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Longitudinal Change in Galectin-3 and Incident Cardiovascular Outcomes

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ABSTRACT

BACKGROUND Galectin-3 (Gal-3) has been associated with heart failure (HF) and poor cardiovascular outcomes. However, the effect of longitudinal changes in Gal-3 on clinical outcomes remains unclear.

OBJECTIVES The authors sought to study clinical determinants of change in Gal-3 among community-dwelling individuals. Further, they sought to examine the role of serial Gal-3 measurements in predicting risk of future HF, cardiovascular disease (CVD), and mortality.

METHODS A total of 2,477 participants in the Framingham Heart Study Offspring cohort underwent measurement of plasma Gal-3 levels at 2 examinations (1995 to 1998 and 2005 to 2008). Linear regression models were used to examine clinical correlates of change in Gal-3. Proportional hazards models were used to relate future clinical outcomes with change in Gal-3.

RESULTS The following clinical correlates were associated with greater longitudinal increases in Gal-3 levels: age, female sex, hypertension, diabetes, body mass index, interim development of chronic kidney disease, and HF ($p < 0.0001$ for all in multivariable model). Change in Gal-3 was associated with future HF (hazard ratio [HR]: 1.39 per 1-SD increase; 95% confidence interval [CI]: 1.13 to 1.71), CVD (HR: 1.29; 95% CI: 1.11 to 1.51), and all-cause mortality (HR: 1.30; 95% CI: 1.17 to 1.46). Change in Gal-3 was associated with both HF with preserved as well as reduced ejection fraction ($p < 0.05$ for both).

CONCLUSIONS Longitudinal changes in Gal-3 are associated with traditional cardiovascular risk factors and renal disease. In turn, change in Gal-3 predicts future HF, CVD, and mortality in the community. Future studies are needed to determine whether serial Gal-3 measures may be useful in disease prevention. (J Am Coll Cardiol 2018;72:3246–54)

Galectin-3 (Gal-3), a chimera-type β-galactoside-binding lectin, is an emerging biomarker of heart failure (HF) (1,2). Expressed by activated macrophages and other cells during myocardial stress, Gal-3 plays an important regulatory role in inflammation and fibrosis (2). Gal-3 binding sites are mainly located in myocardial extracellular matrix and cardiac fibroblasts, where it

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induces fibroblast proliferation, collagen deposition, and cardiac remodeling (3). In animal models, overexpression of myocardial Gal-3 during early preclinical stages of heart failure has been well documented (3,4). Further, intrapericardial infusion of recombinant Gal-3 in healthy animals promotes cardiac fibrosis and induces heart failure (3,4), while genetic disruption or pharmacological inhibition of Gal-3 prevents heart failure from developing in mice and rats (5). In human studies, higher levels of Gal-3 are associated with all-cause and cardiovascular mortality in patients with heart failure as well as in general population (6–9). In addition, circulating Gal-3 predicts incident HF after acute coronary syndrome in the community (10–13).

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Despite relatively low biological variation (14), plasma Gal-3 levels do change over time (15), and Gal-3 trajectory might be independent of baseline levels (16). Systolic blood pressure and urinary excretion of albumin were shown to predict temporal changes in Gal-3 levels (17). Prior studies have shown that serial measurements of Gal-3 provide incremental prognostic information in patients with existing HF (8,18,19). Further, in a recent study, individuals with persistently elevated plasma Gal-3 concentrations had a significantly higher incidence of HF over an 8-year follow-up, with comparable signals for cardiovascular mortality, new-onset atrial fibrillation (AF), and cardiovascular events, suggesting a role for repeated measurements of circulating Gal-3 (16).

The exact triggers that modulate up-regulation of plasma Gal-3 levels remain unclear. In the present study, we aimed to investigate clinical predictors of longitudinal change in Gal-3 levels over the course of 10 years among participants of the FHS (Framingham Heart Study). We also sought to examine the effect of longitudinal changes in Gal-3 levels on clinical outcomes, including incident HF, cardiovascular disease (CVD), and all-cause mortality. We postulated that change in Gal-3 over time would be informative with respect to risk of new-onset HF, CVD, and all-cause mortality.

METHODS

STUDY SAMPLE. The design and enrollment of the FHS Offspring cohort has been described (20). In brief, in 1971, 5,124 individuals, consisting of children and spouses of the children of the FHS original cohort, were enrolled into a prospective, observational, community-based cohort called FHS Offspring. Participants who attended 2 separate examinations and had Gal-3 assayed at both timepoints were included in this analysis (earlier examination: 1995 to 1998, later examination: 2005 to 2008). Of 3,532 attendees at the earlier examination, those with off-site examinations (n = 120), missing Gal-3 (n = 55), outlier Gal-3 levels (n = 8), missing covariates (n = 198), and nonattendance at the later examination (n = 674: 380 deceased, 294 alive but did not attend later examination or did not have Gal-3 measured) were excluded, resulting in a final cohort of 2,477 individuals. The Institutional Review Board at Boston University Medical Center approved the study protocols, and all participants provided written informed consent.

GAL-3 MEASUREMENT. Plasma samples were collected after an overnight fast at each examination cycle, immediately centrifuged, and stored at −80°C. Gal-3 concentrations were measured using an enzyme-linked immunosorbent assay (BG Medicine, Waltham, Massachusetts). The stability of Gal-3 measurement using enzyme-linked immunosorbent assay has been described previously (21–23). The assay showed consistent performance across lots. The respective lower and upper detection limits were 1.32 and 96.6 ng/ml for both examinations. Respective within-run and total coefficients of variation were 2.1% to 5.7% and 4.2% to 12.0% for the earlier examination measurements, and 1.9% to 5.6% and 3.9% to 11.3% for the later examination measurements.

CLINICAL COVARIATES. A thorough clinical assessment was performed at each examination cycle, which included medical history and physical examination. Blood pressures were expressed as the average of 2 separate physician-obtained measurements. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or current use of antihypertensive medication(s). Body mass index (BMI) was calculated using weight and height at each examination cycle (kg/m^2). Diabetes mellitus was defined as fasting glucose ≥126 mg/dl, random nonfasting blood glucose ≥200 mg/dl, use of oral hypoglycemic medication(s) or insulin, or prior history of diabetes. Participants smoking cigarettes regularly during the prior year for each examination were considered current smokers. Total and high-density lipoprotein (HDL) cholesterol levels were measured. Glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (24). Chronic kidney disease (CKD) was defined as eGFR ≤30 ml/min/1.73 m^2. Left ventricular

ABBREVIATIONS AND ACRONYMS

BMI = body mass index

BNP = B-type natriuretic peptide

CKD = chronic kidney disease

CVD = cardiovascular disease

eGFR = estimated glomerular filtration rate

Gal-3 = galectin-3

HDL = high-density lipoprotein

HF = heart failure

HFpEF = heart failure with preserved ejection fraction

HFrEF = heart failure with reduced ejection fraction

LVH = left ventricular hypertrophy

TABLE 1 Characteristics of Participants at Earlier Examination by Quartiles of Longitudinal Change in Galectin-3

	Quartile 1 (n = 619)	Quartile 2 (n = 619)	Quartile 3 (n = 620)	Quartile 4 (n = 619)	Total (n = 2,477)	p Value*
Age, yrs	55 ± 8	56 ± 8	57 ± 9	59 ± 9	57 ± 9	<0.0001
Female	330 (53)	341 (55)	351 (57)	338 (55)	1,360 (55)	0.71
Systolic blood pressure, mm Hg	122 ± 16	125 ± 17	128 ± 17	131 ± 20	126 ± 18	<0.0001
Antihypertensive treatment	108 (17)	117 (19)	168 (27)	197 (32)	590 (24)	<0.0001
Diabetes mellitus	24 (4)	27 (4)	46 (7)	81 (13)	178 (7)	<0.0001
Body mass index, kg/m^2	27 ± 5	27 ± 5	28 ± 5	29 ± 6	28 ± 5	<0.0001
Cholesterol, mg/dl	208 ± 38	206 ± 37	205 ± 38	205 ± 38	206 ± 38	0.50
HDL, mg/dl	52 ± 17	52 ± 16	53 ± 16	51 ± 16	52 ± 16	0.13
Smoking	79 (13)	100 (16)	79 (13)	95 (15)	353 (14)	0.19
Left ventricular hypertrophy	1 (0.2)	1 (0.2)	2 (0.3)	3 (0.5)	7 (0.3)	0.77
eGFR, ml/min/1.73 m^2	97 ± 146	92 ± 49	91 ± 39	89 ± 38	92 ± 82	0.32
Prevalent CKD	24 (4)	29 (5)	48 (8)	46 (7)	147 (6)	0.006
Prevalent CVD	14 (2)	19 (3)	19 (3)	36 (6)	88 (4)	0.004
Prevalent HF	2 (0.3)	1 (0.2)	0 (0.0)	3 (0.5)	6 (0.2)	0.34
Prevalent AF	10 (2)	7 (1)	8 (1)	15 (2)	40 (2)	0.28

Values are mean ± SD or n (%). Median (25th, 75th percentiles) of change in Gal-3 levels for each quartile were: Quartile 1: −3.4 ng/ml (−4.4, −2.7 ng/ml), Quartile 2: −1.5 ng/ml (−2.0, −1.1 ng/ml), Quartile 3: 0 ng/ml (−0.4, 0.4 ng/ml), Quartile 4: 2.4 ng/ml (1.5, 4 ng/ml), Total: −0.8 ng/ml (−2.3, 0.9 ng/ml). *p values derive from statistical tests of equal means (or proportions) across quartiles using analysis of variance for continuous variables and chi-square test for categorical characteristics.

AF = atrial fibrillation; CKD = chronic kidney disease; CVD = cardiovascular disease; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; HF = heart failure.

hypertrophy (LVH) was considered present if there was electrocardiographic evidence of LVH with strain. AF was diagnosed if there was evidence of atrial flutter or AF after reviewing all available electrocardiograms or Holter monitor readings. B-type natriuretic peptide (BNP) levels were measured using high-sensitivity, noncompetitive immunoradiometric assays (Shionogi) (25).

ASCERTAINMENT OF CLINICAL ENDPOINTS. Participants of the FHS cohort undergo close surveillance for development of CVD and HF. Data obtained from medical records, physician visits, and hospitalization are reviewed and new events are confirmed by a panel consisting of 3 FHS physicians, and are documented and updated in the surveillance datasets. FHS criteria were used to define heart failure (26). CVD was defined as myocardial infarction (MI), coronary insufficiency (prolonged angina with documented ECG changes), coronary heart disease death, HF, or stroke. Interim events were considered new events that happened between the earlier and later examinations (i.e., between 1995 and 1998 and 2005 and 2008). Incident events were defined as new events that happened after the later examination (i.e., from 2005 to 2008 onward).

STATISTICAL ANALYSIS. Gal-3 concentrations were natural log-transformed due to skewed distribution. Change in Gal-3 between the earlier and later examinations was calculated as log(Gal-3) at the later examination minus log(Gal-3) at the earlier examination. Baseline characteristics at the earlier examination were summarized across quartiles of the change in Gal-3.

To identify clinical correlates of longitudinal change in Gal-3 (dependent variable), we used linear regression models. We first assessed individual correlates in linear regression models adjusting for age, sex, and baseline levels of Gal-3 at the earlier examination. We then used stepwise selection including all the correlates, forcing in age, sex, and Gal-3 at the earlier examination. We included these clinical correlates ascertained at the earlier examination: systolic blood pressure, current use of antihypertensive medications, diabetes mellitus, BMI, HDL to total cholesterol ratio, smoking, LVH, eGFR, prevalent CKD, CVD, and HF. We ran stepwise selection with and without interim development of CKD, CVD, and HF between the earlier and later exams.

To examine the effect of longitudinal change in Gal-3 levels on clinical outcomes, we used proportional hazard (Cox) regression models. In the primary analysis, we examined whether incident HF occurring after the later exam was associated with change in Gal-3 levels. In secondary analyses, we examined associations with CVD and mortality. We also modeled change in Gal-3 by quartiles; furthermore, we dichotomized Gal-3 levels (at the clinical cutoff of 17.8 ng/ml [8]) to study whether outcomes were associated with persistently low (low-low), persistently high (high-high), progression from low to high

levels (low-high), or progression from high to low levels (high-low). We used 2 models: 1) adjusting for age, sex, and baseline levels of Gal-3; and 2) adjusting for age, sex, baseline levels of Gal-3, systolic blood pressure, current use of antihypertensive medications, diabetes mellitus, BMI, HDL to total cholesterol ratio, smoking, LVH, eGFR, prevalent CVD (except for the CVD models), and prevalent HF. Individuals with prevalent CVD or HF before the later examination were excluded from CVD and HF models, respectively. In secondary analyses, we further adjusted for BNP in multivariable models.

Kaplan-Meier curves were used to depict the relation of change in Gal-3 by quartiles and by classes of change on clinical outcomes. In exploratory analyses, we examined change in Gal-3 levels vis a vis HF subtypes—including heart failure with preserved ejection fraction (HFpEF) (defined as left ventricular ejection fraction ≥50%) and heart failure with reduced ejection fraction (HFrEF) (left ventricular ejection fraction <50%). A 2-sided p value <0.05 was deemed significant. All analyses were performed using SAS version 9.4 for Windows (SAS Institute Inc., Cary, North Carolina).

RESULTS

BASELINE CHARACTERISTICS. A total of 2,477 participants (mean age of 57 ± 9 years, 55% women) were included in our study. Gal-3 levels changed over time in the majority of participants, with >30% showing a >20% change from baseline. When examined using the dichotomous clinical cutoff of 17.8 ng/ml, we found that 6% of the participants changed from low to high and 6% from high to low Gal-3 levels. By contrast, 82% remained persistently low and 5% persistently high. Baseline characteristics of participants by quartiles of change in Gal-3 are summarized in Table 1. Participants with greater increase in Gal-3 were older at baseline, had higher systolic blood pressure, were more likely to be diabetic, and had greater BMI (p value <0.05 for all). In addition, participants with prevalent CVD or CKD had a higher increase in Gal-3 levels at the following examination (p = 0.004 and p = 0.006, respectively).

CLINICAL CORRELATES OF LONGITUDINAL CHANGE IN GAL-3. In linear regression models adjusted for age, sex, and baseline Gal-3 levels, we found that older age, female sex, and lower baseline levels of Gal-3 were significantly associated with greater increases in Gal-3 over a 10-year period (Table 2). Traditional cardiovascular risk factors were similarly associated with greater increases in Gal-3 over time, including systolic blood pressure, antihypertensive use, diabetes, BMI, and smoking. Baseline CVD and CKD were significantly associated with greater increase in Gal-3, whereas prevalent HF was not. In a stepwise selection model, the following clinical covariates remained independent predictors of change in Gal-3: age, female sex, systolic blood pressure, antihypertensive medication use, diabetes, BMI, HDL to cholesterol ratio, eGFR, prevalent CKD, and prevalent CVD (Table 2) (model R-square = 0.16).

TABLE 2 Clinical Correlates of Longitudinal Change in Galectin-3 (n = 2,477)

	Age, Sex, Baseline Gal-3–Adjusted Model			Multivariable-Adjusted Stepwise Model†		
	β*	SE	p Value	β	SE	p Value
Age	0.0065	0.0005	<0.0001	0.0034	0.0005	<0.0001
Female	0.0239	0.0084	0.0046	0.0423	0.0082	<0.0001
Systolic blood pressure	0.0357	0.0045	<0.0001	0.0212	0.0045	<0.0001
Antihypertensive treatment	0.0815	0.0100	<0.0001	0.0384	0.0103	<0.0001
Diabetes	0.1226	0.0160	<0.0001	0.0679	0.0162	<0.0001
Body mass index	0.0335	0.0042	<0.0001	0.0209	0.0042	<0.0001
HDL-to-cholesterol ratio	0.0057	0.0029	<0.0001			
Smoking	0.0341	0.0119	0.004			
Left ventricular hypertrophy	0.0095	0.0777	0.90			
eGFR	−0.0039	0.0041	0.35			
Prevalent CKD	0.0438	0.0179	0.01			
Prevalent CVD	0.0767	0.0225	0.0007			
Prevalent HF	−0.0303	0.0838	0.72			
Interim development of CKD	0.1945	0.0152	<0.0001	0.1692	0.0149	<0.0001
Interim development of CVD	0.1279	0.0201	<0.0001			
Interim development of HF	0.2457	0.0329	<0.0001	0.1634	0.0338	<0.0001

*Estimated β coefficient represents the change in log-Gal3 between earlier and later examinations in the presence of the trait at earlier examination (dichotomous variables) or per 1 SD of the trait at earlier examination (continuous variables). †Step-wise selection forced age, sex, and baseline Gal-3 in the model and selected from all remaining variables listed in Table 2.

Gal-3 = galectin-3; other abbreviations as in Table 1.

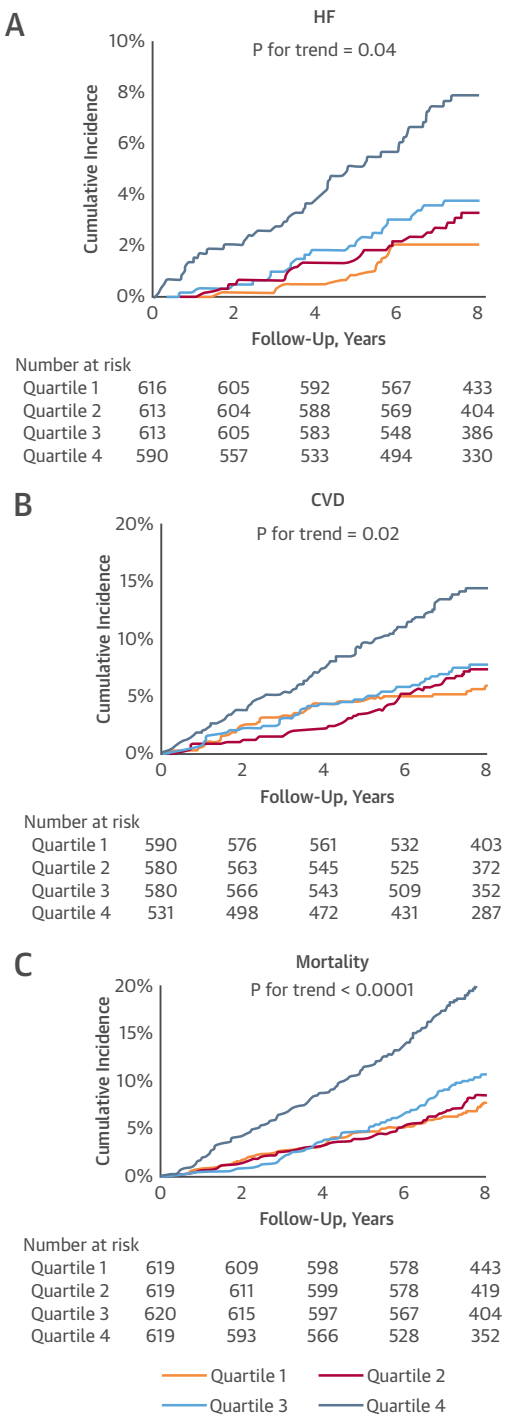
TABLE 3 Association Between Incident Outcomes, Baseline Galectin-3, and Longitudinal Change in Galectin-3

	Baseline Gal-3		Change in Gal-3	
Outcome/Model	HR (95% CI)†	p Value	HR (95% CI)†	p Value
Incident heart failure				
Age, sex, baseline Gal-3–adjusted	1.36 (1.10–1.68)	0.0037	1.60 (1.34–1.93)	<0.0001
Multivariable-adjusted*	1.26 (1.00–1.59)	0.048	1.39 (1.13–1.71)	0.0021
Incident cardiovascular disease				
Age, sex, baseline Gal-3–adjusted	1.29 (1.12–1.50)	0.0006	1.40 (1.29–1.61)	<0.0001
Multivariable-adjusted*	1.20 (1.02–1.41)	0.0257	1.29 (1.11–1.51)	0.001
Mortality				
Age, sex, baseline Gal-3–adjusted	1.38 (1.23–1.55)	<0.0001	1.38 (1.25–1.52)	<0.0001
Multivariable-adjusted*	1.42 (1.25–1.60)	<0.0001	1.30 (1.17–1.46)	<0.0001

*Multivariable models adjusted for baseline Gal-3 levels, age, sex, systolic blood pressure, antihypertensive treatment, diabetes, body mass index, smoking, left ventricular hypertrophy, HDL-to-cholesterol ratio, eGFR, prevalent CVD (except for CVD model). Mortality analysis was also adjusted for prevalent HF. †Hazard ratio and 95% confidence intervals for 1-SD increase in log-Gal3 levels over a 10-year period. This corresponds to 3.3 ng/ml change in Gal-3.

CI = confidence interval; HR = hazard ratio; other abbreviations as in Tables 1 and 2.

FIGURE 1 Cumulative Incidence of HF, CVD, and Mortality by Quartiles of Longitudinal Change in Galectin-3



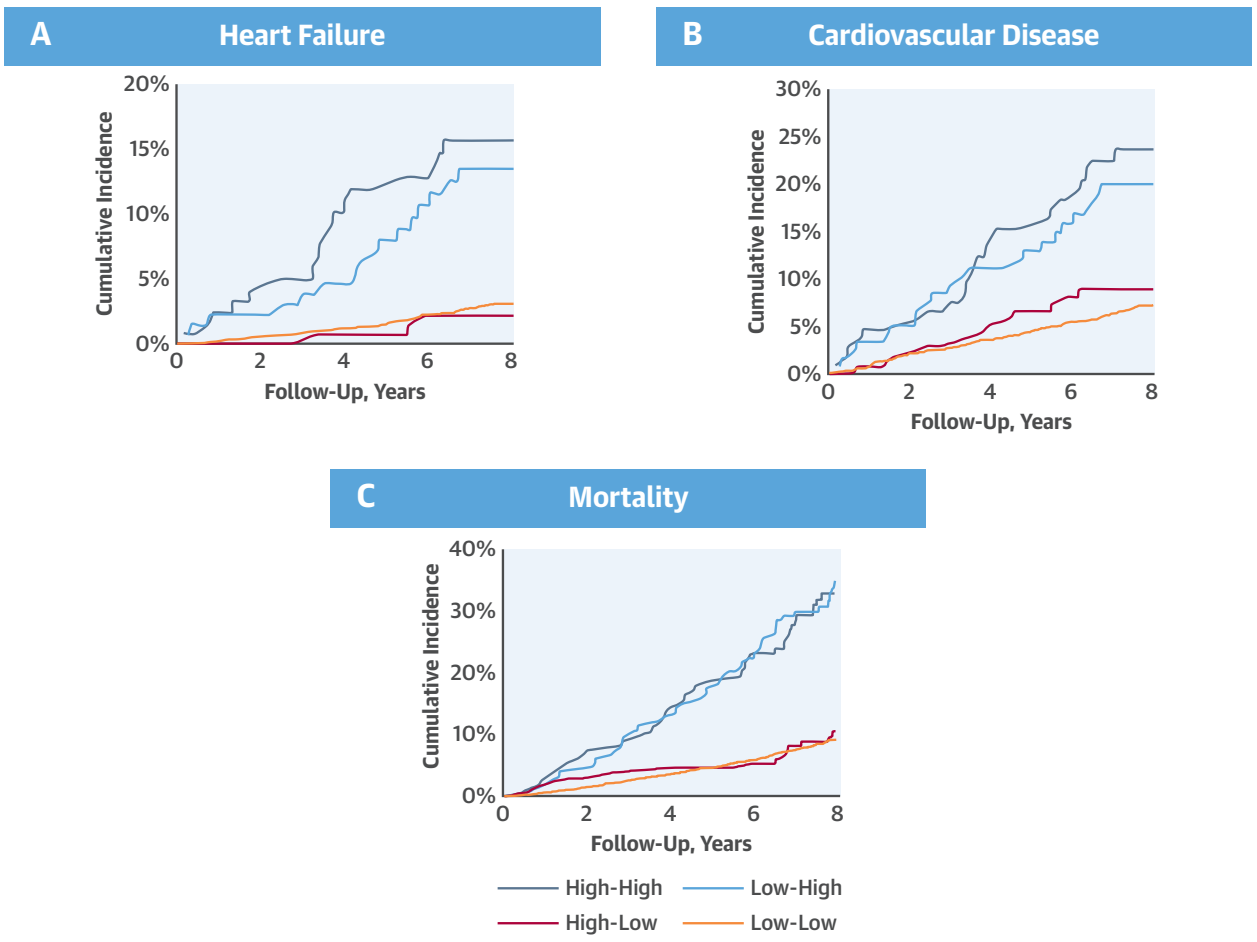
Longitudinal rise in galectin-3 levels was associated with significantly higher risk of developing incident HF **(A)**, incident CVD **(B)**, and all-cause mortality **(C)**. CVD = cardiovascular disease; HF = heart failure.

We examined interim events between the earlier and the later examinations, including 39 individuals who developed HF, 108 individuals who developed CVD, and 198 who developed CKD. We found that the interim development of CHF and CKD was significantly associated with 17% and 19% increases in Gal-3 levels, respectively ($\beta \pm SE$ 0.17 ± 0.01; $p < 0.0001$ for CKD, and $\beta \pm SE$ 0.16 ± 0.03; $p < 0.0001$ for HF). Interim development of CVD did not predict change in Gal-3 ($p > 0.05$).

CHANGE IN GAL-3 OVER TIME IS ASSOCIATED WITH INCIDENT CVD. Heart failure. Of 2,432 individuals free of HF at the later examination, 108 developed incident HF over a mean follow-up of 7.8 years. Whereas baseline Gal-3 was significantly associated with incident HF, longitudinal change in Gal-3 was significantly predictive of HF regardless of baseline Gal-3 levels ($p < 0.05$) (**Table 3**, Online Table 1). In age, sex, and baseline Gal-3-adjusted models, a 1-SD longitudinal increase in log-Gal-3 was associated with a 60% increase in risk of HF (hazard ratio [HR]: 1.60; 95% confidence interval [CI]: 1.34 to 1.93; $p < 0.0001$) (**Table 3**). This association remained significant after adjusting for other risk factors of HF ($p = 0.0021$). Further, longitudinal change in log-Gal-3 remained predictive of HF even after adjustment for baseline BNP levels (Online Table 2). **Figure 1A** depicts the cumulative incidence of HF across quartiles of change in Gal-3. A greater longitudinal increase in Gal-3 was associated with greater incidence of HF (p for trend = 0.04 across quartiles) (Online Table 3). Individuals with persistently high levels of Gal-3 had a 2.3 times higher risk of developing HF compared with those with persistently low levels (HR: 2.28; 95% CI: 1.28 to 4.05; $p = 0.005$) (**Central Illustration panel A**, Online Table 4, Online Figure 1C). Further, those with progression from low levels of Gal-3 at the earlier examination to high levels at the later examination (low-high) showed 82% increased risk of developing HF (HR: 1.82; 95% CI: 1.03 to 3.22; $p = 0.04$). By contrast, those who went from high to low Gal-3 levels on subsequent examination had an HR of 0.47 (95% CI: 0.15 to 1.51; $p = 0.21$).

HF SUBTYPES. A total of 63 individuals developed HFpEF, and 38 developed HFrEF, with 7 unclassified HF cases. Longitudinal change in Gal-3 predicted both HFpEF and HFrEF (**Table 4**). Among patients with HFpEF, 6.3% had previous MI and 15.9% had major CVD prior to the incident HF event. By contrast, among patients with HFrEF, 10.5% had previous MI and 18.4% had antecedent major CVD. In age, sex, and baseline Gal-3-adjusted models, a

CENTRAL ILLUSTRATION **Cumulative Incidence of Heart Failure, Cardiovascular Disease, and Mortality by Classes of Longitudinal Change in Galectin-3**



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Persistently high levels of galectin-3 were associated with a significantly higher risk of incident heart failure **(A)**, incident cardiovascular disease **(B)**, and all-cause mortality **(C)**. High-high, low-high, high-low, and low-low represent classes of change in galectin-3 between the earlier and later examinations using the clinical cut-off of 17.8 ng/ml.

1-SD longitudinal increase in Gal-3 was associated with a 1.59-fold increased hazard of HFpEF, and a 1.73-fold increased risk of HFrEF (HR: 1.59; 95% CI: 1.18 to 2.13; $p = 0.0022$; and HR: 1.73; 95% CI: 1.36 to 2.19; $p < 0.0001$ for HFpEF and HFrEF, respectively). The results remained significant in multivariable-adjusted models ($p < 0.05$).

CARDIOVASCULAR DISEASE. Of 2,281 individuals free of CVD at the later examination, 213 developed incident CVD over a mean follow-up of 7.7 years. A 1-SD longitudinal increase in log-Gal3 was associated with 40% ($p < 0.0001$) and 29% ($p = 0.001$) increase in risk of CVD in age, sex, and baseline Gal-3-adjusted, and multivariable-adjusted models respectively. Change in log-Gal3 predicted CVD even after further adjustment for baseline BNP levels (Online Table 2). Risk of incident CVD significantly increased across quartiles of change in Gal-3 (**Figure 1B**) (p for trend = 0.02). Individuals in the fourth quartile (mean Gal-3 increase of 2.4 ng/ml) showed a 74% higher risk of developing CVD (HR: 1.74; 95% CI: 1.12 to 2.71; $p = 0.01$). Participants with persistently high levels of Gal-3 (high-high) had an 80% higher risk of developing CVD compared with those with persistently low levels (HR: 1.80; 95% CI: 1.13 to 2.86; $p = 0.013$) (**Central Illustration panel B**). Although not significant, progression of Gal-3 levels from low to

TABLE 4 Association Between Incident HF Subtype and Longitudinal Change in Galectin-3

Model	HFpEF (63 events)		HFrEF (38 events)	
	HR (95% CI)†	p Value	HR (95% CI)†	p Value
Age, sex, baseline Gal-3–adjusted	1.59 (1.18–2.13)	0.002	1.73 (1.36–2.19)	<0.0001
Multivariable-adjusted*	1.39 (1.05–1.84)	0.02	1.42 (1.02–1.96)	0.04

*Multivariable models adjusted for baseline Gal-3 levels, age, sex, systolic blood pressure, antihypertensive treatment, diabetes, body mass index, smoking, left ventricular hypertrophy, HDL-to-cholesterol ratio, eGFR, and prevalent CVD (except for CVD model). Mortality analysis was also adjusted for prevalent HF. †Hazard ratio and 95% confidence intervals for 1-SD increase in log-Gal3 levels over a 10-year period. This corresponds to 3.3 ng/ml change in Gal-3.

Abbreviations as in Tables 1 to 3.

high levels was associated with a 43% increase in CVD risk (HR: 1.43; 95% CI: 0.89 to 2.28). The opposite was seen for individuals with high Gal-3 levels at the earlier examination and low Gal-3 levels at the later examination (HR: 0.86; 95% CI: 0.47 to 1.57).

ALL-CAUSE MORTALITY. During a mean follow up of 7.9 years, 348 deaths were reported. Longitudinal change in Gal-3 was associated with higher mortality in both age/sex/baseline Gal-3-adjusted and multivariable-adjusted models (HR: 1.38; 95% CI: 1.25 to 1.52, and HR: 1.30; 95% CI: 1.17 to 1.46, respectively, both p values <0.0001). These results remained similar after further adjustment in baseline BNP levels (Online Table 2). The fourth quartile of the change in Gal-3 was associated with 94% increase in risk of death (p = 0.0002). **Figure 1C** demonstrates the Kaplan-Meier curves for mortality rate. Individuals with persistently high levels of Gal-3 (high-high) had a >2-fold increased rate of mortality compared with those with persistently low levels (HR: 2.25; 95% CI: 1.61 to 3.15; p < 0.0001) (**Central Illustration panel C**).

DISCUSSION

Our main study findings are 3-fold. First, while traditional cardiovascular risk factors, including older age, hypertension, diabetes, and BMI, are associated with rise in Gal-3 over time, the largest increases in Gal-3 occur with intervening events including the development of CKD and HF. Second, we demonstrate that longitudinal change in Gal-3 above and beyond a single baseline measure is independently associated with the development of future HF, CVD, and all-cause mortality (**Central Illustration**). Specifically, participants who had both persistently high Gal-3 levels, and those who had low baseline and high subsequent levels of Gal-3 had a >2-fold increased hazard of future HF compared with individuals with persistently low Gal-3 levels. Third, we demonstrate that change in Gal-3 predicts both HFpEF and HFrEF. These data highlight the potential role of serial Gal-3 measurements in informing cardiovascular risk.

Clinical correlates of change in Gal-3 in our study are in keeping with 2 prior studies examining temporal changes in Gal-3 levels. Among patients with existing HF, Anand et al. (19) found that lower baseline Gal-3 levels, lower eGFR, interim decrease in eGFR, female sex, and higher BMI were associated with greater increases in Gal-3 levels at 4 months. Similarly, in the PREVEND (Prevention of REnal and Vascular ENd-stage Disease) study, investigators showed that in the general population, systolic blood pressure >170 mm Hg and urinary albumin excretion >30 mg/24 h were independent predictors of dynamic increase in Gal-3 levels across a time span of 9 years (17). We validate hypertension as a predictor, but extend these findings by showing that diabetes and obesity are also associated with a rise in Gal-3 over the span of about a decade. Interestingly, we found that women in the community have greater increases in Gal-3 over time compared with men. This is consistent with findings among women and men with HF (19). Whether Gal-3 potentially underlies some of the sex differences observed in HF remains unclear.

Aside from traditional cardiovascular risk factors, we show that interim development of CKD and HF strongly influences change in Gal-3. Previous studies have demonstrated that baseline Gal-3 measures predict incident CKD in the general population independent of HF (27,28). Potential mechanisms could be aldosterone-induced vascular fibrosis mediated by Gal-3 as well as the role of Gal-3 in atherogenesis (29). In turn, we now show that the interim development of CKD further compounds future increases in Gal-3. Similarly, we found that interim development of HF was associated with subsequent increases in Gal-3. In our study, prevalent HF did not predict longitudinal change in Gal-3 given that there were only 6 cases of prevalent HF. Further, prevalent (but not interim) CVD predicted change in Gal-3.

With respect to future outcomes, a single Gal-3 measurement has been shown to predict prognosis both among patients with existing HF and also development of future HF among those in the community (8,12). We now show that serial Gal-3 measures are informative above and beyond a single baseline measurement. Specifically, a steeper rise in Gal-3 over 10 years was independently associated with greater risk of future HF as well as CVD even after accounting for baseline Gal-3. Interestingly, when using the clinical cut-off of 17.8 ng/ml, we found that participants with persistently high levels of Gal-3 on serial examinations (high-high)

and those with progression of Gal-3 from low to high levels (low-high) showed a significantly greater risk of developing future HF. By contrast, participants with initial high levels and subsequent decline to low Gal-3 levels (high-low) showed no increased risk of future HF. Similar findings were previously reported in PREVEND, although associations were attenuated after adjusting for potential clinical confounders in the previous study (16). Finally, we demonstrate that the association of longitudinal change in Gal-3 and future HF extends to both HFpEF and HFrEF. Taken together, these findings highlight the added value of repeated measurements of Gal-3 in identification of individuals at risk for development of HF and CVD in the community.

The mechanisms underlying the association of Gal-3 and HF may be multifold. First, traditional cardiovascular and metabolic risk factors may confound this association as they are both associated with Gal-3 trajectory and clinical outcomes (12,30). However, we show independent associations of change in Gal-3 and outcomes even after accounting for potential confounders. Increased Gal-3 expression has been shown to be an independent determinant of cardiac inflammation and fibrosis in experimental studies (2,31). Up-regulation of Gal-3 in animal models is present prior to development of overt HF and leads to cardiac fibroblast activation and extracellular matrix deposition, and hence cardiac remodeling (2). Our study findings suggest that Gal-3 may contribute to the development of future HF in humans. Whether targeting the Gal-3 pathway may have clinical benefits with respect to disease prevention remains unknown (32).

STUDY LIMITATIONS. The FHS participants are predominantly white, potentially limiting the generalizability of our findings to broader populations, particularly in light of known racial differences in the utility of Gal-3 and HF risk prediction (33). Gal-3 levels were ascertained at 2 timepoints nearly a decade apart, and participants with clinical events in the interim were excluded from survival analyses, potentially biasing our sample toward healthier participants.

CONCLUSIONS

Traditional cardiovascular risk factors, including older age, hypertension, diabetes, and BMI, are associated with rise in Gal-3 levels over time, with the largest changes in Gal-3 in the context of interim development of CKD and HF. Further, change in Gal-3 over time is associated with the development of future HF, CVD, and all-cause mortality, and adds additional information beyond a single baseline measurement. These data highlight the potential role of serial Gal-3 measurements in informing cardiovascular risk. Future studies are needed to elucidate whether Gal-3 may represent a potential therapeutic target in both HF development and disease progression.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Rising plasma levels of Gal-3 are associated with incident heart failure (both HFpEF and HFrEF), cardiovascular disease, and all-cause mortality in community-dwelling individuals.

TRANSLATIONAL OUTLOOK: Further studies are needed to determine the effect of Gal-3 inhibition on clinical outcomes in patients at risk of adverse cardiovascular events.

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APPENDIX For supplemental tables and a figure, please see the online version of this paper.