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Approval Sheet

Race and sex differences in nitroso-redox balance and arterial stiffness in patients with heart failure with reduced ejection fraction

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ABSTRACT

Background: Black patients and women with heart failure with reduced ejection fraction (HFrEF) have worse health status than White patients and men, respectively. We sought to determine if differences in oxidative stress and arterial stiffness in these groups may explain the pathophysiology underlying these disparities.

Methods: Patients with HFrEF (N=205, 51% female, 62% Black) were recruited at Emory University from 2015-2019. At a single study visit, indices of arterial stiffness and wave reflections were measured including carotid-femoral pulse wave velocity (PWV), augmentation index normalized at heart rate of 75 (AIx @ HR 75), and reflection magnitude (RM). Plasma levels of nitrites and aminothiol markers of oxidative stress (OS) reduced (cysteine [Cys] and glutathione [GSH]) and oxidized (cystine [CySS] and glutathione disulphide [GSSG]) were quantified by high performance liquid chromatography to assess systemic nitroso-redox balance. Multivariable linear regression was used to determine the association between OS and arterial stiffness measures. The association between arterial stiffness and a composite clinical endpoint (death, left ventricular assist device implantation, or heart transplant) was assessed using cox proportional hazards analysis. Significant interactions between race, sex, OS biomarkers, and arterial stiffness were tested within adjusted models using natural cubic splines.

Results: Levels of OS and arterial stiffness were similar between Black and White patients. Compared to men, women had lower levels of OS (Cys: 8.2 [6.5, 9.7] v. 7.5 [5.4, 9.5] µM, P=0.034, GSSG: 0.04 [0.01, 0.06] v. 0.05 [0.02, 0.10] µM, P=0.023) and greater wave reflections (AIx @ HR 75: 22.0 [17.3, 30.1] v. 17.3 [5.3, 25.5] %, P=0.007). OS biomarkers were not associated with arterial stiffness. There was no association between any arterial stiffness measures and the composite primary endpoint (PWV: hazard ratio [HR] 0.62, 95% confidence interval [CI] 0.29-1.32, AIx @ 75 HR: 0.91, 95% CI 0.65-1.28, RM: HR 0.77, 95% CI 0.32-1.90).

Conclusion: In a cohort of well-treated patients with HFrEF, OS was not associated with impaired arterial stiffness, and arterial stiffness measures were not predictive of clinical outcomes.

Race and sex differences in nitroso-redox balance and arterial stiffness in patients with heart failure with reduced ejection fraction

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INTRODUCTION

Heart failure (HF) affects nearly 6 million Americans, with approximately 50% of cases classified as heart failure with reduced ejection fraction (HFrEF).[1] Despite increasing availability of pharmacologic and device-based therapies for HFrEF, important disparities exist in the incidence of HFrEF by race and gender. Black patients have a higher incidence of HFrEF compared to other race-ethnic groups and are more likely to have nonischemic etiology with an earlier age of onset.[2,3] Women are less likely to have HFrEF compared to men, are more likely to have nonischemic etiologies, and have higher comorbidity burden than men with HFrEF.[4] Moreover, women and Black patients with HFrEF have been shown to have worse health status, defined by patient symptoms, physical and social limitations, and quality of life.[5] Black patients with HF also have a higher risk of HF hospitalization, morbidity, and mortality compared to White patients, even when accounting for social and clinical characteristics.[6]

Disparities in the incidence of HFrEF by race and sex may be due in part to underlying differences in HF pathophysiology, including impairments in nitric oxide bioavailability and arterial stiffness. Nitric oxide (NO) is a potent vasodilator in the cardiovascular system, regulating mechanisms responsible for the excitation-contraction coupling and calcium regulation of myocytes centrally and endothelial smooth muscle relaxation in the peripheral vasculature.[7] Impaired NO regulation via oxidative stress (OS), termed the nitroso-redox balance, may contribute to cardiomyocyte damage leading to eccentric remodeling seen in HFrEF.[7] Levels of OS can be estimated by examining protein oxidation via aminothiol residues altered by OS, with increased cystine levels reflecting extracellular OS and decreased glutathione levels reflecting intracellular OS (**Figure 1**).[8] Prior analyses have confirmed aminothiol markers of OS are associated with endothelial dysfunction, arterial stiffness, cardiovascular disease (CVD) risk factors, and clinical outcomes.[8-10] Moreover, prior work demonstrates higher levels of OS in Black patients, which may be in part due to impairment in NO-mediated mechanisms, inducing more severe endothelial dysfunction.[9,11]

Disruptions in nitroso-redox balance may exacerbate hemodynamic instability in HFrEF. Widespread endothelial dysfunction disrupts vascular homeostasis, causing increased afterload and resistance to forward blood flow.[12,13] Similarly, coronary endothelial dysfunction can cause myocardial ischemia, impaired contractility and long-term alterations of NO production.[13] Additionally, increased stiffness throughout the arterial tree can increase the velocity of blood flow in both forward and backward directions, necessitating an increase in systolic pressure to overcome reflected waves on the heart.[14]

Black patients with HF have been shown to have worse endothelial function and arterial stiffness compared to White patients.[15,16] Similarly, women have been shown to have worse arterial stiffness than men, though these data are primarily derived from cohorts with heart failure with preserved ejection (HFpEF).[17,18] In this study, we sought to examine both sex- and race-based differences in the nitroso-redox balance in patients with HFrEF. Additionally, we examined the association between nitroso-redox balance, arterial stiffness, and the central pressure-flow relationship, as well as the association of arterial stiffness with clinical outcomes including differences by race and sex.

METHODS

Study population. Self-identified Black and White subjects (age ≥ 18 years) were screened for eligibility from the outpatient HF clinics at the Emory University Hospitals from 2015-2019. Patients were recruited using the following inclusion criteria: 1) EF ≤40% by echocardiogram due to ischemic or non-ischemic etiology; and 2) NYHA class II-IV HF symptoms for 3 months despite guideline-directed medical therapy. Patients were excluded for the following reasons: 1) HF etiology including hypertrophic or restrictive cardiomyopathy, constrictive pericarditis, or complex congenital heart disease; 2) prior heart transplant (HT) or left ventricular assist device (LVAD); 3) any conditions other than HF that are likely to alter the patient's status over 6 months; 4) end-stage HF requiring continuous inotrope infusion; 5) serum creatinine > 3 mg/dL or estimated glomerular filtration rate (eGFR) < 20 ml/min/1.73m^2.

Study visit. After informed consent and enrollment, interviews and medical records were used to collect demographics, medical and procedure history, current medications, and laboratory results. All study visits were carried out in the Emory Clinical Research Center. This study was approved by the Emory University Institutional Review Board.

Measures of HF severity. Severity of illness was assessed using NYHA functional class[19] and B-type natriuretic peptide (BNP, Quest Diagnostics).[20,21]

Biomarkers of nitroso-redox balance. Plasma (~40cc) was collected and processed using standard methodology and stored on a designated rack and shelf at -80°C. Specimens were analyzed in a core lab according to previously described procedures to measure aminothiol markers of OS and plasma nitrite concentrations.[9,22-27] Plasma glutathione (GSH), cysteine CyS), and their oxidized products glutathione disulfide (GSSG) and cystine (CySS) were assayed using high performance liquid chromatography (HPLC)[28] and fluorescence detection following N-dansyl derivatization of plasma. The redox potentials of GSH and CyS (in millivolts)

were calculated using the Nernst equation. Plasma nitrite concentrations were quantified by ion chromatography (ENO20 Analyzer, Eicom).

Measures of arterial stiffness and wave reflections. All measurements were performed prior to venipuncture for biomarkers, in a quiet, temperature-controlled environment set at 22°C after an overnight fast and were made with participants in the supine position after a 10-minute rest period. Patients were asked to hold vasoactive medications on the day of their study visit. Augmentation index (AIx) and pulse wave velocity (PWV) were measured using radial pulse wave analysis (SphygmoCor®, Atcor).[29] In brief, peripheral pressure waveforms are recorded from the radial artery at the wrist, using applanation tonometry with a high-fidelity micromanometer. AIx is derived and normalized for heart rate of 75 bpm (AIx @ HR 75). Carotid-femoral PWV is determined using transcutaneous Doppler flow velocity simultaneously over the common carotid and femoral arteries. A physiologic proximal aortic flow waveform[30] estimated using pulse wave Doppler at the LV outflow tract (LVOT) as an estimate of stroke volume (SV) was used for separation of the pressure wave into forward and reflected waves.[31] Reflection Magnitude (RM) was calculated as [backward wave amplitude / forward wave amplitude X 100].[32]

Clinical outcomes of interest. The primary clinical outcome of interest was a composite of death, HT, or LVAD implantation. The secondary outcome of this study was a composite endpoint of death or hospitalization. All-cause, cardiovascular, and HF-specific hospitalizations were recorded and adjudicated by an independent review committee. Patients were actively followed for the occurrence of clinical events every 6-months after study enrollment. Patients without available follow-up data were contacted by phone. For patients completely lost to follow up, death data was ascertained by the Social Security Death Index query.

Statistical analysis. Data are presented as mean ± standard deviation (SD), median (interquartile range [IQR]), or N (%) of patients. Baseline characteristics were compared between patients according to race and sex using Student's *t* test for normally distributed continuous variables, non-parametric Mann-Whitney U test for non-normally distributed continuous variables, and the chi-square or Fisher's exact test for categorical variables.

Biomarkers of OS and NO and measurements of arterial stiffness were analyzed as continuous variables. Data was log-transformed to achieve near-normal distribution when appropriate. The association of nitroso-redox balance with each measurement of arterial stiffness was analyzed using linear regression. Linear regression models were adjusted for potential confounders selected using a directed acyclic graph (DAG) (**Supplemental Figure 1**). These confounders included age, HF etiology, hypertension, mean arterial pressure (MAP), and

body mass index (BMI). Interactions terms for CySS*race, GSH*race, CySS*sex, and GSH*sex were utilized to determine differences in the association of OS with arterial stiffness measures by race or by sex. Significant interactions were tested using stratification by either sex or race using cubic splines with three degrees of freedom.

The association between arterial stiffness and the primary and secondary composite endpoints was assessed using cox proportional hazards models. Cox models were adjusted for potential confounders selected using a DAG (**Supplemental Figure 1**). These confounders included age, HF etiology, hypertension, MAP, BMI, eGFR, and BNP. Time-dependent covariates were included in the model where the proportional hazards assumption was not fulfilled. Interaction terms for race*arterial stiffness measure and sex*arterial stiffness measure were utilized to determine differences in the association of arterial stiffness measures with the primary outcome by race or sex. Significant interactions by either sex or race were visualized using natural cubic splines with three degrees of freedom.

Data were analyzed using SAS statistical software version 9.4 (SAS Institute Inc.) and RStudio version 1.3.1073 (The Comprehensive R Archive Network: https://cran.r-project.org). A two-sided P-value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics. Baseline characteristics of the 205 HFrEF patients evaluated are displayed in **Table 1**. Compared to White patients, Black patients were more likely to have nonischemic HF, high blood pressure, elevated uric acid, and higher BMI (**Table 2**). Black patients were also more likely to be on outpatient diuretics and hydralazine than White patients. Black patients were less likely to have a history of coronary arterial bypass graft (CABG) and elevated triglycerides. Compared to males, females were younger, have higher HDL levels and higher NYHA class (**Table 3**). Females were less likely to have a history of CABG than males.

Differences in nitroso-redox balance and arterial stiffness by racial group. Levels of GSH, CySS, E_h Cys, E_h GSH, and nitrite metabolites did not differ significantly between Black and White patients (**Table 2A**). Compared to White patients, Black patients had higher diastolic blood pressures both peripherally and centrally, but there was no difference in systolic pressures. There were no significant differences by race in PWV, AIx or RM (**Table 2B**).

Differences in nitroso-redox balance and arterial stiffness by sex group. Females were more likely to have higher levels of Cys, lower levels of GSSG, and higher E_h GSH compared to males (**Table 2A**), but similar nitrite levels and E_h Cys. AIx @ HR 75 was higher in males than

females. Females had lower peripheral and central diastolic blood pressures compared to males (**Table 2B**).

Association of nitroso-redox balance with arterial stiffness. On univariate analysis, there was a significant association between GSH and PWV (β=0.185, P-value=0.03) and between CySS and AIx @ HR 75 (β=0.199, P-value=0.030). Adjusting for covariates eliminated the associations between OS markers and arterial stiffness measures (**Table 3**). For RM, there was a significant interaction between CySS*sex (P=0.015), such that as cystine levels increased, women had an increase in RM while men demonstrated a decrease in RM (**Figure 2**).

Association of arterial stiffness with death, LVAD implantation, and HT. On univariate analysis, AIx @ HR 75 was associated with the primary composite endpoint (Hazard Ratio [HR] 0.68, 95% Confidence Interval [CI] 0.54-0.86), however this was attenuated in the full model (adjusted HR [aHR] 0.91, 95% CI 0.63-1.28) (**Table 4A**). PWV (aHR 0.86, 95% CI 0.70-1.06) and RM (aHR 0.86, 95% CI 0.06-12.63) were not significant predictors of the primary endpoint. For the primary endpoint, there was a significant interaction between PWV*sex (P=0.013), such that as PWV increased, the likelihood of women experiencing the primary endpoint decreased at a higher rate compared to men (**Figure 3A**).

Association of arterial stiffness with death or hospitalization. On univariate analysis, AIx @ HR 75 (HR 0.77, 95% CI 0.63-0.96) and RM (uHR 0.51, 95% CI 0.30-0.89) were found to be significant predictors of the secondary composite endpoint (**Table 4B**). However, these associations were attenuated in the full model for AIx @ HR 75 (aHR 0.93, 95% CI 0.72-1.19) and RM (aHR 0.62, 95% CI 0.34-1.11). For the secondary endpoint, there was a significant interaction between PWV*race (0.047), such that higher PWV in Black patients was associated with an increased likelihood of the secondary endpoint, compared to White patients who experienced a decrease in the likelihood of the secondary endpoint (**Figure 3B**).

DISCUSSION

In a cohort of patients with HFrEF who underwent comprehensive phenotyping to assess nitroso-redox balance and measures of arterial stiffness, we identified that: 1) OS biomarkers were not associated with arterial stiffness measures, 2) any univariate associations between measures of arterial stiffness and clinical events were explained by traditional cardiovascular risk factors after adjustment, 3) despite worse arterial stiffness in women, rates of clinical outcomes were similar among men and women, and 4) there were no differences in arterial stiffness measures and clinical outcomes by race. To our knowledge, this is the first study to

examine the association of OS measures with arterial stiffness measures in a HFrEF population, while taking into account potential differences by race and sex.

Higher levels of OS are a risk factor for both subclinical and clinical cardiovascular disease. Prior work by our group has specifically documented the association of cystine with arterial stiffness and adverse cardiovascular events.[8,33] HF is associated with increased inflammation, arterial stiffness, and lower NO bioavailability, with potentially greater implication of inflammation and arterial stiffness in HFpEF than HFrEF.[34] We were unable to demonstrate these associations in our HFrEF cohort, perhaps due in part to the vasoactive effects of chronic HF medication, such as angiotensin-converting enzyme inhibitors, beta-blockers, and nitrates.

In our cohort, women had lower levels of OS, but worse arterial stiffness than men. Levels of OS increase after menopause, likely mediated by loss of the antioxidant effect of estrogen.[35] Women have also been shown to worse arterial stiffness compared to men, particularly in patients with HFpEF which is more common in older women. However, prior studies defining the association between OS and arterial stiffness in women are limited.[17,36] Our findings are consistent with a prior study by Raad et al., which showed that women with elevated cystine levels exhibit diastolic dysfunction, raising the possibility that post-menopausal women with increased OS and arterial stiffness may be more prone to develop HFpEF compared to HFrEF.[37,38]

In our cohort, there were no demonstrable differences in measures of OS or arterial stiffness between Black and White patients. Prior studies in healthy Black and White patients have demonstrated lower levels of GSH in Black patients, and lower bioavailability and responsiveness to NO. [9,39] Clinically, the nitroso-redox balance has been a target for improving myocardial and peripheral vascular relaxation in Black patients. The A-HeFT clinical trial showed improved survival in patients assigned to the hydralazine-isosorbide dinitrate arm in a completely Black cohort.[40] Isosorbide dinitrate stimulates nitric oxide signaling while hydralazine is an antioxidant that inhibits reactive oxygen species generation, thus impacting both sides of the nitroso-redox balance.[41] Current evidence-based guidelines recommend the use of hydralazine-isosorbide dinitrate in Black patients given the survival benefit.[42] Greater arterial stiffness, as measured by PWV and AIx, has also been demonstrated in Black patients.[43] The reasons for increased arterial stiffness in Black patients are not well defined, but might include differences in elastin and collagen production in the endothelial walls, contributions OS, as well as increased cardiovascular risk factors in Black patients.[43]

Arterial stiffness predicts incident cardiovascular disease, including HF. Chirinos et al. used the Multiethnic Study of Atherosclerosis (MESA) cohort of 6,814 healthy patients to

examine the association of RM with incident HF.[32] Compared to AIx and Pulse Pressure Amplification (PPA), RM was a significant predictor of incident HF. Earlier arrival of the backward wave reflected from stiff arteries back to the heart leads to increased arterial afterload and increased late-systolic load. As the heart increases contractility to accommodate these pressure loads, left ventricular remodeling may ensue and contractile function may be impaired, increasing the risk for incident HF (**Supplemental Figure 2**). Studies examining pulsatile arterial hemodynamics in patients with prevalent HF are limited. Our study adds to the literature in that our cohort included patients with a previously established diagnosis of HF who were on contemporary guideline-directed medical therapy. Though RM was not a significant predictor for primary and secondary outcomes in our cohort, this may be due to the compensatory mechanisms the left ventricle has developed during HFrEF progression, as well as long-term effects of medical therapy on ventricular remodeling. Additionally, in HFrEF patients, loss of systolic function may adversely impact the magnitude of the forward wave as well as the reflected wave, significantly affecting the overall resulting waveform.[44,45]

Finally, social determinants of health may contribute to our findings, particularly by race. While men and women have previously identified differences in hormone expression, Black and White patients have few identified genetic variants that explain worse clinical outcomes.[46] Prior large studies have shown that decreased access to specialized care in Black patients may lead to increased hospitalizations and HF-related death.[47] Black patients are also less likely to be prescribed guideline-directed medical therapy and offered advanced HF therapies compared to White patients.[47] Differences by race in the identification and management of heart failure due to societal failings for Black patients may certainly lead to worse arterial stiffness and uncontrolled HF symptoms compared to White patients. These circumstances may not apply to our cohort, which consists of patients evaluated routinely at a tertiary care center. However, although race is certainly a social construct with many potential confounders including those previously mentioned, we cannot completely exclude genetic variations within ancestral lines in people identifying as Black that may contribute to impaired arterial stiffness and increased oxidative stress. For example, Black patients are more likely to develop dilated cardiomyopathy (DCM) compared to White patients and are estimated to have 33% heritability of dilated cardiomyopathy according to a recent genome-wide association study.[6] As genetic variants among individuals are better characterized, crude categorization of patients by race in medical studies might be replaced.[48]

Our study should be interpreted in the context of important limitations. Our study represents a cross-sectional, single-center experience with a small sample size, which may have limited our

power to detect differences by race and sex. We primarily examined the biologic factors contributing to clinical outcomes, without adjustment for social determinants such as insurance type or income level. Still, strengths include the use of multiple modalities to assess differences in arterial stiffness in a diverse population of patients. Using incremental precision in arterial stiffness measurements, we were able to finely assess the contribution of arterial stiffness in clinical outcomes, better accounting for measurement imprecision compared to studies using only one modality. Additionally, to our knowledge, our study is the first to compare oxidative stress and arterial stiffness measurements in a HFrEF population, as well as the first to relate measurements of arterial stiffness to clinical outcomes in HFrEF patients.

CONCLUSION

Overall, in this HFrEF cohort, OS was not strongly associated with impaired arterial stiffness. Differences in OS and arterial stiffness were more pronounced in men versus women, although these measures were not predictive of clinical events on multivariate analysis. Although prior studies have identified an association between biomarkers of OS, arterial stiffness and clinical outcomes, this association may be more difficult to detect in patients with more advanced disease, such as those with HFrEF. Early identification of oxidative stress and arterial stiffness measures may be most effective when used to employ therapeutic interventions to decrease incident HFrEF and enable risk stratification to avoid cardiovascular events including hospitalization and CV-related death.

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Table 1. Baseline characteristics of cohort overall, stratified by race, and stratified by sex.

Characteristic	Total Cohort N=205 (100%)	Black N=127 (62%)	White N=78 (38%)	P-value	Female N=104 (51%)	Male N=101 (49%)	P-value
Age	54.4 ± 13.0	51.9 ± 12.9	58.5 ± 12.3	0.269	52.4 ± 12.9	56.4 ± 12.9	0.028
Gender							
- Male	104 (50.7)	71 (55.9)	33 (42.3)	0.059	71 (68.3)	56 (55.4)	0.059
- Female	101 (49.3)	56 (44.1)	45 (57.7)		33 (31.7)	45 (44.6)	
Level of Education							
- College graduate / Graduate education	67 (33.5)	32 (26.0)	35 (45.5)	0.018	38 (37.6)	29 (29.3)	0.198
- Some college	66 (33.0)	45 (36.6)	21 (27.3)		35 (34.7)	31 (31.3)	
- High-school graduate or below	67 (33.5)	46 (37.4)	21 (27.3)		28 (27.7)	39 (39.4)	
Income							
- Less than 25,000	55 (26.8)	21 (16.5)	34 (43.6)	<0.001	26 (25.0)	29 (28.7)	0.569
- 25,000-49,999	38 (18.5)	20 (15.7)	18 (23.1)		19 (18.3)	19 (18.8)	
- 50,000 and over	64 (31.2)	49 (38.6)	15 (19.2)		37 (35.6)	27 (26.7)	
- Refused to answer/Did not know	48 (23.4)	37 (29.1)	11 (14.1)		22 (21.2)	26 (25.7)	
Marital Status							
- Married	99 (48.3)	48 (37.8)	51 (65.4)	0.001	39 (37.5)	60 (59.4)	0.019
- Divorced/Separated	56 (27.3)	39 (30.7)	17 (21.8)		32 (30.8)	24 (23.8)	
- Widowed	12 (5.9)	7 (5.5)	5 (6.4)		9 (8.7)	3 (3.0)	
- Single	37 (18.0)	32 (25.2)	5 (6.4)		23 (22.1)	14 (13.9)	
Heart failure type							
- Ischemic	40 (19.5)	16 (12.6)	24 (30.8)	0.004	15 (14.4)	25 (24.8)	0.143
- Nonischemic	159 (77.6)	106 (83.5)	53 (67.9)		85 (81.7)	74 (73.3)	
NYHA Class							
- Class 1	9 (4.4)	6 (5.0)	3 (3.9)	0.924	4 (4.0)	5 (5.1)	0.031
- Class 2	84 (41.0)	49 (40.8)	35 (45.5)		33 (33.3)	51 (52.0)	
- Class 3	91 (44.4)	57 (47.5)	34 (44.2)		56 (56.6)	35 (35.7)	
- Class 4	13 (6.3)	8 (6.7)	5 (6.5)		6 (6.1)	7 (7.1)	
Diabetes	90 (43.9)	60 (49.2)	30 (39.0)	0.158	47 (47.0)	43 (43.4)	0.613
Hypertension	116 (56.6)	84 (68.9)	32 (41.6)	<0.001	59 (59.0)	57 (57.6)	0.839
Dyslipidemia	38 (18.5)	26 (21.3)	12 (15.6)	0.317	14 (14.0)	24 (24.2)	0.066
Myocardial Infarction	54 (26.3)	31 (25.4)	23 (29.9)	0.491	23 (23.0)	31 (31.3)	0.187
Coronary Arterial Bypass Graft	13 (6.3)	4 (3.3)	9 (11.7)	0.019	3 (3.0)	10 (10.1)	0.043
Body mass index (kg/m2)	30.3 (25.9-37.8)	31.2 (26.2-38.4)	30.0 (25.8-36.2)	0.382	30.6 (26.3-38.1)	30.1 (25.6-37.2)	0.408
Average Systolic Blood Pressure (mmHg)	114 ± 18.6	115.4 ± 19.5	111.8 ± 17.1	0.105	113.2 ± 18.1	114.8 ± 19.2	0.541
Average Diastolic Blood Pressure (mmHg)	67.8 ± 12.4	69.6 ± 12.7	65.1 ± 11.4	0.268	66.2 ± 11.7	69.5 ± 12.9	0.067
BNP (pg/mL)	170 (70-492)	217 (73-644)	149 (53-326)	0.590	136 (39-534)	231 (94-483)	0.315
Total Cholesterol (mg/dL)	173 (140-204)	173 (143-209)	173 (139-197.5)	0.943	177 (144-213)	159 (136-197)	0.049

HDL (mg/dL)	43 (36.3-55.8)	44 (38-58)	42 (33-54.5)	0.395	48 (39-61)	41 (34-50)	0.003
Triglycerides (mg/dL)	110 (80-158.5)	99 (78-139)	129 (85-195.5)	0.013	114 (83-146)	106 (77-164)	0.687
LDL (mg/dL)	96 (75-122)	100 (78-127)	89 (74-121)	0.110	100 (78-131)	91 (74-117)	0.31
Uric Acid (mg/dL)	7.4 (5.8-9.2)	8.0 (6.0-10.2)	6.9 (5.5-8.1)	0.047	7.1 (5.4-9.3)	7.6 (6.3-9.2)	0.084
Estimated glomerular filtration rate (mL/min/1.73 m2)	71 (52-90.5)	71 (48-92)	71 (58-85)	0.982	72 (52-97)	70.5 (51-84)	0.137
ACE or ARB use	121 (59.0)	70 (55.1)	51 (65.4)	0.147	65 (62.5)	56 (55.4)	0.305
Beta blocker use	193 (97.0)	118 (96.7)	75 (97.4)	0.784	96 (96.0)	97 (98.0)	0.414
Digoxin use	41 (20.6)	18 (14.8)	23 (29.9)	0.010	16 (16.0)	25 (25.3)	0.107
Diuretic use	180 (90.5)	116 (95.1)	64 (83.1)	0.005	92 (92.0)	88 (88.9)	0.455
Hydralazine use	36 (18.1)	33 (27.0)	3 (3.9)	<0.001	12 (12.0)	24 (24.2)	0.025
Oral nitrate use	31 (15.6)	17 (13.9)	14 (18.2)	0.421	17 (17.0)	14 (14.1)	0.578
ARNI use	30 (15.1)	18 (14.8)	12 (15.6)	0.873	18 (18.0)	12 (12.1)	0.247
LVEF%	23.5 ± 8.9	22.4 ± 9.2	25.2 ± 8.2	0.311	23.1 ± 9.1	23.9 ± 8.8	0.557

Abbreviations: NYHA - New York Heart Association, BNP – B-Type natiuretic peptide, HDL - high density lipoprotein, LDL - low density lipoprotein, LVEF - left ventricular ejection fraction, ARNI: Angiotensin Receptor II Blocker - Neprilysin Inhibitor, ACE: Angiotensin-converting enzyme inhibitor, ARB: Angiotensin Receptor II Blocker

Table 2A & 2B. Oxidative stress metabolites (A) and arterial stiffness measurements (B) stratified by race and sex.

Nitroso-Redox Biomarker	Black N=127 (62%)	White N=78 (38%)	P-value*	Female N=104 (51%)	Male N= 101 (49%)	P-value*
Cysteine (Cys) (µmol/L)	7.9 [6.3, 9.6]	7.5 [6.1, 9.7]	0.595	8.2 [6.5, 9.7]	7.5 [5.4, 9.5]	0.034
E_h Cys (mV)	-63.5 ± 9.1	-62.5 ± 8.9	0.655	-64.3 ± 8.7	-62.0 ± 9.2	0.077
Cystine (Cyss) (µmol/L)	99.2 [82.1, 120.3]	98.8 [82.6, 115.4]	0.955	103.6 [84.3, 130.1]	96.3 [81.8, 113.6]	0.080
Glutathione (GSH) (µmol/L)	1.4 [1.0, 1.9]	1.5 [1.1, 1.9]	0.519	1.5 [1.1, 1.9]	1.4 [1.1, 1.8]	0.923
E_h GSH (mV)	-135.4 ± 12.9	-134.5 ± 13.8	0.447	-137.1 ± 12.8	-133.0 ± 13.5	0.030
Glutathione disulphide (GSSG) (µmol/L)	0.04 [0.02, 0.07]	0.04 [0.02, 0.08]	0.810	0.04 [0.01, 0.06]	0.05 [0.02, 0.10]	0.023
Cyss/GSH Ratio	73.4 [47.0, 101.3]	69.6 [45.2, 101.4]	0.408	71.9 [49.5, 104.0]	71.6 [43.4, 98.6]	0.439
Nitrites	0.21 [0.10, 0.33]	0.26 [0.16, 0.40]	0.126	0.23 [0.10, 0.36]	0.23 [0.15, 0.36]	0.500

*Data are presented as median [interquartile range]. P-value < 0.05 considered significant.

Arterial Stiffness Measurement	Black N=127 (62%)	White N=78 (38%)	P-value*	Female N=104 (51%)	Male N=101 (49%)	P-value*
Peripheral Measures						
Systolic pressure (mmHg)	113 [103, 128]	112 [105, 120]	0.371	111 [105, 122]	113 [102, 131]	0.419
Diastolic pressure (mmHg)	69 [61, 76]	63 [57, 72]	0.019	66 [57, 72]	69 [61, 76]	0.025
Mean pulse pressure (mmHg)	84 [76, 92]	81 [73, 88]	0.235	82 [73, 88]	83 [75, 92]	0.138
Augmentation Index (%)	88 [78, 102]	88 [80, 95]	0.980	91 [81, 103]	86 [77, 94]	0.062
Central Measures						
Systolic pressure (mmHg)	101 [92, 114]	101 [93, 109]	0.759	101 [93, 110]	102 [93, 113]	0.480
Diastolic pressure (mmHg)	71 [61, 77]	63 [58, 72]	0.004	67 [58, 73]	71 [62, 77]	0.017
Mean pulse pressure (mmHg)	83 [76, 92]	81 [73, 88]	0.235	81.5 [73, 88]	83 [75, 92]	0.137
Augmentation Index (%)	129 [115, 146]	133 [124, 145.25]	0.319	135 [120, 151]	130 [114, 140]	0.082
Pulse Wave Analysis Measures						
PWV (ms)	7.0 [5.9, 8.2]	6.5 [5.8, 7.4]	0.218	6.7 [5.6, 7.6]	7 [6.1, 7.8]	0.354
Augmentation Index @ HR 75 (%)	19.4 [10.0, 28.2]	21.8 [14.6, 27.1]	0.304	22.0 [17.3, 30.1]	17.3 [5.3, 25.5]	0.007
Forward Wave Amplitude (mmHg)	25.2 [18.7, 33.3]	27.9 [21.3, 35.0]	0.213	26.7 [21.6, 35.3]	25.2 [18.6, 33.8]	0.167
Backward Wave Amplitude (mmHg)	12.3 [8.2, 16.7]	12.1 [9.2, 16.3]	0.660	12.8 [9.1, 17.9]	11.3 [7.9, 15.8]	0.093
Reflection Magnitude (%)	0.48 [0.39, 0.56]	0.45 [0.40, 0.52]	0.154	0.48 [0.40, 0.56]	0.47 [0.39, 0.52]	0.157

*Data are presented as median [interquartile range]. P-value < 0.05 considered significant.
Abbreviations: PWV - pulse wave velocity, HR – heart rate

Table 3. Linear Regression Analysis of Nitroso-Redox Biomarkers and Arterial stiffness Measurements.

	PWV (m/s)†				AIx @ 75 HR (%)†				Reflection Magnitude (%)†			
	Univariate		Multivariate*		Univariate		Multivariate*		Univariate		Multivariate*	
	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value	Beta	P-value
Cyss	-0.120	0.162	-0.031	0.742	0.199	0.030	0.092	0.323	-0.001	0.992	-0.008	0.928
Age	-0.045	0.600	-0.017	0.855	0.202	0.025	0.296	0.004	0.043	0.621	0.033	0.725
MAP	0.253	0.003	0.243	0.012	0.140	0.125	0.279	0.003	0.130	0.130	0.206	0.021
GSH	0.185	0.030	0.162	0.060	-0.053	0.568	-0.018	0.838	-0.003	0.975	-0.008	0.922
Age	-0.045	0.600	-0.007	0.938	0.202	0.025	0.327	0.001	0.043	0.621	0.029	0.750
MAP	0.253	0.003	0.249	0.006	0.140	0.125	0.272	0.004	0.130	0.130	0.206	0.020

*Full model also adjusted for male gender, Black race, ischemic heart failure etiology, hypertension, and body mass index
†Continuous variable required log-transformation to obtain normal distribution
Abbreviations: Cyss – cystine, GSH – glutathione, MAP – mean arterial pressure, PWV – pulse wave velocity, AIx – augmentation index

Table 4A and 4B. Cox Proportional Hazards Models for Arterial Stiffness Measurements and Composite Outcomes.

Table 4A. Primary Composite Outcome: Death / LVAD Implantation / HT

Variable	Pulse Wave Velocity (m/s)				Augmentation Index @ HR 75 (%)				Reflection Magnitude (%)			
	Unadjusted HR (95% CI)	P-value	Adjusted HR (95% CI)	P-value	Unadjusted HR (95% CI)	P-value	Adjusted HR (95% CI)	P-value	Unadjusted HR (95% CI)	P-value	Adjusted HR (95% CI)	P-value
Race - White - Black	Ref. 1.12 (0.69-1.82)	0.658	Ref. 0.98 (0.49-1.94)	0.953	Ref. 1.12 (0.69-1.82)	0.658	Ref. 0.57 (0.27-1.18)	0.132	Ref. 1.12 (0.69-1.82)	0.658	Ref. 0.66 (0.34-1.29)	0.224
Age	0.92 (0.75-1.10)	0.356	0.61 (0.45-0.82)	0.001	0.92 (0.75-1.10)	0.356	0.74 (0.54-1.01)	0.059	0.92 (0.75-1.10)	0.356	0.68 (0.51-0.90)	0.007
Sex - Female - Male	Ref. 1.75 (1.08-2.83)	0.022	Ref. 1.99 (1.01-3.90)	0.047	Ref. 1.75 (1.08-2.83)	0.022	Ref. 1.92 (0.94-3.92)	0.076	Ref. 1.75 (1.08-2.83)	0.022	Ref. 1.85 (0.98-3.52)	0.060
Heart Failure Etiology - Nonischemic - Ischemic	Ref. 2.01 (1.20-3.39)	0.009	Ref. 2.98 (1.37-6.48)	0.006	Ref. 2.01 (1.20-3.39)	0.009	Ref. 1.88 (0.82-4.27)	0.135	Ref. 2.01 (1.20-3.39)	0.009	Ref. 2.04 (0.94-4.43)	0.071
Hypertension	1.10 (0.68-1.79)	0.69	0.79 (0.34-1.83)	0.581	1.10 (0.68-1.79)	0.69	0.81 (0.33-2.02)	0.658	1.10 (0.68-1.79)	0.69	1.02 (0.45-2.29)	0.965
Mean Arterial Pressure (MAP)	0.69 (0.55-0.85)	<0.001	0.65 (0.40-1.06)	0.084	0.69 (0.55-0.85)	<0.001	0.55 (0.33-0.93)	0.025	0.69 (0.55-0.85)	<0.001	0.56 (0.35-0.90)	0.016
MAP*time	1.00 (1.00-1.00)	0.027	1.00 (1.00-1.00)	0.544	1.00 (1.00-1.00)	0.027	1.00 (1.00-1.00)	0.243	1.00 (1.00-1.00)	0.027	1.00 (1.00-1.00)	0.238
Body Mass Index	1.00 (0.98-1.03)	0.889	0.98 (0.94-1.03)	0.432	1.00 (0.98-1.03)	0.889	1.00 (0.96-1.05)	0.963	1.00 (0.98-1.03)	0.889	1.00 (0.96-1.05)	0.944
Arterial stiffness Measurement	0.64 (0.34-1.19)	0.157	0.62 (0.29-1.32)	0.217	0.68 (0.54-0.86)	0.001	0.91 (0.65-1.28)	0.590	0.61 (0.29-1.26)	0.180	0.77 (0.32-1.90)	0.577
Diabetes	1.11 (0.69-1.78)	0.672	1.64 (0.72-3.73)	0.244	1.11 (0.69-1.78)	0.672	1.33 (0.51-3.46)	0.564	1.11 (0.69-1.78)	0.672	1.22 (0.53-2.82)	0.644
B-Type Natriuretic Peptide	1.01 (1.00-1.01)	<0.001	1.01 (1.00-1.01)	<0.001	1.01 (1.00-1.01)	<0.001	1.01 (1.00-1.02)	0.012	1.01 (1.00-1.01)	<0.001	1.01 (1.00-1.02)	0.001
Estimated Glomerular Filtration Rate (eGFR)	0.86 (0.78-0.95)	0.003	0.80 (0.63-1.01)	0.062	0.86 (0.78-0.95)	0.003	0.82 (0.62-1.09)	0.179	0.86 (0.78-0.95)	0.003	0.79 (0.62-1.01)	0.065
eGFR*time	1.00 (1.00-1.00)	0.027	1.00 (1.00-1.00)	0.174	1.00 (1.00-1.00)	0.027	1.00 (1.00-1.00)	0.424	1.00 (1.00-1.00)	0.027	1.00 (1.00-1.00)	0.224

Table 4B. Secondary Composite Outcome: Death / Hospitalization

Variable	Pulse Wave Velocity (m/s)				Augmentation Index @ HR 75 (%)				Reflection Magnitude (%)			
	Unadjusted HR (95% CI)	P-value	Adjusted HR (95% CI)	P-value	Unadjusted HR (95% CI)	P-value	Adjusted HR (95% CI)	P-value	Unadjusted HR (95% CI)	P-value	Adjusted HR (95% CI)	P-value
Race - White - Black	Ref. 1.09 (0.77-1.54)	0.620	Ref. 0.83 (0.51-1.36)	0.457	Ref. 1.09 (0.77-1.54)	0.620	Ref. 0.93 (0.57-1.53)	0.783	Ref. 1.09 (0.77-1.54)	0.620	Ref. 1.07 (0.67-1.70)	0.778
Age†	1.00 (0.88-1.14)	0.964	0.87 (0.70-1.07)	0.178	1.00 (0.99-1.01)	0.964	0.78 (0.62-0.98)	0.034	1.00 (0.88-1.14)	0.964	0.79 (0.65-0.98)	0.030
Sex - Female - Male	Ref. 1.22 (0.87-1.70)	0.247	Ref. 1.28 (0.82-2.02)	0.282	Ref. 1.22 (0.87-1.70)	0.247	Ref. 1.58 (0.94-2.64)	0.082	Ref. 1.22 (0.87-1.70)	0.247	Ref. 1.37 (0.87-2.15)	0.178
Heart Failure Etiology - Nonischemic - Ischemic	Ref. 1.74 (1.18-2.56)	0.006	Ref. 1.76 (1.00-3.09)	0.048	Ref. 1.74 (1.18-2.56)	0.006	Ref. 2.59 (1.42-4.37)	0.002	Ref. 1.74 (1.18-2.56)	0.006	Ref. 2.26 (1.34-3.84)	0.002
Hypertension	1.10 (0.78-1.55)	0.598	1.39 (0.77-2.51)	0.269	1.10 (0.78-1.55)	0.598	1.22 (0.62-2.40)	0.567	1.10 (0.78-1.55)	0.598	1.41 (0.76-2.63)	0.281
Mean Arterial Pressure†	0.98 (0.97-0.99)	0.026	0.89 (0.72-1.07)	0.196	0.98 (0.97-0.99)	0.026	0.95 (0.79-1.15)	0.623	0.98 (0.97-0.99)	0.026	0.98 (0.82-1.17)	0.808
Body Mass Index	1.01 (0.99-1.03)	0.344	1.00 (0.97-1.04)	0.836	1.01 (0.99-1.03)	0.344	1.03 (1.00-1.06)	0.040	1.01 (0.99-1.03)	0.344	1.02 (0.99-1.05)	0.154
Arterial stiffness Measurement	1.11 (0.67-1.84)	0.682	1.40 (0.86-2.30)	0.178	0.77 (0.63-0.96)	0.018	0.93 (0.72-1.19)	0.546	0.51 (0.30-0.89)	0.017	0.62 (0.34-1.11)	0.106
Diabetes	1.27 (0.91-1.78)	0.163	1.11 (0.66-1.86)	0.709	1.27 (0.91-1.78)	0.163	0.78 (0.40-1.50)	0.458	1.27 (0.91-1.78)	0.163	0.71 (0.39-1.27)	0.247
B-Type Natriuretic Peptide†	1.01 (1.01-1.01)	<0.001	1.01 (1.01-1.02)	<0.001	1.01 (1.01-1.01)	<0.001	1.01 (1.00-1.02)	0.002	1.01 (1.01-1.01)	<0.001	1.01 (1.01-1.02)	<0.001
Estimated Glomerular Filtration Rate†	0.88 (0.82-0.94)	<0.001	0.93 (0.83-1.04)	0.198	0.88 (0.82-0.94)	<0.001	0.90 (0.81-1.01)	0.083	0.88 (0.82-0.94)	<0.001	0.90 (0.81-1.00)	0.990

†Age, Mean Arterial Pressure, eGFR, and BNP hazard ratios are presented as change per unit of 10 for both outcomes.

Figure 1. Diagram of nitroso-redox balance and surrogate measurements.

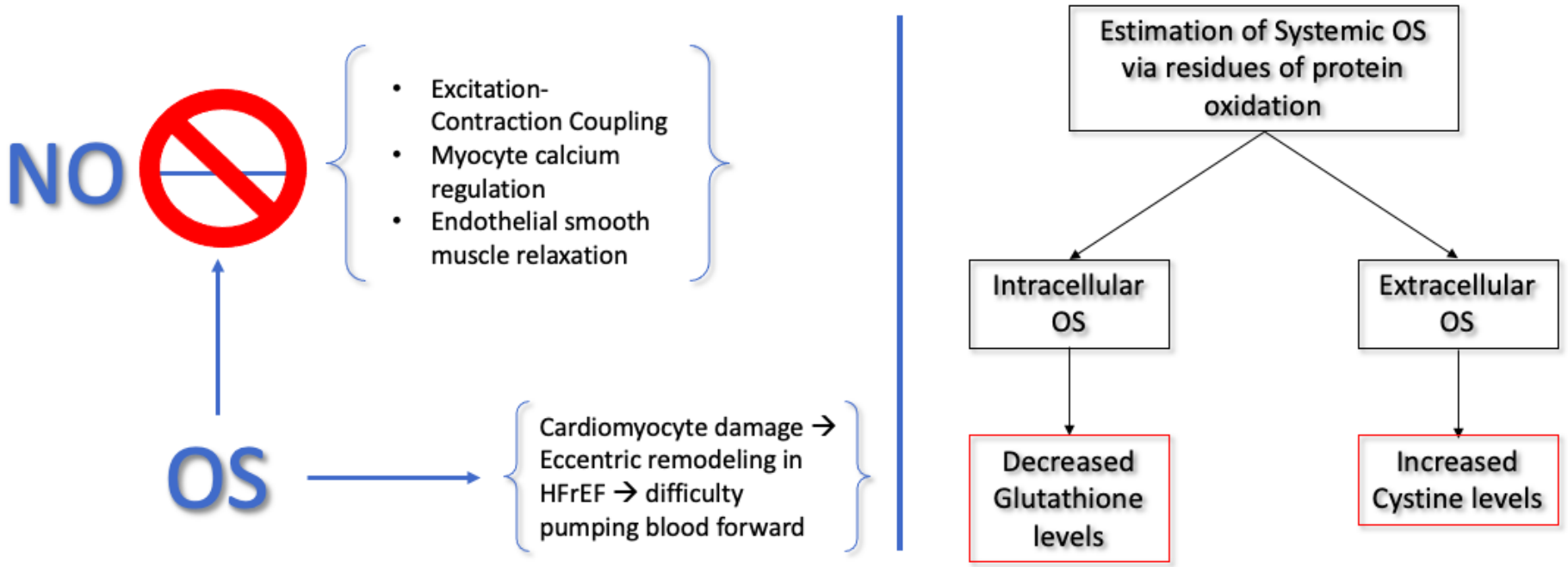


Figure 2. Cubic spline model of interaction between cystine and reflection magnitude by sex.

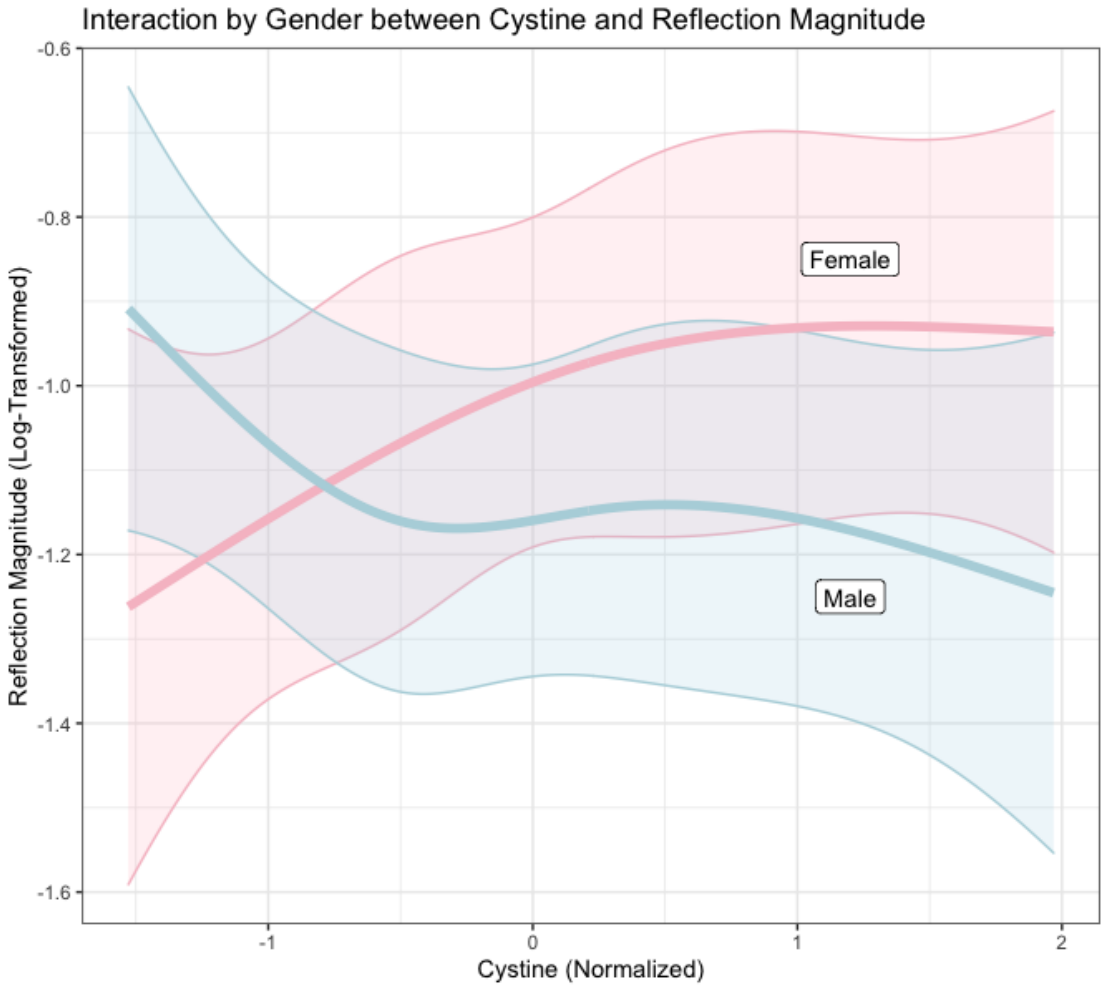
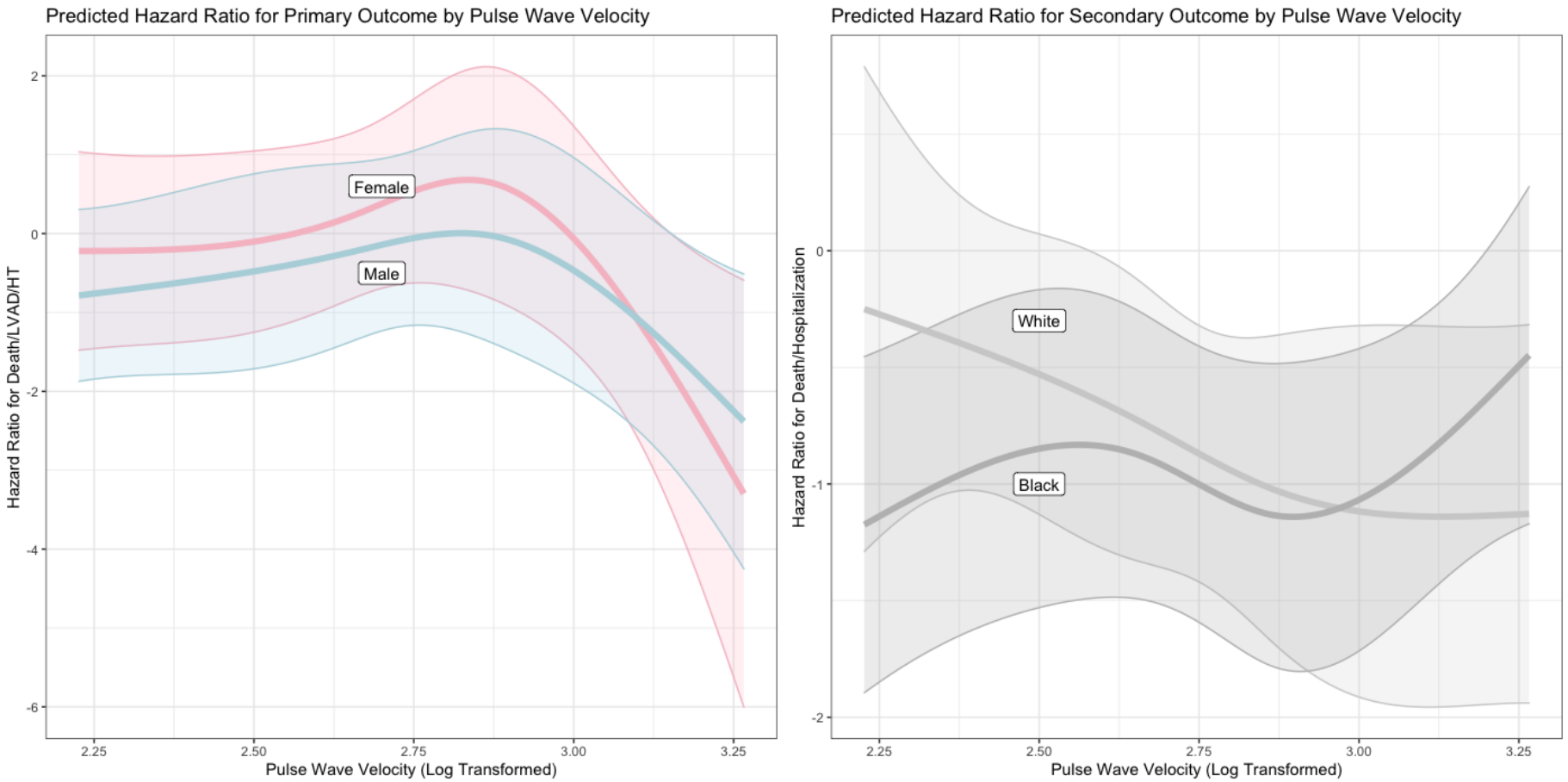


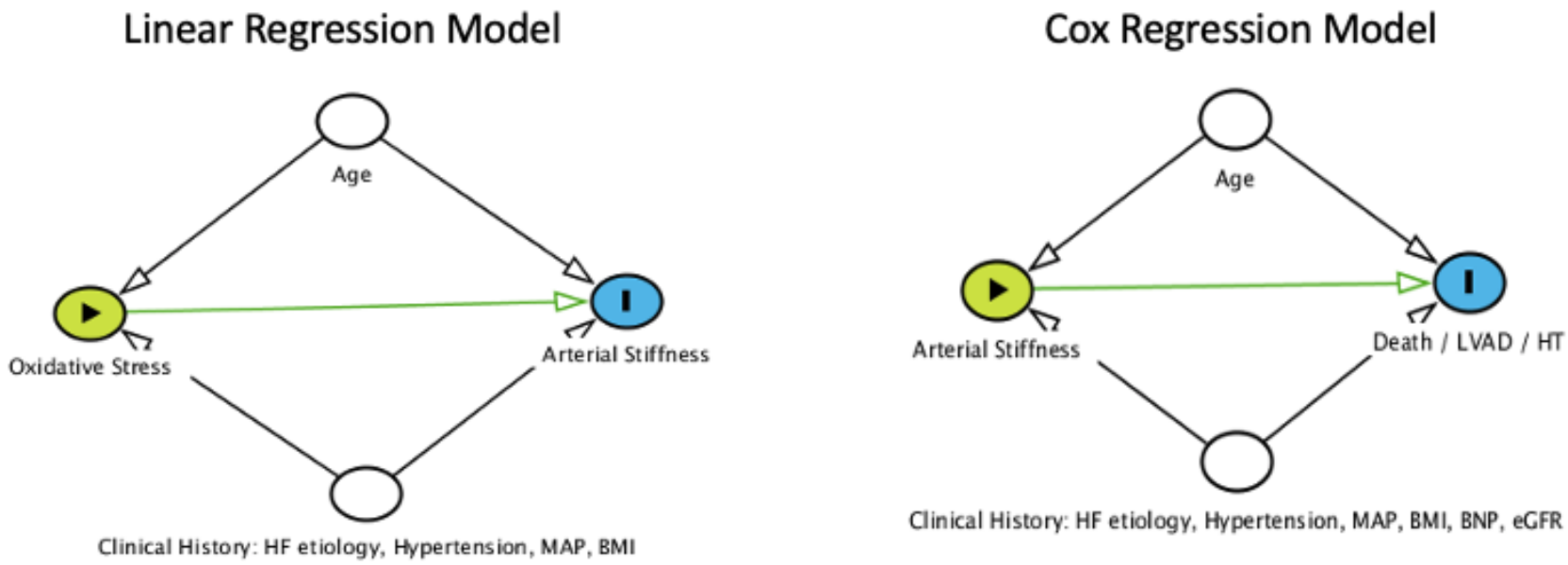
Figure 3A and 3B. Cubic spline model of interaction between pulse wave velocity and primary and secondary endpoints by sex (A) and race (B).



Supplemental Table 1. Terminology dictionary for measures of arterial stiffness.

Arterial stiffness Measurement	Definition
Allometric Flow-Mediated Dilation (%)	Largest percent change in arterial diameter during an ischemic challenge, scaled to reduce bias toward baseline diameter, a measure of endothelial function
Pulse Wave Velocity (m/s)	Speed of at which arterial pulse propagates along the arterial wall (incident wave), a measure of arterial stiffness
Reactive Hyperemia Index (%)	Non-invasive measure of microvascular endothelial dysfunction, thought to be less operator dependent than flow-mediated dilation
Ejection Duration (m/s)	Duration of ventricular ejection
Systolic pressure (mmHg)	Amount of pressure blood exerts against arterial walls during ventricular contraction
Diastolic pressure (mmHg)	Amount of pressure blood exerts against arterial walls during ventricular relaxation
Mean pulse pressure (mmHg)	Relative difference between systolic and diastolic blood pressures
Augmentation Index (%)	Measure of arterial stiffness derived from difference between first and second systolic peaks of the aortic waveform
Aortic Impedance	Slope of pressure-flow relation in the absence of wave reflections, represents pulsatile load imposed by the proximal aorta
Stroke Volume	Volume of blood ejected from the ventricle during systole
Pulse Pressure Amplitude	Difference between systolic and diastolic blood pressure
Mean Arterial Pressure	Average arterial pressure during one cardiac cycle, derived from diastolic and systolic blood pressures
1st Systolic Peak (P1)	First systolic inflection of the aortic waveform
2nd Systolic Peak (P2)	Systolic peak of the aortic waveform
Augmentation Pressure (P2-P1)	Relative difference between systolic peak and first systolic inflection of aortic waveform, used to derive augmentation index
Augmentation Index @ HR 75 (%)	Measure of arterial stiffness derived from difference between first and second systolic peaks, normalized to a heart rate of 75 to reduce confounding on arterial stiffness measurement
Forward Wave Amplitude (mmHg)	Forward component of aortic pressure wave
Backward Wave Amplitude (mmHg)	Backward component of aortic pressure wave
Reflection Magnitude	Ratio of amplitudes of forward and backward waves, thought to have fewer confounding variables in its derivation than augmentation index

Supplemental Figure 1. Directed acyclic graph for linear regression and cox proportional hazards models.



Supplemental Figure 2. Diagram showing effect of arterial stiffness on cardiac function, adapted from Weber and Chirinos.