- The study paves way for further investigation into the prognostic and therapeutic implications of oxidative stress in diastolic dysfunction and heart failure with preserved ejection fraction.

Nonstandard Abbreviations and Acronyms

CMD	coronary microvascular dysfunction
CMRI	cardiac magnetic resonance imaging
HFpEF	heart failure with preserved ejection fraction
INOCA	ischemia and no obstructive coronary artery disease
LVEDP	left-ventricular end-diastolic pressure
OS	oxidative stress
PFR	peak filling rate
WISE-CVD	Women's Ischemia Syndrome Evaluation-Coronary Vascular Dysfunction

with preserved ejection fraction (HFpEF),[7] decreased ejection fraction,[8] and increased mortality.[7–9] While there are well-defined associated clinical risk factors for the development of diastolic dysfunction,[8] the specific pathophysiology is not completely understood.

Oxidative stress (OS) occurs when cellular pro-oxidants overwhelm cellular anti-oxidant defense mechanisms and disrupt redox signaling and control. Systemic OS can be readily estimated by measurement of circulating aminothiol levels.[10] In the intracellular space, glutathione plays an essential role to help eliminate peroxides and other oxidants and preserve the redox state of numerous biomolecules.[11] In the extracellular space, cysteine plays a critical role as an antioxidant, and cystine, its oxidized disulfide form, serves as an important measure of systemic OS. Therefore, lower circulating levels of glutathione and higher levels of cystine reflect a relative deficiency of the anti-oxidants and overproduction of oxidants and vice versa.[12–14] Higher OS using these measures is associated with increased age, diabetes mellitus, smoking, impaired endothelial function, increased arterial stiffness, carotid atherosclerosis, myocardial stiffness, and higher rates of adverse cardiovascular events.[13–19]

We investigated the hypothesis that OS, measured using circulating levels of aminothiols cystine and glutathione, is associated with diastolic dysfunction measured invasively and noninvasively in women with evidence of INOCA.

METHODS

Study Population

Women with INOCA and normal left ventricular ejection fraction (defined as ejection fraction ≥55%), referred for coronary angiography, were enrolled as part of the National Heart, Lung, and Blood Institute–sponsored WISE-CVD study (NCT00832702). The data that support the findings of this study are available from the corresponding author upon reasonable request. INOCA is defined as symptoms (usually angina and/or dyspnea) with signs suggesting ischemia (ECG changes during exercise, and wall motion or perfusion abnormalities during echocardiography or nuclear imaging)[20] and absence of obstructive coronary artery disease by core laboratory (epicardial coronary artery diameter stenosis <50%). Invasive functional measurements (ie, fractional flow reserve and instantaneous wave-free ratio) were performed, as clinically indicated, at the discretion of the interventional cardiologist. Women with primary cardiomyopathies and valvular heart disease were excluded.[21] A subgroup of subjects (n=75 women) who had complete cardiac catheterization, CMRI, and OS assessment were included in this analysis. The definition of clinical risk factors is as previously published in the WISE-CVD study.[22]

Subjects provided written informed consent as approved by the institutional review board at Cedars-Sinai Medical Center, Los Angeles and the University of Florida, Gainesville. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's research committee.

Diastolic Function Assessments

Subjects undergoing catheterization were referred for evaluation of their symptoms and comprehensive coronary reactivity assessment.[23] The catheterization preparation protocols are as previously described.[23,24] Subjects fasted for 12 hours and

withheld any nicotine or sublingual nitroglycerin for 4 hours and any vasoactive agents or caffeine for 24 hours before catheterization. After confirming the absence of obstructive coronary artery disease by clinically indicated angiography, women underwent evaluation of resting LVEDP using a pig-tail catheter positioned in the left ventricle. Left ventricular diastolic dysfunction is defined as LVEDP >12 mm Hg. Diastolic function was also assessed noninvasively by CMRI. All CMRIs were performed using the same equipment and protocol at both centers (1.5 T Magnetom Avanto, Siemens Healthcare, Erlangen, Germany) with ECG gating and a phase-array surface coil (CP Body Array Flex, Siemens Medical Systems, Erlangen, Germany). Analyses of CMRI data were performed at the WISE-CVD CMRI Core Lab at Cedars-Sinai Medical Center, Los Angeles using the Argus software (Siemens Medical) by 1 experienced analyst (L.E. Thomson) who traced the endocardial contours manually from end-systole to end-diastole and measured end-systolic volume and end-diastolic volume; protocol details have been previously published.[21] End-diastolic frames were used to calculate septal wall thickness and left ventricle mass, while stroke volume was calculated as the difference between end-diastolic volume and end-systolic volume, and ejection fraction was calculated as stroke volume divided by end-diastolic volume. Volume–time curves were used to derive indices of diastolic dysfunction, including early peak filling rate (PFR). PFR increases with diastolic dysfunction grade, similar to E/e' by echocardiography.[25,26] The exception is the progression from normal to grade I diastolic dysfunction, as PFR mildly decreases (compared with echocardiography, where E/e' ratio mildly increases).[27] Therefore, PFR normalized for end-diastolic volume is used and has been demonstrated to be a useful index for evaluating diastolic function.

Measurement of OS

At the time of cardiac catheterization, arterial blood was collected from the femoral sheath. Blood samples were transferred into Eppendorf tubes containing preservatives to prevent auto-oxidation, centrifuged, and stored at −80°C. We have shown previously that samples are stable under these conditions for ≈1 year.[15,28,29] Plasma levels of cystine and glutathione were measured using high-performance liquid chromatography after dansyl derivatization on a 3-aminopropyl column with fluorescence detection.[29] Metabolites were identified by co-elution with standards and quantified by integration relative to the internal standards, with validation relative to external standards.[15,28,29] The coefficients of variation for these metabolites are 5% for glutathione and 3.2% for cystine.

Statistical Analysis

Clinical variables are summarized using mean±SD or a count (%) for categorical variables. Data normality were evaluated using the Kolmogorov–Smirnov criterion. Differences between groups were assessed using the *t* test. For non-normally distributed variables, the Mann–Whitney *U* test was used to compare groups in unadjusted analyses. Relationships between the plasma aminothiols and diastolic function measurements were examined using the Spearman's correlation analyses. Tests to see whether our data met the assumption of collinearity indicated that multicollinearity was not a concern (none of the tolerance values were <0.1 and all the variance inflation factors were <1.5). Multivariate linear regression analyses were performed to examine the relationship between OS, diastolic function, and clinical variables. Clinical

Table 1. Baseline Characteristics

Variables	Mean±SD or %
Age, y	54±11
Race (nonwhite), N (%)	21 (28)
Diabetes mellitus, N (%)	8 (11)
Hypertension, N (%)	26 (35)
History of smoking, N (%)	34 (45)
Body mass index, kg/m^2	30±8
Cholesterol, mg/dL	182±34
Low-density lipoprotein, mg/dL	100±36
High-density lipoprotein, mg/dL	57±15
Triglyceride, mg/dL	120±67
Oxidative stress	
Cystine, μmol/L	70±21.7
Glutathione, μmol/L	2.5±1.4
Left ventricular function	
Ejection fraction, %	69±7
EDV, mL/m^2	126±22
PFR (EDV/s)	3.1±0.6
Time to PFR, ms	197±39.3
LVEDP, mm Hg	16±6
Medications	
Aspirin, N (%)	60 (80)
Statin, N (%)	41 (55)
Beta blockers, N (%)	36 (48)
Calcium channel blockers, N (%)	15 (20)
ACEI/ARB, N (%)	19 (25)
Nitrates, N (%)	37 (79)

ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; EDV, end-diastolic volume; LVEDP, left ventricular end-diastolic pressure; and PFR, peak filling rate.

variables entered in this regression model were age, body mass index, race, history of hypertension, diabetes mellitus, smoking, hyperlipidemia, and cardiovascular medications. A significance level of 0.05 was used for all analyses. Analyses were performed using IBM SPSS software version 24 (IBM, New York, NY).

RESULTS

Baseline characteristics are summarized in Table 1. The mean age was 54±11 years, 11% had diabetes mellitus, 35% hypertension, and 45% a history of previous smoking. The cystine, but not glutathione level, correlated with age (r=0.38; P<0.001) and hypertension (r=0.25; P=0.031).

Diastolic Function

Forty-six subjects (61%) had an elevated LVEDP of >12 mm Hg at cardiac catheterization. There was no correlation between the LVEDP and PFR (r=−0.04, P=0.74). PFR was inversely correlated with age (r=−0.36; P=0.002) and hyperlipidemia (r=−0.23; P=0.047). LVEDP was not associated with age, history of hypertension, diabetes mellitus, or hyperlipidemia.

Relationship Between OS and Diastolic Function

Cystine levels inversely correlated with the PFR (r=−0.31, P=0.007) and positively correlated with LVEDP (r=0.25; P=0.038) (Figure 1), indicating that higher OS is associated with increased diastolic dysfunction. Cystine levels were significantly higher in subjects with high LVEDP (>12 mm Hg) versus those with low LVEDP (both P=0.02) (Figure 2). In multivariate regression analyses adjusted for the aforementioned clinical covariates, the plasma cystine level remained associated with a higher LVEDP (Table 2). For every unit increase in the PFR, the plasma cystine was lower by 14.5 µmol/L and for every unit increase in LVEDP, plasma cystine was higher by 0.8 µmol/L. We found no significant associations between plasma glutathione level and diastolic parameters (ie, PFR and LVEDP) in this cohort using both univariate and multivariate analyses.

DISCUSSION

We demonstrate that systemic OS, measured as cystine levels, is associated with diastolic dysfunction, assessed invasively and noninvasively, even after adjusting for common comorbidities affecting diastolic function. Glutathione levels did not correlate with PFR or LVEDP. To our knowledge, this is the first study to investigate the relationship between OS and diastolic dysfunction using PFR derived from CMRI in humans.

We have previously reported that women with evidence of INOCA have impaired diastolic dysfunction compared with healthy reference subjects as assessed by CMRI.[4] We also have shown that 40% of these women have elevated LVEDP.[3] We have now extended our prior findings by demonstrating that subclinical diastolic dysfunction is frequently prevalent in women with INOCA and that it is associated with higher levels of systemic OS.

The role of OS in the pathophysiology of diastolic function is poorly understood in humans. Oxidative stress is also a complex with multiple interplays between extracellular and intracellular (both cytosolic

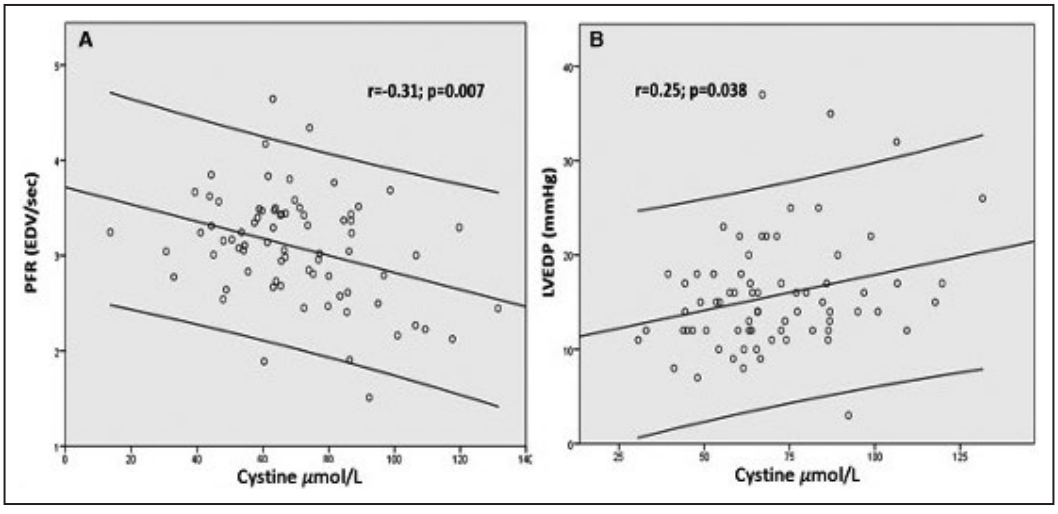

Figure 1. Correlations between (A) plasma cystine level and PFR, (B) plasma cystine level and LVEDP.
EDV indicates end diastolic volume; LVEDP, left ventricular end-diastolic pressure; and PFR, peak flow rate.

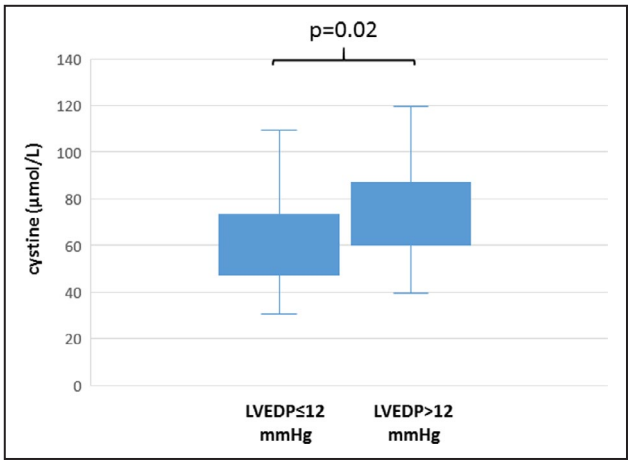

Figure 2. Differences in cystine levels between subjects with high and low LVEDP.
LVEDP indicates left ventricular end-diastolic pressure.

and compartmental) levels of different OS markers. In cardiac myocytes, experimental studies demonstrate that with increasing OS, myocytes exhibit alterations in myocardial structure and function, increased myocardial collagen content, elevated passive myocyte stiffness, increased left ventricular end-diastolic stiffness, and increased E/A ratio as measured by CMRI.[16,30] The sarcoplasmic proteins, including myosin binding protein-C, are phosphorylated by reactive oxygen species sensitive enzymes[31] that in turn leads to increased myofilament Ca^{2+} sensitivity[32,33] and impaired cardiac relaxation.[34] This is also seen with decreased levels of nitric oxide, which modulates cardiac relaxation via phosphorylation of downstream molecules and subsequent sarcomere stiffening[35,36] and hypertrophic signaling.[37]

In our study, LVEDP did not correlate with PFR, as previously observed.[3] The most likely explanation is that the LVEDP and PFR were measured at 2 different time-points. Given that our study excluded patients with clinical heart failure, both LVEDP and PFR were likely in their early phase and small fluctuations in other physiological parameters at 2 different time-points may explain why they did not correlate.

We have previously demonstrated that the majority of women with INOCA in the original WISE cohort with no obstructive coronary artery disease had coronary microvascular dysfunction (CMD).[38] Other work has demonstrated that CMD is associated with OS[39] and is also associated with diastolic dysfunction and development of clinical HFpEF.[40] Our current study results demonstrate a correlation between OS and diastolic dysfunction. Given the cross-sectional nature of our study, we cannot confer causality between OS and diastolic dysfunction. Furthermore, it is unclear whether OS and CMD lie within the same linear sequence to develop diastolic dysfunction. While 1 previous study has shown that OS precedes CMD,[39] this is an area that requires further prospective investigation, as the link between OS, CMD, and development of HFpEF will be critical to define as it is.

Table 2. Relationship Between Clinical Parameters and Diastolic Function Measures, and Plasma Cystine Level After Multivariate Regression Analyses

	Multivariate Analysis	
	β Value	*P* Value
Age	0.32	0.24
Body mass index	−0.17	0.59
History of hypertension	5.5	0.35
Diabetes mellitus	−6.3	0.41
History of smoking	−3.9	0.44
Hypercholesterolemia	0.13	0.96
Medications		
Aspirin	−4.7	0.46
Beta-blockers	−1.2	0.8
Calcium channel blockers	4.6	0.5
Statin use	−6.3	0.19
Nitroglycerine use	2.1	0.59
ACEI/ARB	11.7	0.08
Left ventricular end-diastolic pressure	0.8	0.047*
Peak filling rate	−14.6	0.009*

ACEI indicates angiotensin-converting enzyme inhibitor; and ARB, angiotensin receptor blocker.
**P* value is statistically significant.

Strengths and Limitations

This is the first study in humans to demonstrate that high systemic OS, measured by plasma aminothiol, cystine, is associated with measures of diastolic dysfunction using CMRI. Limitations of the study are that the cohort consists of only female subjects with signs and symptoms of ischemia undergoing clinically indicated cardiac catheterization, and thus the findings may not generalize to other groups. Furthermore, we did not find an association between glutathione levels and diastolic function, perhaps because it is an intracellular moiety and accurate measurements are not possible in a study like ours. We only measured cystine and glutathione as an indicator of OS. However, these 2 markers have been shown to be correlated with subclinical cardiovascular disease and its progression.[15,19,41] During angiography, LVEDP can be affected by various vasoactive and sedative agents. Per protocol,[23] women were instructed to hold off taking long-acting nitrates, ranolazine, short-acting calcium, channel blockers, α-blockers, β-blockers,

aldosterone inhibitors, and angiotensin-converting enzyme I/angiotensin II receptor antagonists for 24 hours, and long-acting calcium channel blockers were held for 48 hours before invasive coronary angiography. Additionally, most women received moderate sedation as clinically indicated during cardiac catheterization. However, previous study showed that conscious sedation does not alter indices of the left ventricular diastolic function in healthy individuals and those with pre-existing diastolic dysfunction.[42] Finally, because of the cross-sectional nature of our study, we cannot confer causality between OS and diastolic dysfunction. Therefore, future investigations are needed to prove causality. The strength of the study includes addressing the area of development of diastolic dysfunction and HFpEF in patients with CMD, which remains poorly understood.

CONCLUSIONS

OS, measured as elevated plasma cystine levels, is associated with diastolic dysfunction, measured by resting LVEDP and CMRI PFR in women with INOCA. Whether OS is related mechanistically to progression to HFpEF in women with evidence of INOCA needs further exploration.

ARTICLE INFORMATION

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