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Endothelin-1, cardiac morphology, and heart failure: The MESA Angiogenesis Study

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Abstract

Background—Circulating levels of endothelin-1 (ET1) are elevated in heart failure and predict poor prognosis; however, it is not clear whether ET1 elevation is an adaptive response, maladaptive response, or an epiphenomenon of heart failure. In the current study, we evaluated relationships between ET1, cardiac morphology, and incident heart failure or cardiovascular death in participants with no evidence of clinical cardiovascular disease at the time ET1 was measured.

Methods and Results—ET1 was measured 1,361 participants in the Multi-Ethnic Study of Atherosclerosis Angiogenesis Sub-Study. As suggested by linear regression, participants with lower circulating ET1 levels tended to be older, non-white, more likely to have smoked heavily, and less likely to report intentional exercise. Participants with higher ET1 levels had smaller left ventricular end-diastolic volumes (8.9 mL smaller per log increase in ET1, 95% CI 17.1 to 0.7,

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p=0.03) with an increased left ventricular ejection fraction (2.8% per log increase in ET1, 95% CI 0.5 to 5.2%, p=0.02). As suggested by Cox Proportional Hazards estimates, participants with higher ET1 levels had a lower risk for the composite outcome of heart failure or cardiovascular death in models that were unadjusted or had limited adjustment (p=0.03 and 0.05 respectively). Lower risk for heart failure could not be clearly shown in a model including health behaviors.

Conclusions—These results suggest, but do not confirm that elevated levels of circulating ET1 are associated with a more favorable cardiac phenotype. The relationship between ET1 and outcomes was not fully independent of one or more covariates.

Subject terms:

Endothelin-1; heart failure; cardiac morphology

Introduction

Endothelin signaling is a treatment target in pulmonary arterial hypertension.[1] Endothelin-receptor antagonists lower pulmonary vascular resistance, improve right heart function, and reduce morbidity and mortality in patients with pulmonary arterial hypertension.[2–4] Endothelin signaling has also been hypothesized to be a marker or mediator of combined pre- and post- capillary pulmonary hypertension and elevated levels of Endothelin-1 (ET1) are associated with worse morbidity and mortality in individuals with left heart disease.[5–10] Despite this observation and in counterpoint to individuals with pulmonary arterial hypertension, endothelin-receptor antagonism in patients with left heart failure has not shown benefit and may be harmful.[11–13] Furthermore, some animal models suggest endothelin-receptor agonism may benefit the heart in the absence of excessive right ventricular afterload and antagonism may therefore be harmful.[14–17]

As such, while perturbed endothelin signaling is commonly observed in heart failure, the casual role of endothelin signaling in the pathogenesis and progression of heart failure is less clear. ET1 elevation in patients with extant heart failure could represent an adaptive response, a maladaptive or contributing factor to disease progression, or an epiphenomenon with no causal role in disease (Figure 1). Measurement before disease onset may improve our ability to understand the underlying role of a biomarker in disease pathogenesis.

We examined relationships between ET1, cardiac morphology, and incident heart failure or cardiovascular death in a multi-ethnic cohort of adults free of clinical cardiovascular disease at baseline. We hypothesized that increased ET1 levels at baseline would be associated with right ventricular dilation and decreased function. In addition, we hypothesized that ET1 would be associated with increased risk for incident heart failure or cardiovascular death over follow-up.

Methods

The Multi-Ethnic Study of Atherosclerosis (MESA) is a cohort study sponsored by the National Heart, Lung, and Blood Institute and designed to investigate subclinical

cardiovascular disease. From 2000 to 2002, MESA recruited participants without clinical cardiovascular disease aged 45–84 years old from six US communities.

The current study included participants in the MESA-Angiogenesis sub-study. Of 6,814 participants enrolled in MESA, 4,204 with interpretable right ventricular morphology on cardiac magnetic resonance imaging (cMRI) were included in the MESA-Right Ventricle Study (MESA-RV). As previously reported participants in MESA-RV were slightly younger and had slightly lower body mass indices than the parent study.[18] A random subset of the total MESA-RV cohort was randomly selected as the MESA-Angiogenesis cohort with event rates and characteristics similar to the parent MESA-RV cohort. The American Heart Association funded measurement of biomarkers including endothelin-1, angiopoietin, and vascular endothelial growth factor in the MESA-Angiogenesis sub-study. Institutional Review Boards of participating institutions approved MESA protocols. All participants provided informed consent.

Cardiac magnetic resonance imaging

cMRI was obtained at the baseline examination for all participants in MESA-RV. The cMRI protocol and interpretation of LV and RV parameters in MESA have been previously described.[19–21] Briefly, endocardial and epicardial borders of the RV and LV were traced on MRI short axis fast gradient recalled (FGRE) cine images using a semi-automated method at end-systole and end-diastole from (MASS 4.2, Medic, Leiden, the Netherlands). The outflow tract was included in RV volume. Papillary muscles and trabeculae were included in volumes and excluded from mass in both the RV and LV. End-systolic and end-diastolic volumes were calculated using Simpson's rule by summation of areas multiplied by the sum of slice thickness and image gap. Difference between epicardial and endocardial volumes of the RV or LV free wall at end-diastole multiplied by the specific gravity of the heart (1.05g/mL) was used to estimate mass. Ejection fraction was calculated by dividing stroke volume by end-diastolic volume.

Endothelin-1 Assay

ET1 was measured using EMD Millipore's MILLIPLEX MAP Human Angiogenesis Growth Factor Magnetic Bead Panel 1 (Lot# 2802970). Seventy-eight samples were assayed in duplicate. The reliability coefficient was 0.83 and inter-assay coefficient of variation was 4.8% on control samples in this study. There is another commercially available assay (R&D systems ELISA assay; used in the Jackson Heart Study[7]). To place our results in context with these other results: each method is reliable; however, the absolute level is discrepant. When both methods were used on the same pool of 20 control participants by the MESA coordinating center, the mean level using the R&D Elisa was 1.6 pg/mL (standard deviation 0.6 pg/mL) and the Millipore assay was 46.6 pg/mL (standard deviation 6.3 pg/mL). ET1 was log-transformed for all regression analyses.

Ascertainment of events

Full details of event ascertainment and definition are available in MESA's manual of procedures (MOP).[22] Briefly, clinical outcomes were assessed at MESA study examinations and by telephone interview every 9 to 12 months. Records were obtained for approximately

99% of hospitalizations and 97% of outpatient cardiovascular diagnostic encounters through calendar year 2014. Incident heart failure required heart failure symptoms, a physician diagnosis of heart failure and an objective feature of heart failure. Cardiovascular death was any death adjudicated as related to cardiac or vascular disease. If a participant developed incident heart failure and subsequently died, the time to first event (heart failure) was used for analyses. Two physicians from the MESA events committee independently reviewed all medical records for classification and dating of events. If reviewers disagreed, they adjudicated differences. If disagreement persisted, the full events committee made the final classification.

Statistical Analysis

We used linear regression to estimate associations between ET1 levels and cardiac morphology or predictors of ET1 level at the baseline exam. Covariates were assessed at the initial MESA exam and chosen *a priori*.[22] In limited models, we adjusted for age, sex, race, height, weight, and study site. In adjusted models, we included participants' education and cardiovascular risk factors including smoking status, pack-years of smoking, hypertension, diabetes mellitus, and cholesterol. In exploratory models, we further adjusted for estimated glomerular filtration rate (GFR), co-medication use, or intentional exercise.

Cox proportional hazards was used to estimate unadjusted and adjusted associations between ET1 level at the baseline exam and incident heart failure or cardiovascular death. Limited and adjusted models were evaluated. Because a test of Schoenfeld residuals in unadjusted analyses suggested a non-zero slope with relation to time (p=0.05), all models included a time varying covariate of ET1 level (interaction of time with ET1 level) to account for non-proportional hazards. Exploratory models also evaluated whether age, sex, or hypertension modified significant associations between ET1 and clinical outcomes. Parsimonious models are included in the online supplement and were created using sequential backward elimination of the least significant covariate until are included adjustments were statistically significant (threshold of significance, p-value <0.05) to evaluate whether coefficients were stable and whether pre-specified primary models were overly adjusted. Analyses were performed using STATA 15.1 (StataCorp, College Station, TX, USA).

Results

1,538 participants had ET1 sampled as part of the MESA-Angiogenesis case-cohort study. Of these, 1,383 participants were in the MESA-Angiogenesis cohort and 1,364 had all available covariates. Three additional participants were excluded from primary analyses (but included in analyses in the online supplement) given concern for measurement error since these individuals had measured ET1 levels of 348.8 pg/mL, 368.4pg/mL, and 621.1pg/mL. These values were >30 standard deviations above the mean (>14 standard deviations when these values were included) and >16 standard deviations from the closest observation (> 8 standard deviations when these observations were included). The final study sample included 1,361 participants of whom 1,280 participants did not have an event during follow-up, 53 participants had incident heart failure over follow-up, and 28 participants suffered cardiovascular death during follow-up (Figure 2). The mean age of the sample was 61.2

years, 52.4% were women, and 40.3% were white. The average ET1 level was 51.3 ± 9.7 pg/mL (Table 1). Individuals with higher ET1 levels tended to be younger, more likely to be non-white, more likely to exercise regularly and were less likely to have smoked heavily (Table 2 and Table E1). Median follow-up was 13.1years, maximum follow-up was 14.5 years, and total follow-up was 16,212 person-years. The incidence of heart failure or cardiovascular death among members of the *cohort* was 5.0 events per 1,000 person-years.

After adjustment for covariates, higher levels of ET1 were associated with a smaller LV end-diastolic volume (−8.9 mL smaller per log increase in ET1, 95% CI 17.1 to 0.7, p=0.03), and an increased LV ejection fraction (2.8% per log increase in ET1, 95% CI 0.5 to 5.2%, p=0.02)(Table 3; shown graphically in Figures E1 and E2 of the online supplement). Relationships between ET1 and the RV were sensitive to adjustment and were less compelling. Bi-ventricular relationships were similar with further adjustment for co-medication use, when accounting for renal function, or when accounting for intentional exercise (Table E2 in the supplemental material).

Strata of participants by ET1 level were created. Fourteen participants with the lowest ET1 levels (<44 pg/mL) had incident heart failure or cardiovascular death over 2,123 person years (6.6 per 1,000 person-years), 36 participants with medium-low ET1 levels (44–50 pg/mL) experienced an event over 6,338 person-years (5.7 per 1,000 person-years), 19 participants with medium-high ET1 levels (50–56 pg/mL) had incident heart failure or cardiovascular death over 4,566 person-years of follow-up (4.2 per 1,000 person-years), and 12 participants with the highest ET1 levels (>56 pg/mL) had incident heart failure or cardiovascular death over 3,185 person-years of follow-up (3.8 per 1,000 person-years). This suggests a risk difference between the highest and lowest cohort of 2.8 episodes of heart failure or cardiovascular death per 1,000 person-years or a relative risk of 0.58.

In unadjusted models using cox proportional hazards, a log increase in ET1 level was associated with decreased hazard of heart failure or cardiovascular death (hazard ratio 0.09, 95% CI 0.01 to 0.73, p=0.03). The association was similar with limited adjustment (hazard ratio 0.06, 95% CI 0.00 to 1.03, p=0.05) and slightly less strong with full adjustment (hazard ratio 0.07, 95% CI 0.00 to 1.30, p=0.08) where results were not statistically significant (Table 4). Although the trend was similar, associations between ET1 and heart or cardiovascular death separately were not statistically significant in these smaller groups. There was no suggestion of a relationship between ET1 and non-cardiovascular death (Table 4).

In exploratory analyses, there was no suggestion that age or sex modified relationships between ET1 and incident heart failure or cardiovascular death (p-value for the interactions= 0.63 & 0.75). Systemic hypertension may modify the association between ET and incident heart failure or cardiovascular death with a stronger relationship among individuals with systemic hypertension (p-value for the interaction= 0.03; hazard ratio in participants with hypertension was 0.01 per log increase in ET1, 95% CI 0.00 to 0.53, p=0.02; hazard ratio in participants WITHOUT hypertension was 0.76, 95% CI 0.02 to 28.2, p=0.88). Inclusion of the three participants with markedly elevated ET1 levels did not impact results in any

analysis (Tables E3 & E4) and parsimonious models for all relationships were similar to the full models (Table E5).

Discussion

We observed associations between higher ET1 levels, increased ejection fraction, and reduced risk for incident heart failure or cardiovascular death. We did not observe a clear relationship between ET1 and incident heart failure or cardiovascular death in the fully adjusted model. This suggests the relationship between ET1 and outcomes was not fully independent of one or more covariates. ET1 levels appeared to be associated with age, race/ethnicity, smoking, and intentional exercise.

These results are different than we expected, but not necessarily incongruous with previous studies. In participants with *existing* heart disease, several studies have shown higher ET1 levels are associated with worse outcomes.[6,8–10] ET1 is a pulmonary vasoconstrictor and we anticipated higher ET1 levels would be associated with right ventricular dilation. Although they did not report on right ventricular dilation, this hypothesis was reinforced by the Jackson Heart Study, which observed that elevated ET1 was associated with increased pulmonary artery systolic pressure, heart failure and death.[7] The key difference between previous studies and the current analysis is the absence of clinical cardiovascular disease at the time ET1 was assayed.

MESA participants in the current study were enrolled with the intent of excluding individuals with clinically detectable cardiovascular disease at the time baseline bloodwork was measured.[23] The absence of overt cardiovascular disease is likely important. By measuring ET1 levels in the absence of clinical cardiovascular disease, the potential for reverse causation to cause associations is diminished.[24,25] Ideally, previous observational studies in individuals with heart failure would have evaluated associations between ET1 and outcomes in individuals with otherwise similar heart failure severity; however, in practice such a comparison is difficult and residual confounding may exist despite adjustment. This is especially true when the marker of interest itself may reflect severity.

Adaptive response, maladaptive response, and epiphenomena all increase in the setting of active disease and can be proportional to disease severity. Because of this, even beneficial responses can be associated with increased mortality if they are measured in the setting of more severe disease. In heart failure this paradigm has been seen with IL-33. Increased IL-33 is associated with heart failure severity, but is actually likely to be cardioprotective.[26,27] Allegorically, while the number of firefighters at a fire are certainly associated with the severity of the fire, no one doubts that the firefighters are there to help. We intuitively understand the causal pathway in this example, but cannot be as certain for observed biologic pathways in disease.

Endothelin signaling is already a target in pulmonary arterial hypertension where elevation is harmful and blockade is clearly beneficial. This benefit is predominantly thought to be related to the action of endothelin-receptor blockade in the diseased pulmonary vasculature. This is different than left heart failure where endothelin-receptor blockade remains an active

area of investigation, the pulmonary vasculature is not the primary problem, and results are less encouraging.[11] The MELODY-1 trial of endothelin receptor-blockade in individuals with significant pre and post capillary disease was recently published and did not suggest benefit. Instead, there was worse volume retention in treated individuals and no significant impact on pre-capillary parameters.[11,28] Some have speculated that endothelin-receptor blockade may be similar to beta-blockade where short-term worsening may be offset by long-term disease stability and improved mortality.[29–32] None of the participants in the current study are known to have pulmonary arterial hypertension and our results, suggesting high levels of ET1 may prevent heart failure, cautiously argue against the idea that reduced ET1 levels or ET1 blockade will lead to a long term benefit in the absence of pulmonary arterial hypertension.

The possibility that low ET1 levels can lead to heart failure and higher ET1 levels may be cardioprotective is supported by animal models. Acutely, endothelin-receptor antagonism results in negative inotropy in animal models, which agrees with our observation that lower ET1 levels were associated with reduced LV ejection fraction.[14,17,28] To evaluate chronic impacts of ET1 difference, genetically modified mice with variable ET1 expression (20%, 65%, wild-type, and 350%) have been studied. Mice with lower ET1 expression develop a dilated cardiomyopathy and die more quickly than wild-type mice (20% expression- death at 560 days; 65% expression- death at 632 days; wild-type/100% expression- death at 841 days). Mice with over-expression of ET1 (350%) had slightly more cardiac hypertrophy, normal cardiac function, and lived 876 days.[16] It may be possible to have too much ET1 as mice with 1,500% over-expression of ET1 developed heart failure in a separate study.[33] This pre-clinical research agrees with our observation that lower ET1 levels (in a population with few extreme values) are associated with increased incidence of heart failure and cardiovascular death compared to higher levels.

The mechanism by which elevated ET1 levels may offer benefit is unknown. Though both pulmonary arterial and venous remodeling may occur in PH due to left heart disease, in most instances the majority of the pre-capillary component is functional in nature, as evidenced by the rapid reduction of pre-capillary parameters after left ventricular assist device implantation and LV unloading.[34–36] This functional component may in part be mediated by elevated ET1 levels.[37] This paradigm may be supported by our observation that increased ET1 levels are associated with smaller LV volumes and a higher LV ejection fraction. One might speculate that modest elevations in pulmonary vascular resistance could in fact “protect” the diseased left ventricle from further elevations in preload. Alternatively, there may be a direct action of ET1 on cardiac myocytes contributing to a greater tolerance for physiologic stress.[16]

This study has limitations. Our results do not definitively inform mechanism. It is noteworthy that statistical significance in adjusted models was not consistent, which increases the possibility for residual or unmeasured confounding to explain associations between elevated ET1 and cardiovascular outcomes. Alternatively the loss of significance could suggest that ET1 could mediate some of the effects of age, exercise, or cigarette smoking on cardiac health. Other causal explanations are also possible and would need to be investigated in mechanistic studies. For instance, elevated ET1 levels could be a

compensation for poorly functioning endothelin-receptors. Our description of circulating levels of ET1 do not necessarily reflect differences in endothelin signaling at the cellular level. Our results in isolation may raise more questions than they answer; however, our observations reinforce mechanistic studies in mice and align with recent tepid results in the randomized trials of endothelin receptor antagonists in left heart disease. The questions raised are important as we consider whether endothelin-receptor antagonism has a role in the treatment of left heart disease.

Summary

Our results also suggest, but do not confirm that higher ET1 levels measured in the absence of cardiovascular disease may be associated with a lower risk for incident heart failure or cardiovascular death. This agrees with previous results in pre-clinical animal models that suggest ET1 prevents heart failure. Understanding whether ET1 elevation is adaptive, maladaptive, or merely a bystander response in left heart failure is important because there are ongoing plans to target this pathway in diseases of left heart failure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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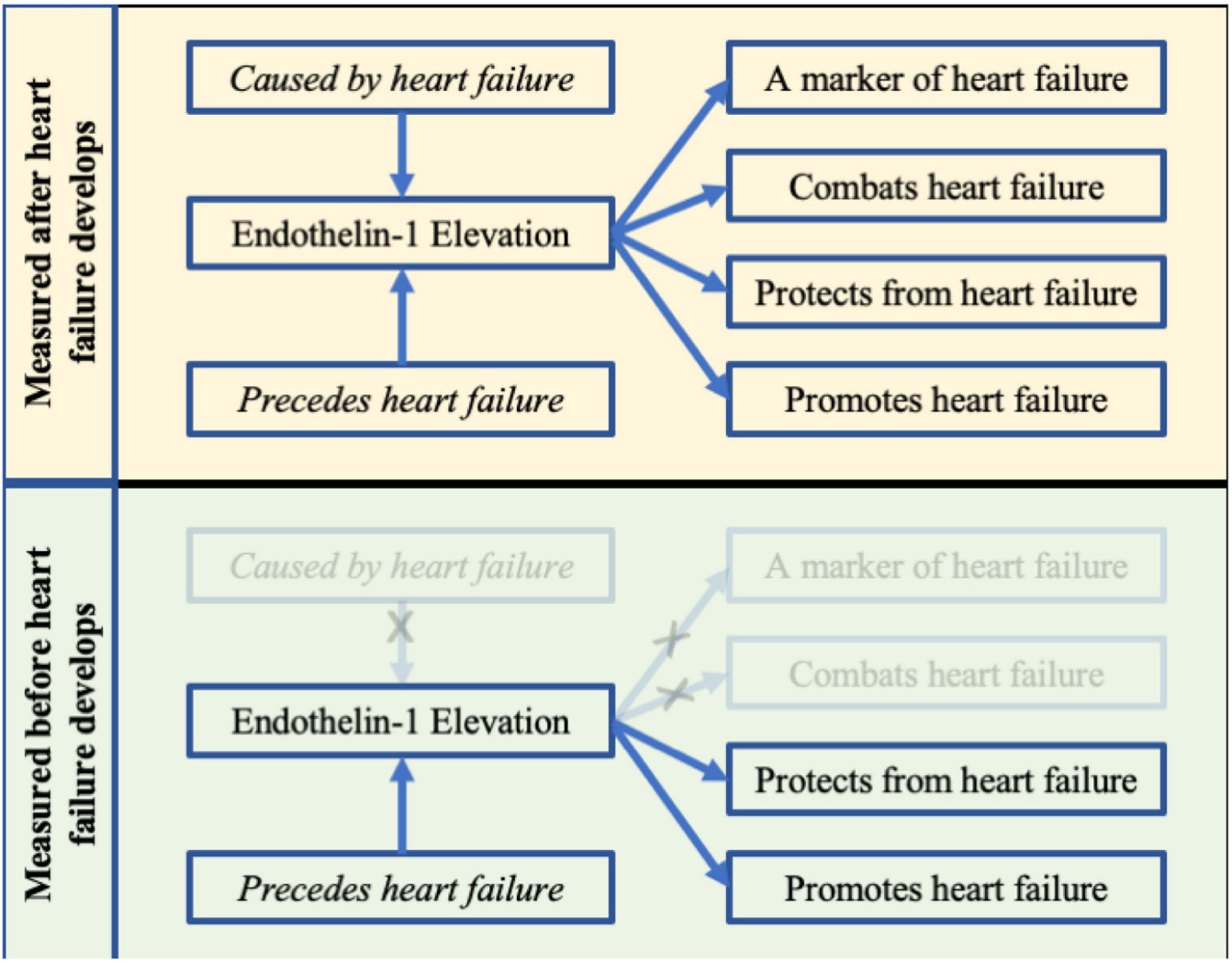

Figure 1. Timing of endothelin-1 measurement.

If endothelin-1 is associated with heart failure, there are fewer possible explanations if endothelin-1 is measured before the development of disease in a prospective cohort design.

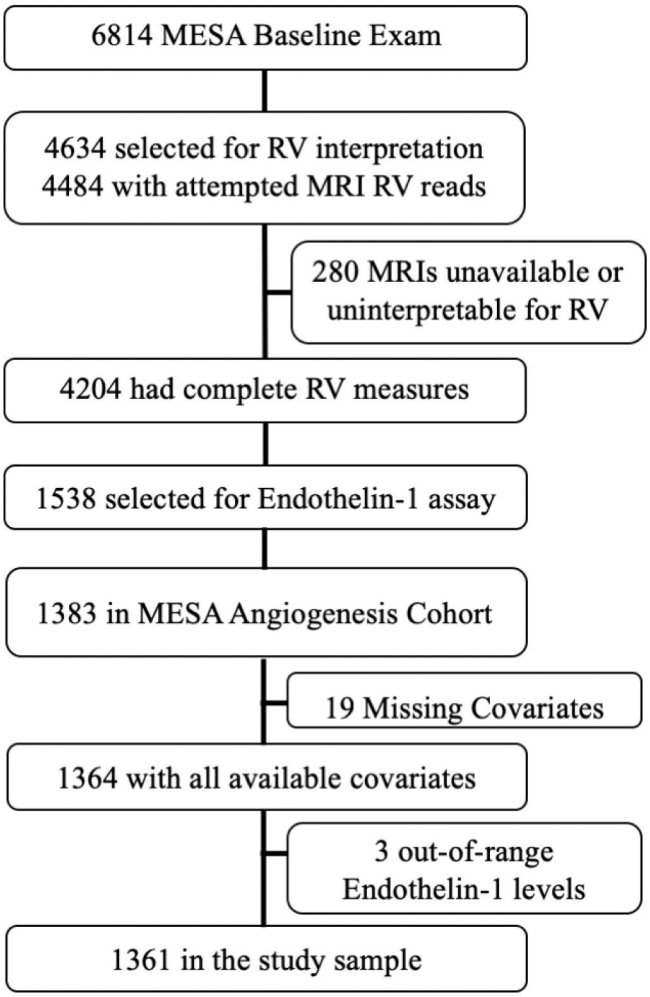

Figure 2.
Study Design

Table 1.

Characteristics of the MESA Angiogenesis *Cohort*

	n=1,361
Mean Endothelin-1 (pg/mL)	51.3 ± 9.7
Endothelin-1 range (pg/mL)	34.6 − 189.1
Age (years)	61 ± 10
Female (%)	52
Race (%)	
White	40
Chinese	12
African-American	27
Hispanic	21
Height (cm)	166 ± 10
Weight (kg)	77 ± 16
Body mass index (kg/m^2)	28 ± 5
Educational attainment (%)	
No high school degree	16
High school degree	19
Some college or certificate	28
Bachelor's Degree	17
Higher than bachelor's degree	20
Insurance Status (%)	
No insurance	7
Medicare	33
Private insurance	76
Cigarette smoking status (%)	
Never	53
Former	36
Current	11
Pack-years	10 ± 21
Metabolic Syndrome (%)	33
Hypertension (%)	43
Systolic blood pressure (mmHg)	125 ± 21
Diabetes mellitus (%)	13
Glucose (mg/dL)	97 ± 31
Cholesterol (mg/dL)	194 ± 33
NT-ProBNP (pg/mL)	93 ± 147
Medications (%)	
NSAIDs	42
Beta-blockers	9
ACE-inhibitors/ARBs	17
Any diuretic	12

Data presented as mean ± standard deviation or percentage as appropriate Abbreviations: pg=picogram, mL=milliliter, cm=centimeters, kg=kilograms, m^2=meters squared, mmHg=millimeters of mercury, mg=milligram, dL=deciliter, NT-ProBNP=amino-terminal fragment of pro-B-type natriuretic peptide NSAIDs=non-steroidal anti-inflammatory medications, ACE-inhibitors= angiotensin converting enzyme inhibitors, ARBs=angiotensin II receptor blockers

Table 2.

Unadjusted and multivariable linear regression estimating associations of demographic and cardiac risk factors with endothelin-1 levels (n=1,361)

	Difference in Endothelin-1 Level (pg/mL)					
	Univariate			Multivariate		
	Difference	**(95% CI)**	**p-value**	**Difference**	**(95% CI)**	**p-value**
Age (per 10 years)	−0.8	(−1.3, −0.3)	0.002	−0.8	(−1.4, −0.2)	0.007
Female	0.1	(−0.9, 1.1)	0.84	−0.2	(−1.2, 0.9)	0.75
Body mass index (per 5 kg/m^2)	−0.2	(−0.7, 0.3)	0.50	−0.2	(−0.8, 0.4)	0.46
Race						
White			Referent			Referent
Chinese	−1.5	(−3.3, 0.2)	0.07	−3.3	(−5.5, −1.2)	0.002
African-American	−0.6	(−1.9, 0.7)	0.36	−0.8	(−2.3, 0.6)	0.26
Hispanic	−0.7	(−2.1, 0.7)	0.35	−2.3	(−4.1, −0.6)	0.008
Educational attainment						
< high school degree			Referent			Referent
High school degree	0.9	(−0.9, 2.7)	0.31	0.4	(−1.4, 2.2)	0.67
Some college	0.5	(−1.1, 2.1)	0.53	−0.6	(−2.2, 1.2)	0.54
Bachelor's Degree	−0.7	(−2.5, 1.0)	0.42	−1.9	(−3.9, 0.0)	0.06
≥ bachelor's degree	0.1	(−1.7, 1.8)	0.93	−1.6	(−3.5, 0.4)	0.12
Exercise (per 500 MET/min/week)	0.1	(0.0, 0.2)	0.02	0.1	(0.0, 0.2)	0.06
Pack-years (per 10 years)	−0.4	(−0.6, −0.1)	0.003	−0.4	(−0.6, −0.1)	0.003
GFR (per 10 mL/min/1.73m^2)	0.2	(−0.2, 0.5)	0.32	0.1	(−0.2, 0.5)	0.46
Hypertension	−0.6	(−1.7, 0.4)	0.24	0.2	(−1.0, 1.3)	0.79
Diabetes mellitus	−1.4	(−2.9, 0.2)	0.09	−1.0	(−2.6, 0.6)	0.22

Multivariate model included all listed variables and also included study site.

Data presented as mean ± standard deviation or percentage as appropriate

Abbreviations: pg=picogram, mL=milliliter, CI= confidence interval, kg=kilograms, m^2=meters squared, MET/min/week= Metabolic equivalents per minute per week

A parsimonious backward elimination multivariate model of predictors of endothelin-1 level included only age (−0.8 pg/mL per 10 additional years of age, p=0.004), intentional exercise (0.1 pg/mL per 500 additional MET/min/week, p=0.02), and pack-years of smoking (−0.3 pg/mL per 10 pack-years, p=0.008).

Table 3.

Multivariable linear regression estimating associations between endothelin-1 level and cardiac structure and function (n=1,361)

	Per log increase in Endothelin-1		
	Difference	**(95% CI)**	**p-value**
Right Ventricle			
RV mass, g (Unadjusted)	0.5	(−1.1,2.0)	0.57
RV mass, g (Limited model *)	−0.8	(−1.9,0.4)	0.18
RV mass, g (Full Model †)	−0.8	(−2.0, 0.3)	0.17
RVEDV, mL (Unadjusted)	1.9	(−8.7, 12.5)	0.72
RVEDV, mL (Limited model)	−6.0	(−13.2, 1.2)	0.10
RVEDV, mL (Full Model)	−6.9	(−14.0, 0.4)	0.06
RVEF, % (Unadjusted)	0.0	(−2.2, 2.3)	0.99
RVEF, % (Limited model)	0.2	(−1.9,2.3)	0.85
RVEF, % (Full Model)	−0.1	(−2.0, 2.2)	0.91
Left Ventricle			
LV mass, g (Unadjusted)	−1.8	(−15.5,11.8)	0.79
LV mass, g (Limited model *)	−3.0	(−12.5, 6.6)	0.53
LV mass, g (Full Model †)	−1.9	(−11.0,7.1)	0.67
LVEDV, mL (Unadjusted)	−0.1	(−10.8, 10.6)	0.98
LVEDV, mL (Limited model)	−8.2	(−16.4,0.0)	0.05
LVEDV, mL (Full Model)	−8.9	(−17.1,−0.7)	0.03
LVEF, % (Unadjusted)	2.5	(0.0,5.1)	0.05
LVEF, % (Limited model)	2.8	(0.5, 5.2)	0.02
LVEF, % (Full Model)	2.8	(0.5, 5.2)	0.02

Abbreviations: SD=standard deviation, CI=confidence interval, RV=right ventricular,

GFR=glomerular filtration rate, EDV=end-diastolic volume, and EF=ejection fraction, LV=left ventricular, g=grams, mL=milliliters

* Limited model: age, sex, race/ethnicity, height and weight, study site

† Full model: Limited + education, smoking status, pack-years, hypertension, systolic blood pressure, diabetes, and cholesterol

Table 4.

Cox proportional hazard regression estimating the relationship of log of endothelin-1 level at the baseline exam with clinical outcomes (n=1,361)

Hazard Ratio per log increase in endothelin-1	**level**	**(95% CI)**	**p-value**
Hazard of heart failure or cardiovascular death			
Unadjusted	0.09	(0.01, 0.73)	0.03
Limited model *	0.06	(0.00, 1.03)	0.05
Full Model †	0.07	(0.00, 1.30)	0.08
Hazard of heart failure			
Unadjusted	0.04	(0.00, 1.20)	0.06
Limited model *	0.10	(0.01, 1.90)	0.13
Full Model †	0.14	(0.01, 2.91)	0.20
Hazard cardiovascular death			
Unadjusted	0.02	(0.00, 2.09)	0.10
Limited model *	0.06	(0.00, 3.01)	0.16
Full Model †	0.07	(0.00, 3.21)	0.17
Hazard of non-cardiovascular death			
Unadjusted	0.37	(0.02, 6.54)	0.50
Limited model *	0.60	(0.07, 5.09)	0.64
Full Model †	0.76	(0.08, 6.83)	0.80

Definition of abbreviations: CI-confidence interval

Because initial models suggested a non-proportional hazard, all models accounted included a term accounting for the possibility of a time-varying relationship between endothelin-1 and the hazard of heart failure or death. Three influential endothelin-1 outliers were excluded from the primary analysis. Inclusion of these outlier strengthened the association with mortality

* Limited model: age, sex, race/ethnicity, height and weight, study site

† Full model: Limited + education, smoking status, pack-years, hypertension, systolic blood pressure, diabetes, and cholesterol