

Proteomics and the Role of
HIV Infection in Cardiac Remodeling

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Abstract

Cardiac remodeling, defined as structural and/or functional alterations of the heart, is an early and pivotal feature within the pathophysiologic spectrum of heart failure (HF). Improved detection of cardiac remodeling could aid in earlier HF intervention, more refined risk prediction, more precise characterization of clinical disease, and more effective mitigation of HF progression. This is true both in the general population and higher risk populations, such as persons living with HIV (PLWH). There is a large and growing burden of cardiovascular disease (CVD) among PLWH, and even in the context of effective, modern antiretroviral treatment, PLWH have a higher risk of HF relative to uninfected populations. Characterization of cardiac structural phenotypes among clinically relevant HIV patient populations is, however, both inconsistent and limited.

In the first chapter, we analyzed data from the Multi-Ethnic Study of Atherosclerosis and report proteomic profiling of left ventricular structural phenotypes. Our results highlight the promise of more recently identified biomarker candidates for cardiac remodeling and HF. In the second chapter, we sought to externally validate previously identified cardiac remodeling biomarker candidates using data from the Veterans Aging Cohort Study, which has a high representation of PLWH. In the third chapter, we utilized data on over 19,000 veterans referred for echocardiography within Veterans Affairs healthcare system, the largest single provider of HIV care in the United States, to better characterize cardiac structure among PLWH. We found that HIV infection and measures of HIV disease severity were cross-sectionally associated with adverse cardiac structural phenotypes. Among a subsample of participants with proteomic profiling, we also observed associations between HIV serostatus and candidate biomarkers of cardiac remodeling.

This dissertation ultimately leveraged data from unique and classical epidemiologic sources to advance research on candidate biomarkers and the role of HIV infection in cardiac remodeling.

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A. Introduction

Heart failure (**HF**) is a substantial public health and economic burden, expected to increase in both prevalence and cost over the next 10 years.¹⁻³ Unfortunately, the diagnosis of HF, a complex clinical syndrome, is largely limited to symptomatic stages,^{4, 5} before which preclinical alterations in cardiac structure often occur.⁶⁻⁹ These diagnostic limitations taken together with high prevalence of asymptomatic disease¹⁰⁻¹³ and high mortality at diagnosis¹⁴ are highly problematic, posing a considerable obstacle to early treatment, which could reduce poor outcomes.^{15, 16}

HF is highly heterogeneous in its pathology. However, cardiac remodeling—which is independently associated with cardiovascular events and mortality in multiple population-based cohorts¹⁷⁻²¹—is an early and pivotal player in dysfunction across this pathophysiologic spectrum.²²⁻²⁵ Cardiac remodeling is defined as alterations in regional or global geometry or function of the heart occurring as a physiologic adaptation either to stressors that persistently elevate myocardial workload or to the effect of disease that may reduce contractility or alter tissue composition of the myocardium. Direct consequences of cardiac remodeling include malignant arrhythmias and cardiac dysfunction, two of the primary underlying pathologies of HF.

Although cardiac remodeling has been relatively well-characterized biologically, very few factors have been identified that contribute to remodeling in cardiac diseases, particularly on a population level. Further, detection of subclinical HF markers such as cardiac remodeling is imperative to early intervention, and a reliable, cost-effective, and simple clinical tool remains largely an unmet need.

This necessity and accompanying knowledge gaps extend from the general population to higher risk populations, such as persons living with HIV (**PLWH**). There is a large and growing burden of cardiovascular disease (**CVD**) among PLWH,²⁶⁻²⁹ and it is currently well-recognized that even in the context of effective, modern antiretroviral treatment (**ART**), PLWH experience a higher risk of CVD—including HF—relative to uninfected populations.³⁰⁻³⁹ This excess risk is independent of traditional risk factors, making a more complete understanding of contributing mechanisms imperative to providing adequate care for an aging population of PLWH.

Though some mechanisms for CVD among PLWH have been largely agreed upon, the understanding of what may drive the observed excess risk remains incomplete, and

there are currently no means by which to mitigate it. Further, treatment for CVD among PLWH is currently guided by data from clinical trials conducted among HIV-uninfected persons, despite knowledge that pathophysiology may in some cases be distinct between these populations.

The scope of the extant literature on the population-level biology of cardiac remodeling is limited in that cellular and soluble biomarkers are often few and inconsistent between studies. Further, explored mechanisms are often complex and overlapping, and biomarkers used to represent such processes cannot clearly or fully reflect hypothesized biology. This poses a considerable impediment to developing novel clinical tools for its detection and strategies to mitigate risk by limiting the field's ability to choose well-informed biomarker and therapeutic target candidates.

Proteomics is a method growing in feasibility and popularity that facilitates the large-scale exploration of protein concentrations in biospecimens, and pathway analysis utilizes databases containing content on protein function, pathways, and systems models to place proteomic findings in a broader biological context. Together with cardiac imaging, these tools could facilitate the much-needed hypothesis generation for the expansion of our understanding of cardiac remodeling, both in the general population and among PLWH.

The objective of this dissertation is to leverage data available from both unique and classical epidemiologic sources to meaningfully progress research on the population-level associated biology of cardiac remodeling and the role HIV infection may play in that process.

B. Background and Rationale

B.1. HEART FAILURE

Heart failure (HF) is a clinical syndrome characterized by higher intracardiac pressure and/or lower cardiac output resulting from impaired ventricular contractility (systolic dysfunction) or relaxation (diastolic dysfunction).^{4, 5} HF is classified broadly into four subtypes defined by left ventricular ejection fraction (**LVEF**), the percentage of blood ejected from the left ventricle with each cardiac contraction:

- HF with reduced ejection fraction (**HFrEF** or systolic HF; LVEF ≤40%)
- HF with preserved ejection fraction (**HFpEF** or diastolic HF; LVEF ≥50%)
- HF with mid-range ejection fraction (**HFmEF**; LVEF 41-49%)
- HF with preserved ejection fraction, improved (HFpEF improved; a subset of HFpEF patients with previous HFrEF who may be clinically distinct; LVEF >40%)

HF is further characterized into stages⁴ and functional classes,⁴⁰ which respectively reflect progression through and patient functional status with the syndrome. The defining features of each stage and functional classification, including some treatment considerations, are outlined below in **Figure 1**.

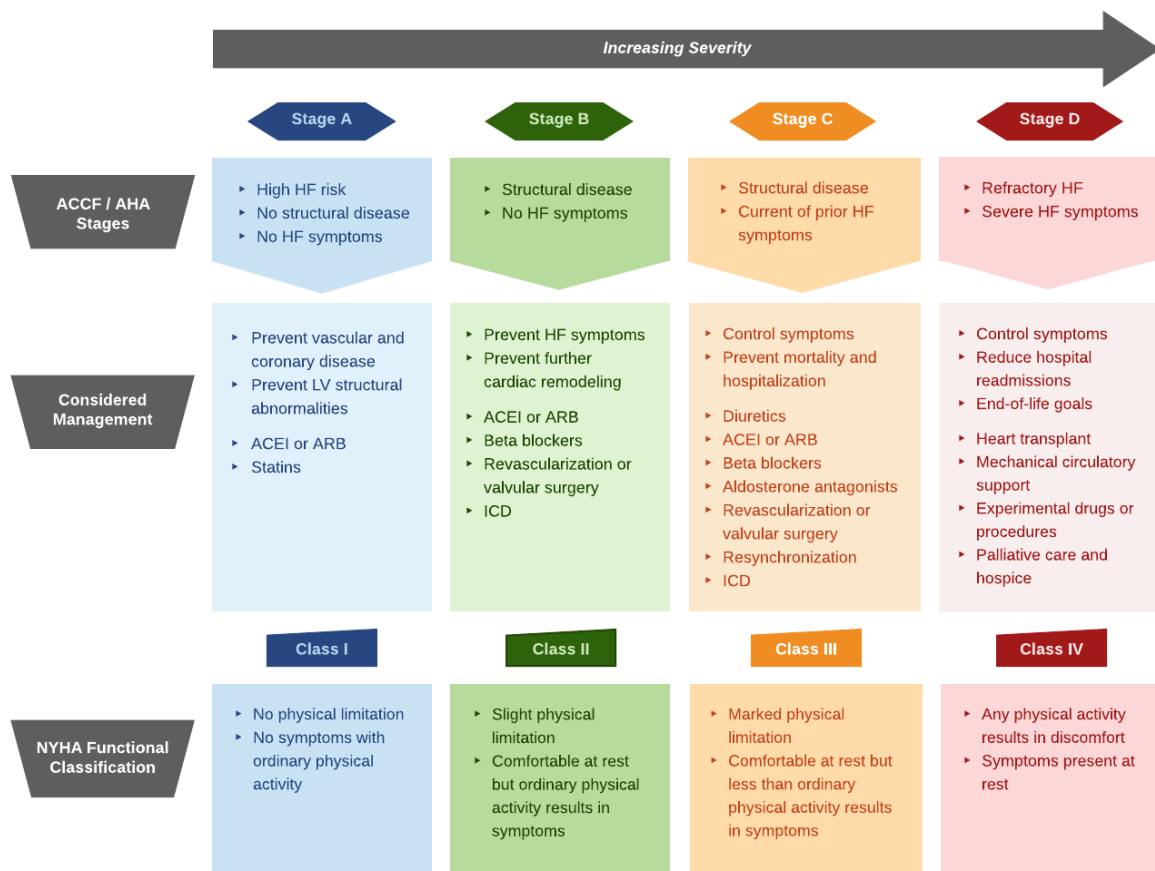


Figure 1. American College of Cardiology Foundation (ACCF) / American Heart Association (AHA) heart failure stages and New York Heart Association (NYHA) heart failure functional classification system. Considered Management includes treatment goals and possible therapeutic strategies; adapted from ACCF / AHA Guidelines for Management of Heart Failure.²

HF=heart failure; LV=left ventricular; ACEI=angiotensin-converting enzyme inhibitor; ARB=angiotensin II receptor blocker; ICD=implantable cardioverter-defibrillator.

Burden, Prognosis, and Challenges in Current Care

Diagnosed HF currently affects over 26 million people globally¹ and, due in part to an aging population, is rising in prevalence.^{2,3} Lifetime risk of HF in the United States is over 20% after the age of 40 years with over 870,000 new cases diagnosed annually and a roughly 50% 5-year survival rate at diagnosis.² Though outcomes have improved with advances in management, 12-month post-diagnosis hospitalization and mortality remain high,¹⁻³ making it both a substantial economic^{2,41} and public health burden.

Diagnosis of HF can be challenging, given its syndromic definition. Diagnosis ends up largely limited to symptomatic clinical contexts (Stage C/D),^{4,5} before which structural and/or functional cardiac abnormalities are typically present.⁶⁻⁹ Population-based studies have in fact estimated prevalence of subclinical, Stage A/B HF at 68% among individuals aged 47-62 years¹⁰ and 82% among older individuals, aged 67-91 years.¹¹ The diagnostic limitations, high mortality rate at diagnosis, and prevalence of asymptomatic disease is—taken together—highly problematic. Improved detection of subclinical abnormalities could lead to earlier treatment, which could in turn reduce poor outcomes, including HF mortality.^{15,16}

Treatment for HF is dependent on stage (**Figure 1**), with goals in asymptomatic stages (A/B) being largely prevention-oriented and goals in symptomatic stages (C/D) being management-oriented. Because several cardiovascular complications can pathologically contribute to HF risk, general cardiovascular disease (**CVD**) risk factors are the primary targets for early HF intervention. This might include standard management of comorbidities such as hypertension or hyperlipidemia, which play a critical role in the development of cardiovascular complications across the life-course. Other common HF comorbidities include obesity, diabetes mellitus, valvular disease, lung disease, and kidney disease—all of which can play a role in treatment strategy.^{4,5}

Later stage treatment of HF focuses on symptom management and prevention of hospitalization. Examples include management of edema via diuretics as well as treatment of contributing pathologies—including arrhythmias via cardiac resynchronization therapies or coronary heart disease via revascularization.^{4,5} Earlier intervention is substantially less invasive and impactful on quality of life than later stage intervention, and there is evidence early intervention is more effective at prolonging the

HF disease course.^{15, 16, 42, 43} This highlights the importance of subclinical HF detection and intervention.

B.2. CARDIAC REMODELING

As discussed above, diagnosis of HF is often limited to symptomatic clinical contexts, before which preclinical alterations in cardiac structure and function are often present. This is problematic, given the high prevalence of early stage, asymptomatic HF as well as the benefit of early treatment. Further, HF is a highly heterogeneous condition both in underlying etiologies and responses to therapies.^{4, 5} Cardiac remodeling, however, is a process preceding both systolic and diastolic dysfunction^{6-9, 23, 24} across this spectrum and has been independently associated with cardiovascular events and mortality in multiple population-based cohorts.¹⁷⁻²¹

Cardiac remodeling is defined as alterations in regional or global geometry or function of the heart resulting from molecular, cellular, or interstitial changes following acute or chronic insult. It occurs as a physiologic adaptation either to stressors that persistently elevate myocardial workload or the effect of disease that may reduce contractility or alter tissue composition of the myocardium. Cardiac remodeling is classically characterized as either physiological (adaptive) or pathological (maladaptive), which may differ in stimuli, manifestation, and consequences.

Quantification and Characterization

Though failure of the right heart does occur, it is a less prevalent condition and typically preceded by failure of the left heart via pressure and volume overload.⁴⁴ Further, though there has been a growing recognition of the effect of right heart alterations on cardiac dysfunction, fewer population-based studies have focused on these measures, particularly in defining cardiac remodeling. Remodeling of the left ventricle (**LV**) was therefore the focus of this dissertation—specifically, structural characteristics of the left ventricle that may capture both clinical and subclinical disease as well as changes in those features over time.

The law of LaPlace states that tension or stress within a chamber wall is proportional to the pressure within and radius of that chamber and inversely proportional to its wall thickness. Applied to cardiac remodeling, this suggests that in the context of pressure

overload, the ventricle must compensate to some degree with constriction or increase in wall thickness (and therefore mass) to maintain constant stress within the cardiac wall.⁴⁵ Left ventricular mass and volumetric measures are therefore commonly used to characterize cardiac structure and remodeling. It is important to acknowledge, however, these measures merely represent general phenotypes of a variety of complex pathological processes taking place during cardiac remodeling.

The American Heart Association defines cardiac remodeling using LV mass and volume (**Figure 2**) measured at end-diastole,⁴ which occurs at the end of cardiac relaxation (or the beginning of contraction) and represents the time point at which LV blood volume is at its maximum and chamber pressure is at its minimum. LV mass at end-diastole was explored in this dissertation, indexed to body surface area per recommendations⁴⁶ due to normal physiologic differences in cardiac size governed by body size. This parameter aims to capture hypertrophy generally. Additionally, the ratio of LV mass and volume at end-diastole—the LV mass-to-volume ratio (**M/V**)—was also evaluated to capture geometry, eccentric to concentric, defined in detail below. There are multiple noninvasive approaches to estimate these cardiac structural parameters, the primary methods for which are transthoracic echocardiography and cardiac magnetic resonance (**CMR**) imaging.

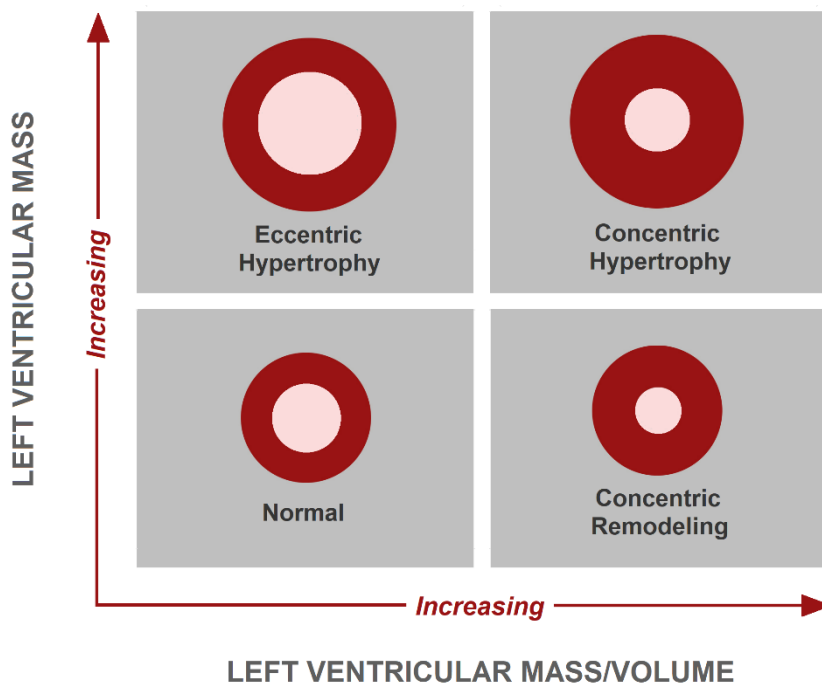


Figure 2. Patterns of left ventricular remodeling defined by mass and volume measured at end-diastole, adapted from Gjesdal, et al.

Echocardiography employs ultrasound and is used most widely due to its clinical availability and anatomic and prognostic validation.^{4, 5, 47, 48} Performance and limitations of echocardiography in estimating LV mass and volumetric measures varies by method: M-mode (one-dimensional echocardiography or **1-DE**), two-dimensional (**2-DE**), or three-dimensional (**3-DE**). The recommended formulae for calculating LV mass and volume via M-mode and 2-DE rely on assumptions regarding the geometry of the left ventricle as a fixed, prolate ellipsoid, which may be a source of measurement error.⁴⁶ LV mass calculated according to the formula endorsed by the American Society of Echocardiography (**ASE**) have, however, been shown to strongly correlate with LV mass measured at autopsy.⁴⁶ Alternatively, 3-DE does not require geometric assumptions, and, with the aid of contour tracking algorithms, can measure LV mass and volumes with greater accuracy and reproducibility.⁴⁹⁻⁵¹

Cardiac magnetic resonance, like 3-DE, also produces three-dimensional images and does not rely on geometric assumptions of chamber geometry for mass and volumetric estimations. The reproducibility and accuracy of volumetric measurements using cardiac MRI is superior to that of 2-DE, and it is superior image resolution over 3-DE that has made CMR the current gold standard for both structural and functional cardiac assessments.⁵²⁻⁵⁴ The trade-off, however, is clinical inaccessibility due to cost, examination time, and required expertise.

Reference values for the LV mass and volumes have been established for both CMR and echocardiography and are presented for those used in this dissertation (**Table 1**).

Table 1. Reference values for left ventricular mass and volume phenotypes in the general population by imaging modality and sex at birth.				
<i>Parameter</i>	<i>2-D Echocardiography</i> ^{A6}		<i>Cardiac MRI</i> ⁵⁵	
	<i>Male</i>	<i>Female</i>	<i>Male</i>	<i>Female</i>
LV mass, g	156 ± 34	115 ± 24	164 ± 36	114 ± 24
LV mass indexed to BSA, g/m ²	82 ± 17	69 ± 13	85 ± 15	67 ± 11
LV EDV, mL	106 ± 22	76 ± 15	142 ± 34	109 ± 23
LV EDV indexed to BSA, mL/m ²	54 ± 10	45 ± 8	74 ± 15	65 ± 11
Mean ± standard deviation presented. MRI=magnetic resonance imaging; LV=left ventricular; BSA=body surface area; EDV=end-diastolic volume.				

Population-Level Risk Factors

Left ventricular remodeling—as with most chronic, progressive diseases—is a multifactorial process. Several population-level predictors of LV remodeling have been identified, many of which are those for general cardiovascular disease. LV remodeling can both precede and follow other cardiovascular events or complications—including infarction, arrhythmia, and valvular disease⁵⁶⁻⁵⁹—which not only have shared risk factors but can also directly lead to heart failure.

Age-related cardiac remodeling has been studied extensively with varying results. Longitudinal cardiac MRI data from the Multi-Ethnic Study of Atherosclerosis (**MESA**) has shown LV mass and volumes decreased with age, though LV volumes declined more rapidly than mass, resulting in an increase in LV mass-to-volume ratio.^{55, 60} Longitudinal echocardiography data from Framingham has, however, suggested LV mass *increases* with age, while volumes decrease.⁶¹ These discrepancies extend to several cross-sectional studies⁶²⁻⁶⁵ and have been hypothesized to be due to differences in disease prevalence and assessment methodologies between studies.

Male sex at birth has repeatedly been shown to be associated with higher LV mass and volume compared to females, independent of other clinical characteristics, including height and weight.^{17, 62, 66} Evidence from the Cardiovascular Health Study actually suggests sex differences in LV mass are greater than differences observed between persons with and without clinical heart failure.⁶⁶

Current evidence suggests persons of both Black^{62, 65, 67, 68} race and Hispanic⁶⁹ ethnicity have higher LV mass compared to white persons in the United States, independent of clinical characteristics. These differences mirror that of disparities in general cardiovascular disease risk and are likely driven by deep-rooted social inequities.

Anthropometric measures also strongly and independently predict LV structural phenotypes. Obesity,^{61, 63, 70, 71} body mass index,^{68, 72} waist circumference,⁶⁵ bodyweight,⁶² and subscapular skin-fold thickness⁶² have each been associated with LV mass and volumes in the positive direction, with evidence from both cross-sectional and longitudinal studies.

Unsurprisingly, numerous studies have identified common cardiovascular disease comorbidities as risk factors for LV remodeling, as well. Perhaps the most valuable

independent clinical predictor of LV mass and volumes is hypertension, which leads to increased afterload, cardiac wall stress, and—in the long-term—hypertrophy. Epidemiologic evidence shows hypertension and use of blood-pressure lowering medication is associated with higher LV mass and volumes both cross-sectionally and longitudinally.⁶¹ More granularly, several studies have shown systolic blood pressure is positively associated with LV mass and end-diastolic volume, and diastolic blood pressure is negatively associated with LV end-diastolic volume.^{62, 63, 73, 74} Lower kidney function measured via Cystatin C^{75, 76} and glomerular filtration rate⁷⁷ has also been associated with higher LV mass, perhaps by way of its strong associations with hypertension. There has also been consistent evidence of higher LV mass and lower LV volume in persons with diabetes compared to persons without.^{68, 73, 78, 79} Finally, it is worth noting the relationship between blood cholesterol levels and cardiac remodeling has been studied extensively, and, though there is no consensus, mostly weak associations have been observed.^{80, 81}

Lifestyle factors are associated with LV phenotype, as well. Importantly, a higher level of self-reported physical activity is associated with higher LV mass^{73, 82, 83} due to physiological remodeling that occurs as a result of elevated cardiac workload, observed among both athletes and in the general population. Increases in LV mass and end-diastolic volume are proportional to the level of activity, but the ratio is static, highlighting the distinction between physiologic and pathologic remodeling (discussed in detail below) as well as the value of the LV mass-to-volume ratio parameter. Higher LV mass has also been consistently associated with current tobacco use in both cross-sectional and longitudinal studies,^{72-74, 82} as well as use of other substances, including alcohol,⁸⁴ cocaine,⁸⁵ and amphetamines.⁸⁶ Lastly, social factors—including low educational attainment and self-reported social isolation—have been associated with higher LV mass, independent of other important risk factors.⁸⁷

Outcomes and Response to Therapy

Largely due to its value in clinical event prediction, assessing LV mass and volumes has become integral to diagnosis, prognosis, and management of patients with a variety of cardiovascular diseases.⁸⁸⁻⁹¹ Such structural measures are highly predictive of cardiac dysfunction as well as incident cardiovascular events, including heart failure.¹⁷⁻²¹ Though effect estimates have varied, evidence from MESA specifically have suggested a 1.4

(95% CI: 1.2–1.5) times higher hazard of HF per 10% increment in LV mass and a 2.3 (0.8–6.1) times higher hazard of HF per 1 g/mL increment in LV M/V, adjusted for traditional CVD risk factors.¹⁷ Further, LV remodeling has been shown to add significant information to event prediction beyond that of traditional cardiovascular disease risk factors.²¹ Reduction of LV mass observed with some therapeutics has also been demonstrated to reduce cardiovascular events,⁹²⁻⁹⁵ further highlighting the value of LV structure as a subclinical disease marker.

Treatment of pathologic remodeling aims to slow or reverse the adaptive processes contributing to structural and functional phenotype alterations. Therapeutic targets have largely been indirect, i.e., centered on reduction of risk factors, and have involved both pharmacologic and device-based treatments.

In the Losartan Intervention for Endpoint Reduction in Hypertension trial,⁹³ blood pressure reduction was associated with a significant lowering of LV mass and LV mass index over a mean (SD) follow-up of 4.8 (1.0) years. This reduction in LV mass among participants on losartan was also associated with lower incidence cardiovascular events and mortality. Though studies of device-based interventions are limited to study populations with symptomatic heart failure, they have suggested approaches such as biventricular pacing,⁹⁶ LV assist devices,⁹³ and aortic valve replacement⁹⁷ may each result in beneficial reductions in LV mass and volume in such populations.

The clinical and biological relevance of LV remodeling outlined above makes characterization of left ventricular structure—and particularly change in structural phenotypes over time—a valuable measure of disease across the pathophysiologic spectrum of heart failure.

Pathophysiology

Cardiomyocytes are the cells responsible for the contractile function of the myocardium. They are terminally differentiated shortly following birth, so adaptation of the cardiac muscle in response to elevated workload does not generally occur via cell proliferation but instead via cell hypertrophy, classically characterized as either physiological or pathological (**Figure 3**).²²⁻²⁵ During physiological hypertrophy, cardiomyocytes grow in both length and thickness, manifesting clinically as mild, coordinated increases in both ventricular volume and wall thickness (eccentric hypertrophy).²²⁻²⁵ This type of

remodeling occurs in response to stimuli such as postnatal growth, pregnancy, and endurance training. During pathological hypertrophy, cardiomyocytes grow more in thickness than in length, manifesting clinically as reduction in ventricular volume with increases in wall thickness (concentric hypertrophy).²²⁻²⁵ Maladaptation to low volume may then lead to further lengthening of cardiomyocytes and chamber dilation (eccentric hypertrophy).²²⁻²⁵ Insults that trigger pathological remodeling may be acute or chronic and include pressure overload due to hypertension or aortic stenosis; volume overload due to mitral regurgitation, aortic regurgitation, or chronic kidney disease; and myocardial hypoxia due to myocardial infarction (MI) or metabolic syndrome.²²⁻²⁵

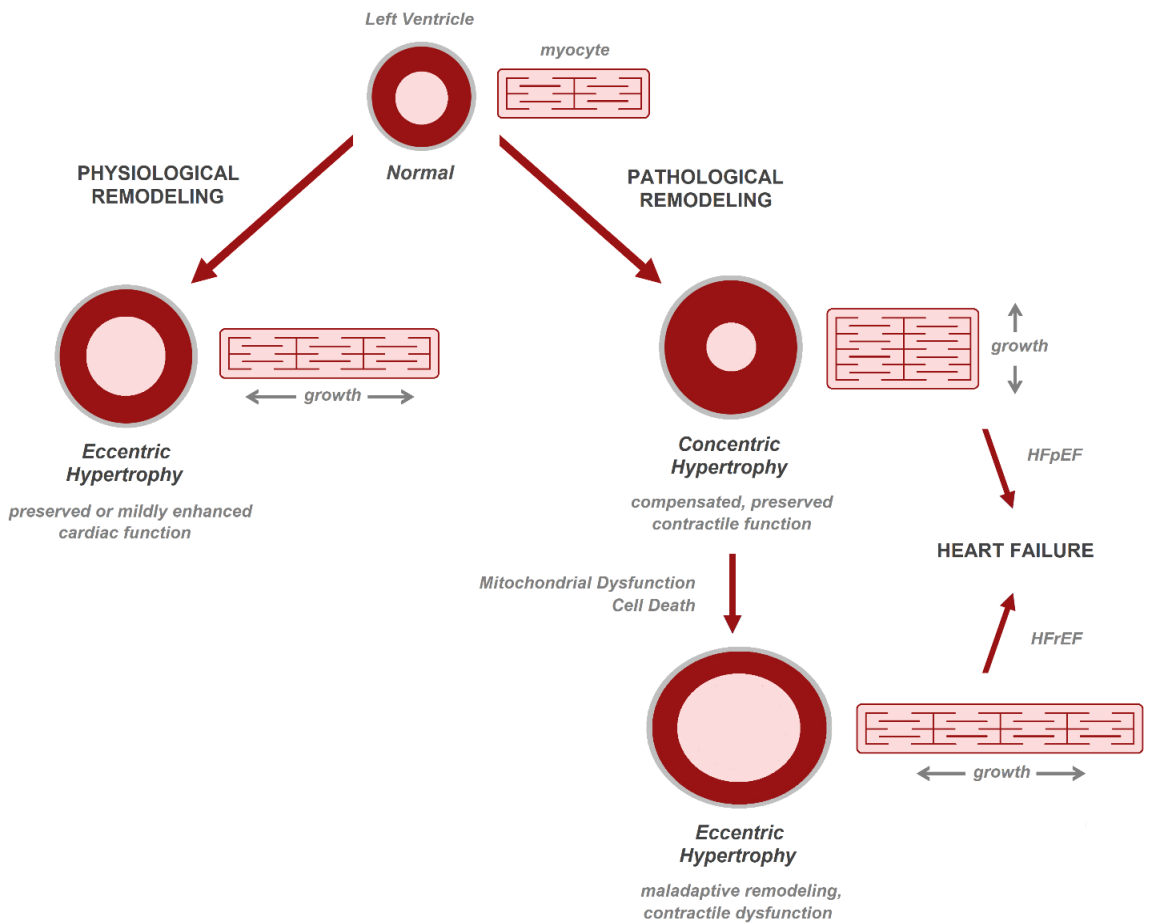


Figure 3. General overview of physiological and pathological cardiac remodeling processes. HFpEF=heart failure with preserved ejection fraction; HFrEF=heart failure with reduced ejection fraction. Adapted from Nakamura et. al.²³

Cardiac remodeling occurs via signaling pathways involved in cellular growth, tissue repair, fluid regulation, and inflammation.⁹⁸⁻¹⁰⁰ The remodeling process therefore not only affects cardiomyocytes but also other cell types (fibroblasts, endothelial cells, immune cells), contents of the interstitial space, and the influence of these factors on myocardial architecture. Specific mechanisms involved depend on the stimuli initiating remodeling and therefore likely differ between physiological and pathological subtypes and further by pathological etiology. The general pathophysiology, however, is relatively well-characterized.

In physiological remodeling, the adaptive response to increased workload induces cell survival signaling, increased energy production and efficiency, angiogenesis that is proportional to the growth of the ventricular wall, antioxidative control mechanisms, mitochondrial quality control, and cardiomyocyte regeneration.²²⁻²⁵ In pathological remodeling, these processes begin to fail, leading to cell death, calcium transport dysregulation, metabolic reprogramming, reactivation of fetal gene expression, fibrosis, altered myocyte structure, impaired protein and mitochondrial quality control, and insufficient angiogenesis.²²⁻²⁵

Though knowledge is undoubtedly incomplete, several mechanisms have been implicated in pathological remodeling and how it may ultimately contribute to cardiac dysfunction. Most of these processes are governed by neurohormonal activation of either the sympathetic or renin-angiotensin-aldosterone system. Both systems activate signaling pathways involved in protein synthesis and growth factors within myocytes and fibroblasts that lead to hypertrophy and fibrosis, vasoconstriction and water retention, as well as oxidative stress and cytotoxicity that leads to cell death.²²⁻²⁵

First, impairment and imbalance in mechanisms of apoptosis, necrosis, and autophagy during remodeling leads to a progressive loss of myocytes, which generally do not proliferate or regenerate.¹⁰¹⁻¹⁰⁵ The architecture of the heart is also modified via imbalance of collagen synthesis and degradation in the interstitium. The interstitial space is largely comprised of cross-linked type I and type III collagen fibers that act to maintain structural alignment while regulating distensibility of the cardiac muscle. In cardiac remodeling, an abnormal accumulation of collagen (type I and type III) has been observed, resulting in fibrosis and ultimately decreases in contractility as well as interference of electrical conduction and reentry.¹⁰⁶⁻¹⁰⁹ Energy metabolism is also altered during remodeling in a manner that results in an energy deficit. Specifically, oxidation of

free fatty acids—the primary cardiac energy substrate—decreases while glucose oxidation increases, resulting in lower myocardial energy availability and overproduction of reactive oxygen species (**ROS**).¹¹⁰⁻¹¹² Oxidative stress resulting from imbalance in ROS production and antioxidant defense can then lead to several conditions that may contribute to remodeling—including cellular dysfunction, DNA damage, metalloproteinase activation, fibroblast proliferation, induction of apoptosis, changes in calcium-transport proteins, and activation of signaling pathways involved in hypertrophy.¹¹³⁻¹¹⁶ Lastly, both the adaptive and innate immune systems are thought to be intimately involved in remodeling with similar effects—including cellular growth, metalloproteinase activation, fibroblast proliferation, and induction of apoptosis.¹¹⁷⁻¹¹⁹

Current Biomarkers

It is true and perhaps highlighted above that cardiac remodeling has been relatively well-characterized with respect to cellular effectors and signaling. However, very few factors have been identified at a population level that contribute to initiation or perpetuation of remodeling in cardiovascular diseases. Screening for cardiac remodeling using circulating biomarkers is therefore not currently recommended under any American or European guidelines.^{4, 5} However, several circulating protein biomarkers are currently used in cardiac remodeling and heart failure research, with varying degrees of clinical value or promise (**Table 2**). These biomarkers generally reflect biological processing of myocardial stress, myocardial injury, remodeling of the extracellular matrix, and inflammation.

Perhaps the most valuable biomarker used in research and diagnostics is N-terminal prohormone of brain natriuretic peptide (**NT-proBNP**). NT-proBNP is a protein secreted in response to myocyte stretch due to increased ventricular preload¹²⁰ and is elevated with cardiac dysfunction. In several population-based cohorts, this biomarker improves risk prediction for both CVD outcomes and mortality¹²¹⁻¹²⁵ and is in fact used in clinical routine to rule out diagnosis of HF (cut-point NPV: 0.94-0.98)—though it should not be used to establish it (cut-point PPV: 0.44-0.67).^{4, 5} NT-proBNP has had suboptimal performance as a screening method for cardiac remodeling, however, particularly in unselected individuals with somewhat improved performance in high-risk populations.^{126, 127} Another limitation of NT-proBNP utility is its lack of pathologic specificity; it cannot reliably discriminate CVD pathologies^{4, 5, 120} and is often elevated in the context of

extracardiac conditions such as renal insufficiency or systemic inflammation.¹²⁸

Several of the other biomarkers currently explored suffer from the challenge of non-cardiospecificity. Circulating biomarkers of collagen turnover or metabolism, for example, may be elevated due to extracardiac fibrotic activity, e.g., in the liver. Ultimately, though several biomarkers have been evaluated in the extant literature, none have yet shown strong clinical utility in screening or identifying patients at high risk of cardiac remodeling and preclinical HF.

Table 2. Circulating molecules proposed as biomarkers of cardiac remodeling. ¹²⁹	
<i>Primary Biological Process</i>	<i>Biomarkers</i>
Myocardial stress, stretch	natriuretic peptides (NT-proBNP, BNP, ANP forms), ST2, GDF15
Myocardial injury, apoptosis	troponins (I, T), myosin light-chain kinase I, sFas, creatinine kinase-MB
Extracellular matrix remodeling	matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), galectin-3, cystatin C, osteopontin, collagen propeptides (PICP, PINP, PIIINP, CITP)
Proliferation and differentiation	TGF- β , syndecans (1, 4)
Inflammation	interleukins, C-reactive protein, TNF- α , MCP-1, GDF15, osteoprotegerin
Oxidative stress	malondialdehyde, myeloperoxidase, thiobarbituric acid reactive substances (TBARs), oxidized low-density lipoproteins, superoxide dismutase, catalase, glutathione forms, total plasma polyphenols
Neurohormonal activation	norepinephrine, angiotensin II, renin, aldosterone, copeptin, endothelin, prolactin
NT-proBNP=N-terminal pro b-type natriuretic peptide; BNP=brain natriuretic peptide; ANP=atrial natriuretic peptide; ST2=suppression of tumorigenicity 2; GDF15=growth differentiation factor 15; PICP=procollagen type I carboxy-terminal propeptide; PINP=procollagen type I N-terminal propeptide; PIIINP=procollagen type III N-terminal propeptide; CITP= Carboxy-terminal telopeptide of type I collagen; TGF- β =transforming growth factor- β sFas=soluble Fas receptor; TNF- α =tumour necrosis factor- α ; MCP-1=monocyte chemo-attractant protein-1	

B.3. HIV INFECTION

Human immunodeficiency virus (**HIV**) is a single-stranded, positive-sense RNA retrovirus transmitted via virus-containing blood, semen, pre-seminal fluid, rectal fluid, vaginal fluid, or breast milk contact with a mucous membrane, damaged tissue, or the bloodstream. HIV infection is currently incurable and has heavily impacted the world since its emergence in the early 1980s—not only from a public health and economic perspective but also socially by way of stigma and discrimination.

Burden, Prognosis, and Current Care

HIV infection burden, prognosis, and care vary substantially between and among world regions, but it remains a significant public health concern in both lower and higher income nations. In the United States, an estimated 1.2 million people over the age of 12 years were living with HIV at the end of 2018, an estimated 14% of whom had not yet been diagnosed.¹³⁰

If left untreated, HIV infection leads to progressive loss of cellular immunity and eventually acquired immunodeficiency syndrome (**AIDS**), a condition in which opportunistic infection and malignancy can thrive without substantial immune challenge. One of the greatest feats in modern medicine, however, was the development of antiretroviral therapy (**ART**), which has transformed an HIV diagnosis from a death sentence into that of a manageable, chronic condition among those with access to care. This has shifted the spectrum of morbidity among this population from AIDS to chronic non-AIDS health complications—including CVD, which has become a major contributor to mortality among PLWH in the United States.²⁶

Current care for PLWH is focused on early diagnosis as well as early initiation of and strong adherence to ART, all of which are associated with lower rates of disease progression and all-cause mortality.¹³¹⁻¹³³ Though there are several clinical considerations involved in regimen selection, current guidelines¹³⁴ recommend most PLWH initiating ART are prescribed a specific combination of two nucleoside reverse transcriptase inhibitors (**NRTI**) (i.e., tenofovir alafenamide [TAF] or tenofovir disoproxil fumarate [TDF] plus emtricitabine [FTC] or lamivudine [3TC], or 3TC plus abacavir [ABC]) with one integrase strand transfer inhibitor (**INSTI**) (i.e., dolutegravir [DTG], bictegravir [BIC], or Raltegravir [RAL]). Important to consider here are the toxicity profiles

Table 3. Toxicities associated with antiretrovirals prescribed in HIV care. ¹³⁵		
<i>Antiretroviral Class</i>	<i>Agent(s)</i>	<i>Documented Adverse Effects</i>
nucleoside reverse transcriptase inhibitor (NRTI)	abacavir (ABC)	associated with higher risk of CVD in some observational studies
	tenofovir (TDF, TAF)	<ul style="list-style-type: none"> ▪ renal toxicity, including proximal tubulopathy and acute or chronic insufficiency ▪ osteomalacia has been reported as a consequence of proximal tubulopathy ▪ TDF associated with decreased bone mineral density
integrase strand transfer inhibitor (INSTI)	bictegravir (BIC), dolutegravir (DTG), raltegravir (RAL), elvitegravir/ cobicistat (EVG/c)	<ul style="list-style-type: none"> ▪ inhibits tubular secretion of creatinine ▪ weight gain ▪ possible neural tube defects in infants born to women on DTG, unknown class effect of INSTIs ▪ depression and suicidal ideation have been reported
	raltegravir (RAL)	Increases in creatinine kinase, myopathy, and rhabdomyolysis have been reported
non-nucleoside reverse transcriptase inhibitor (NNRTI)	efavirenz (EFV), rilpivirine (RPV)	<ul style="list-style-type: none"> ▪ QTc interval prolongation ▪ Short- and long-term neuropsychiatric effects; depression, suicidality, catatonia, ataxia, and encephalopathy have been reported
	efavirenz (EFV)	dyslipidemia
protease inhibitor (PI)	atazanavir (ATV/c, ATV/r)	<ul style="list-style-type: none"> ▪ causes indirect hyperbilirubinemia ▪ nephrolithiasis, cholelithiasis, nephrotoxicity ▪ adverse gastrointestinal effects ▪ ATV/c inhibits tubular secretion of creatinine
	darunavir (DRV/c, DRV/r)	<ul style="list-style-type: none"> ▪ associated with higher risk of CVD in one observational study ▪ hepatotoxicity reported ▪ adverse gastrointestinal effects ▪ DRV/c inhibits tubular secretion of creatinine

and adverse events associated with these commonly prescribed antiretrovirals (outlined in **Table 3**). Other classes of antiretrovirals, i.e., protease inhibitors (**PI**) and non-nucleoside reverse transcriptase inhibitors (**NNRTI**), are prescribed less frequently for reasons such as more severe adverse effects, greater potential for emergence of viral resistance mutations, potential drug interactions, and less evidence from randomized clinical trials. Though there is no dispute that the benefits of ART far outweigh the risks, long-term exposure to these toxicities may have cumulative effects on end organs.

Also of note, regular clinic visits to monitor HIV viral load, kidney function, liver function, and other basic chemistries is currently recommended every 6 months among virologically suppressed PLWH in the United States.¹³⁴ Under these guidelines, PLWH in care may be, on average, seen more frequently than uninfected persons with similar comorbidities.

Predictors of HIV Acquisition and Progression

Several predictors of HIV acquisition have been identified—both sociobehavioral and biological—several of which are complexly interrelated. Many of these risk factors are also important to contextualize, as they are highly dependent on social and structural environments, including accessibility of appropriate prevention tools and services, current public policy, and stigma.

Disparities in HIV acquisition among gender and sexual identities remain in the United States. Men reporting male-to-male sexual contact are still disproportionately affected by HIV compared to other identities or sexual contact types. In 2018, approximately 69% of new HIV diagnoses were among self-identified gay and bisexual men.¹³⁰ Transgender women are also disproportionately affected by HIV infection.¹³⁰ A recent report from the Centers for Disease Control and Prevention estimated prevalence of HIV among transgender women in the United States may be as unacceptably high as 19% based on a meta-analysis of studies conducted between 2006 and 2017.¹³⁶ Disparities also exists among racial and ethnic subgroups. Specifically, Black as well as Hispanic persons have substantially higher risk of HIV diagnosis in the United States compared to other racial and ethnic subpopulations.¹³⁰ As with cardiovascular diseases, this is undeniably driven at its foundation by social inequity that may have effects such as lower access of prevention and care services. It has also been suggested that higher prevalence among these subpopulations may be self-perpetuating in that social and risk networks tend to

have low racial heterogeneity, leading to higher probability of encountering HIV despite having comparable or even lower engagement in risk behaviors.¹³⁷

The primary high-risk behaviors associated with HIV acquisition are unprotected sex—particularly with multiple and concurrent partners^{138, 139}—and sharing of injection drug equipment.¹⁴⁰ Substance use disorder is associated with higher risk of HIV acquisition.^{141, 142} This is due to direct transmission risk among persons who inject drugs, which is unaided by punitive attitudes that have limited implementation of prevention strategies such as opioid substitution treatment and needle exchange programs. High-risk behavior associated with altered judgment from methamphetamine, cocaine, opioid, or alcohol use may also contribute.¹⁴³⁻¹⁴⁵

Low educational attainment, unemployment, and unstable housing have also been associated with HIV acquisition risk,¹⁴⁶ likely lying on several of the complex causal pathways involving behavior, social inequities, and stigma that elevates that risk.

There are also biological contributors to HIV acquisition risk. First, it has been estimated that ulcerative sexually-transmitted diseases—e.g., genital herpes (due to HSV-1 or HSV-2), syphilis, or chancroid—may more than double risk of acquisition among HIV-uninfected persons.^{147, 148} Second, with the advent of pre-exposure prophylaxis (**PrEP**) came a pharmacologic approach to HIV prevention; consistent, daily use of certain combination antiretrovirals reduces risk of HIV acquisition by as much as 99% among high-risk uninfected persons.^{149, 150}

Outcomes among PLWH are dependent on several HIV-specific factors, but major prognostic indicators are timing of HIV diagnosis relative to the infection event, timing of ART initiation, as well as ART access, adherence, and retention in care. Higher CD4+ T-cell count and lower HIV viral load at diagnosis (typically observed with earlier diagnosis) as well as more rapid ART initiation have been associated with substantially lower rates of AIDS-defining illness, non-AIDS defining morbidity, and all-cause mortality.^{131, 132, 151} ART adherence also predicts prognosis, with interrupted use associated with higher viral load and its immunologic consequences, development of viral ART resistance, incidence of both AIDS-defining and non-AIDS-defining illnesses, and mortality.¹⁵²⁻¹⁵⁴ Related is retention in care, which is a major barrier to optimal HIV care through lower ART adherence, less frequent monitoring of therapeutic response, less successful treatment of comorbidities, and ultimately poorer outcomes.¹⁵⁵⁻¹⁵⁹

Basic Pathology and Natural History

The primary target of HIV is activated CD4+ T-cells, entry into which is mediated by virion interaction with CD4 and the chemokine coreceptors, CXCR4 and CCR5.¹⁶⁰ Other cells bearing these receptors are also sensitive to infection—including resting CD4+ T-cells, monocytes, macrophages, and dendritic cells.¹⁶⁰

In acute or primary infection, HIV replication rises rapidly with a concurrent flood of inflammatory cytokine and chemokine production and an innate immune response largely mediated by natural killer cells.¹⁶¹ A strong adaptive immune response also occurs shortly following infection in which HIV-specific CD8+ T-cells kill productively infected cells.¹⁶² Approximately three months post-infection, neutralizing antibodies are produced,¹⁶³ and this seroconversion can often be accompanied by flu-like symptoms, such as fever and fatigue.¹⁶⁴ These innate and adaptive immune responses will decrease viral load initially and partially recover CD4+ T-cells, but mutations in key viral epitopes are selected for in these processes, leading to viral immune evasion, exhaustion of HIV-specific T-cells, and loss of effector function.^{162, 165, 166}

The hallmark of HIV infection is progressive loss of CD4+ T-cells due not only to direct infection but also immunologic bystander effects.¹⁶⁷ There is a particularly large depletion in activated CD4+ T-cells in the gastrointestinal tract observed in early infection, which is not recovered following ART initiation.¹⁶⁸ The relative composition of T-cell subsets is altered, as well, including disproportionate loss of cells imperative to bacterial defense—including T-helper-17 (**Th-17**) cells and mucosal-associated invariant T-cells.^{169, 170} Lastly, permanent HIV-mediated damage and fibrosis of lymphatic tissue can lead to depletion of naïve T-cells.¹⁷¹

The chronic phase of infection begins at approximately six months post-infection and is characterized by slower progression relative to the acute phase (**Figure 4**). Over time, however, HIV viral load will steadily increase with a concurrent reduction in CD4+ T-cell count. Without intervention, this eventually leads to severe deficiency in cell-mediated immunity and progression to AIDS.

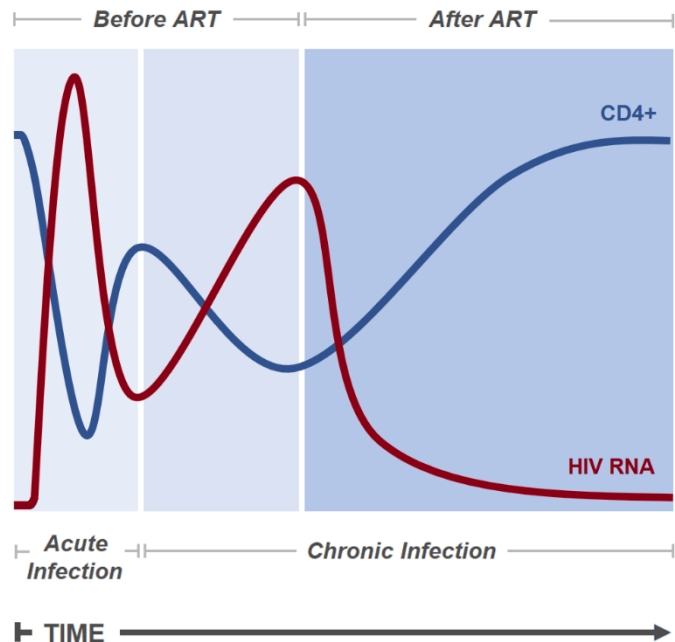


Figure 4. Approximate relative CD4+ T-cell count and viral load through the natural history of HIV infection, assuming initiation of antiretroviral (ART), specifically in the chronic phase of infection.

ART initiation can be but is not often initiated during the acute phase of infection, and

irreparable damage to the immune system is largely unavoidable, even in the current treatment era. Though incomplete and variable, immune recovery does occur with initiation of ART, as does a rapid decline in plasma HIV viral load. The virus persists in ART-treated PLWH, however, which is hypothesized to be due to low-level residual replication,¹⁷² latent infection of resting memory T-cells,¹⁷³ and anatomical viral reservoirs in lymphoid tissue, the gastrointestinal (**GI**) tract,¹⁷⁴ and the central nervous system.¹⁷⁵ This persistence can lead to continued immune dysfunction, chronic inflammation, and their consequences.

B.4. CONVERGENCE OF HIV INFECTION AND CARDIOVASCULAR PATHOLOGIES

Among PLWH, prolonged life expectancy with the advent of ART brought with it higher prevalence of age-related diseases, including CVD and its commonly observed comorbidities. It has been estimated that by the year 2030, 73% of PLWH will be over the age of 50 years, and 78% will have CVD.²⁹ Such morbidities among PLWH have

been observed with earlier onset and higher risk relative to uninfected comparators and may be pathophysiologically distinct from those occurring in the general population.¹⁷⁶

Epidemiologic Evidence

It is currently well-recognized that even in the context of effective, modern ART regimens, PLWH have a higher risk of cardiovascular complications relative to uninfected populations, independent of traditional risk factors. There is evidence of this elevated risk with incident events—including heart failure^{30, 33, 38} and related pathologies, such as MI,^{30-32, 177} sudden cardiac death,³⁴ and atrial fibrillation³⁰—as well as subclinical disease markers—including coronary artery calcium,¹⁷⁸⁻¹⁸⁰ carotid intima-media thickness,^{179, 181} arterial stiffness,^{182, 183} left ventricular hypertrophy,¹⁸⁴⁻¹⁸⁷ and cardiac dysfunction.^{184-186, 188} Longitudinal studies conducted in the United States have yielded HIV effect size estimates for events that are comparable to that of traditional CVD risk factors, such as hypertension or hyperlipidemia. In fact, a recent meta-analysis including 793,635 PLWH and 3.5 million person-years yielded a pooled hazard ratio estimating PLWH have a 2.2 (95% CI: 1.7-2.8) times higher hazard of any CVD event compared to uninfected persons.²⁷

Heart failure among ART-treated PLWH has garnered specific concern due to consistent and relatively large HIV effect estimates, some of which are independent of myocardial infarction and exclude participants with hypertension and alcohol, tobacco, or cocaine abuse.^{30, 33, 38} Specifically, longitudinal data with adjudicated events in the Veterans Aging Cohort Study (**VACS**) has suggested PLWH have a 1.4 (95% CI: 1.3-1.5) times higher risk of incident HF over a median follow-up of 7 years, independent of demographics, smoking, hypertension, LDL-c, HDL-c, triglycerides, statin use, Hepatitis C infection, renal disease, BMI, substance use, atrial fibrillation, and major depression.³³ A recent analysis within a large US healthcare database (MarketScan) estimated an even larger independent effect with a hazard ratio of 3.2 (2.4-4.2).³⁰ This excess risk has been observed for both diastolic HF (HFpEF) and systolic HF (HFrEF) and may be greater among younger persons; Black persons; those with comorbidities such as obesity, hypertension, or diabetes; those who smoke or have alcohol dependence; and PLWH with higher HIV viral load or lower CD4+ T-cell count.³³

There is also evidence from a small study conducted among women in the United States suggesting HF prognosis may be poorer among PLWH, with higher rates of HF hospitalization and mortality observed relative to uninfected comparators.¹⁸⁹

Hypothesized Mechanisms

HIV infection likely contributes to risk of HF through augmentation of mechanisms that translate from the general population as well as via HIV-specific mechanisms. However, the pathophysiology of HF among PLWH remains poorly defined, particularly in the modern ART era.

Differing prevalence in more traditional CVD risk factors between PLWH and uninfected persons undoubtedly contributes in part to excess risk. Smoking is one of the most important modifiable CVD risk factors among PLWH,^{190, 191} with prevalence estimates in the United States as high as 42% for current smokers and 20% for former smokers from a large, nationally representative sample.¹⁹² Heavy alcohol use is also highly prevalent and associated with CVD among PLWH in the United States.¹⁹³ Use of other substances—particularly methamphetamine and cocaine—is also relatively common among PLWH¹⁹⁴⁻¹⁹⁶ and may contribute to higher risk of HF¹⁹⁷ and MI.¹⁹⁸ Mental health disorders, such as anxiety and mood disorders, also disproportionately affect PLWH and may also play a role.¹⁹⁴⁻¹⁹⁶ Evidence on the role of hypertension is inconsistent, with some studies suggesting ART-treated PLWH have a higher prevalence relative to uninfected comparators and other studies concluding no association.¹⁹⁹⁻²⁰¹ Physical activity and cardiorespiratory fitness have been reported low among PLWH and are associated with vascular dysfunction as well as risk for CVD and mortality within this population.^{202, 203} Though these are important contributors, most HIV effects on incident CVD and related cardiac abnormalities have been estimated independent of many of these factors.

Prevalence of other viral infections—e.g., herpes simplex viruses, hepatitis B (HBV), Hepatitis C (HCV)—are high among PLWH compared to the general population.^{204, 205} Such co-pathogens could contribute to a chronically activated immune state that initiates or perpetuates common CVD pathologies, particularly in the context of HIV-mediated immune dysfunction (discussed further below).²⁰⁶⁻²⁰⁹

Care disparities may also contribute. There is literature suggesting differences in access to or receipt of cardiovascular care among PLWH in the United States, relative to uninfected comparators. Specifically, lower utilization of secondary prevention measures such as aspirin/anti-platelet and lipid-lowering therapy have been described among those with indication,²¹⁰⁻²¹² and this may certainly contribute to higher risk of both subclinical and clinically overt CVD.

Long-term antiretroviral use may play a role in risk of HF and related pathologies among PLWH, as well, despite safety profiles markedly improving and the benefits of ART far outweighing risks. Earlier generation antiretrovirals—including many protease inhibitors—were associated with increases in triglyceride levels as well as fat redistribution to the abdomen and deposition of adipose tissue in the muscles and liver.²¹³⁻²¹⁵ Though current first-line antiretrovirals have been shown to have minimal lipid effects,¹³⁵ body composition changes have been associated with ART initiation of any type, specifically increases in both subcutaneous and visceral fat.²¹⁶ Weight gain following initiation of INSTIs has recently been of particular concern.²¹⁷⁻²¹⁹ Many antiretrovirals have been associated with higher risk of CVD events, as well, including not only early generation protease inhibitors²¹⁶ but also a current-generation protease inhibitor (darunavir) and nucleoside reverse transcriptase inhibitor (abacavir),²²⁰⁻²²³ both in relatively widespread clinical use. Despite these possible ART-mediated mechanisms, the impact of ART toxicities on CVD risk is currently thought to be low.²²⁴

Biological effects of HIV itself appear to play a major role in the pathophysiology of CVD among ART-treated PLWH, and—though knowledge is certainly incomplete—the interconnected mechanisms of immune dysfunction, persistent immune activation, and hypercoagulation have been implicated. Though antiretrovirals do improve these conditions, they remain abnormal relative to uninfected persons, and together, they create the perfect storm for development and exacerbation of vascular disease that could lead to HF and related cardiovascular pathologies.

There is consistent evidence among ART-treated PLWH demonstrating abnormally high levels of circulating inflammatory biomarkers—including interleukin (IL)-6—and immune activation biomarkers—including soluble cluster of differentiation (CD)163 and CD14—that are also strongly predictive of subclinical CVD markers, CVD events, and all-cause mortality.²²⁵⁻²³⁷ Inflammation and immune activation are recognized as major pathological contributors to initiation and perpetuation of atherosclerosis and have been

associated with lower high-density lipoprotein cholesterol, higher oxidized low-density lipoprotein cholesterol, and microvascular dysfunction among PLWH.²³⁸⁻²⁴⁰ Immune activation is also intimately involved in the pathology of fibrosis. Persistent systemic inflammation, in particular, contributes to fibrotic activity in the myocardium via chronic insult and activation of inflammatory pathways within cardiac tissue.¹¹⁷

There has also been consistent evidence among ART-treated PLWH demonstrating elevated levels of coagulation—measured primarily by D-dimer—compared to uninfected persons, also strongly predictive of CVD events and all-cause mortality among PLWH.^{225, 226, 228, 230, 236, 237} Coagulation is a well-characterized process involved in thrombotic cardiovascular pathologies, e.g., MI, and is closely related to inflammation and immune activation.²⁴¹ Hypercoagulation in the context of HIV infection is thought to be the result of elevated tissue factor activity and decreased anti-coagulant activity, triggered ultimately by persistent systemic inflammation and its consequences, e.g., microvascular disease.²⁴²

Chronic immune activation among PLWH is undoubtedly multifactorial, and several contributing factors have been hypothesized. Even in the context of successful viral suppression to undetectable levels in the blood, HIV persists in reservoirs throughout the body, activating both innate and adaptive immune responses at low but continuous levels.²⁴³⁻²⁴⁵ HIV infection can also lead to permanent immunologic damage such as fibrosis within lymphatic tissues,²⁴⁶ which can impair immune recovery and compromise the immune response to HIV itself as well as co-pathogens, contributing to a persistently activated immune state.^{206, 207, 247} There has also been work among ART-treated PLWH highlighting the potential role of immune activation at the level of mucosal surfaces, particularly in the GI tract.^{169,248-252} Reduced ratios of Th-17 and possibly regulatory T-cells have been observed (as previously noted), as well as damage to the integrity of the mucosal epithelium, chronic translocation of bacterial antigens, and alterations in the GI microbiome.

B.5. IMPORTANT GAPS IN KNOWLEDGE

Among the General Population

Pathological cardiac remodeling is well-established to play a critical role in the natural history of several CV pathologies that lead to HF, thereby making it a focus of research

efforts in areas of risk stratification, diagnosis, treatment efficacy monitoring, and novel therapeutics.

Detection of subclinical markers of HF such as LV remodeling is necessary if the goal of early stage intervention is to be achieved in the future, and better defining who undergoes assessment based on risk factors is imperative to ensuring cost-effectiveness of screening strategies. There is currently no reliable, fully validated biomarker of LV remodeling, making this a greater challenge in detection of subclinical HF.

Although LV remodeling has been relatively well-characterized with respect to cellular effectors and signaling, very few factors have been identified that contribute to initiation and/or perpetuation of remodeling in cardiac diseases. Several biomarkers for early detection have been proposed in recent years—including carboxy-terminal propeptide of procollagen type I (PICP), soluble ST2, and galectin-3—few of which have shown true clinical promise. Of importance, several identified candidates lack cardiospecificity and are instead involved in general biological processes. A frequently discussed example of this challenge are biomarkers of fibrosis, which occurs in response to injury across tissue types and organs; these markers may be clinically problematic in the presence of elevated fibrotic activity outside the myocardium, e.g., in the liver. Further, apart from classical factors that have been reviewed above, there may be novel factors of importance currently unidentified.

Among Persons Living with HIV Infection

The knowledge gaps outlined above extend from the general population to higher risk populations, such as PLWH. Further, though some mechanisms for CVD among PLWH have been largely agreed upon, the understanding of what may drive the observed excess risk remains incomplete, and there are currently no means by which to mitigate it. Also of note, treatment for CVD among PLWH is currently guided by data from clinical trials conducted among HIV-uninfected persons, despite knowledge that pathophysiology may in some cases be distinct between these populations.

The general consistency of epidemiologic findings on the potential role of inflammation contributing to excess risk for CVD among ART-treated PLWH has motivated research on novel anti-inflammatory treatment strategies to be given in adjunct to ART. There are several completed trials of candidate treatments, with most failing to demonstrate effects

on inflammatory biomarkers that might warrant further study in clinical outcome trials.²⁵³

28-34

The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) was a clinical outcome trial recently conducted among HIV-uninfected persons to evaluate the use of canakinumab—a therapeutic IL-1 β -specific monoclonal antibody—to prevent adverse cardiac events. Results demonstrated canakinumab significantly reduced risk of MI, stroke, or CVD death as well as fatal cancer events compared to placebo via a mechanism that lowers IL-6 and hsCRP levels.²⁵⁴ Importantly, however, there was also significantly higher observed risk of death due to infection or sepsis in those receiving canakinumab.²⁵⁴ Considering both the unique features of HIV-associated inflammation as well as the potential for infection with immune deficiency, ongoing and future research will need to consider balancing risks and benefits associated with anti-inflammatory strategies in the context of HIV infection. This may also support the need for an alternative approach to risk mitigation via pathways that have not yet been identified.

Meanwhile, the population of ART-treated PLWH is aging, rapidly increasing the need for solutions to these unanswered questions.

Challenges with Biomarkers

Protein biomarkers measured in epidemiologic studies provide a means to evaluate physiologic states in individuals at a given point in time. They do, however, have several non-trivial limitations in terms of their ability to help define pathophysiologic mechanisms that may contribute to disease risk. In general, biomarkers assayed must be hand-selected by investigators, typically informed by current literature, which is subject to publication bias and largely conducted in the general population. This may be problematic in translation to other populations, e.g., ART-treated PLWH, where pathology may reasonably differ. This process is also inherently biased by our incomplete knowledge of biology. Further, biomarkers presently used to evaluate mechanisms implicated in cardiac remodeling or HF—both among persons living with and without HIV—cannot clearly and fully reflect complex and overlapping biology that may be at play. This is of particularly critical consideration when few biomarkers are being evaluated. Taken together, these limitations pose a substantial impediment on developing better strategies for cardiac remodeling and HF risk mitigation by limiting the

field's ability to select novel and well-informed candidates for screening and therapeutic strategies.

B.6. PROTEOMICS AND PATHWAY ANALYSIS TO FILL THE GAPS

Improved detection of subclinical markers of heart failure such as left ventricular remodeling is imperative to early-stage HF intervention, and improvement in intervention strategies will always be a goal. These concepts extend from the general population to PLWH, who are at greater risk of HF and related pathologies due to mechanisms that are incompletely understood.

Such knowledge gaps are due in part to the fact plasma proteins explored for disease associations in the extant epidemiologic literature represent a miniscule proportion of the peripheral blood proteome—the entire complement of circulating proteins. Proteomics is an emerging research method that facilitates the large-scale exploration of these proteins in biospecimens, offering a more expansive approach to mechanistic hypothesis generation.²⁵⁵⁻²⁵⁷

Protein expression is dependent on a host of stimuli and other factors, including both type and location of the cell of protein origin. Proteomics can be applied to a variety of biospecimens, including specific cell types and tissues. This has advantages in pathophysiology studies, specifically the ability to measure protein signatures within a specific system that may be directly involved in a given pathology. This dissertation, however, did not focus on protein expression within cell or tissue subsets but instead evaluated circulating protein abundance in plasma. This approach is systemic in nature—i.e., the source of many circulating proteins may be undeterminable—which may result in pathologically non-specific results. The advantage of this approach, however, is that measurement is non-invasive and is more easily translatable to a clinic setting, e.g., for use as a biomarker in screening or treatment monitoring.

Pathway analysis is a general term used in bioinformatics to describe the use of statistical approaches or software programs to identify biologic processes over- or under-represented in the results of high throughput -omics analyses. In its use in this dissertation, it pooled statistical results of protein-phenotype associations by pathway using databases with content on protein function, pathways, and systems models. This helped place analysis results in a broader biological context.

Together with cardiac imaging, proteomics and pathway analysis tools could facilitate the next steps in closing the highlighted knowledge gaps. The studies in this dissertation leveraged available proteomics and cardiac imaging data from a well-described population-based cohort (MESA) and the largest cohort of PLWH in the current ART era (VACS) to generate hypotheses for better defining biological drivers of LV remodeling and the role of HIV in this process at the population level.

C. Chapter 1: *Proteomics of Cardiac Remodeling in the Multiethnic Study of Atherosclerosis*

C.1. OVERVIEW

Introduction

Plasma proteomic profiling may offer a more expansive approach to novel biomarker discovery and mechanistic hypothesis generation for cardiac remodeling, a critical component of a range of cardiovascular pathologies. This study utilized a large-scale, high-throughput aptamer-based proteomic platform to identify circulating biomarkers associated with left ventricular (LV) structure in a diverse, population-based cohort.

Methods

Plasma abundances of 1,953 proteins were quantified in participants of the Multi-Ethnic Study of Atherosclerosis at baseline (2000-2002) and year 10 of follow-up using an aptamer-based proteomic profiling platform. LV mass index and LV mass-to-volume ratio were assessed by gold-standard cardiac magnetic resonance (CMR). Linear regression with robust variance was used to assess associations between protein levels and LV structure cross-sectionally, as well as with their change over ten years.

Results

This study included 763 participants (mean age 60 ± 10 years at baseline; 53% female; 19% Black race; 31% Hispanic ethnicity) with both CMR and proteomic profiling. Following adjustment for kidney function and traditional risk factors, 4 proteins were associated with LV mass index and 8 with LV mass-to-volume ratio (false discovery rate <0.10), replicated both at baseline and year 10 of follow-up.

Conclusion

This study reports proteomic profiling of LV structural characteristics, the results of which both reinforce previous findings and highlight the promise of more recently identified biomarker candidates for cardiac remodeling and heart failure.

C.2. INTRODUCTION

The diagnosis of heart failure (HF), a complex clinical syndrome with substantial public health and economic burden, is largely limited to symptomatic stages,^{4, 5} before which preclinical alterations in cardiac structure often occur.⁶⁻⁹ These diagnostic limitations taken together with high prevalence of asymptomatic disease¹⁰⁻¹³ and high mortality at diagnosis¹⁴ are highly problematic, posing a considerable obstacle to early treatment, which could reduce poor outcomes.^{15, 16}

Cardiac remodeling—which is independently associated with cardiovascular events and mortality in multiple population-based cohorts¹⁷⁻²¹—is an early and pivotal player in dysfunction across the pathophysiologic spectrum of HF.²²⁻²⁵ Although cardiac remodeling has been relatively well-characterized biologically, very few factors have been identified that contribute to remodeling in cardiac diseases, particularly on a population level. Further, detection of subclinical HF markers such as cardiac remodeling could aid in earlier intervention, more refined risk prediction, more precise characterization of clinical disease, and ultimately effective mitigation of HF progression.

Aptamer-based proteomics arrays are growing in feasibility and popularity and facilitate systematic profiling of plasma protein signatures. These methods have previously been used to identify novel candidate biomarkers of cardiovascular disease risk.^{256, 258-260} Together with cardiac imaging, these tools could facilitate the much-needed hypothesis generation for the expansion of our understanding of cardiac remodeling.

The objective of this study was to use the aptamer-based SOMAScan platform (SomaLogic, Boulder, CO) and gold standard cardiac magnetic resonance (CMR) imaging to identify plasma proteins cross-sectionally associated with left ventricular structure among a diverse U.S. population in the Multi-Ethnic Study of Atherosclerosis.

C.3. METHODS

Study Population

The Multiethnic Study of Atherosclerosis (MESA) is a prospective population-based cohort initiated in 2000 to study the characteristics and progression of subclinical cardiovascular disease among a diverse population in the United States.²⁶¹ MESA participants were recruited from six geographic areas across the U.S.—Baltimore City and Baltimore County, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los

Angeles County, California; Northern Manhattan and the Bronx, New York; and St. Paul, Minnesota. At the time of enrollment, participants were 45-84 years of age and had no history of clinical cardiovascular disease—including coronary artery disease, peripheral vascular disease, cerebrovascular disease, and heart failure.²⁶¹

This study utilized data on a subset of MESA participants from baseline (Exam 1) and year 10 of follow-up (Exam 5). Proteomics was completed at both time points in 983 randomly selected MESA participants consenting to use of their data and specimens by commercial enterprises. At baseline, 763 of these participants had both proteomics that passed quality control checks and technically adequate CMR images from which LV phenotypes were ascertained, 652 of whom also had this data at Exam 5. Primary confounder data was missing in 13 and 10 participants at baseline and Exam 5, respectively, for final complete case analysis sample sizes of $n=750$ for baseline analyses and $n=642$ for all analyses utilizing Exam 5 values.

Outcome Definitions and Ascertainment

In MESA, cardiac magnetic resonance was used to quantify volume and dimensions of all four cardiac chambers. At Exam 1, CMR was conducted among consenting participants a median of 16 days following baseline evaluations (including blood draws used for proteomic assay), and 95% of imaging assessments were completed within 11 weeks. At Exam 5, CMR was conducted within a median of 15 days of specimen collection, with 95% completed within 10 weeks.²⁶²

CMR imaging at both Exam 1 and 5 was performed using 1.5-T scanners (Magnetom Avanto and Magnetom Espree, Siemens Medical Systems, Erlangen, Germany) with six-channel anterior and posterior phased-array torso coil elements. The protocol included acquisition of one cine horizontal long-axis section (i.e., four-chamber view), at least 12 cine short-axis sections from the atria to the cardiac apex, and one cine vertical long-axis section (i.e., two-chamber view).

All obtained CMR imaging data were analyzed and interpreted at a central location (Johns Hopkins Hospital, Baltimore, MD) by trained readers overseen by DA Bluemke and JAC Lima, each of whom have over 20 years of experience in clinical and research CMR interpretation. Readers were blinded to participant clinical information, including CVD risk factors. LV end-diastolic mass and volume were quantified using CIM software

(version 6.2; Auckland MR Imaging Research Group, University of Auckland, Auckland, New Zealand).²⁶³

Each reader independently analyzed every 10th consecutive CMR as a quality control measure. At Exam 1, the overall interobserver intraclass correlation (**ICC**) coefficients were 0.98 for both LV mass and LV end-diastolic volume (EDV), and technical errors of measurement were 6% and 4%, respectively.⁵⁵ At Exam 5, ICC coefficients for LV mass and LV EDV were similar at 0.95 and 0.96, respectively, and technical errors of measurement were 6% and 5%, respectively.²⁶⁴

LV structure was assessed at Exam 1 using a fast gradient-echo pulse sequence technique. The imaging protocol at Exam 5, however, utilized a magnetic resonance pulse sequence with steady-state free precession. The latter is the current standard but was unavailable at the baseline examination. Steady-state free precession allows for a faster examination of higher quality compared to the fast gradient-echo pulse sequence²⁶⁵ but has been shown to produce smaller LV mass measurements.²⁶⁶ To adjust for this difference in MESA, calibration curves were previously fitted using data from 498 randomly selected participants with both Exam 1 and Exam 5 imaging studies. Apart from differences in utilized pulse sequences, these calibration curves allow adjustment for variability between visits due to differences in reader, software, and imaging equipment.

Proteomics

Relative plasma protein abundances of each of approximately 2,000 proteins were captured using the SOMAscan platform (SomaLogic, Boulder, CO). SOMAscan has exhibited high precision and reproducibility^{257, 267} and has been described in detail previously.^{257, 268} Briefly, the SOMAscan assay utilizes single-stranded DNA aptamers (SOMAmers) with chemically modified nucleotides to bind target protein antigen, which can then be quantified using relative fluorescence on microarrays.²⁶⁹ Each SOMAmer has been validated for its specificity, upper and lower limits of detection, and intra- and inter-assay variability.²⁷⁰ Previous work indicates median intra- and inter-run coefficients of variation of approximately 5% and a median intra-class correlation coefficient of 0.96 among split sample duplicates.^{259, 271}

This platform has been completed at a central laboratory on EDTA plasma samples stored at -70°C from a subset of MESA participants. The central laboratory applied

standard SOMALogic quality control methods for normalization and calibration of protein analyte data at SOMAmer, sample, and plate levels. Data was first normalized using hybridization control SOMAmers on the microarray, correcting for systematic errors during hybridization at the sample level. Median signal normalization was then applied to within-plate values to correct for sample or assay errors from sources such as pipetting, reagent concentration, and assay timing variation. Finally, plate scale calibration samples were used to calculate calibration scaled factors to further normalize, correcting for protein-specific plate-to-plate variation.

Following completion of quality control, 1,953 aptamers were analyzed. Protein analyte values, which are measured in relative fluorescence units (RFU) and were predominantly heavily right-skewed, were \log_2 -transformed, and outliers greater than 5 standard deviations from the sample mean on the \log_2 scale were winsorized.

Additional Clinical and Laboratory Data

Standardized instruments were used in all patient questionnaires. Data collected by patient survey includes demographics (age, sex, race/ethnicity), medical and family history, current use of medication, and health behaviors (e.g., exercise frequency, tobacco and alcohol use).

Height and weight were measured to the nearest 0.1 cm and 0.5 kg, respectively, and were used to derive body mass index (kg/m^2). Resting blood pressure was measured in the right arm using an automated oscillometric method (Dinamap) after being seated for five minutes; three readings were taken, the second and third of which were averaged for reporting. Diabetes mellitus was defined as fasting glucose ≥ 126 mg/dL, non-fasting glucose ≥ 200 mg/dL, treatment for diabetes mellitus, or self-reported physician diagnosis of diabetes.

Laboratory data included a lipid panel, creatinine, glucose, insulin, and HgA1C, which were measured using standard protocols. Glucose, triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol levels were measured after 12 hours of fasting, and low-density lipoprotein (LDL) cholesterol levels were calculated with the Friedewald equation.²⁷² Serum creatinine was measured by rate reflectance spectrophotometry using thin film adaptation of the creatine amidinohydrolase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics Inc, Rochester, NY).

The CKD-EPI creatinine equation²⁷³ was then used to estimate glomerular filtration rate (eGFR).

Statistical Analysis

Demographic and basic clinical characteristics of participants in the analysis samples were summarized overall and by exam using medians (interquartile ranges [IQR]) for continuous variables and proportions (counts) for categorical variables. Complete case analyses were performed to estimate the cross-sectional effects of protein abundances on LV structural parameters at baseline (Exam 1). Inference was made using Huber-White robust variance estimators, adjusting for clinic center, demographics (age, sex at birth, race/ethnicity), total:HDL-cholesterol, lipid-lowering therapy, systolic blood pressure, blood pressure-lowering therapy, diabetes, body mass index, physical activity, substance use (tobacco, alcohol), and kidney function (eGFR). The Benjamini and Hochberg (BH) false discovery rate (FDR) approach was used to correct for multiple comparisons. A later life replication analysis was then conducted at year 10 of follow-up (Exam 5), limited to proteins with fully adjusted associations that were statistically significant at baseline (FDR-adjusted $p < 0.10$). Phenotypic change over ten years was also analyzed using a follow-up score approach, where LV structure at year 10 was modeled, further adjusting for baseline structure. Sensitivity analyses included further adjustment for immunoassay-measured NT-proBNP, anticoagulant use, and antiarrhythmic use, as well as categorizing protein abundance values into tertiles and a subgroup analysis excluding participants with prior CVD events in analyses conducted at year 10. Proteins that were strongly and consistently associated with LV structural characteristics at each exam were then further analyzed for independent associations in one model per outcome and evaluated for associations with basic demographic and clinical characteristics using linear regression. All analyses were conducted using R software version 4.0.2.

Pathway Analysis

Pathway analysis aims to highlight key pathophysiologic mechanisms contributing to a particular phenotype and is thus a powerful tool in system-level hypothesis generation. Ingenuity Pathway Analysis (IPA) (Qiagen) is a software and database search tool that can identify over-represented biological pathways in a wide variety of statistical analysis results.²⁷⁴ IPA utilizes a curated database consisting of over 6 million content items,

representing individual relationships between proteins, genes, complexes, cells, tissues, drugs, and diseases. This content is updated weekly and originates from public databases, pathway and systems models, and manually curated scientific literature.

Network pathway analyses were performed for each outcome by uploading fully-adjusted modeling results and restricting analysis to proteins associated with LV characteristics at an FDR adjusted p -value <0.10 . The Core Analysis pipeline was then used to estimate the degree to which certain canonical pathways, protein networks, and upstream regulators were over-represented in the results.

C.4. RESULTS

A total of 763 MESA participants had both CMR imaging and proteomics at baseline (Exam 1) following exclusions due to poor cardiac image quality ($n=86$), laboratory technical error ($n=4$), and protein analyte values outside normalization bounds ($n=2$). Thirteen participants had missing data on one or more primary confounders, so complete case analysis sample size was $n=750$. At year 10 (Exam 5), 652 participants had complete CMR images and proteomics following exclusions due to technical error ($n=3$). Ten participants had missing data on one or more primary confounders, so complete case analysis sample size was $n=642$.

Demographic and clinical characteristics of participants are summarized in **Table 4**. Median [IQR] age at baseline was 59 [15] years, 53% were female, 19% were of Black race, 31% were of Hispanic ethnicity, 14% were current smokers, 36% were hypertensive, 10% were hyperlipidemic, and 8% were diabetic. Median [IQR] MRI-derived LV ejection fraction was 62.7 [8.0]%, LV end-diastolic volume index was 69.9 [16.0] mL/m², LV end-systolic volume index was 25.7 [7.7] mL/m², LV mass index was 63.4 [15.4] g/m², and 10% were categorized as exhibiting LV hypertrophy.

The distribution of results of primary proteomics analyses are visually depicted by outcome and exam in **Figure 5**. At baseline and following full adjustment for clinic center, age, sex at birth, race/ethnicity, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, lipid-lowering therapy, diabetes, body mass index, physical activity, smoking status, alcohol use status, and eGFR, the abundances of 14 proteins were significantly associated with LV mass index and 30 proteins were associated with LV mass-to-volume ratio. Proteins are listed in **Table 5** and **Table 6**,

ordered by ascending Benjamini-Hochberg adjusted p -values. When assessed by both FDR-adjusted p -value and absolute standardized effect size, the protein with the strongest association with LV mass index at baseline was leptin; on average, LV mass index was 5.21 g/m² lower per standard deviation (SD) increment in log-transformed leptin (95% CI: -6.63 to -3.78 and BH p -value: 9.91×10^{-10}) after adjustment for important confounders. The protein with the strongest association with LV mass-to-volume ratio at baseline was observed with complement component-1r (C1r). On average, LV mass-to-volume ratio was 0.04 g/mL higher per two-fold increment in C1r (95% CI: 0.02 to 0.05 and BH p -value: 5.02×10^{-5}) after adjustment for important confounders. Upon further adjustment for immunoassay-measured NT-proBNP, no effect estimates for associated proteins were substantially altered when evaluating either outcome.

Pathway analysis was used to detect possible canonical pathways, upstream regulators, protein networks, and shared molecular and physiologic functions. Results from LV mass index analyses at Exam 1 yielded no plausible canonical pathway with none in the database represented at more than 2.5% of molecules. The top shared upstream regulator was MAPK8, and two primary networks were identified—one associated with organ development and morphology (**Figure 6a**) and one associated with connective tissue disorders. Results from LV mass-to-volume analyses at Exam 1 yielded one pathway of interest, leukocyte extravasation signaling. The top shared upstream regulator was TGF β 1, and five primary networks were identified—the top of which was associated with cell morphology and cellular assembly and organization (**Figure 6b**), while others were associated with organ morphology, cell-to-cell interaction, and cellular movement.

In a later life replication analysis at year 10 of follow-up (Exam 5), proteins that remained significantly associated with LV mass index in consistent directions were leptin, renin, NT-proBNP, and cathepsin D, following adjustment for important confounders.

Associations with LV mass-to-volume that were replicated were tumor necrosis factor-inducible gene 6 protein (TSG-6), bone morphogenetic protein receptor type-1A (BMPR1A), chordin-like protein 1 (CRDL1), b-endorphin, transforming growth factor beta receptor type 3 (TGF- β R III), cGMP-stimulated phosphodiesterase 2A (PDE2A), and repulsive guidance molecule B (RGMB). **Figure 7** depicts fully adjusted parameter estimates for proteins significantly associated with LV structural characteristics at baseline plotted against parameter estimates for the same associations at year 10 of

follow-up. Proteins that were strongly and consistently associated with LV characteristics at each exam were then analyzed simultaneously in one model for each outcome at baseline. When assessing LV mass index, leptin, renin, and NT-proBNP remained strongly associated with very similar effect estimates as when assessed individually, while the effect of cathepsin D was diminished but still statistically significant (effect estimate [95% CI]: -0.98 [-1.74 to -0.23]; unadjusted $p=0.01$). Together these proteins described roughly 8% of the variance in LV mass index beyond the traditional risk factors included in the model. When assessing LV mass-to-volume ratio, all effect estimates were in consistent directions but were substantially reduced. TSG-6 and b-endorphin remained significantly associated (unadjusted $p<0.05$), while BMPR1A, RGMB, TGF- β R III, PDE2A, and CRDL1 were not independently associated. Together these proteins described 5% of the variance in LV mass-to-volume ratio beyond traditional risk factors.

Proteomics analyses of change in left ventricular phenotypes over ten years yielded no fully adjusted, statistically significant associations. Effect estimates for associations between change in structural phenotypes and proteins replicated in cross-sectional analyses are listed in *Supplemental Table S2*. Six of these target proteins yielded relatively large and/or moderately precise estimates in expected directions—specifically leptin and cathepsin D as they relate to LV mass index in this data and CRDL1, PDE2A, BMPR1A, and RGMB as they relate to LV mass-to-volume ratio. Baseline cross-sectional associations between participant characteristics and these proteins are presented in *Supplemental Table A3*.

Finally, year 10 cross-sectional parameter estimates were largely unchanged in a sensitivity analysis that excluded participants with prior cardiovascular events, and all primary proteomic findings were consistent when categorizing protein abundances into tertiles.

C.5. DISCUSSION

In this diverse population-based cohort of adults with no history of cardiovascular disease, plasma abundances of 1,953 proteins were evaluated for associations with left ventricular structural characteristics, LV mass index and LV mass-to-volume. Following

adjustment for kidney function and traditional CVD risk factors as well as correction for multiple comparisons, 14 and 30 proteins were significantly cross-sectionally associated with these respective characteristics. Among the same participants ten years later, 4 proteins exhibited consistent and statistically significant cross-sectional effects with LV mass index—specifically NT-proBNP, leptin, renin, and cathepsin D—while 7 proteins exhibited consistent and significant effects on LV mass-to-volume ratio—specifically TSG-6, BMPR1A, chordin-like protein 1, b-endorphin, TGF- β RIII, PDE2A, and RGMB. In analyses of change in LV phenotype over ten years, baseline leptin and cathepsin D had relatively strong effects on change in LV mass index, and baseline RGMB, CRDL1, and PDE2A maintained relatively strong effects on change in LV mass-to-volume ratio, though all estimates were imprecise.

Importantly and as expected, NT-proBNP—an important clinical biomarker secreted in response to myocyte stretch¹²⁰—was positively and consistently associated with LV mass index across all cross-sectional analyses.

Leptin is an extensively studied protein hormone produced predominantly by adipocytes that functions to provide feedback to the central nervous system regarding the status of peripheral energy reserves.²⁷⁵ Apart from its important role in regulating appetite²⁷⁶ and insulin sensitivity,²⁷⁷ leptin regulates clearance of free fatty acids by stimulating their oxidation and uptake in non-adipocyte tissues, such as the myocardium, preventing their deposit.²⁷⁸ Findings reported here are consistent with this biologically and other observational cohorts reporting a protective effect of leptin on LV mass,²⁷⁹⁻²⁸² including a recent proteomics analysis in the Framingham Heart Study.²⁶⁰ However, several studies have also shown detrimental effects of leptin on LV size,^{281, 283, 284} which has been hypothesized to be due to development of leptin resistance among those with higher body mass index and adiposity.^{285, 286}

Less studied are cathepsin D, repulsive guidance molecule domain family member B (RGMB), chordin-like protein 1 (CRDL1), and phosphodiesterase 2A (PDE2A). Cathepsin D is an important lysosomal protease required in certain epithelial cells for tissue remodeling and renewal.²⁸⁷ Although little data exists on Cathepsin D and cardiac remodeling, specifically, cathepsins play many roles within the vascular system—including extracellular matrix degradation and regulation of inflammatory cell adhesion, migration, proliferation, and activation.²⁸⁸ Upregulation of cathepsin D induced by myocardial infarction has also recently been suggested to be protective against cardiac

remodeling in a murine model.²⁸⁹ Three proteins with replicated LV mass-to-volume associations are involved in regulation of bone morphogenic proteins (BMPs), which are critical to development and maintenance of tissue architecture, including that of the heart and vasculature,²⁹⁰ and some studies have reported a protective role of BMPs during cardiac remodeling post-infarction.²⁹¹⁻²⁹³ The RGM family of proteins function as co-receptors for BMPs, and a recent proteomics analysis of HF identified RGMB as well as other members of this protein family (RGMA and RGMC) as being negatively associated with incident events, independent of traditional risk factors.²⁶⁰ Also involved in regulation of BMPs are CRDL1 and BMPR1A, both of which are antagonists to BMP4, which has been suggested to mediate cardiac hypertrophy via apoptosis, fibrosis, and ion channel remodeling.^{294, 295} In murine models, BMP4 has been shown to be elevated in cardiovascular pathologies such as hypertension, atherosclerosis, valvular abnormality, and ischemic myocardial injury.²⁹⁶⁻²⁹⁸

Lastly, cGMP-stimulated PDE2A is a phosphodiesterase that controls the degradation of guanosine 3',5'-cyclic monophosphate (cGMP), a critically important secondary messenger to a multitude of cellular processes that has been shown to inhibit pathologic cardiac remodeling.²⁹⁹ Most clinical and epidemiologic research to date on phosphodiesterases has involved PDE1, which has been identified as an important regulator of cardiomyocyte physiology.³⁰⁰⁻³⁰³ PDE1 is closely related to PDE2, both of which are expressed in cardiomyocytes³⁰³ and show evidence of upregulation in the context of cardiac hypertrophy.^{302, 304, 305}

Strengths and Limitations

There are limitations to this study. Specific to protein measurement, it should be considered in interpretation that the SOMAScan platform used in this study is not agnostic, representing only a small proportion of the proteome. Protein levels measured via the SOMAScan platform are also semi-quantitative and can neither be directly interpreted nor compared to other assays. Additionally, since protein levels were measured on biospecimens in long-term storage and across two time points separated by ten years, the possibility of differential protein degradation during that time cannot be excluded. One study has suggested, however, absence of widespread protein degradation in samples stored over 20 years.²⁷¹ This study was also limited to evaluation of proteins circulating in plasma, so cardiospecificity cannot be established. Moreover,

this assay binds protein epitopes that are not all fully characterized and with affinity that may be sensitive to alterations in protein structure. All proteins of interest therefore require validation using other methods, such as immunoassay or mass spectrometry.

Specific to analytic approach, the method used for multiple testing correction assumes independence of tests—in this case, proteins—which is unreasonable and conservative.

Finally, there are general limitations of the outcome measures themselves, particularly their inability to differentiate between the variable pathologies that may lead to LV remodeling, meaning identified protein candidates for future study may be more or less relevant to a clearly defined etiologic context. Furthermore, predictors of HF are not limited to those associated with cardiac structural phenotypes, which may alone be insufficient to result in clinical HF.

There are several strengths of this study. First, the outcome is measured using gold standard cardiac imaging methods and were extensively controlled for quality, as are data on important covariables. Second, this nationwide population-based sample is relatively large for such imaging studies and is diverse in represented races and ethnicities. Finally, two observations spanning 10 years have been captured, allowing both for internal later-life replication as well as inference beyond cross-sectional associations.

Conclusion

In conclusion, this study reports proteomic profiling of left ventricular structural characteristics in a population-based cohort of adults without prior cardiovascular disease at baseline. The presented results both reinforce previous findings and highlight the promise of more recently identified biomarker candidates for cardiac remodeling and heart failure, warranting further investigation. If successfully and repeatedly validated in external populations and in relation to incident manifest HF, these proteins may help refine current HF risk prediction and identify novel therapeutic targets to more effectively mitigate risk for HF progression.

Table 4a. Participant characteristics by exam, restricted to those with CMR and proteomics.		
	<i>Median [25, 75] or % (n)</i>	
	<i>Exam 1 (n=763)</i>	<i>Exam 5 (n=652)</i>
<i>Demographics</i>		
Age, years	59 [52, 67]	68 [61, 77]
Sex at birth, female	53% (403)	52% (337)
Race and ethnicity	--	--
White	43% (326)	45% (298)
Black	19% (145)	18% (120)
Chinese	7% (55)	7% (47)
Hispanic	31% (237)	29% (187)
<i>Behavioral Characteristics</i>		
Moderate to vigorous physical activity, MET-min/week	5033 [2595, 8460]	3240 [1658, 5933]
Smoking status	--	--
Current	14% (104)	8% (52)
Former	38% (293)	41% (260)
Current alcohol use	71% (458)	43% (277)
<i>Clinical Characteristics</i>		
Body mass index, kg/m ²	27.7 [24.9, 30.9]	27.9 [24.9, 31.6]
Systolic blood pressure, mmHg	118 [108, 135]	120 [109, 136]
Diastolic blood pressure, mmHg	71 [65, 78]	69 [63, 75]
Hypertension	36% (276)	56% (364)
Any blood pressure-lowering therapy use	31% (239)	50% (327)
Beta-blocker use (+/- diuretic)	10% (69)	17% (93)
ACE inhibitor use (+/- diuretic)	12% (79)	22% (116)
Angiotensin receptor blocker use (+/- diuretic)	6% (40)	13% (76)
Total cholesterol, mg/dL	195 [170, 216]	185 [160, 206]
HDL-cholesterol, mg/dL	49 [40, 59]	53 [44, 64]
LDL-cholesterol, mg/dL	119 [96, 139]	106 [83, 128]
Hyperlipidemia	10% (78)	8% (49)
Any lipid-lowering therapy use	16% (124)	37% (243)
Statin use	15% (113)	35% (232)
Metabolic syndrome	33% (252)	36% (233)
Diabetes	8% (60)	15% (96)
Diabetes therapy use	7% (50)	13% (82)
eGFR (CKD-EPI), mL/min/1.73m ²	78.2 [66.8, 89.0]	81.2 [68.6, 91.9]
CMR=cardiac magnetic resonance; ACE=angiotensin-converting enzyme; HDL=high-density lipoprotein; LDL=low-density lipoprotein; eGFR=estimated glomerular filtration rate.		

Table 4b. Participant characteristics by exam, restricted to those with CMR and proteomics.		
	<i>Median [25, 75] or % (n)</i>	
	<i>Exam 1 (n=763)</i>	<i>Exam 5 (n=652)</i>
<i>Left Ventricular Characteristics</i>		
LV ejection fraction, %	62.7 [58.1, 66.1]	61.6 [56.8, 66.3]
LV stroke volume, mL/beat	80.5 [67.6, 94.8]	69.5 [58.3, 81.2]
LV stroke volume index	43.8 [37.6, 49.3]	38.4 [32.7, 44.2]
LV end-diastolic volume, mL	129 [111, 151]	114 [94, 136]
LV end-diastolic volume index	69.9 [62.3, 78.3]	61.6 [53.7, 71.7]
LV end-systolic volume, mL	47.7 [39.5, 57.9]	42.5 [34.2, 55.2]
LV end-systolic volume index	25.7 [22.3, 30.0]	22.9 [18.9, 29.1]
LV hypertrophy	10% (79)	--
LV end-diastolic mass, g	118 [100, 141]	120 [98, 146]
LV mass index, g/m ²	63.4 [56.7, 72.1]	64.4 [56.6, 74.8]
LV mass/volume ratio, g/mL	0.913 [0.820, 1.041]	1.048 [0.907, 1.216]
<i>Change in Left Ventricular Characteristics Over 10 Years</i>		
LV ejection fraction, %	--	-0.80 [-5.43, 4.42]
LV stroke volume index	--	-3.81 [-9.72, 1.84]
LV end-diastolic volume index	--	-5.25 [-12.29, 1.85]
LV end-systolic volume index	--	-1.59 [-5.57, 2.49]
LV mass index, g/m ²	--	1.42 [-4.29, 7.61]
LV mass/volume ratio	--	0.10 [-0.01, 0.23]
CMR=cardiac magnetic resonance; LV=left ventricular.		

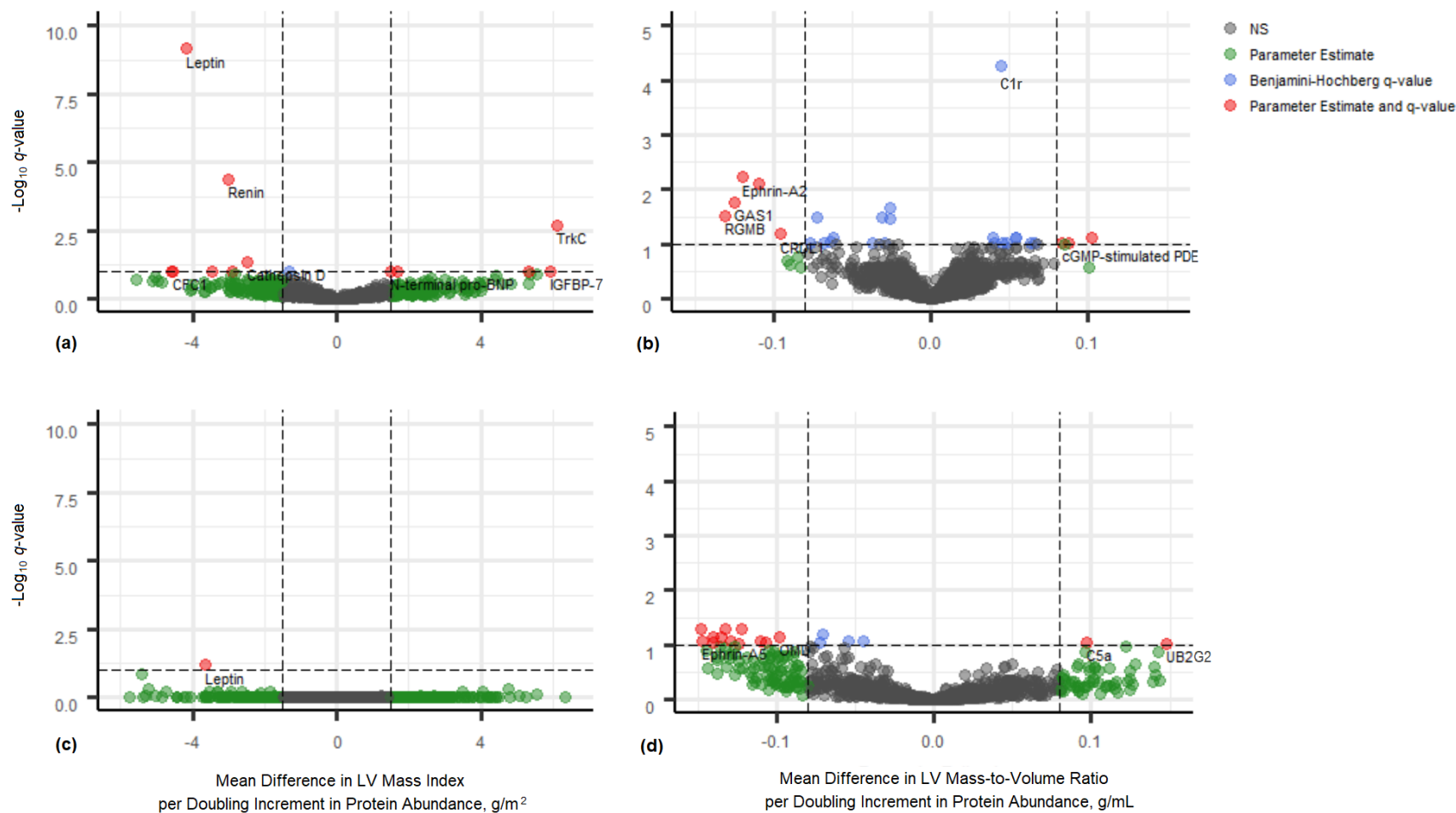


Figure 5. Volcano plots of proteomics parameter estimates vs. Benjamini-Hochberg q -values resulting from generalized estimating equations, adjusting for clinic center, age, sex at birth, race/ethnicity, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, diabetes, body mass index, physical activity, smoking status, alcohol use status, and eGFR. **(a)** Exam 1 left ventricular mass index; **(b)** Exam 1 left ventricular mass-to-volume ratio; **(c)** Exam 5 left ventricular mass index; **(d)** Exam 5 left ventricular mass-to-volume ratio.

Table 5. Proteins with significant cross-sectional associations with left ventricular mass index at baseline in MESA ($n=750$).

<i>Protein</i>	<i>Mean Difference in LVMI (95% CI)</i>	<i>BH p-value</i>
Leptin	-5.21 (-6.63 to -3.78)	9.91e-10
Renin	-2.39 (-3.26 to -1.53)	3.55e-05
NT-3 growth factor receptor	1.82 (1.03 to 2.60)	0.002
Cathepsin D	-1.56 (-2.36 to -0.76)	0.043
Activin receptor type-1B	-1.44 (-2.23 to -0.65)	0.097
C-reactive protein	-1.50 (-2.35 to -0.65)	0.097
N-terminal pro-BNP	1.77 (0.73 to 2.82)	0.097
Heat shock cognate 71 kDa protein	-1.27 (-2.01 to -0.52)	0.097
Insulin-like growth factor-binding protein 7	1.46 (0.61 to 2.32)	0.097
S-formylglutathione hydrolase	1.13 (0.46 to 1.81)	0.097
TATA-box-binding protein	-1.21 (-1.93 to -0.49)	0.097
Contactin-4	1.49 (0.63 to 2.34)	0.097
Heterogeneous nuclear RNP A/B	-1.29 (-2.07 to -0.51)	0.097
Cryptic protein	-1.17 (-1.86 to -0.47)	0.097

Mean difference in LVMI per standard deviation increment in log-transformed protein abundance, estimated using generalized estimating equations, adjusting for clinic center, age, sex at birth, race/ethnicity, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, lipid-lowering therapy, diabetes, body mass index, physical activity, smoking status, alcohol use status, and estimated glomerular filtration rate.

LVMI=left ventricular mass index; CI=confidence interval; BH=Benjamini-Hochberg; HDL=high-density lipoprotein.

Table 6. Proteins with significant cross-sectional associations with left ventricular mass-to-volume ratio at baseline in MESA ($n=750$).

<i>Protein</i>	<i>Mean Difference in LVMV (95% CI)</i>	<i>BH p-value</i>
Complement C1r subcomponent	0.04 (0.02 to 0.05)	5.03e-05
Ephrin-A2	-0.03 (-0.04 to -0.02)	0.006
TGF- β receptor type 3	-0.03 (-0.04 to -0.02)	0.009
Growth arrest-specific protein 1	-0.03 (-0.04 to -0.01)	0.018
Stromelysin-1, MMP-3	-0.03 (-0.04 to -0.01)	0.019
Nectin-like protein 1	-0.02 (-0.04 to -0.01)	0.028
Scavenger receptor class F member 2	-0.02 (-0.03 to -0.01)	0.028
RGM domain family member B	-0.03 (-0.05 to -0.02)	0.028
Aurora kinase A	-0.02 (-0.03 to -0.01)	0.031
Chordin-like protein 1	-0.03 (-0.04 to -0.01)	0.064
Brain-derived neurotrophic factor	0.03 (0.01 to 0.04)	0.075
TNF-inducible gene 6	-0.03 (-0.04 to -0.01)	0.075
Inorganic pyrophosphatase	0.03 (0.01 to 0.04)	0.075
Stabilin-2	0.03 (0.01 to 0.04)	0.080
Collectin Kidney 1	0.02 (0.01 to 0.03)	0.086
Glypican 3	-0.02 (-0.03 to -0.01)	0.088
Myoglobin	-0.03 (-0.04 to -0.01)	0.088
Myokinase	0.03 (0.01 to 0.04)	0.088
Peroxiredoxin-1	0.02 (0.01 to 0.04)	0.088
Periostin	-0.02 (-0.03 to -0.01)	0.088
Galectin-7	0.02 (0.01 to 0.04)	0.088
cGMP-phosphodiesterase 2A	0.02 (0.01 to 0.04)	0.088
PK3CG	0.03 (0.01 to 0.04)	0.088
Cyclin-dependent kinase 1/cyclin B	0.02 (0.01 to 0.04)	0.088
OX-2 membrane glycoprotein	-0.02 (-0.03 to -0.01)	0.088
Dual phosphodiesterase 11A	0.02 (0.01 to 0.04)	0.088
Amyloid precursor protein	0.02 (0.01 to 0.04)	0.088
BMP receptor type 1A	-0.02 (-0.03 to -0.01)	0.090
β -Endorphin	-0.02 (-0.03 to -0.01)	0.095
Thrombospondin-4	-0.02 (-0.03 to -0.01)	0.099
Focal adhesion kinase 1	-0.02 (-0.03 to -0.01)	0.099

Mean difference in LVMV per standard deviation increment in log-transformed protein abundance, estimated using generalized estimating equations adjusting for clinic center, age, sex at birth, race/ethnicity, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, lipid-lowering therapy, diabetes, body mass index, physical activity, smoking status, alcohol use status, and estimated glomerular filtration rate.

LVMV=left ventricular mass-to-volume ratio; CI=confidence interval; BH=Benjamini-Hochberg; HDL=high-density lipoprotein.

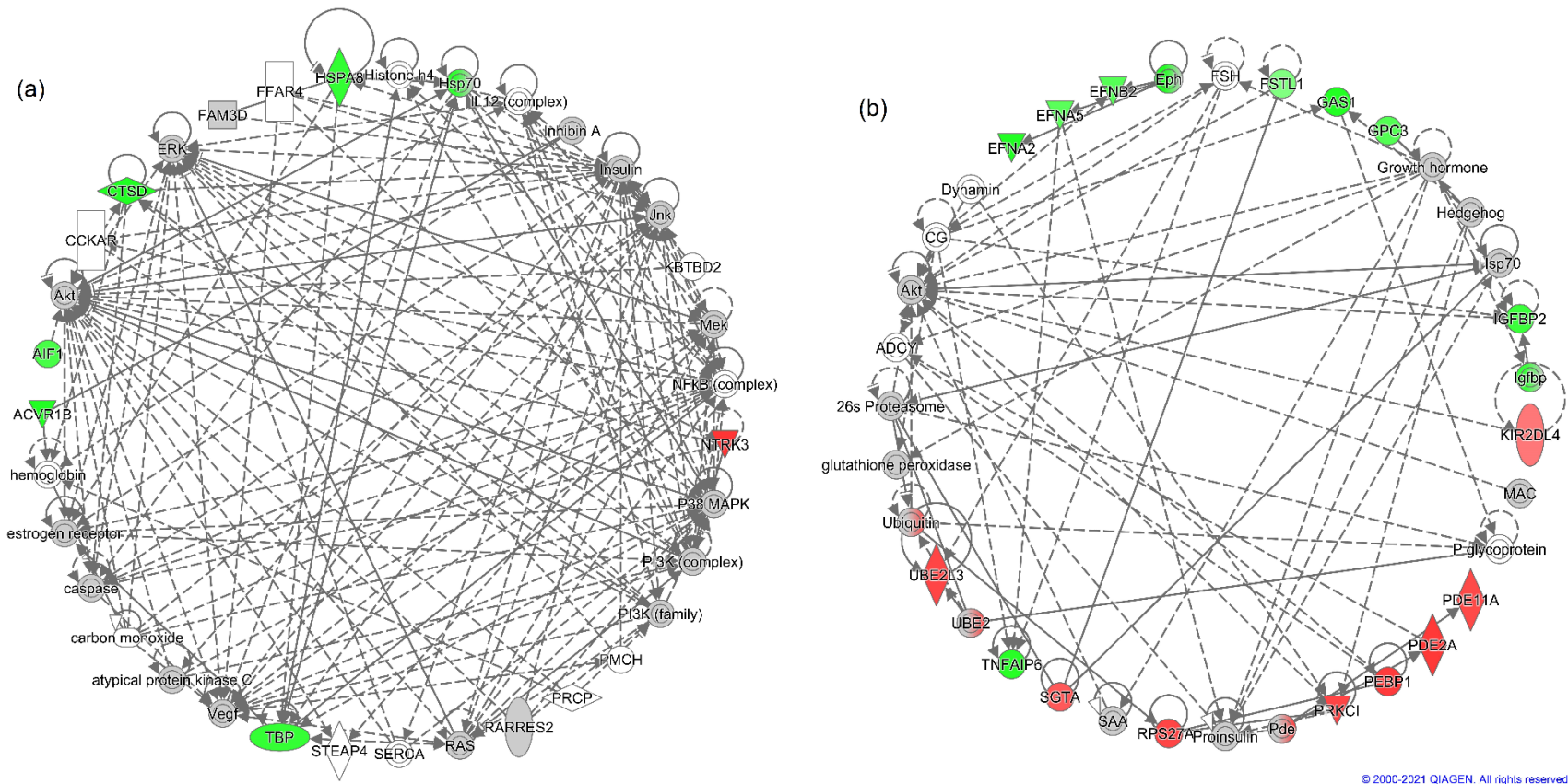


Figure 6. Top protein networks with left ventricular (LV) structure associations identified using Ingenuity Pathway Analysis. Green represents proteins positively associated with the outcome and red represents those negatively associated. **(a)** LV mass index, network associated with organ development and morphology; **(b)** LV mass-to-volume ratio, network associated with cell morphology and cellular assembly and organization.

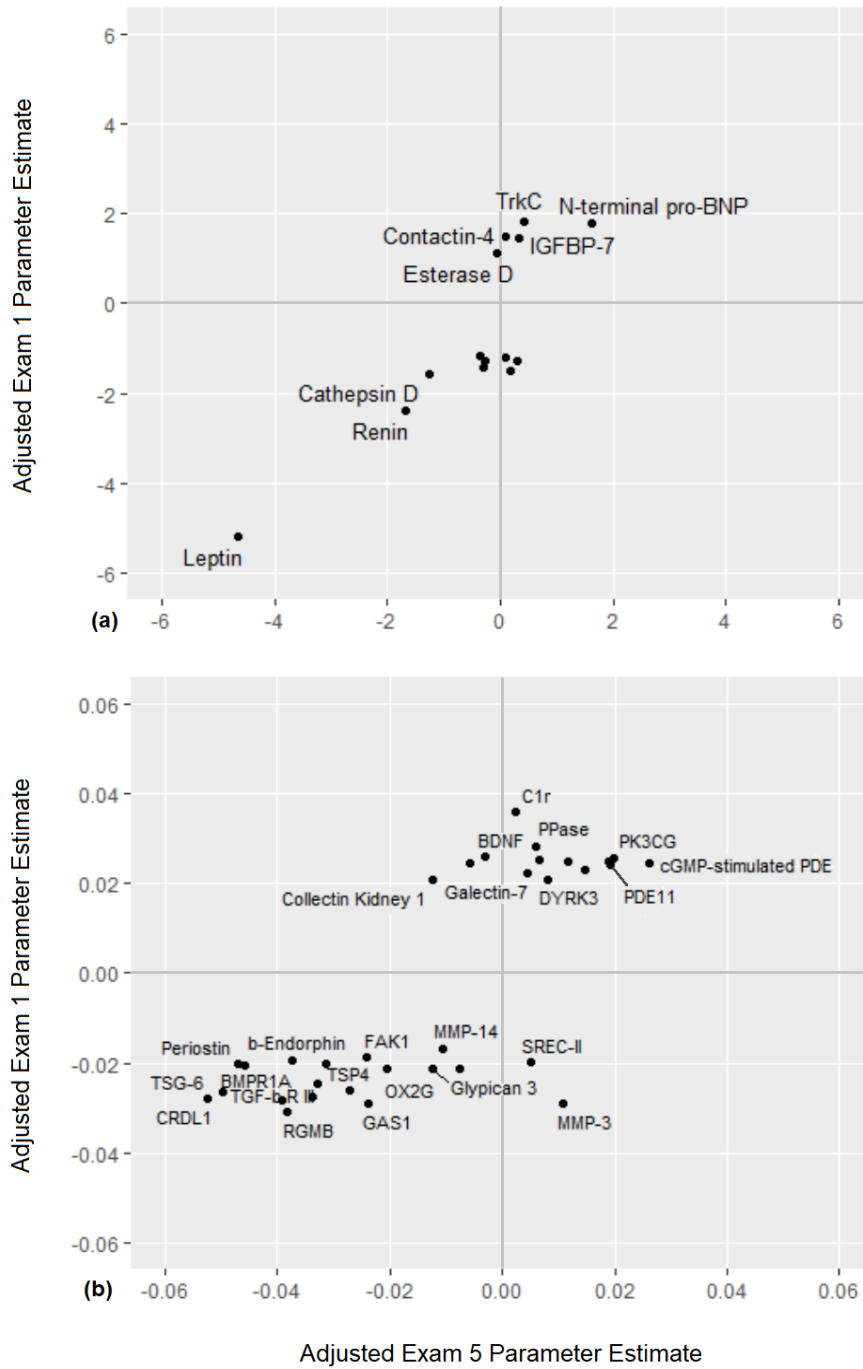


Figure 7. Scatterplots of standardized parameter estimates for protein abundances from generalized estimating equations at baseline (Exam 1) vs. year 10 (Exam 5), each adjusted for clinic center, age, sex at birth, race/ethnicity, body mass index, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, lipid-lowering therapy, diabetes, physical activity, smoking status, alcohol use status, and eGFR. Plotted proteins limited to those with Benjamini-Hochberg p -values < 0.10 at baseline. **(a)** left ventricular mass index; **(b)** left ventricular mass-to-volume ratio.

Supplemental Tables and Figures

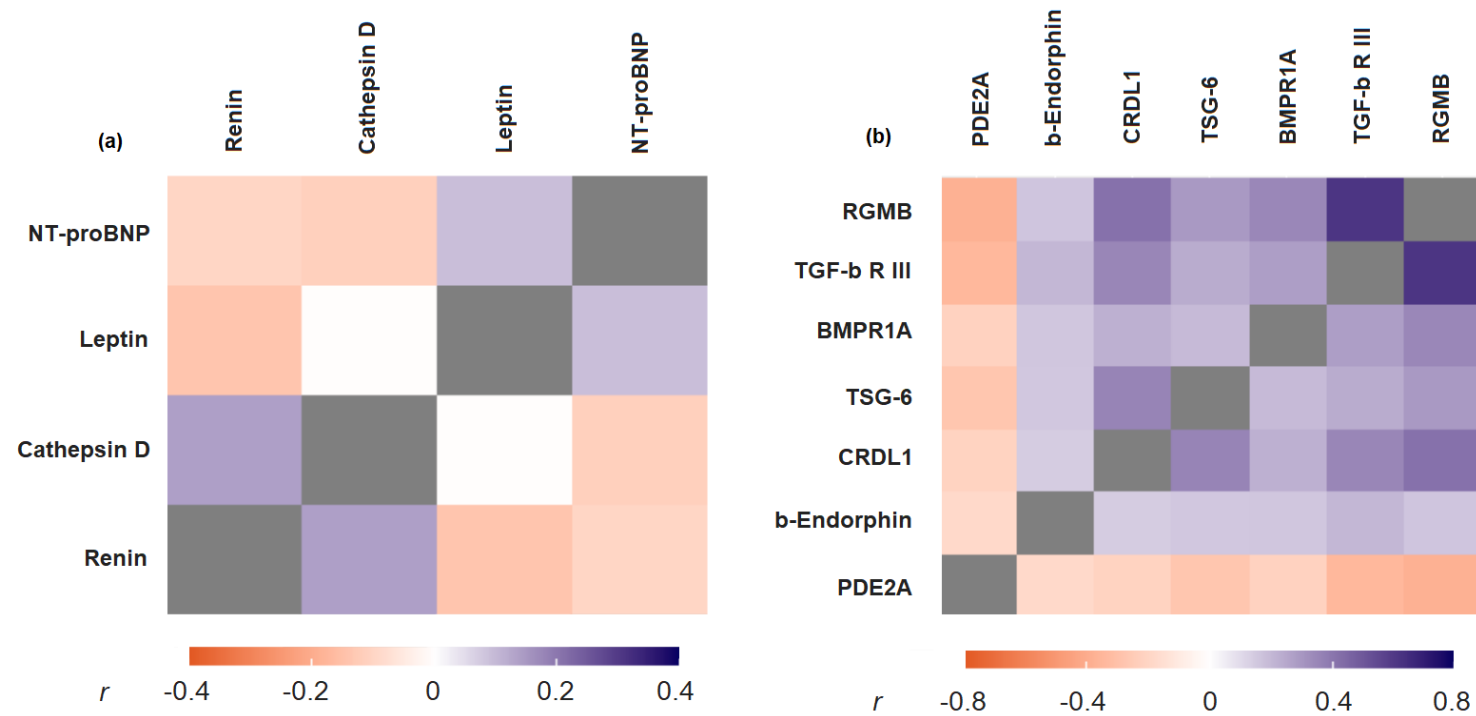


Figure S1. Spearman rank order correlations of proteins with replicated cross-sectional associations at MESA Exam 1 and Exam 5, each adjusted for clinic center, age, sex at birth, race/ethnicity, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, lipid-lowering therapy, diabetes, body mass index, physical activity, smoking status, alcohol use status, and eGFR. All correlations are $p \leq 0.05$. (a) left ventricular mass index, g/m^2 and (b) left ventricular mass-to-volume ratio, g/mL . RGMB=repulsive guidance molecule B; TGF-b R III=tumor necrosis factor-beta receptor type 3; BMPR1A=bone morphogenetic protein receptor type-1A; TSG-6=tumor necrosis factor-inducible gene 6; CRDL1=chordin-like protein 1; PDE2A=cGMP-stimulated phosphodiesterase-2A.

Table S1. Unadjusted associations between baseline demographic and clinical characteristics and change in left ventricular structure over ten years in MESA ($n=646$).

	<i>Mean Change in LV Mass Index</i>		<i>Mean Change in LV Mass-to-Volume Ratio</i>	
	<i>Estimate (95% CI)</i>	<i>p</i>	<i>Estimate (95% CI)</i>	<i>p</i>
<i>Demographics</i>				
Age, per 10 years	0.25 (-0.14 to 0.64)	0.214	0.001 (0.000 to 0.001)	0.210
Sex at birth, male	4.79 (4.08 to 5.50)	<0.001	0.036 (0.020 to 0.052)	0.000
Race and ethnicity (vs. white)	--	--	--	--
Black African American	1.76 (0.80 to 2.72)	<0.001	0.006 (-0.015 to 0.027)	0.591
Chinese	0.03 (-1.07 to 1.13)	0.960	-0.028 (-0.052 to -0.005)	0.018
Hispanic	-0.21 (-1.17 to 0.75)	0.668	-0.019 (-0.044 to 0.005)	0.120
<i>Clinical Characteristics</i>				
Smoking status (vs. never)	--	--	--	--
Current	-0.83 (-1.98 to 0.31)	0.154	0.049 (0.025 to 0.073)	<0.001
Former	0.63 (-0.15 to 1.40)	0.113	0.019 (0.002 to 0.036)	0.029
Current alcohol use	-0.28 (-1.20 to 0.64)	0.555	-0.002 (-0.022 to 0.018)	0.855
Body mass index, per kg/m ²	0.15 (0.07 to 0.22)	<0.001	0.005 (0.003 to 0.006)	<0.001
Obesity	1.43 (0.62 to 2.24)	0.001	0.041 (0.023 to 0.058)	<0.001
Hypertension	-0.21 (-0.96 to 0.54)	0.587	0.005 (-0.011 to 0.022)	0.527
Hyperlipidemia	-1.33 (-2.58 to -0.08)	0.038	0.002 (-0.025 to 0.029)	0.894
Metabolic syndrome	0.49 (-0.32 to 1.29)	0.234	0.028 (0.011 to 0.046)	0.001
Diabetes	1.24 (-0.06 to 2.55)	0.062	0.037 (0.008 to 0.065)	0.011
Renal insufficiency	-0.47 (-1.89 to 0.96)	0.518	-0.011 (-0.041 to 0.020)	0.505

Table S2. Associations between baseline protein abundance and change in left ventricular structure over ten years in MESA, restricted to proteins replicated in cross-sectional analyses ($n=646$).		
<i>Protein</i>	<i>Mean Difference (95% CI)</i>	<i>Robust p-value</i>
<i>Change in left ventricular mass index</i>		
Leptin	-1.35 (-2.93 to 0.22)	0.092
Cathepsin D	-0.21 (-0.93 to 0.51)	0.567
Renin	0.12 (-0.78 to 1.02)	0.796
NT-proBNP	-0.01 (-0.84 to 0.82)	0.981
<i>Change in left ventricular mass-to-volume ratio</i>		
Chordin-like protein 1	-0.03 (-0.05 to 0.00)	0.053
cGMP-stimulated PDE2A	0.02 (0.00 to 0.04)	0.079
Bone morphogenetic protein receptor type-1A	-0.02 (-0.04 to 0.00)	0.098
RGM domain family member B	-0.02 (-0.04 to 0.00)	0.099
TGF- β receptor type 3	-0.01 (-0.03 to 0.01)	0.168
TNF-inducible gene 6 protein	-0.01 (-0.03 to 0.01)	0.316
<p>Mean difference per standard deviation increment in log-transformed protein abundance, estimated using generalized estimating equations, adjusting for clinic center, age, sex at birth, race/ethnicity, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, lipid-lowering therapy, diabetes, body mass index, physical activity, smoking status, alcohol use status, and estimated glomerular filtration rate.</p> <p>LVMI=left ventricular mass index; CI=confidence interval; HDL=high-density lipoprotein.</p>		

Table S3 (a). Unadjusted cross-sectional associations between baseline clinical characteristics and proteins with strong and replicated left ventricular mass index associations (<i>n</i> =750).		
<i>Characteristic</i>	<i>Mean Standardized Difference (95% Confidence Interval)</i>	<i>p-value</i>
<i>Leptin</i>		
Age, per 10 years	0.05 (-0.02 to 0.13)	0.16
Male sex	-1.39 (-1.49 to -1.29)	<0.001
Black race (vs. white)	0.37 (0.18 to 0.57)	<0.001
Hispanic ethnicity (vs. white)	0.09 (-0.07 to 0.26)	0.26
Current smoking (vs. never)	-0.44 (-0.65 to -0.22)	<0.001
Obesity	1.04 (0.91 to 1.17)	<0.001
Hypertension	0.38 (0.24 to 0.53)	<0.001
Hyperlipidemia	0.15 (-0.09 to 0.38)	0.21
Diabetes	0.03 (-0.23 to 0.30)	0.81
<i>Cathepsin D</i>		
Age, per 10 years	0.12 (0.06 to 0.18)	<0.001
Male sex	0.18 (0.06 to 0.30)	0.01
Black race (vs. white)	0.11 (-0.06 to 0.29)	0.21
Hispanic ethnicity (vs. white)	0.26 (0.12 to 0.39)	<0.001
Current smoking (vs. never)	-0.05 (-0.25 to 0.14)	0.60
Obesity	0.20 (0.07 to 0.33)	0.003
Hypertension	0.16 (0.03 to 0.29)	0.02
Hyperlipidemia	-0.10 (-0.31 to 0.10)	0.32
Diabetes	0.33 (0.10 to 0.56)	0.01
Effect estimated using linear regression, mean standardized difference in log-transformed protein per increment indicated or yes vs. no for continuous or dichotomous characteristics, respectively.		

Table S3 (b). Unadjusted cross-sectional associations between baseline clinical characteristics and proteins with strong and replicated left ventricular mass-to-volume associations (<i>n</i> =750).		
<i>Characteristic</i>	<i>Mean Standardized Difference (95% Confidence Interval)</i>	<i>p-value</i>
<i>RGM domain family member B (RGMB)</i>		
Age, per 10 years	0.04 (-0.03 to 0.11)	0.29
Male sex	0.46 (0.32 to 0.60)	<0.001
Black race (vs. white)	-0.54 (-0.73 to -0.35)	<0.001
Hispanic ethnicity (vs. white)	-0.49 (-0.64 to -0.34)	<0.001
Current smoking (vs. never)	-0.22 (-0.44 to 0.00)	0.05
Obesity	-0.38 (-0.53 to -0.23)	<0.001
Hypertension	-0.23 (-0.39 to -0.08)	0.002
Hyperlipidemia	-0.22 (-0.46 to 0.01)	0.06
Diabetes	-0.58 (-0.84 to -0.32)	<0.001
<i>Chordin-like protein 1 (CRDL1)</i>		
Age, per 10 years	0.55 (0.49 to 0.61)	<0.001
Male sex	-0.24 (-0.38 to -0.10)	0.001
Black race (vs. white)	-0.39 (-0.58 to -0.21)	<0.001
Hispanic ethnicity (vs. white)	-0.47 (-0.63 to -0.31)	<0.001
Current smoking (vs. never)	-0.18 (-0.40 to 0.03)	0.09
Obesity	-0.37 (-0.52 to -0.22)	<0.001
Hypertension	0.16 (0.01 to 0.31)	0.04
Hyperlipidemia	-0.12 (-0.35 to 0.12)	0.33
Diabetes	-0.28 (-0.54 to -0.01)	0.04
Effect estimated using linear regression, mean standardized difference in log-transformed protein per increment indicated or yes vs. no for continuous or dichotomous characteristics, respectively.		

Table S3 (b) Continued. Unadjusted cross-sectional associations between baseline clinical characteristics and proteins with strong and replicated left ventricular mass-to-volume associations (<i>n</i> =750).		
<i>Characteristic</i>	<i>Mean Standardized Difference (95% Confidence Interval)</i>	<i>p-value</i>
<i>Phosphodiesterase 2A (PDE2A)</i>		
Age, per 10 years	0.03 (-0.04 to 0.01)	0.34
Male sex	-0.17 (-0.31 to -0.04)	0.01
Black race (vs. white)	0.18 (0.00 to 0.37)	0.05
Hispanic ethnicity (vs. white)	0.27 (0.12 to 0.42)	<0.001
Current smoking (vs. never)	0.07 (-0.14 to 0.27)	0.50
Obesity	0.21 (0.07 to 0.36)	0.004
Hypertension	0.19 (0.05 to 0.33)	0.001
Hyperlipidemia	0.07 (-0.15 to 0.29)	0.54
Diabetes	0.35 (0.10 to 0.60)	0.01
<i>Bone morphogenetic protein receptor type-1A (BMPR1A)</i>		
Age, per 10 years	0.02 (-0.04 to 0.08)	0.45
Male sex	-0.02 (-0.15 to 0.10)	0.70
Black race (vs. white)	0.12 (-0.05 to 0.28)	0.17
Hispanic ethnicity (vs. white)	0.05 (-0.10 to 0.19)	0.53
Current smoking (vs. never)	0.03 (-0.16 to 0.22)	0.74
Obesity	0.02 (-0.11 to 0.16)	0.74
Hypertension	0.06 (-0.07 to 0.19)	0.38
Hyperlipidemia	-0.09 (-0.29 to 0.12)	0.40
Diabetes	0.02 (-0.21 to 0.25)	0.88
Effect estimated using linear regression, mean standardized difference in log-transformed protein per increment indicated or yes vs. no for continuous or dichotomous characteristics, respectively.		

D. Chapter 2: *External Validation of Candidate Protein Biomarkers for Cardiac Remodeling Among US Veterans Living with and without HIV*

D.1. OVERVIEW

Introduction

Detection of subclinical heart failure markers such as cardiac remodeling is critical to early intervention, and a reliable, cost-effective, and simple clinical tool remains largely an unmet need. This study utilized a high-throughput aptamer-based proteomic platform to externally validate recent cardiac remodeling biomarker candidates among a diverse population of U.S. veterans in care.

Methods

Plasma abundances of 75 proteins were quantified in participants of the Veterans Aging Cohort Study (VACS) between 2005 and 2006 using an aptamer-based proteomic profiling platform. Echocardiographic LV mass index and LV mass-to-volume ratio were extracted from electronic medical records using a custom, validated natural language processing algorithm. Linear regression with robust variance was used to assess cross-sectional associations between protein levels and LV structure.

Results

This study included 107 participants (median [IQR] age 56 [11] years; 97% male; 63% Black race) with proteomic profiling and LV structural characteristics obtained within the following 5 years. Following adjustment for kidney function and traditional risk factors, one protein—Cathepsin D—was significantly associated with LV structure (false discovery rate <0.10). Standardized effect estimates of several evaluated proteins, however, were of similar magnitude and direction compared to prior literature.

Conclusion

This study reports external validation of protein biomarker candidates for cardiac remodeling that may warrant further investigation.

D.2. INTRODUCTION

Heart failure (HF) is a complex, heterogeneous syndrome with a high public health and economic burden. HF is clinically diagnosed in over 26 million people globally¹ and, due in part to an aging population, is rising in prevalence.^{2,3} However, HF diagnosis is largely limited to symptomatic stages, and detection of subclinical HF markers—including abnormal or altered left ventricular structure—is critical to earlier disease detection and intervention.

Alterations in left ventricular mass and volumetric measures play early and pivotal roles in dysfunction across the pathophysiologic spectrum of HF,²²⁻²⁵ and these structural characteristics have been independently associated with cardiovascular events and mortality in multiple population-based cohorts.¹⁷⁻²¹ Efforts have been made in recent years to identify candidate novel protein biomarkers for detection of cardiac structural disease, including recent proteomic profiling conducted in the Framingham Heart Study²⁶⁰ and the Multiethnic Study of Atherosclerosis (Chapter 1). The majority of the extant literature, however, is limited in that cellular and soluble biomarkers are often few and inconsistent between studies. This poses a considerable impediment to developing novel clinical tools and strategies to mitigate HF risk by limiting the field's ability to choose well-informed biomarker and therapeutic target candidates.

The objective of this study, conducted among a diverse U.S. population in the Veterans Aging Cohort Study, was to use the aptamer-based SOMAScan platform (SomaLogic, Boulder, CO) and electronic medical record (EMR)-derived LV phenotypes to externally validate cross-sectional associations between plasma protein levels and left ventricular structure observed in the extant literature.

D.3. METHODS

Study Population

The Veterans Aging Cohort Study (VACS) is a prospective study initiated in 1998 to study the role of HIV and long-term antiretroviral treatment in aging and comorbid disease among patients seen in Veterans Administration Medical Center (VAMC) infectious disease and general medical clinics.³⁰⁶ This study takes advantage of the infrastructure in place within the VAMC healthcare system to combine electronic medical

record (EMR) and administrative data with patient and provider surveys, in-depth telephone interviews, and biospecimen banking.

VACS participants were recruited from nine geographic areas across the U.S.— Los Angeles, CA; Atlanta, GA; Dallas, TX; Houston, TX; Bronx, NY; Manhattan/Brooklyn, NY; Baltimore, Maryland; Pittsburgh, Pennsylvania; and Washington, DC. This study utilized data on a subset of VACS participants enrolled in a biomarker study with EDTA plasma collected and stored between 2005 and 2006. The analysis sample was defined as any VACS participant with proteomics data and LV mass and volumetric data captured ≤ 5 years following the blood draw used for proteomics.

Left Ventricular Structure Ascertainment

The response variables for this study are LV mass index and LV mass-to-volume ratio. Mass and volume at end-diastole were extracted from unstructured and semi-structured text fields within the VA health system EMR using a previously developed and validated custom pipeline of natural language processing algorithms built using the Leo framework.^{33, 307} On manual validation, the precision of extracted phenotype values across different report formats ranged from 86 to 100%, and recall ranged from 71 to 100%. LV mass values outside the range of 50-350g and LV end-diastolic volume values outside the range of 30-250mL were assumed erroneous and excluded from analysis. LV mass was indexed to body surface area prior to analyses, per current recommendations.⁴⁶

Protein Measurement

Relative plasma protein abundances of each of 75 target proteins were captured using the SOMAscan platform (SomaLogic, Boulder, CO). SOMAscan has been described in detail previously^{257, 268} and has exhibited high precision and reproducibility.^{257, 267} It utilizes single-stranded DNA aptamers with chemically modified nucleotides that bind epitopes on target proteins, which is then quantified using relative fluorescence on microarrays.²⁶⁹ Previous work indicates median intra- and inter-run coefficients of variation for each aptamer of approximately 5% and a median intra-class correlation coefficient of 0.96 among split sample duplicates.^{259, 271}

This platform was completed at a central SOMALogic laboratory on EDTA plasma samples stored at -70°C from a subset of VACS participants. The central laboratory

applied standardized quality control methods for normalization and calibration of protein analyte data at the aptamer, sample, and plate level. This procedure corrects for systemic errors during sample hybridization, sample or assay technical errors, and protein-specific plate-to-plate variation.

Due to power limitations, only a subset of 75 out of roughly 5,000 proteins measured on the SOMAScan platform were analyzed in this study, selected based on prior literature. A full list of targeted proteins can be found in *Supplemental Table S4*. Protein analyte values, which are measured in relative fluorescence units (RFU) and were predominantly heavily right-skewed, were \log_2 -transformed, and outliers greater than 5 standard deviations from the sample mean on the \log_2 scale were winsorized.

Additional Clinical and Laboratory Data

All covariate data was ascertained using a combination of clinical, laboratory, survey, administrative, pharmacy, and/or ICD-9 code data collected closest to and within 180 days of the date of proteomics sample collection, as previously described.^{33, 308-311} Standardized instruments were used in all patient questionnaires, which were adapted from the Veterans Health Survey³¹² and included questions regarding health behaviors, e.g., exercise frequency, detailed substance use and patterns of use.

Statistical Analysis

Demographic and basic clinical characteristics of participants in the analysis samples were summarized using medians (interquartile ranges [IQR]) for continuous variables and proportions (counts) for categorical variables. Complete case analyses were performed to estimate the effects of protein abundances on LV structural parameters ascertained within the following five years. Inference was made using Huber-White robust variance estimators, adjusting for clinic center, age, sex at birth, race/ethnicity, total:HDL-cholesterol, statin, systolic blood pressure, blood pressure-lowering therapy, diabetes, body mass index, physical activity, current smoking, hazardous alcohol use, cocaine use within the previous month, prevalent cardiovascular disease, and estimated glomerular filtration rate. The Benjamini and Hochberg (BH) false discovery rate (FDR) approach was used to correct for multiple comparisons. Proteins with relatively consistent effects to those estimated in Chapter 1 were then evaluated for associations with basic demographic and clinical characteristics among a larger sample of VACS

participants using linear regression. All analyses were conducted using R software version 4.0.2.

D.4. RESULTS

A total of 107 VACS participants had proteomics and target LV imaging parameters within the target time frame following exclusions due to inappropriate or missing LV parameter units ($n=26$), physiologically implausible LV parameter values ($n=2$), duplicate LV parameter entries from different EMR field sources ($n=20$), and laboratory flag due to technical error or protein analyte values outside normalization bounds ($n=1$).

Demographic and clinical characteristics of participants are summarized in **Table 7**. Median [IQR] age at baseline was 56 [11] years, 97% were male, 63% were Black, 47% were current smokers, 74% were hypertensive, 59% were hyperlipidemic, 48% were diabetic, and 59% were living with HIV infection. Median [IQR] EMR-derived LV mass index was 91 [36] g/m², LV end-diastolic volume index was 53 [25] mL/m², and LV mass-to-volume ratio was 1.76 [0.64] g/mL.

Following full adjustment for clinic center, age, sex at birth, race/ethnicity, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, statin, diabetes, body mass index, physical activity, smoking status, alcohol use status, HIV infection, and eGFR, none of the 75 protein abundances tested was significantly associated with LV mass index and one was associated with LV mass-to-volume ratio (cathepsin D; mean difference per SD increment in log₂-protein abundance [95% CI]: 0.21 [0.09 to 0.34] g/mL; FDR-adjusted $p=0.08$).

Results of proteins replicated in cross-sectional analyses within the Multi-Ethnic Study of Atherosclerosis study population (Chapter 1) are listed in **Table 8**, ordered by descending standardized parameter estimates. Among this targeted list of proteins, effects on LV mass index were consistent in direction and magnitude with those estimated in MESA for NT-proBNP, leptin, renin, and cathepsin D, though imprecise. When evaluating LV mass-to-volume ratio, effect estimates were consistent for repulsive guidance molecule domain family member B (RGMB) and cGMP-stimulated phosphodiesterase 2A (PDE2A). A comparison of standardized effect estimates from the current study and a cross-sectional analysis at baseline in MESA is depicted graphically in **Figure 8**.

Four of these proteins have shown relatively consistent results in analyses from Chapter 1 and Chapter 2—specifically leptin and cathepsin D as they relate to LV mass index and its change over ten years, as well as RGMB and PDE2A as they relate to LV mass-to-volume ratio and its change over ten years. Unadjusted associations between these proteins and basic demographic and clinical characteristics within the VACS Biomarker Cohort ($n=2,237$) are presented in *Supplemental Table S5*.

D.5. DISCUSSION

In this diverse sample of veterans referred for echocardiography within the largest integrated health system in the United States, plasma abundances of 75 previously identified cardiac remodeling biomarker candidates were evaluated for associations with EMR-derived left ventricular structural characteristics. Following adjustment for kidney function and traditional CVD risk factors as well as correction for multiple comparisons, cathepsin D was positively and significantly associated with LV mass-to-volume ratio, while none of these proteins were significantly associated with LV mass index. Due to power limitations affecting estimate precision, however, a select set of proteins were examined for consistency in direction and magnitude of effect estimates compared to prior analyses. By this assessment, leptin and cathepsin D showed consistency in their associations with LV mass index and repulsive guidance molecule domain family member B (RGMB) and phosphodiesterase 2A (PDE2A) in their associations with LV mass-to-volume ratio.

Cathepsin D is an important lysosomal protease required in certain epithelial cells for tissue remodeling and renewal.²⁸⁷ Little data exists on Cathepsin D and cardiac remodeling, but cathepsins play many roles within the vascular system—including extracellular matrix degradation and regulation of inflammatory responses.²⁸⁸ Its negative effect in relation to LV mass index and positive effect in relation to LV mass-to-volume ratio in the current study is not necessarily inconsistent, given the two parameters capture different biological processes. This does, however, support further study of cathepsins in cardiac remodeling on the population level.

Leptin is a protein hormone studied predominantly for its role in regulating appetite²⁷⁶ and insulin sensitivity.²⁷⁷ Leptin does, however, contribute to a wide range of physiologic processes, including regulation of free fatty acid clearance, preventing their deposition in non-adipocyte tissues, such as the myocardium.²⁷⁸ Findings reported here are consistent

with this biologically as well as observational cohort data reporting a protective effect of leptin on LV mass²⁷⁹⁻²⁸² This includes a recent proteomics analysis in the Framingham Heart Study.²⁶⁰ However, several studies have also shown detrimental effects of leptin on LV size,^{281, 283, 284} which has been hypothesized to be due to leptin resistance among those with higher body mass index and adiposity.^{285, 286}

The RGM family of proteins function as co-receptors for bone morphogenic proteins (BMPs), which are critical to development and maintenance of tissue architecture.²⁹⁰ Some studies have reported a protective role of BMPs during cardiac remodeling post-infarction,²⁹¹⁻²⁹³ and a recent proteomics analysis of HF identified RGMB as well as other members of this protein family (RGMA and RGMC) as being negatively associated with incident events, independent of traditional risk factors.²⁶⁰

Lastly, cGMP-stimulated PDE2A is a phosphodiesterase that controls the degradation of guanosine 3',5'-cyclic monophosphate (cGMP), a critically important secondary messenger that has been shown to inhibit pathologic cardiac remodeling.²⁹⁹ Some phosphodiesterases, including PDE2, are thought to be important regulators of cardiomyocyte physiology,³⁰⁰⁻³⁰³ and there is evidence to suggest PDE2, specifically, is both expressed in cardiomyocytes³⁰³ and upregulated in the context of cardiac hypertrophy.^{302, 304, 305}

Strengths and Limitations

There are several limitations to this study. First, inclusion into the analysis sample is dependent on receipt of echocardiography within the VA healthcare system, provider documentation of target parameters, and successful extraction of these parameters by the employed NLP algorithm. This may have resulted in selection bias if the probability of cardiac imaging is dependent on both proteomic profile and LV structure, both of which are possible.

Second, the use of EMR-derived phenotypes is generally suboptimal, not collected with the aim of providing robust scientific data. Specifically, though imaging protocols are well-standardized in VA clinical practice and performed only by trained technicians, there is no quality control for outcome measurement or data entry, and the algorithm used for value extraction is imperfect. This may result in greater measurement error relative to

more standardized data collection methods in imaging studies, though such error would likely be non-differential.

Third, relative timing of cardiac imaging and proteomics assessment vary substantially in this analysis sample. Effort was made to limit the variability in this timing by imposing a threshold for EMR variable extraction of ≤ 5 years from the blood draw used for protein measurement, but this time frame is wide. Current data on the stability of the proteome measured using SOMAScan has, however, suggested a large proportion of assayed proteins have high within-person correlation over a period of years.²⁷¹ Additionally, data from Chapter 1 suggests high within-person stability of proteins highlighted in this study over 10 years (all Spearman's $\rho > 0.4$, p -values $< 1.0 \times 10^{-6}$) as well as relatively small average changes in LV phenotypes over 10 years (*Table 4b*), twice the duration of the imposed time window.

Regarding protein quantification, levels measured via the SOMAScan platform are semi-quantitative and can neither be directly interpreted nor compared to other assays. Further, this assay binds protein epitopes that are not all fully characterized and with affinity that may be sensitive to alterations in protein structure. All proteins of interest therefore require further validation using an alternative method, such as standard immunoassay or mass spectrometry.

Finally, following full data quality control, power to detect associations was low. This not only affected the precision of presented results but also led to the inability to agnostically evaluate proteins, both of which should be considered when interpreting results.

This study does have some strengths, including being conducted in a unique nationwide sample with a high representation of PLWH and persons of Black race. This emphasizes the promise of highlighted proteins, given their consistency in effect among what may be a population at higher risk for cardiac remodeling and related pathologies.

Conclusion

In conclusion, this study reports associations between levels of 75 circulating plasma protein levels and left ventricular structural characteristics among a diverse population of U.S. veterans in care. Given the major limitations in this study, results should not be over-interpreted. However, they do provide some support for continued study of a number of candidate cardiac remodeling biomarkers. If successfully and repeatedly

validated in external populations and in relation to incident manifest HF, these proteins may help refine current HF risk prediction and identify novel therapeutic targets to more effectively mitigate risk for HF progression.

Table 7. Participant demographic and clinical characteristics overall at the time of blood draw used for proteomics ($n=107$).

	<i>Median (25th, 75th Percentile) or % (n)</i>
<i>Demographics</i>	
Age, years	56 (50, 61)
Sex at birth, male	97.2% (104)
Race and ethnicity	--
White	29.0% (31)
Black	62.6% (67)
Hispanic	3.7% (4)
Other	4.7% (5)
<i>Clinical Characteristics</i>	
Body mass index, kg/m ²	26.9 (23.5, 32.6)
Systolic blood pressure, mmHg	128 (119, 142)
Diastolic blood pressure, mmHg	77 (71, 83)
Hypertension	73.8% (79)
Any blood pressure-lowering therapy use	88.8% (95)
Total cholesterol, mg/dL	171 (147, 207)
HDL-cholesterol, mg/dL	41 (34, 56)
LDL-cholesterol, mg/dL	93 (73, 125)
Hyperlipidemia	63.2% (67)
Statin use	62.1% (66)
Warfarin use	14.0% (15)
Diabetes	47.7% (51)
Diabetes therapy use	40.2% (43)
eGFR (CKD-EPI), mL/min/1.73m ²	88.0 (67.7, 113.1)
FIB-4 score	1.42 (0.94, 2.06)
White blood cell count, x1000 cells/mL	5.6 (4.3, 7.2)
D-dimer, µg/mL	0.38 (0.23, 0.73)
sCD14, ng/mL	1915 (1554, 2321)
Interleukin-6, pg/mL	2.53 (1.44, 3.93)
HDL=high-density lipoprotein; LDL=low-density lipoprotein; eGFR=estimated glomerular filtration rate.	

Table 7 Continued. Participant demographic and clinical characteristics overall at the time of blood draw used for proteomics ($n=107$).

	Median (25 th , 75 th Percentile) or % (n)
<i>Behavioral Characteristics</i>	
Frequency of moderate physical activity	--
< 1 time per week	33.9% (36)
1-2 times per week	17.9% (19)
≥ 3 times per week	48.1% (51)
Smoking status	--
Current	46.7% (50)
Former	30.8% (33)
Hazardous alcohol use or dependency	46.7% (50)
Cocaine use	--
Current use ≥ once per month	16.8% (18)
History of cocaine abuse	33.6% (36)
<i>Prior or Prevalent Conditions</i>	
HIV infection	58.9% (63)
Hepatitis C infection	21.5% (23)
Major depression	24.3% (26)
Acute myocardial infarction	7.5% (8)
Unstable angina	7.5% (8)
Revascularization	8.4% (9)
Heart failure	20.6% (22)
Atrial fibrillation	7.5% (8)
Ischemic stroke	5.6% (6)
<i>Left Ventricular Structural Parameters</i>	
LV mass index, g/m ²	98 (79, 119)
LV end-diastolic volume index, mL	56 (42, 70)
LV mass/volume ratio, g/mL	1.76 (1.45, 2.11)
LV=left ventricular.	

Table 8. Associations between plasma protein abundance and left ventricular structure, restricted to proteins replicated in cross-sectional Chapter 1 analyses ($n=107$).

<i>Protein</i>	<i>Mean Difference (95% CI)</i>	<i>Robust p-value</i>
<i>Left ventricular mass index</i>		
Leptin	-2.62 (-9.37 to 4.13)	0.447
Renin	-3.18 (-11.21 to 4.85)	0.437
Cathepsin D	-1.39 (-7.13 to 4.35)	0.636
N-terminal pro-BNP	3.84 (-1.83 to 9.51)	0.185
<i>Left ventricular mass-to-volume ratio</i>		
Cathepsin D	0.21 (0.09 to 0.34)	0.001
Chordin-like protein 1	0.08 (-0.08 to 0.25)	0.310
cGMP-stimulated PDE2A	-0.03 (-0.14 to 0.08)	0.598
Bone morphogenetic protein receptor type-1A	-0.01 (-0.13 to 0.11)	0.845
RGM domain family member B	-0.03 (-0.18 to 0.12)	0.703
TGF- β receptor type 3	0.01 (-0.14 to 0.16)	0.904
TNF-inducible gene 6 protein	0.02 (-0.12 to 0.17)	0.741
<p>Mean difference in structural parameter per standard deviation increment in log-transformed protein abundance, estimated using generalized estimating equations, adjusting for clinic center, age, sex at birth, race/ethnicity, HIV infection status, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, statin, diabetes, body mass index, physical activity, smoking status, alcohol use status, and estimated glomerular filtration rate.</p> <p>CI=confidence interval; BNP=brain natriuretic peptide; PDE=phosphodiesterase; RGM=repulsive guidance molecule; TGF=transforming growth factor; TNF=tumor necrosis factor.</p>		

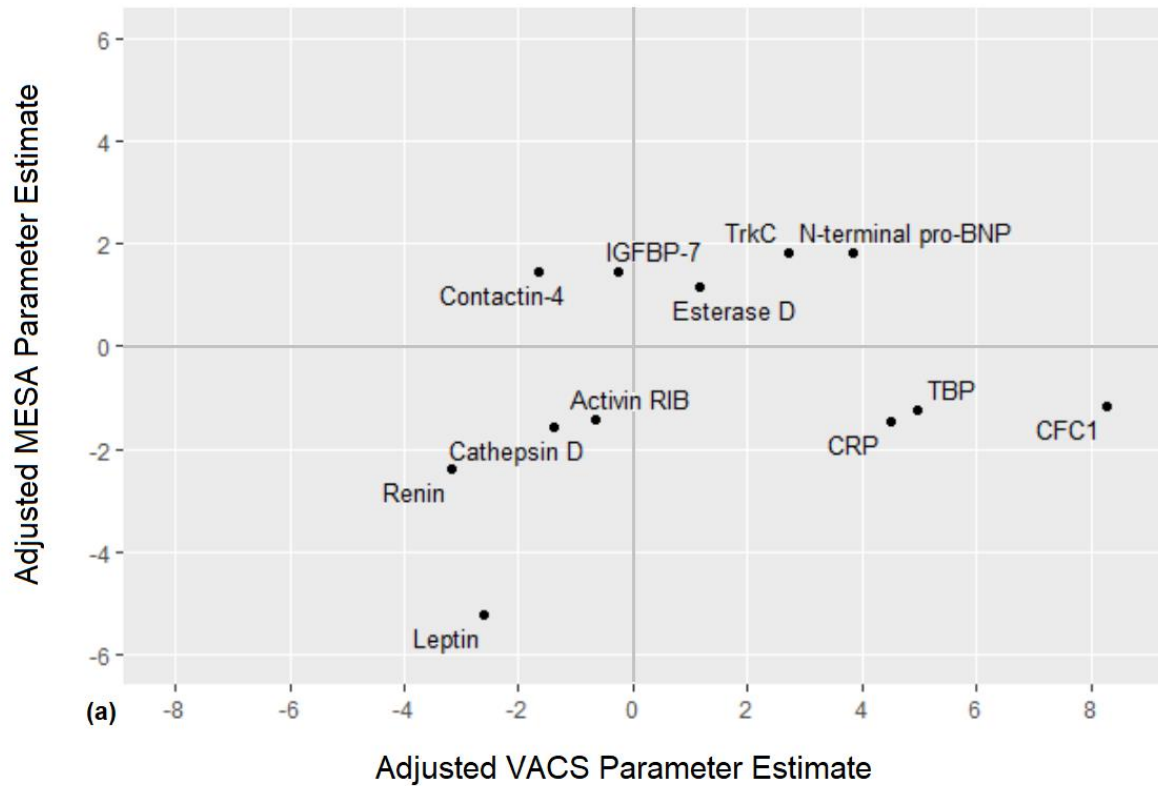


Figure 8 (a). Scatterplot of standardized parameter estimates for protein abundances from generalized estimating equations modeling left ventricular mass index at baseline in MESA vs. VACS, each adjusted for clinic center, age, sex at birth, race/ethnicity, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, statin, diabetes, body mass index, physical activity, smoking status, alcohol use status, and eGFR. Plotted proteins limited to those with Benjamini-Hochberg p -values <0.10 in MESA.

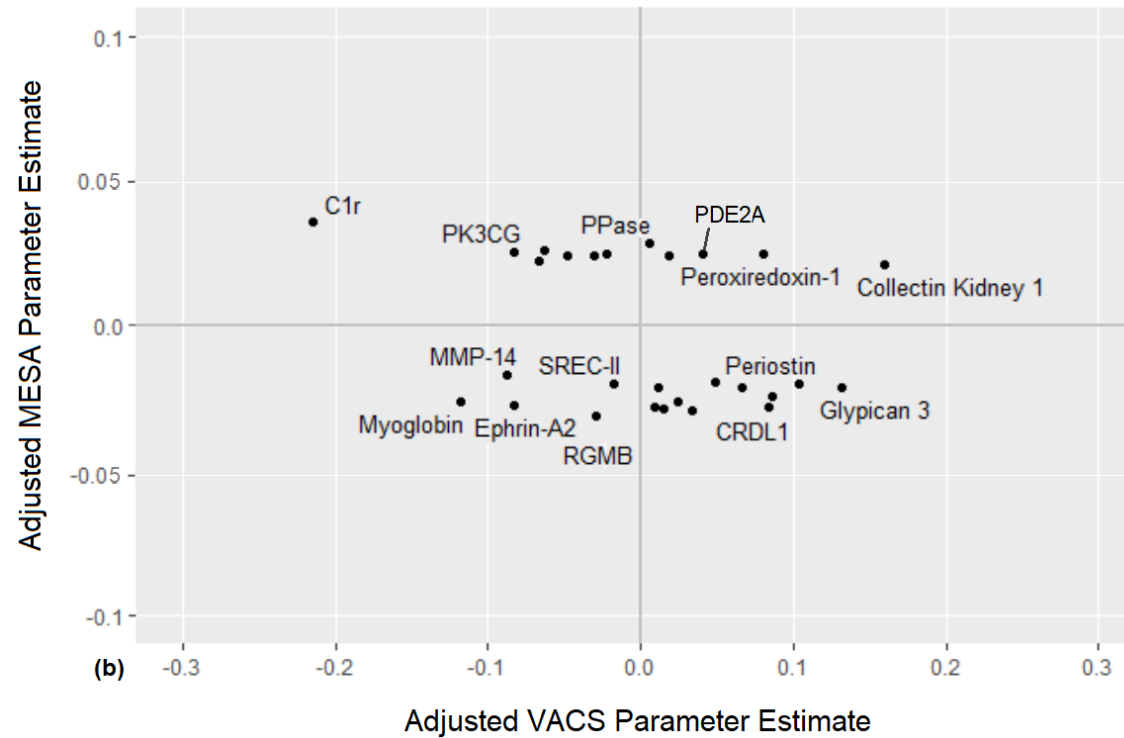


Figure 8 (b). Scatterplot of standardized parameter estimates for protein abundances from generalized estimating equations modeling left ventricular mass-to-volume ratio at baseline in MESA vs. VACS, each adjusted for clinic center, age, sex at birth, race/ethnicity, systolic blood pressure, blood pressure-lowering therapy, total:HDL-cholesterol, statin, diabetes, body mass index, physical activity, smoking status, alcohol use status, and eGFR. Plotted proteins limited to those with Benjamini-Hochberg p -values <0.10 in MESA.

Supplemental Tables and Figures

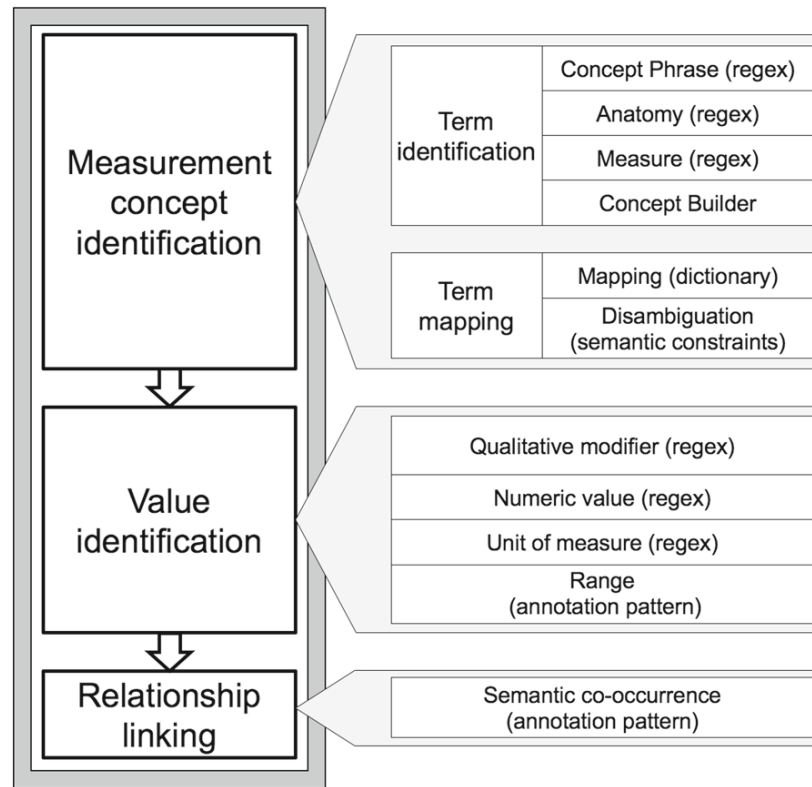


Figure S2: Overall system design of natural language processing algorithm used to extract left ventricular phenotype data; figure published previously by Patterson et al.²⁵⁶

Table S4. Proteins assessed in the current study ($n=75$).

<i>Protein Name</i>	<i>Entrez Gene Symbol</i>
Angiotensin-converting enzyme 2	ACE2
Activin receptor type-1B	ACVR1B
Adenylate kinase isoenzyme 1	AK1
Amyloid beta A4 protein	APP
Aurora kinase A	AURKA
Brain-derived neurotrophic factor	BDNF
Bone morphogenetic protein receptor type-1B	BMPR1B
Complement C1r subcomponent	C1R
Cell adhesion molecule 3	CADM3
Catalase	CAT
C-C motif chemokine 2	CCL2
OX-2 membrane glycoprotein	CD200
Cyclin-dependent kinase 1:G2/mitotic-specific cyclin-B1	CDC2/CCNB1
Cryptic protein	CFC1
Chordin-like protein 1	CHRDL1
Contactin-4	CNTN4
Collectin-11	COLEC11
C-reactive protein	CRP
Cystatin-C	CST3
Cathepsin D	CTSD
Ephrin-A2	EFNA2
S-formylglutathione hydrolase	ESD
Tumor necrosis factor receptor superfamily member 6	FAS
Growth arrest-specific protein 1	GAS1
Growth/differentiation factor 15	GDF15
Glypican-3	GPC3
Insulin-like growth factor-binding protein 7	IGFBP7
Interleukin-1 receptor type 1	IL1R1
Interleukin-2	IL2
Interleukin-4	IL4
Interleukin-6	IL6
Leptin	LEP
Galectin-3	LGALS3
Galectin-7	LGALS7
Myoglobin	MB
Interstitial collagenase	MMP1
72 kDa type IV collagenase	MMP2
Stromelysin-1	MMP3

Table S4 Continued. Proteins assessed in the current study (*n*=75).

<i>Protein Name</i>	<i>Entrez Gene Symbol</i>
Neutrophil collagenase	MMP8
Matrix metalloproteinase-9	MMP9
Stromelysin-2	MMP10
Macrophage metalloelastase	MMP12
Collagenase 3	MMP13
Matrix metalloproteinase-14	MMP14
Matrix metalloproteinase-16	MMP16
Matrix metalloproteinase-16	MMP16
Matrix metalloproteinase-19	MMP19
Myeloperoxidase	MPO
Atrial natriuretic factor	NPPA
Natriuretic peptides B	NPPB
N-terminal pro-BNP	NPPB
NT-3 growth factor receptor	NTRK3
cGMP-dependent 3',5'-cyclic phosphodiesterase	PDE2A
Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit gamma isoform	PIK3CG
Beta-endorphin	POMC
Periostin	POSTN
Inorganic pyrophosphatase	PPA1
Peroxiredoxin-1	PRDX1
Prolactin	PRL
Renin	REN
Repulsive guidance molecule domain family member B	RGMB
Scavenger receptor class F member 2	SCARF2
Superoxide dismutase [Cu-Zn]	SOD1
Osteopontin	SPP1
Stabilin-2	STAB2
TATA-box-binding protein	TBP
Transforming growth factor beta-1	TGFB1
Transforming growth factor beta receptor type 3	TGFBR3
Metalloproteinase inhibitor 1	TIMP1
Metalloproteinase inhibitor 2	TIMP2
Metalloproteinase inhibitor 3	TIMP3
Metalloproteinase inhibitor 4	TIMP4
Tumor necrosis factor	TNF
Tumor necrosis factor-inducible gene 6 protein	TNFAIP6
Troponin I, cardiac muscle	TNNI3
Troponin T, cardiac muscle	TNNT2

Table S5 (a). Unadjusted cross-sectional associations between baseline clinical characteristics and proteins with moderately strong and replicated left ventricular mass index associations ($n=2,237$).

<i>Characteristic</i>	<i>Mean Standardized Difference (95% Confidence Interval)</i>	<i>p-value</i>
<i>Leptin</i>		
Age, per 10 years	0.13 (0.08 to 0.17)	<0.001
Male sex	-1.20 (-1.38 to -1.02)	<0.001
Black race (vs. white)	-0.20 (-0.31 to -0.10)	<0.001
Hispanic ethnicity (vs. white)	-0.02 (-0.18 to 0.14)	0.830
Current smoking (vs. never)	-0.43 (-0.53 to -0.33)	<0.001
Obesity	1.25 (1.18 to 1.33)	<0.001
Hypertension	0.47 (0.39 to 0.55)	<0.001
Hyperlipidemia	0.46 (0.37 to 0.54)	<0.001
Diabetes	0.49 (0.39 to 0.58)	<0.001
Coronary artery disease	0.32 (0.14 to 0.50)	<0.001
Heart failure	0.33 (0.16 to 0.50)	<0.001
Atrial fibrillation	0.32 (0.07 to 0.56)	0.011
HIV infection	-0.69 (-0.77 to -0.61)	<0.001
HCV infection	-0.26 (-0.35 to -0.17)	<0.001
<i>Cathepsin D</i>		
Age, per 10 years	0.07 (0.02 to 0.11)	0.007
Male sex	0.24 (0.06 to 0.43)	0.011
Black race (vs. white)	0.14 (0.04 to 0.25)	0.009
Hispanic ethnicity (vs. white)	0.23 (0.07 to 0.39)	0.005
Current smoking (vs. never)	0.38 (0.28 to 0.49)	<0.001
Obesity	-0.19 (-0.28 to -0.10)	<0.001
Hypertension	0.02 (-0.07 to 0.10)	0.699
Hyperlipidemia	-0.32 (-0.40 to -0.23)	<0.001
Diabetes	0.18 (0.08 to 0.27)	<0.001
Coronary artery disease	0.03 (-0.16 to 0.20)	0.787
Heart failure	-0.02 (-0.19 to 0.15)	0.787
Atrial fibrillation	-0.22 (-0.47 to 0.02)	0.074
HIV infection	0.33 (0.24 to 0.41)	<0.001
HCV infection	1.03 (0.94 to 1.11)	<0.001
Effect estimated using linear regression, mean standardized difference in log-transformed protein per increment indicated or yes vs. no for continuous or dichotomous characteristics, respectively.		

Table S5 (b). Unadjusted cross-sectional associations between baseline clinical characteristics and proteins with moderately strong and replicated left ventricular mass-to-volume associations ($n=2,237$).

<i>Characteristic</i>	<i>Mean Standardized Difference (95% Confidence Interval)</i>	<i>p-value</i>
<i>Repulsive guidance molecule domain family member B (RGMB)</i>		
Age, per 10 years	0.10 (0.05 to 0.14)	<0.001
Male sex	0.48 (0.29 to 0.66)	<0.001
Black race (vs. white)	-0.05 (-0.15 to 0.06)	0.377
Hispanic ethnicity (vs. white)	-0.04 (-0.18 to 0.11)	0.610
Current smoking (vs. never)	-0.17 (-0.27 to -0.06)	0.001
Obesity	-0.12 (-0.21 to -0.02)	0.015
Hypertension	0.17 (0.09 to 0.25)	<0.001
Hyperlipidemia	-0.05 (-0.14 to 0.03)	0.214
Diabetes	0.02 (-0.08 to 0.12)	0.692
Coronary artery disease	0.15 (-0.03 to 0.33)	0.098
Heart failure	0.66 (0.49 to 0.82)	<0.001
Atrial fibrillation	0.15 (-0.09 to 0.40)	0.225
HIV infection	-0.20 (-0.28 to -0.11)	<0.001
HCV infection	0.17 (0.08 to 0.26)	<0.001
<i>cGMP-stimulated phosphodiesterase 2A (PDE2A)</i>		
Age, per 10 years	0.00 (-0.05 to 0.04)	0.940
Male sex	0.09 (-0.09 to 0.26)	0.339
Black race (vs. white)	0.05 (-0.05 to 0.15)	0.369
Hispanic ethnicity (vs. white)	-0.08 (-0.23 to 0.06)	0.267
Current smoking (vs. never)	-0.05 (-0.15 to 0.05)	0.357
Obesity	-0.01 (-0.09 to 0.08)	0.914
Hypertension	-0.01 (-0.09 to 0.07)	0.864
Hyperlipidemia	0.00 (-0.08 to 0.08)	0.939
Diabetes	-0.10 (-0.19 to 0.00)	0.045
Coronary artery disease	-0.05 (-0.22 to 0.12)	0.544
Heart failure	-0.05 (-0.20 to 0.11)	0.580
Atrial fibrillation	-0.03 (-0.26 to 0.20)	0.808
HIV infection	-0.01 (-0.09 to 0.08)	0.924
HCV infection	-0.06 (-0.15 to 0.02)	0.151
Effect estimated using linear regression, mean standardized difference in log-transformed protein per increment indicated or yes vs. no for continuous or dichotomous characteristics, respectively.		

E. Chapter 3: *The Effect of HIV Infection on Left Ventricular Structure in the Veterans Aging Cohort Study*

E.1. OVERVIEW

Introduction

Persons living with HIV (PLWH) are at higher risk of incident heart failure compared to persons without HIV (PWOH), but the characterization of cardiac phenotypes among clinically relevant patient populations is limited. The drivers of this excess risk are also incompletely understood. This study utilized data from the largest contemporary cohort of PLWH and PWOH with electronic medical record (EMR)-derived left ventricular (LV) structural phenotypes to better characterize the cross-sectional relationship between HIV infection and LV structure.

Methods

Echocardiographic LV mass index and LV mass-to-volume ratio were extracted from electronic medical records using a custom, validated natural language processing algorithm. Linear and log-binomial regression was used to assess cross-sectional associations between LV structure and both HIV serostatus and, among veterans with HIV, measures of HIV disease severity.

Results

This study included 19,053 veterans (median [IQR] age 58 [12] years; 97% male; 49% of Black race, 30% PLWH) with LV structural characteristics obtained at any time between 2003 and 2015. Following adjustment for demographics, substance use, and hepatitis C infection, as well as major factors leading to echocardiography referral within this health system, PLWH had greater mean LV mass index and similar mean LV mass-to-volume ratio compared to PWOH. Among PLWH, higher LV mass index was associated with measures of greater HIV disease severity, including lower CD4+ cell counts and CD4+:CD8+ ratio.

Conclusion

Among this veteran population referred for echocardiography, adverse cardiac structural phenotypes were associated with HIV infection and measures of greater HIV disease severity.

E.2. INTRODUCTION

There is a large and growing burden of cardiovascular disease (CVD) among persons living with HIV (PLWH),²⁶⁻²⁹ and it is currently well-recognized that even in the context of effective antiretroviral treatment (ART), PLWH experience a higher risk of CVD relative to persons without HIV (PWOH).³⁰⁻³⁹ Heart failure among ART-treated PLWH has garnered specific concern, with evidence of strong, independent HIV effects on incident heart failure with both reduced and preserved ejection fraction^{30, 33, 38} as well as related pathologies such as myocardial infarction,^{30-32, 177} sudden cardiac death,³⁴ and atrial fibrillation.³⁰ This elevated risk is hypothesized to not only be due to differences in prevalence of traditional risk factors but also due to HIV-specific factors, including chronic inflammation and immune dysfunction.

Cardiac remodeling is an early and pivotal player in dysfunction across the pathophysiologic spectrum of HF.²²⁻²⁵ Cardiac remodeling is defined as alterations in regional or global geometry or function of the heart occurring as a physiologic adaptation either to stressors that persistently elevate myocardial workload or to the effect of disease that may reduce contractility or alter tissue composition of the myocardium. Among the general population, structural cardiac remodeling strongly and consistently predicts both systolic and diastolic dysfunction^{6-9, 23, 24} as well as incident clinical HF.¹⁷⁻²¹ However, cardiac remodeling among PLWH remains poorly characterized among clinical populations with inconsistent effects observed across studies, several of which are limited by sample size.^{184-187,313}

The objective of this study was to leverage data from the largest contemporary cohort of PLWH and PWOH with electronic medical record (EMR)-derived left ventricular structural phenotypes to better characterize the cross-sectional relationship between cardiac structure and both HIV infection and measures of HIV disease severity.

E.3. METHODS

Study Population

The Veterans Aging Cohort Study (VACS) is a prospective study initiated in 1998 to study the role of HIV and long-term antiretroviral treatment in aging and comorbid disease among patients seen in Veterans Administration Medical Center (VAMC)

infectious disease and general medical clinics.³⁰⁶ The VA is the largest integrated healthcare system with over 1,700 hospitals, clinics, and nursing homes, and it is the largest single provider of HIV care in the United States.

VACS has enrolled over 150,000 participants through the VA Healthcare System—50,000 PLWH matched on clinic center, age, race, and year of enrollment 1:2 to 100,000 HIV-uninfected participants. The analysis sample in the current study included all VACS participants enrolled on or after April 1, 2003 who were referred for a transthoracic echocardiogram with target LV structural data reported in the EMR. Baseline was a participant's first echocardiogram with all target parameters, and clinical and laboratory measurements closest to and within 180 days of this date.

Left Ventricular Structure Ascertainment

The response variables for this study are LV mass index and LV mass-to-volume ratio. Mass and volume at end-diastole were extracted from unstructured and semi-structured text fields within the VA health system EMR using a previously developed and validated custom pipeline of natural language processing algorithms built using the Leo framework,^{33, 307} described in more detail in the *Supplemental Methods*. On manual validation, the precision of extracted phenotype values across different report formats ranged from 86 to 100%, and recall ranged from 71 to 100%. LV mass and end-diastolic volume values outside the range of 50-350g and 30-250mL, respectively, were assumed erroneous and excluded from analysis. LV mass was indexed to body surface area prior to analyses, per current recommendations.⁴⁶ Extracted parameters were also used to define conditions using clinical thresholds, specifically LV hypertrophy (LVH) (defined as LV mass index >115 g/m² for males and >95 g/m² for females), which was then further categorized into concentric (LVH plus LV mass-to-volume ratio >2 g/mL) or eccentric (<2 g/mL) in geometry.⁴⁶

HIV Status Ascertainment

HIV status in the greater VACS is determined based on a validated metric including at least one inpatient or two or more outpatient ICD ninth revision (ICD-9) codes for HIV and if the participant was included in the VA Immunology Case Registry.³¹⁴

Additional Clinical and Laboratory Data

Administrative data was used to determine age, sex, and race/ethnicity. Body mass index (BMI), hypertension, lipid levels, and diabetes were defined using a combination of clinical outpatient and laboratory data collected closest to the date of echocardiogram. Prescribed medication was based on pharmacy data and defined as a prescription filled within the interval of -180 to +7 days of the echocardiogram date. Smoking was measured from health factor data that are collected in a standardized form within the VA health system, as previously described and validated.³⁰⁹ Hypertension was defined as recently prescribed antihypertensive medication or blood pressure $\geq 140/90$ mmHg³¹⁵ using the mean of 3 routine outpatient clinical measurements closest to the date of the echocardiogram. Diabetes was defined using a validated metric that considers glucose measurements, antidiabetic agent use, and at least 1 inpatient or at least 2 outpatient ICD-9 codes.³¹⁶ Hepatitis C virus (HCV) infection was defined as a positive HCV antibody test result or at least 1 inpatient or at least 2 outpatient ICD-9 codes at any time prior to the echocardiogram.^{308, 316} Finally, atrial fibrillation as well as history of alcohol and cocaine abuse or dependence were defined using ICD-9 codes.³¹⁰

Among PLWH in this sample, data on CD4+ cell counts, CD8+ cell counts, and HIV-1 RNA were pulled from laboratory files closest to and within 180 days of the echocardiogram date. Finally, the VACS index, a score that assesses 5-year all-cause mortality risk using indicators of HIV disease and organ system injury, was also calculated using measures collected within the same time period.³¹⁷

Statistical Analysis

Demographic and basic clinical characteristics of participants in the analysis sample were summarized overall and by HIV status using medians (interquartile ranges [IQR]) for continuous variables and proportions (counts) for categorical variables. Complete case analyses were performed using linear regression to estimate mean differences in LV mass index, LV end-diastolic volume index, and LV mass-to-volume ratio by HIV status, and log-binomial regression was used to estimate relative risks for dichotomized characteristics. Model 1 adjusts for hypothesized confounders, including clinic center, age, sex at birth, race/ethnicity, current smoking, history of alcohol abuse, and history of cocaine abuse. Other possible predictors of LV structural characteristics that are related to HIV infection—e.g., hypercoagulation, inflammation, body mass index, hypertension,

diabetes, prior myocardial infarction (MI)—are hypothesized to be mediators on the causal pathway of interest rather than confounders and thus were not adjusted for. However, due to concerns regarding internal validity in this study (depicted in *Supplemental Figure S3*), estimates in Model 2 are further adjusted for the most frequent indications for echocardiography within the VA healthcare system³¹⁸—specifically prior or prevalent MI, unstable angina, revascularization, heart failure, atrial fibrillation, and hypertension.

LV structural characteristics that show evidence of a difference by HIV status were then assessed for associations with HIV-related characteristics among participants with HIV. Finally, in a subsample of VACS participants with data on circulating protein abundances, associations between HIV-related characteristics and plasma levels of NT-proBNP, a widely used clinical biomarker used to rule out HF diagnosis,¹²¹⁻¹²⁵ and leptin, a protein consistently associated with LV mass and hypertrophy in the literature,²⁷⁹⁻²⁸² were analyzed using linear regression. All analyses were conducted using R software version 4.0.2.

E.4. RESULTS

A total of 19,053 veterans ($n=5,767$ living with HIV and $n=13,286$ living without HIV) with reported left ventricular structural parameters on echocardiography were identified following exclusions due to inappropriate or missing LV parameter units ($n=2,414$) or physiologically implausible LV parameter values ($n=911$). Missing covariate data was limited to laboratory measures, which were not used in primary analyses.

Demographic and clinical characteristics of participants are summarized in **Table 9**. Median [IQR] age at baseline was 58 [12] years, 97% were male, 35% were current smokers, 54% were diabetic, 86% had hypertension, 93% had hyperlipidemia, and 44% had prevalent or prior clinical cardiovascular disease. Median [IQR] EMR-derived LV mass index among PLWH and PWOH was 96 [42] and 96 [40] g/m², LV end-diastolic volume index was 53 [25] and 53 [24] mL/m², and LV mass-to-volume ratio was 1.82 [0.73] and 1.83 [0.75] g/mL, respectively.

HIV-related characteristics are reported in **Table 10** among participants living with HIV. Median [IQR] CD4+ cell count was 412 [400] cells/ μ L and HIV viral load was 75 [766] copies/mL. Seventy percent were prescribed antiretrovirals within the prior 6 months,

87% were prescribed a nucleoside reverse transcriptase inhibitor (60% of total PLWH), and 59% of which were prescribed a protease inhibitor (41% of total PLWH). Median [IQR] VACS Index 5-year all-cause mortality risk was 39 [32]%.

The cross-sectional effect of HIV infection on cardiac structure among those referred for echocardiography are reported in **Table 11**. Veterans living with HIV had, on average, an LV mass index 1.42 g/m² higher than their uninfected counterparts (95% CI: 0.49 to 2.34; $p=0.003$), following full adjustment for age, sex at birth, race/ethnicity, smoking status, history of alcohol use disorder, history of cocaine use disorder, HCV infection, hypertension, and prior or prevalent CVD. Similar fully adjusted LV mass-to-volume ratio was observed among veterans living with and without HIV in the same sample (0.010 g/mL lower among PLWH compared to PWOH; 95% CI: -0.030 to 0.009; $p=0.294$). In a subgroup analysis comparing only virally suppressed PLWH to PWOH, however, there was no evidence of a difference in mean LV mass index (estimate [95% CI]: -0.65 [-1.82 to 0.52]; $p=0.27$), and estimates for other parameters were also attenuated.

Figure 9 depicts the unadjusted mean difference in LV mass index by HIV-related characteristics among veterans living with HIV in this sample. Lower CD4+ cell count ($p<0.001$) and CD4:CD8 ($p<0.001$) as well as no HIV viral suppression ($p<0.001$) or recently prescribed antiretroviral therapy ($p=0.014$) were each associated with higher LV mass index, on average. The mean difference in LV mass index among those recently prescribed antiretrovirals with more cardiotoxic profiles, specifically abacavir and darunavir compared to no ART, was approximately 1.6 g/m² and 1.7 g/m², respectively, although estimates were imprecise. Finally, higher LV mass index was associated with a higher VACS index score; an average 1.2 g/m² increment in LV mass index was associated with a 5-point increment in all-cause mortality risk score (95% CI: 1.06 to 1.44; $p<0.001$).

Among a subset of VACS participants with circulating protein abundance measured, the cross-sectional effect of HIV infection on plasma NT-proBNP and leptin levels were estimated and are presented in **Table 12**, as are the effects of HIV-related characteristics among veterans living with HIV. HIV infection was cross-sectionally associated with lower leptin ($p<0.001$) but NT-proBNP levels were similar between the two groups ($p=0.20$). Among veterans living with HIV, lower leptin levels were associated

with measures of greater HIV disease severity, including lower CD4+ cell counts and higher HIV viral load.

E.5. DISCUSSION

In this national sample of veterans referred for echocardiography, PLWH had higher mean LV mass index and similar mean LV mass-to-volume ratio compared to PWOH, independent of demographics, substance use, HCV infection, and primary factors leading to echocardiography referral within this health system. Taken together, these and results of dichotomized characteristics suggest a higher risk among PLWH of LV hypertrophy overall and of hypertrophy with eccentric geometry, compared to PWOH. Eccentric hypertrophy is typically the result of volume overload, commonly observed in the context of systolic dysfunction. Though recent studies among PLWH have suggested a shift in cardiac dysfunction phenotype from systolic to diastolic, prevalence of systolic dysfunction among PLWH in the ART era is still thought to be high.¹⁸⁸

Among PLWH, higher LV mass index was associated with measures of greater HIV disease severity, e.g., lower CD4+ cell counts and CD4+:CD8+ ratio, higher viral load, and no prescribed antiretroviral therapy within the prior six months. In fact, in a subgroup analysis comparing only PLWH with viral suppression to PWOH, the effect of HIV infection on LV mass index was nullified, suggesting well-managed HIV infection among this patient population may mitigate any excess risk of subclinical structural disease. Finally, each of the evaluated LV structural characteristics were associated with higher VACS index scores among PLWH, supporting the clinical relevance of these indices within this population.

Advanced HIV disease is classically associated with systolic dysfunction and dilated cardiomyopathy, but with the advent of effective ART came a transition from this phenotype to one characterized more by subclinical cardiac remodeling.^{188, 319, 320} There has, however, been some inconsistency in findings regarding the effect of HIV infection on various cardiac structural indices, which may be due in part to differences in source population disease prevalence and cardiac assessment methodologies between what have all been cross-sectional studies. Consistent with findings in this study among veterans in care, HIV infection has been previously associated with higher LV mass in the United States.¹⁸⁴⁻¹⁸⁷ However, these studies have largely not detected differences in

LV end-diastolic volume by HIV status and have also varied in their detection of difference by measures of HIV disease severity.

The role leptin may play in cardiac remodeling among PLWH is unclear and could not be fully assessed in the present study. However, several observational studies have reported a protective effect of leptin on LV mass,²⁷⁹⁻²⁸² including recent proteomics analyses in the Framingham Heart Study²⁶⁰ and the Multi-Ethnic Study of Atherosclerosis (reported in Chapter 1). This motivated its evaluation among a subset of participants in the current study, which suggested lower plasma leptin level was associated with both HIV infection and measures of HIV disease severity. Among ART-treated PLWH, altered levels of circulating adipokines, including leptin, have been associated with changes in body fat composition and other adverse cardiometabolic risk factors.³²¹⁻³²⁵ Many of these studies were motivated by HIV-associated lipodystrophy observed as a consequence of older generation antiretrovirals, which are no longer in widespread use. However, HIV infection in the current ART era remains associated with metabolic abnormalities and adipose tissue inflammation.^{326, 327} Moreover, leptin is currently hypothesized to play a role in cardiac remodeling independent of its more traditionally studied metabolic effects, specifically via prevention of free fatty acid deposition in the myocardium.²⁷⁸

Strengths and Limitations

There are limitations to this study. First, the use of EMR-derived LV phenotypes is suboptimal. Specifically, there is no quality control for measurement or data entry, and the algorithm employed to extract target parameters is imperfect. This may result in greater measurement error relative to more standardized data collection methods in imaging-focused studies. There is, however, some strength in pragmatism, assessing differences in clinically interpreted echocardiograms from which management decisions might be made. There are also concerns regarding external validity in this study. First, an inherent limitation of drawing inference from a veteran source population is low representation of women. Additionally, it is undoubtedly true that persons in care differ from those out of care in several aspects of health, particularly among PLWH where retention in care is a critical component of prognosis. Lastly, there are concerns regarding internal validity, as analysis sample inclusion is conditional on presence of a documented echocardiogram with target parameters within the VA health system

database, which cannot be assumed independent of HIV status or LV structural phenotype. Effort was made to diminish bias that may result from this by adjusting estimates for the most frequent factors leading to echocardiography referral in the VA healthcare system, although we were unable to ascertain true indications. This approach also would have theoretically resulted in estimation of a partial HIV effect on structural indices within the source population.

There are strengths to this study. This is the largest study evaluating the effect of HIV infection on LV structure conducted to date, representing a nationwide source population with high representation of persons of Black race from the largest single provider of HIV care in the United States.

Conclusion

In this large national sample of veterans in care, HIV infection and measures of HIV disease severity were associated with differences in cardiac structure. These findings support the need for continued study of cardiac remodeling among PLWH and future work examining the mechanisms through which this may contribute to the excess risk of heart failure among this population.

Table 9. Participant demographic and clinical characteristics overall and by HIV status.			
	<i>Median (25th, 75th Percentile) or % (n)</i>		
	<i>PLWH (n=5,767)</i>	<i>PWOH (n=13,286)</i>	<i>Overall (n=19,053)</i>
<i>Demographics</i>			
Age, years	57 (51, 64)	58 (52, 64)	58 (52, 64)
Sex at birth, male	98.0% (5651)	97.1% (12906)	97.4% (18557)
Race and ethnicity	--	--	--
White	37.8% (2179)	36.7% (4872)	37% (7051)
Black	48.8% (2813)	49.6% (6584)	49.3% (9397)
Hispanic	10.7% (615)	11.9% (1582)	11.5% (2197)
Other	2.8% (160)	1.9% (248)	2.1% (408)
<i>Behavioral Characteristics</i>			
Smoking status	--	--	--
Current	38.0% (2191)	33.2% (4416)	34.7% (6607)
Former	15.3% (880)	19.2% (2550)	18.0% (3430)
History of alcohol use disorder	29.2% (1684)	30.9% (4104)	30.4% (5788)
History of cocaine use disorder	23.3% (1341)	17.1% (2268)	18.9% (3609)
<i>Clinical Characteristics</i>			
Body mass index, kg/m ²	26.0 (22.8, 29.7)	30.0 (26.1, 34.4)	28.7 (24.8, 33.2)
Systolic blood pressure, mmHg	129 (119, 141)	132 (122, 143)	131 (121, 143)
Diastolic blood pressure, mmHg	77 (70, 84)	77 (71, 84)	77 (71, 84)
Any blood pressure-lowering therapy use	74.9% (4319)	85.1% (11306)	82.0% (15625)
HDL-cholesterol, mg/dL	40 (32, 50)	42 (35, 51)	41 (34, 51)
LDL-cholesterol, mg/dL	93 (72, 118)	95 (74, 122)	95 (74, 120)
Statin use	57.0% (3289)	71.2% (9462)	66.9% (12751)
Warfarin use	13.5% (776)	16.5% (2197)	15.6% (2973)
Diabetes	45.1% (2601)	58.1% (7718)	54.2% (10319)
eGFR (CKD-EPI), mL/min/1.73m ²	75 (57, 95)	77 (60, 94)	76 (60, 95)
FIB-4 score	1.61 (1.10, 2.45)	1.28 (0.91, 1.82)	1.37 (0.96, 2.01)
<i>Prior or Prevalent Conditions</i>			
Hepatitis C infection	16% (916)	8% (1100)	11% (2016)
Acute myocardial infarction	12% (714)	11% (1493)	12% (2207)
Unstable angina	9% (533)	12% (1613)	11% (2146)
Revascularization	12% (703)	13% (1748)	13% (2451)
Heart failure	25% (1448)	28% (3716)	27% (5164)
Atrial fibrillation	13% (724)	14% (1919)	14% (2643)
PLWH=persons living with HIV; PWOH=people living without HIV; HDL=high-density lipoprotein; LDL=low-density lipoprotein; eGFR=estimated glomerular filtration rate.			

Table 10. HIV-related characteristics among participants living with HIV (*n*=5,767).

	Median (25 th , 75 th Percentile) or % (n)
<i>HIV-Related Characteristics</i>	
CD4+ cell count, cells/ μ L	412 (229, 629)
% CD4+ cells	24.4 (15.0, 34.0)
CD8+ cell count, cells/ μ L	819 (528, 1194)
% CD8+ cells	49.9 (38.0, 61.0)
HIV viral load, copies/mL	75 (40, 806)
Antiretroviral use	69.7% (4017)
Antiretroviral regimen at baseline contains	--
Protease inhibitor	41.2% (2376)
Nucleoside reverse transcriptase inhibitor	60.3% (3477)
Non-nucleoside reverse transcriptase inhibitor	22.2% (1280)
Abacavir	7.8% (449)
Darunavir	8.3% (478)
Veterans Aging Cohort Study (VACS) index score	39 (23, 55)
VACS index is an extensively validated 5-year all-cause mortality risk score based on indications of HIV disease and organ system injury ³¹⁷	

Table 11. Cross-sectional effect of HIV infection on cardiac structure among veterans referred for echocardiography within the Veterans Affairs healthcare system ($n=19,053$).

<i>Left Ventricular Parameter</i>	<i>Model 1</i>		<i>Model 2</i>	
	<i>Effect Estimate (95% CI)</i>	<i>p-value</i>	<i>Effect Estimate (95% CI)</i>	<i>p-value</i>
<i>Mean Difference, PLWH vs. PWOH</i>				
LV mass index, g/m ²	0.24 (-0.71 to 1.19)	0.618	1.42 (0.49 to 2.34)	0.003
LV end-diastolic volume index, mL/m ²	0.95 (0.36 to 1.53)	0.002	1.28 (0.71 to 1.85)	<0.001
LV mass/volume ratio, g/mL	-0.023 (-0.042 to -0.004)	0.018	-0.010 (-0.030 to 0.009)	0.294
<i>Relative Risk, PLWH vs. PWOH</i>				
LV hypertrophy	1.02 (0.97 to 1.07)	0.43	1.07 (1.02, 1.13)	0.003
Concentric LV hypertrophy	0.98 (0.91 to 1.06)	0.66	1.03 (0.96 to 1.11)	0.37
Eccentric LV hypertrophy	1.07 (0.98 to 1.15)	0.12	1.13 (1.04 to 1.22)	0.003
<p>Modeled using linear and log-binomial regression for continuous and dichotomous parameters, respectively. Model 1 adjusts for sex at birth, continuous age, race/ethnicity, smoking status, history of alcohol use disorder, history of cocaine use disorder, and HCV infection. Model 2 further adjusts for primary factors leading to echocardiography referral within the Veterans Affairs healthcare system—hypertension and prior/prevalent cardiovascular disease (myocardial infarction, unstable angina, revascularization, atrial fibrillation, or heart failure). Hypertrophy defined as LV mass index >115 g/m² for males and >95 g/m² for females. Concentric hypertrophy defined as hypertrophy and LV mass-to-volume >2 g/mL. Eccentric hypertrophy defined as hypertrophy and LV mass-to-volume <2g/mL.</p> <p>CI=confidence interval; PLWH=persons living with HIV; PWOH=persons without HIV; LV=left ventricular.</p>				

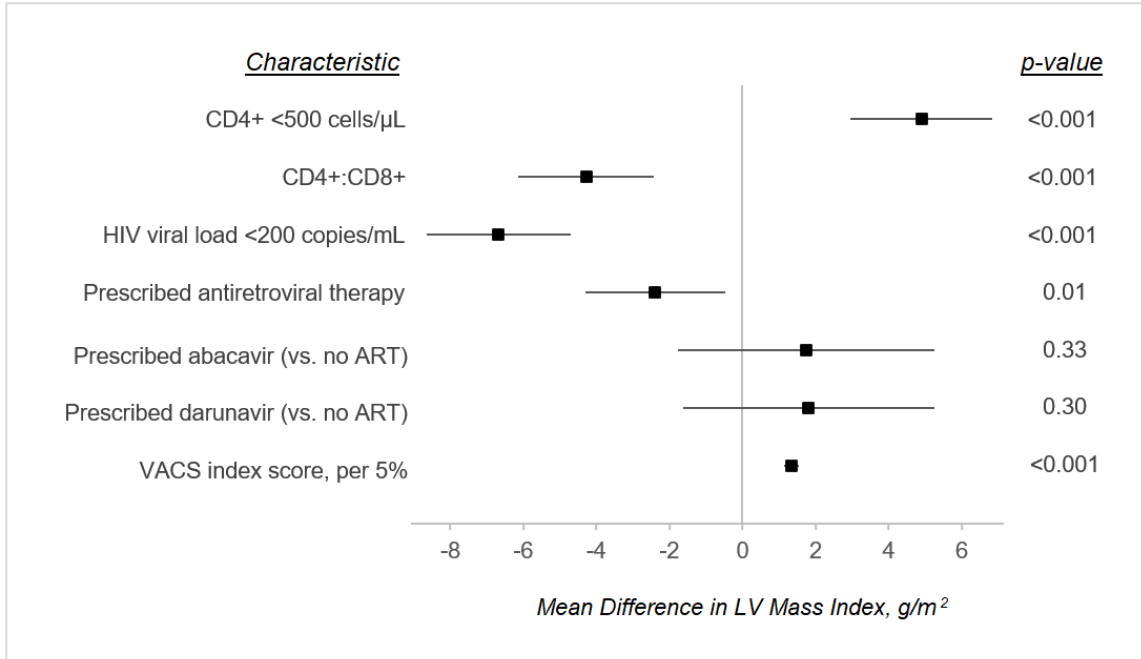


Figure 9. Unadjusted mean difference in left ventricular (LV) mass index by HIV characteristic among persons living with HIV receiving echocardiography within the Veterans Affairs Healthcare System ($n=5,767$). Estimate for CD4:CD8 per one-unit increment; estimate for VACS index score per 10% increment; all other estimates for yes vs. no.

Table 12. Cross-sectional effects of HIV infection and measures of HIV disease severity on plasma leptin and NT-proBNP levels.

Characteristic	Mean Standardized Difference in log ₂ -Leptin		Mean Standardized Difference in log ₂ -NT-proBNP	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
<i>Among All Biomarker Cohort Participants (n=2237)</i>				
HIV infection*	-0.79 (-0.90 to -0.68)	<0.001	-0.06 (-0.14 to 0.03)	0.202
<i>Among Biomarker Cohort Participants Living with HIV (n=1467)</i>				
CD4+ cell count, per 100 cells/uL	0.044 (0.027 to 0.060)	<0.001	-0.052 (-0.069 to -0.034)	<0.001
CD4+ cell count <500 cells/uL	-0.19 (-0.29 to -0.09)	<0.001	0.26 (0.16 to 0.37)	<0.001
% CD4+ lymphocytes	0.005 (0.001 to 0.009)	0.026	-0.005 (-0.010 to -0.001)	0.018
CD8+ cell count, per 100 cells/uL	0.022 (0.011 to 0.033)	<0.001	-0.018 (-0.030 to -0.001)	0.001
% CD8+ lymphocytes	-0.001 (-0.005 to 0.003)	0.676	0.004 (0.000 to 0.008)	0.073
HIV viral load <200 copies/mL	0.24 (0.15 to 0.34)	<0.001	-0.03 (-0.14 to 0.07)	0.518
Prescribed antiretroviral therapy	0.11 (-0.02 to 0.23)	0.096	-0.04 (-0.17 to 0.09)	0.591
Prescribed protease inhibitor	0.13 (0.04 to 0.23)	0.006	0.03 (-0.07 to 0.13)	0.610
Prescribed NRTI	0.10 (-0.03 to 0.22)	0.140	-0.01 (-0.14 to 0.13)	0.934
Prescribed NNRTI	-0.05 (-0.15 to 0.05)	0.318	-0.01 (-0.11 to 0.10)	0.921
Prescribed abacavir	0.12 (-0.06 to 0.30)	0.204	0.12 (-0.07 to 0.31)	0.214

Modeled using linear regression. *Estimate adjusts for sex at birth, continuous age, race/ethnicity, smoking status, history of alcohol use disorder, history of cocaine use disorder, and hepatitis C infection. Other estimates are unadjusted.

NT-proBNP=N-terminal pro-B-type natriuretic peptide; CI=confidence interval; NRTI= nucleoside reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor

Supplemental Figure and Methods

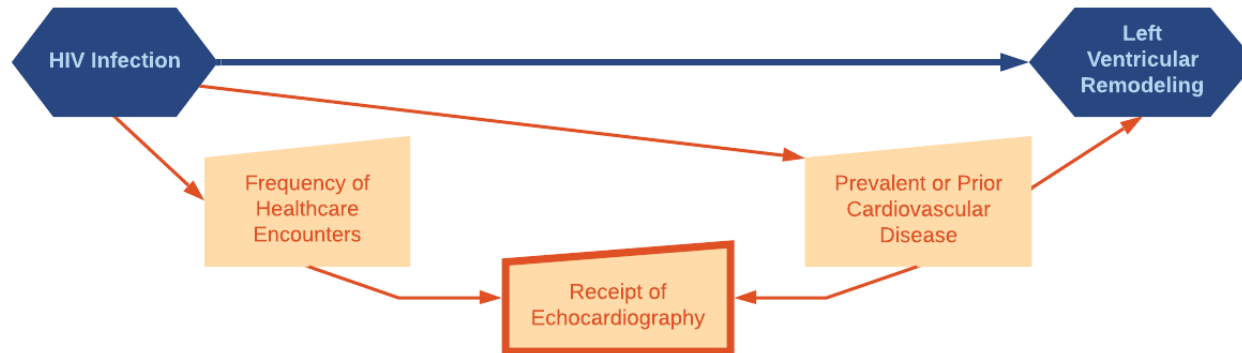


Figure S3. Directed acyclic graph depicting hypothesized source of selection bias in this research. Analysis sample inclusion is conditional on presence of a documented echocardiogram with target parameters within the VA health system database. Receipt of echocardiography, though common, is not routine and requires an indication, the top of which in this health system are ischemic heart disease, heart failure, atrial fibrillation, and hypertension. These health factors are also strongly associated with cardiac remodeling. Additionally, due to frequent healthcare encounters among persons living with HIV (PLWH) in care per HIV treatment guidelines and widespread recognition of their elevated risk of cardiovascular disease, the probability of referral for echocardiography is likely dependent on HIV status. Together, this can impose an artificial association between HIV infection and echocardiography-derived cardiac phenotypes in this sample. Further conditioning on top indications for echocardiography may diminish this bias, although it will also result in estimation of a partial target HIV effect.

Custom Natural Language Processing Algorithm

The outcomes in this study are LV mass index and LV mass-to-volume ratio, the data on which originated from the VA Healthcare System electronic medical records. One of the major challenges of utilizing EMR data in research is the inability to easily incorporate unstructured data. Cardiac phenotypic data are generally entered into the EMR as an unstructured or semi-structured text document that limits measurement retrievability. Natural language processing (NLP) is a general approach used to extract information from such unstructured data forms using algorithms trained to process and analyze natural human language data. A pipeline of NLP algorithms (described below) has been previously developed for extraction of cardiac phenotypes embedded in the VA Health System EMR and was used in this study.

The VA is a national integrated healthcare system that utilizes a comprehensive all-electronic medical record called Veterans Information Systems and Technology Architecture (VistA). The majority of care across the VA healthcare system is recorded through VistA, which currently contains billions of records on over 20 million patients, October 1999 to present.³²⁸

The method for LV parameter acquisition was predominantly 2-D echocardiography, due both to popularity in clinical practice and facets of the employed NLP pipeline. Within the VA healthcare system, echocardiography is performed by trained technicians, and several thoracic measurements are generally obtained, either manually by the technician or automatically by software. The values of these measures are then entered into the EMR in one or more of three formats:³⁰⁷

- A structured record in a VistA “Echocardiogram” file. These files contain coded data for a subset of measurements as well as clinician-entered narrative text, which may be either a brief supplement to the coded data or a full echocardiogram report.
- A semi-structured record in a VistA “Radiology/Nuclear Medicine” file. These files, which may be entered following any imaging procedure, contain structured meta-data in narrative text only. Procedure descriptions and impressions are stored in separate fields.

- A semi-structured text template or record in a VistA “Text Integration Utilities” (TIU) file. These files are effectively a text catch-all, which may include clinic notes or discharge summaries.

As outlined above, results of cardiac imaging are typically embedded within the VA health system EMR in the form of text reports. To extract values from such unstructured or semi-structured content, a natural language processing (NLP) pipeline was developed.³⁰⁷ The primary alternative to an algorithm-based approach to this challenge is manual chart review, which may be more flexible in handling highly variable text but is exceedingly inefficient.

The previously designed and validated NLP pipeline that was employed in this study was built using Leo, a program of libraries and services that aid in efficient development of NLP algorithms using the Apache Unstructured Information Management Architecture Asynchronous Scaleout (UIMA AS) framework. This pipeline (outlined in **Figure S2**) consists of three primary computational algorithms used for measurement-value pair extraction.³⁰⁷

- First is identification of a measurement concept in text using term identification and concept mapping. Identification utilized both a lexicon of terms associated with target concepts as well as data-driven term discovery to capture variations in features such as spelling and word order. Mapping was performed in one of two ways: either via exact match of identified term and mapped concept; or via splitting the identified term into “tokens” delimited by space, matching the tokens to concepts, combining the results into a score equivalent to the number of matched tokens per concept, and then selecting the concept with the highest score as the final mapping.
- Second is identification of a measurement value in text. Values may be quantitative or qualitative; however, this study utilized quantitative values only. Possible values for target concepts are relatively well-defined and were extracted using regular expressions.
- Third is relationship linking of concepts obtained in (i) and values obtained in (ii). Patterns of the form < Term > < separating string > < Value> < Unit> were identified and applied using the Leo annotation pattern annotator to link target concepts to their recorded value.

F. Summary

Heart failure is a complex clinical syndrome with substantial public health burden. Cardiac remodeling, defined as structural and/or functional alterations of the heart, is an early and pivotal feature within the pathophysiologic spectrum of HF. Improved detection of cardiac remodeling could aid in earlier HF intervention, more refined risk prediction, more precise characterization of clinical disease, and more effective mitigation of HF progression. This is true both in the general population and higher risk populations, such as PLWH. There is a large and growing burden of CVD among PLWH, and even in the context of effective, modern antiretroviral treatment, PLWH have a higher risk of HF relative to uninfected populations. Characterization of cardiac structural phenotypes among clinically relevant HIV patient populations is, however, both inconsistent and limited. The objective of this dissertation was to progress research on candidate biomarkers of cardiac remodeling and to better characterize the role HIV infection may play in that process.

In the first chapter, data from the population-based cohort, the Multi-Ethnic Study of Atherosclerosis, was used to report proteomic profiling of left ventricular structural phenotypes. Results emphasized the promise of more recently identified biomarker candidates for cardiac remodeling and HF—including leptin, cathepsins, phosphodiesterases, and regulators of bone morphogenetic proteins.

In the second chapter, external validation of previously identified cardiac remodeling biomarker candidates was performed using data from the Veterans Aging Cohort Study, which has a high representation of PLWH. This chapter highlighted challenges in validation, specifically how difficult inconsistency can be to interpret when the external validation study is of suboptimal design. Despite this as well as sample size limiting the precision of effect estimates, several biomarker candidates had effects consistent in direction and magnitude with those estimated in the first chapter of this dissertation.

The third chapter utilized data on over 19,000 veterans referred for echocardiography within Veterans Affairs healthcare system, the largest single provider of HIV care in the United States. In this study, HIV infection and measures of HIV disease severity were cross-sectionally associated with adverse cardiac structural phenotypes, specifically hypertrophy of eccentric geometry. Among a subsample of participants with proteomic profiling, we also observed associations between HIV serostatus and a candidate

biomarker identified in the first two dissertation chapters. This chapter highlighted the analytic challenges of electronic health record data, specifically when relying on data from procedures that require clinical referral. Lack of internal validity will be a major concern in most such research. It also underscored the need to interpret cardiac phenotype data in concert, as the clinical picture cannot be clearly defined based on any one parameter, even when focused on structural characteristics alone.

Though this dissertation was not poised to propose surrogates of cardiac remodeling or offer definitive evidence of the effect of HIV infection on cardiac structure, it leveraged data from both unique and classical epidemiologic sources to meaningfully progress research in these domains, both of which warrant continued investigation.

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