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ORIGINAL RESEARCH

Obesity, Galectin-3, and Incident Heart Failure: The ARIC Study

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BACKGROUND: Laboratory data suggest obesity is linked to myocardial inflammation and fibrosis, but clinical data are limited. We aimed to examine the association of obesity with galectin-3, a biomarker of cardiac inflammation and fibrosis, and the related implications for heart failure (HF) risk.

METHODS AND RESULTS: We evaluated 8687 participants (mean age 63 years; 21% Black) at ARIC (Atherosclerosis Risk in Communities) Visit 4 (1996–1998) who were free of heart disease. We used adjusted logistic regression to estimate the association of body mass index (BMI) categories with elevated galectin-3 (≥75th sex-specific percentile) overall and across demographic subgroups, with tests for interaction. We used Cox proportional hazards models to assess the combined associations of galectin-3 and BMI with incident HF (through December 31, 2019). Higher BMI was associated with higher odds of elevated galectin-3 (odds ratio [OR], 2.32; 95% CI, 1.88–2.86) for severe obesity ([BMI ≥35 kg/m^2] versus normal weight [BMI 18.5-<25 kg/m^2]). There were stronger associations of BMI with elevated galectin-3 among women versus men and White versus Black participants (both *P*-for-interaction <0.05). Elevated galectin-3 was similarly associated with incident HF among people with and without obesity (HR, 1.49; 95% CI, 1.18–1.88; and HR, 1.71; 95% CI, 1.38–2.11, respectively). People with severe obesity and elevated galectin-3 had >4-fold higher risk of HF (HR, 4.19; 95% CI, 2.98–5.88) than those with normal weight and galectin-3 <25th percentile.

CONCLUSIONS: Obesity is strongly associated with elevated galectin-3. Additionally, the combination of obesity and elevated galectin-3 is associated with marked HF risk, underscoring the importance of elucidating pathways linking obesity with cardiac inflammation and fibrosis.

Key Words: biomarkers ■ galectin-3 ■ heart failure ■ obesity

Obesity, defined in population studies as a body mass index (BMI) ≥30 kg/m^2, is highly prevalent, affecting more than one third of adults in the United States, and is associated with increased risk of heart failure (HF).[1,2] Although the association of obesity with coronary heart disease and stroke may be largely explained by the comorbid conditions of hypertension, dyslipidemia, and diabetes, the excess risk of HF associated with obesity is largely independent of these traditional cardiovascular risk factors.[3] Prior studies indicate direct adverse effects of adiposity on the myocardium, resulting in cardiac dysfunction and subsequent HF.[4] In preclinical studies, cardiac inflammation and myocardial lipotoxicity related to accumulation of free fatty acids, triglycerides, and toxic metabolites in cardiac myocytes result in cellular dysfunction and death, contributing to the development of cardiac hypertrophy, fibrosis, and dysfunction.[5] Even in the

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CLINICAL PERSPECTIVE

What Is New?

- In the present study we demonstrate strong graded associations of higher body mass index with elevated galectin-3, a biomarker of inflammation and fibrosis.

What Are the Clinical Implications?

- Higher body mass index and galectin-3 were each independently associated with heart failure risk, and the combination of severe obesity and elevated galectin-3 identified a subgroup with 4-fold higher risk of future heart failure who may benefit from aggressive preventive strategies.

absence of marked metabolic derangement, obesity is associated with hemodynamic alterations characterized by increased blood volume and cardiac output that contribute to adverse cardiac remodeling and is associated with increased risk of HF.[6]

Despite growing appreciation of direct adverse effects of adiposity on the myocardium that predispose to HF risk, there has been limited evaluation of these associations in population studies until recently, with the emergence of cardiac biomarkers.[7] Biomarkers reflecting subclinical myocardial abnormalities provide prognostic information regarding HF risk and may also contribute to our understanding of the pathogenesis of HF.[8] Galectin-3 is a soluble b-galactoside-binding lectin that has important regulatory roles in inflammation and fibrosis and is associated with risk of future HF.[9]

Preclinical studies have indicated that galectin-3 plays an active role in the development of myocardial remodeling, fibrosis, and ultimately in the development of HF.[10,11] Furthermore, genetic knockouts or pharmacologic inhibition of galectin-3 halts these processes, protecting against the development of HF, with possible implications for HF prevention.[11,12] Epidemiological studies demonstrate consistent associations of circulating levels of galectin-3 with cardiac remodeling,[13] incident HF,[14] and HF prognosis.[15,16] However, despite the well-recognized associations of galectin-3 with HF, its relationship with obesity is not well understood.

To further investigate the link between adiposity and myocardial dysfunction, we sought to evaluate the associations of obesity with inflammation and myocardial fibrosis, as reflected by elevated galectin-3 levels, among participants in the community-based ARIC (Atherosclerosis Risk in Communities) Study. We additionally assessed the implications of elevated galectin-3 among individuals with obesity for incident HF risk.

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Deidentified data from the ARIC study are available through the National Heart, Lung, and Blood Institute Biologic Specimen and Data Repository Information Coordinating Center. Additionally, researchers who are interested in accessing these data may contact the ARIC study Coordinating Center.

The ARIC Study is an ongoing community-based, prospective cohort of predominantly Black and White participants from 4 US communities: Washington County, MD; the northwestern suburbs of Minneapolis, MN; Jackson, MS; and Forsyth County, NC. Cohort enrollment started in 1987 to 1989, at which time 15 792 study participants aged 45 to 64 years were enrolled and followed prospectively. Participants were evaluated through serial study visits (Visit 1: 1987–1989; Visit 2: 1990–1992; Visit 3: 1993–1995; Visit 4: 1996–1998; Visit 5: 2011–2013; Visit 6: 2016–2017; and Visit 7: 2018–2019) involving comprehensive assessments of cardiovascular risk factors and have been followed continuously for cardiovascular disease events by annual or semiannual (since 2012) telephone interviews, active surveillance of ARIC community hospitals, and review of death certificates. Additional study design details have been previously published.[17] All study participants provided written informed consent and the study was approved by the institutional review board of each participating center.

For the present study, we considered 11 656 participants who attended ARIC Visit 4 (1996–1998), a time point where galectin-3 was measured cohort wide. We excluded 70 participants who were not of Black or White race because of limited numbers, as well as the small number of Black adults from the Minnesota and Washington County field centers; 1606 participants with baseline HF or coronary heart disease (prevalent coronary heart disease or HF at Visit 1, adjudicated cases of nonfatal myocardial infarction or coronary revascularization procedure, silent myocardial infarction by electrocardiographic criteria, or hospitalizations related to HF at or before visit 4); 940 participants without galectin-3 measurements, with missing information on BMI, or with BMI <18.5 due to the confounding association with underweight; and 353 participants with missing information on the covariates of interest. After exclusions, 8687 participants were included in the final analysis. The baseline characteristics of participants who were excluded from the study because of missing information on galectin-3 and those included in the study are presented in Table S1.

BMI was the main exposure in cross-sectional analyses and was calculated based on weight and height measured at Visit 4 and reported in kg/m^2. We categorized BMI as: normal weight (BMI 18.5 to <25), overweight (25 to <30), obesity (30 to <35), and severe

obesity (BMI ≥35). We conducted additional analyses categorizing BMI as obesity absent (BMI 18.5 to <30) and obesity present (BMI ≥30) and modeling BMI as a continuous variable scaled per 5 kg/m^2. In secondary analyses, waist circumference was used as an alternative measure of adiposity. Waist circumference was measured at the umbilical level by trained personnel and categorized into quartiles.

All covariates were assessed at baseline (Visit 4). Information on participants' demographics, smoking status, and alcohol intake was obtained through an interviewer administered questionnaire. Seated blood pressure was measured after a 5-minute rest by trained personnel and the average of 2 measurements recorded. Diabetes was defined based on self-reported prior physician diagnosis, use of hypoglycemic medication, a fasting glucose ≥126 mg/dL, or nonfasting glucose ≥200 mg/dL. Insulin use was self-reported. Prevalent atrial fibrillation at Visit 4 was determined by review of ECGs obtained during study visits and of medical records. Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured using enzymatic assays in plasma samples from Visit 4. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.[18] In sensitivity analyses, we used an estimation of eGFR based on serum creatinine and cystatin C.[19] Hs-CRP (high-sensitivity C-reactive protein), NT-proBNP (N-terminal pro brain natruretic peptide, and hs-cTnT (high-sensitivity cardiac troponin T) were measured from stored blood collected at Visit 4 using previously described methods.[20,21]

Galectin-3 was measured from EDTA-plasma samples collected at Visit 4 and stored at –70 °C, using a chemiluminescent microparticle immunoassay on an Architect i 2000sr instrument (Abbott, Abbott Park, IL). The limit of detection of the Architect assay has been previously reported as 1.1 ng/mL and the limit of quantitation as 4.0 ng/mL. Intraassay coefficients of variation were 5.2%, 3.3%, and 2.3% at mean galectin-3 levels of 8.8, 19.2, and 72.0 ng/mL, respectively.

Prior studies have shown that rs4644, a common single-nucleotide polymorphism in the gene encoding galectin-3 (LGALS3), lies within the epitope of the binding region for the antibody used to measure galectin-3 in ARIC and therefore can alter assay performance having significant associations with measurement of plasma galectin-3 concentrations.[22,23] Given the variable prevalence of this variant across different populations, all of our analyses were conducted with adjustment for rs4644, as in prior ARIC analyses.[24] In ARIC, exome genotyping for common variants was performed using the Human Exome BeadChip Array (Illumina, San Diego, CA).[25]

Previously reported differences in the distribution of galectin-3 by sex[14] were confirmed in our data set; therefore, all analyses were performed using sex-specific percentiles of galectin-3, as has been done in prior analyses. When galectin-3 was considered the outcome of interest in cross-sectional analyses, elevated galectin-3 was defined as ≥75th sex-specific percentile (≥15.3 and 17.6 ng/mL for men and women, respectively). When galectin-3 was an exposure of interest in prospective analyses, it was categorized per sex-specific quartiles.

Incident HF was the main outcome of prospective analyses, and was defined as hospitalization or death due to HF, from Visit 4 through 2018 (available only through 2017 from Jackson, MS field center). Incident HF was defined based on review of hospital discharge diagnoses and/or cause of death on death certificates. HF events included first hospitalizations with an *International Classification of Diseases, Ninth Revision* (*ICD-9*) code 428 (early follow-up) or *ICD, Tenth Revision (ICD-10)* code I-50 (later follow-up), in any position, or an underlying cause of death related to HF.

Statistical Analysis

Baseline (Visit 4) characteristics of the study population were examined across categories of BMI and also across quartiles of galectin-3. We used the chi-square test to compare categorical variables and ANOVA for continuous variables.

For all regression analyses, we constructed models with progressive adjustment. Our first adjustment model included demographic variables (age, sex, and a combined race-ARIC center variable [as done in prior work, owing to demographic differences across ARIC centers]), smoking status, alcohol drinking status, and rs4644 genotype. Our second regression model included Model 1 variables plus potential mediators of the association between obesity and HF including systolic blood pressure, use of antihypertensive medication, total cholesterol, high-density lipoprotein cholesterol, triglycerides, diabetes, and eGFR. All covariates were selected a priori based on review of the published literature.

In cross-sectional analyses, we used multivariable logistic regression to assess the association of BMI, modeled both categorically (normal weight, overweight, obesity, severe obesity) and continuously (per 5 units), with elevated galectin-3 (≥75th sex-specific percentile). We used restrictive cubic splines to allow for deviations from linearity in examining the continuous association of BMI with the odds of elevated galectin-3. We conducted secondary analyses examining the association of waist circumference quartiles, as another measure of adiposity, with elevated galectin-3. We performed these analyses in the overall study population and stratified a priori by age (<65 or ≥65 years), sex, and race and used the likelihood ratio test to assess for

interactions between waist circumference quartiles and these demographic variables.

We conducted additional analyses using Spearman's rank correlation coefficient to assess the correlation of galectin-3 with biomarkers of inflammation (hs-CRP), myocardial stress (NT-proBNP), and injury (hs-cTnT). We then examined the associations of obesity with elevated galectin-3 in a third adjustment model that included the variables in Model 2 as well as these additional biomarkers (Model 3). In sensitivity analyses, we evaluated the association of BMI categories with elevated galectin-3 considering eGFR based on both serum creatinine and cystatin C (Models 2a and 3a).

In prospective analyses, we used adjusted Cox proportional hazard models to estimate hazard ratios and 95% CIs for the associations of galectin-3 quartiles with incident HF in the overall study population (with additional adjustment for BMI) and within obesity categories (normal weight, overweight, obesity, and severe obesity). The proportional hazards assumption was confirmed via Schoenfeld residuals. Person-years of follow-up accrued from Visit 4 baseline until incident HF, death from other cause, loss to follow-up, or end of follow-up (December 31, 2018). We also tested for multiplicative interaction between categories of obesity and galectin-3 on the outcome of incident HF.

In addition to our 2 main regression models, we conducted sensitivity analyses using additional adjustment models to assess the robustness of our findings. For these analyses, we considered BMI as 2 categories (obesity absent and obesity present). Model 2a included the variables in Model 2 but used eGFR based on serum creatinine and cystatin C; Model 3 included Model 2 variables as well as the biomarkers of HF risk as described previously; Model 4 included the variables in Model 3 as well as prevalent atrial fibrillation and insulin use; and Model 5 included the variables in Model 4 plus time-varying coronary heart disease. Given the long follow-up time, we performed additional sensitivity analysis considering the competing risk of death. For competing risk regression, we used the Fine and Gray proportional subhazards method to account for noncardiovascular mortality as a competing risk to incident HF.[26]

Finally, to assess whether obesity and galectin-3 provided complementary prognostic information regarding HF risk, we created cross-categories of BMI and galectin-3 sex-specific quartiles to assess their combined associations with incident HF.

Analyses were conducted using STATA version 15.1 and SAS 9.4. All *P* values presented are 2 sided.

RESULTS

The median age was 63 years, 59% were women, and 21% were of Black race. Twenty-six percent of participants had normal weight, 40% overweight, 23% mild obesity, and 12% severe obesity. Compared with participants with normal weight, those with higher BMI were less likely to be current smokers but had an otherwise more adverse cardiovascular risk profile (Table 1). Individuals in higher galectin-3 quartiles were older, more likely of Black race, had a higher BMI, and had a less favorable cardiovascular risk profile (Table S2).

We observed higher median galectin-3 levels for those in higher BMI categories. Median galectin-3 was 13.9 ng/mL among participants with normal weight and 15.7 among those with severe obesity (Table 1). The proportion of participants with elevated galectin-3 (≥75th sex-specific percentile) rose from 21% in those with normal weight, to 23% in those with overweight, to 28% in those with mild obesity, to 37% in those with severe obesity (Figure 1).

In cross-sectional analyses using multivariable adjusted models, compared with people with normal weight, individuals in higher BMI categories were more likely to have elevated galectin-3 (Table 2). Using Model 1, the odds ratio (OR) for elevated galectin-3 for severe obesity versus normal weight was 2.60 (95% CI, 2.15–3.15). This was only slightly attenuated after adjustment for other cardiovascular risk factors (Model 2; OR, 2.32; 95% CI, 1.88–2.86 for severe obesity versus normal weight; Table 2). When assessing the continuous association of BMI with the odds of elevated galectin-3, we found a curvilinear direct association of higher BMI with higher odds of elevated galectin-3 (Figure 2). Each 5 kg/m^2 higher BMI was associated with 1.27 (95% CI, 1.20–1.35; Model 2) higher odds of elevated galectin-3.

In analyses stratified by age, race, and sex, we found significant associations between higher BMI and the odds of elevated galectin-3 across demographic subgroups. However, there were significant differences in the magnitude of associations across demographic subgroups (Table 3). The association of severe obesity with galectin-3 compared with normal weight was stronger among women (OR, 3.00; 95% CI, 2.34–3.85) than men (OR, 1.32; 95% CI, 0.90–1.92; *P* for interaction <0.01), and among White (OR, 2.57; 95% CI, 2.01–3.28) versus Black adults (OR, 1.50; 95% CI, 1.01–2.22; *P* for interaction=0.04). We did not find the association between BMI categories and elevated galectin-3 differed significantly by age group (*P* for interaction=0.08), although there was a tendency toward stronger associations between severe obesity and elevated galectin-3 for those <65 versus ≥65 years.

In secondary analyses, we observed similar associations between quartiles of waist circumference and elevated galectin-3 (Table S3). Compared with those in the lowest quartile of waist circumference, those in the highest quartile had 1.65 (95% CI, 1.38–1.98)

Table 1. Baseline (Visit 4) Characteristics of the Study Population Stratified by BMI Category

Variable*	Normal weight (BMI 18.5 to <25) n=2247	Overweight (BMI 25 to <30) n=3454	Obesity (BMI 30 to <35) n=1954	Severe obesity (BMI ≥35) n=1032	*P* value†
Age, y	62.9 (5.8)	62.8 (5.7)	62.4 (5.5)	61.5 (5.4)	<0.001
Female sex	1471 (65.5%)	1741 (50.4%)	1087 (55.6%)	779 (75.5%)	<0.001
Black race	301 (13.4%)	682 (19.7%)	507 (25.9%)	369 (35.8%)	<0.001
Current smoker	481 (21.4%)	474 (13.7%)	193 (9.9%)	92 (8.9%)	<0.001
Current alcohol use	1273 (56.7%)	1838 (53.2%)	912 (46.7%)	386 (37.4%)	<0.001
Systolic blood pressure, mm Hg	122.9 (19.4)	126.7 (18.2)	129.5 (18.0)	132.6 (17.9)	<0.001
Antihypertensive medication use	571 (25.4%)	1238 (35.8%)	945 (48.4%)	613 (59.4%)	<0.001
Diabetes	125 (5.6%)	427 (12.4%)	414 (21.2%)	315 (30.5%)	<0.001
Waist circumference, cm	87.1 (7.7)	99.3 (7.0)	110.1 (7.3)	125.4 (11.3)	<0.001
Total cholesterol, mg/dL	200.9 (35.3)	202.4 (35.8)	202.5 (38.3)	199.5 (34.4)	0.064
High-density lipoprotein cholesterol, mg/dL	58.1 (18.1)	49.4 (15.6)	46.7 (14.8)	46.7 (13.5)	<0.001
Triglycerides, mg/dL‡	103.0 (75.0–143.0)	123.0 (90.0–174.0)	134.0 (100.0–192.0)	133.5 (100.0–185.0)	<0.001
Estimated glomerular filtration rate	89.9 (77.9–96.0)	89.0 (76.7–95.6)	88.8 (76.3–96.4)	92.1 (78.5–100.6)	<0.001
Galectin-3, ng/mL	13.9 (11.9–16.2)	13.7 (11.6–16.3)	14.4 (12.1–16.8)	15.7 (13.0–18.5)	<0.001
Galectin-3 Q1	612 (27.2%)	892 (25.8%)	433 (22.2%)	166 (16.1%)	<0.001
rs4644—AA	345 (15.4%)	560 (16.2%)	276 (14.1%)	124 (12.0%)	0.002
rs4644—AC	1074 (47.8%)	1595 (46.2%)	921 (47.1%)	462 (44.8%)	
rs4644—CC	828 (36.8%)	1299 (37.6%)	757 (38.7%)	446 (43.2%)	
Prevalent atrial fibrillation	25 (1.1%)	53 (1.5%)	26 (1.3%)	20 (1.9%)	0.27
Insulin use	28 (1.2%)	62 (1.8%)	86 (4.4%)	83 (8.0%)	<0.001

BMI indicates body mass index.

*Continuous variables are presented as means (SD) unless indicated; categorial variables are presented as number (proportion).

†*P* values for continuous variables are based on ANOVA tests if normally distributed, or Kruskal-Wallis tests if not normally distributed. *P* values for categorical variables are based on chi-square tests.

‡Presented as median (interquartile range).

higher odds of elevated galectin-3. There were similar demographic differences in the associations of waist circumference with elevated galectin-3, with stronger associations among White participants versus Black participants (*P* for interaction=0.03) and trends toward stronger associations among women versus men (*P* for interaction=0.06; Table S3).

Galectin-3 had modest to poor correlations with hsCRP, NTproBNP, and hs-cTnT (Spearman's correlation 0.24, 0.16, and 0.05, respectively). The associations of higher BMI categories with elevated galectin-3 were attenuated but remained significant after including hsCRP, NTproBNP, and hs-cTnT in the adjustment model (OR, 1.72; 95% CI, 1.38–2.1.5 for severe obesity versus normal weight; Table 2, Model 3). After additional adjustment for these biomarkers, each 5 kg/m^2 increase in BMI was associated with 1.17 (1.10–1.24) higher odds of elevated galectin-3. Results were attenuated but remained significant for the association of severe obesity with elevated galectin-3 in Models 2a and 3a, using GFR estimation based on both serum creatinine and cystatin C (Table S4).

In prospective analyses, over a median follow-up time of 20.5 years (range 0.14–22.9 years), there were 1675 cases of incident HF (1610 [96%] HF hospitalizations and 65 [4%] deaths related to HF). We observed a graded association between higher quartiles of galectin-3 and incident HF in the overall study population, with galectin-3 ≥75th sex-specific percentile being associated with 68% (HR, 1.68; 95% CI, 1.41–2.00; Model 2) greater risk of HF compared with the lowest quartile. The association of the highest versus lowest galectin-3 quartile with HF remained significant after additional adjustment for BMI (HR, 1.51; 95% CI, 1.27–1.81; Model 2+BMI). Galectin-3 was similarly associated with incident HF across BMI categories, with no significant interaction between quartiles of galectin-3 and BMI categories on incident HF (Table 4). Results were attenuated but remained significant in analyses using eGFR based on serum creatinine and cystatin C (Model 2a, Table S5), and in analyses adjusted for

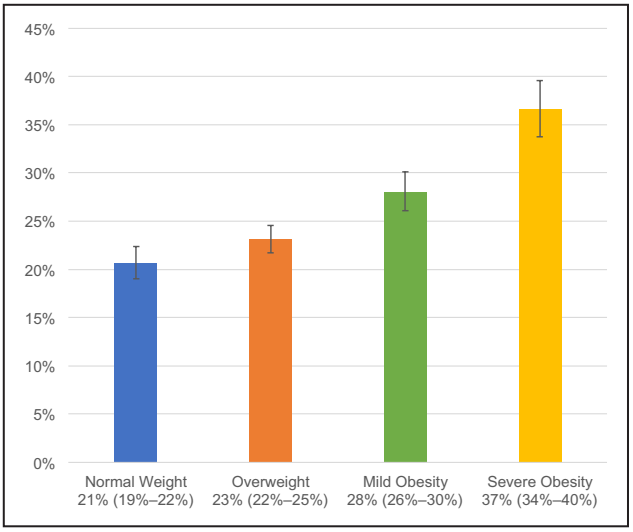

Figure 1. Proportion of participants with elevated galectin-3 (≥75th sex-specific percentile) across BMI categories.
Normal weight=BMI 18.5 to <25; overweight=BMI 25 to <30; mild obesity=BMI 30 to <35; severe obesity=BMI ≥35. BMI indicates body mass index.

biomarkers of HF risk (Model 3), for prevalent atrial fibrillation and insulin use (Model 4), and for time-varying coronary heart disease (Model 5). Results were minimally attenuated and remained significant when considering the competing risk of death (Table S6).

When considering the combined associations of BMI and galectin-3 with HF risk, we found that higher galectin-3 was associated with greater HF risk within each BMI category, and similarly higher BMI was associated with increased HF risk within each galectin-3 quartile (Figure 3 and Table S7). Importantly, we did not find a significant interaction between BMI categories and galectin-3 quartiles on the outcome of incident HF. Thus, higher BMI and galectin-3 provided independent prognostic information regarding HF risk. Compared with people with normal weight and galectin-3 in the lowest quartile, the combination of severe obesity and galectin-3 ≥75th sex-specific percentile was associated with a >4-fold higher risk of future HF (HR, 4.19; 95% CI, 2.98–5.88).

DISCUSSION

In this analysis from the ARIC cohort, we found that higher BMI was independently associated with higher odds of elevated galectin-3, a biomarker of inflammation and fibrosis. Importantly, although the association between BMI and galectin-3 was robust across demographic subgroups, the strength of the association varied significantly by sex and race. Our results were robust and remained significant after adjustments for additional biomarkers of HF risk. In prospective analyses, obesity and galectin-3 provided independent prognostic information about HF risk, with the combination of severe obesity and elevated galectin-3 linked to a >4-fold higher risk than the combination of normal weight and low galectin-3.

Prior population-based studies have shown that galectin-3 is independently associated with increased risk of future HF in healthy individuals and following acute myocardial infarction.[14,27] In addition to levels at a single time point, longitudinal changes in galectin-3 have also been linked to higher risk of HF and provide incremental prognostic information in people with existing HF.[28] Our study adds to this body of literature by demonstrating strong associations of obesity with galectin-3 and marked HF risk associated with galectin-3 elevation among people with excess weight. These findings suggest potent associations of obesity with cardiac inflammation and fibrosis and that the presence of both may have important prognostic implications for the development of HF.

Preclinical studies demonstrate that myocardial expression of galectin-3 is low in normal hearts but rapidly increases preceding the development of HF.[9] Galectin-3 is secreted by cardiac macrophages and promotes fibroblast proliferation and collagen deposition leading to adverse cardiac remodeling and fibrosis.[10] Galectin-3 may also amplify inflammation through macrophage activation and monocyte chemoattraction.[29] Animal studies suggest that obesity upregulates galectin-3 in the cardiovascular system, and that its pharmacological inhibition decreases cardiac and

Table 2. Odds Ratios and 95% CI for the Association of Categories of BMI With Elevated Galectin-3 (≥75th Sex-Specific Percentile) in the Overall Study Population at Visit 4

Adjustment model	Normal weight (BMI 18.5 to <25)	Overweight (BMI 25 to <30)	Obesity (BMI 30 to <35)	Severe obesity (BMI ≥35)
Model 1*	Reference (1)	1.21† (1.05–1.40)	1.64† (1.39–1.93)	2.60† (2.15–3.15)
Model 2‡	Reference (1)	1.08 (0.93–1.27)	1.34† (1.12–1.60)	2.32† (1.88–2.86)
Model 3§	Reference (1)	0.98 (0.84–1.16)	1.14 (0.95–1.38)	1.72† (1.38–2.15)

ARIC indicates Atherosclerosis Risk in Communities; and BMI, body mass index.
*Model 1: age, sex, race-ARIC center, smoking status, alcohol intake, and rs4644.
†$P<0.05$.
‡Model 2: Model 1 variables plus systolic blood pressure, use of anti-hypertensive medication, total cholesterol, high-density lipoprotein-cholesterol, triglycerides, diabetes, and estimated glomerular filtration rate.
§Model 3: Model 2 variables plus high-sensitivity C-reactive protein, N-terminal pro-B-type hormone brain natriuretic peptide, and high-sensitivity cardiac troponin T.

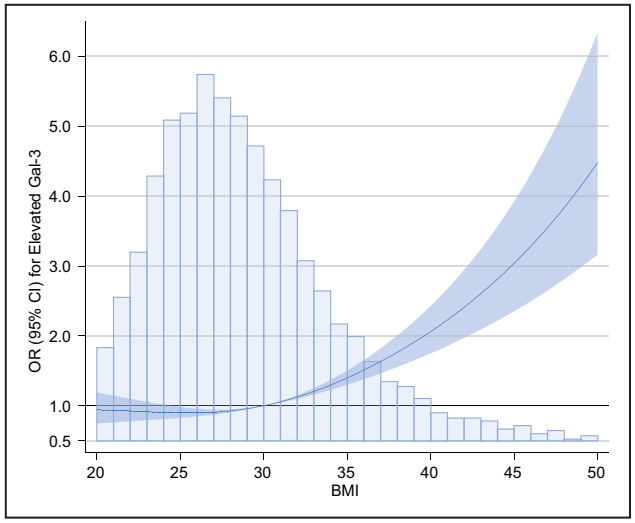

Figure 2. Continuous association of BMI (kg/m^2) with the odds of elevated galectin-3 (≥75th sex-specific percentile) using restricted cubic spline.
Adjusted for age, sex, race-ARIC center, smoking status, alcohol use, hypertension, total cholesterol, HDL-c, triglycerides, diabetes, eGFR, and rs4644. ARIC indicates Atherosclerosis Risk in Communities; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL-c, high-density lipoprotein cholesterol; and OR, odds ratio.

vascular inflammation and fibrosis, ultimately preventing HF.[30] Our study further corroborates the concept that excess adiposity may be independently linked to cardiac inflammation and fibrosis, as reflected by elevated circulating levels of galectin-3 within a community-based population.

Galectin-3 levels are also inversely related to renal function,[31] which can itself promote volume overload and HF development. Recently, it has been suggested that GFR estimates based on both serum creatinine and cystatin C may more accurately reflect renal function, particularly among people with obesity.[31,32] In our study, we found the association of BMI with elevated galectin-3 was attenuated when considering eGFR based on both serum creatinine as well as cystatin C, suggesting that renal impairment may contribute to the BMI and galectin-3 association.

In our diverse study population, we found significant differences in the associations of adiposity with elevated galectin-3 according to demographics, with stronger associations among women and White adults. These findings may underlie known differences in the epidemiology and pathogenesis of HF across demographic subgroups. For example, obesity and diabetes have stronger associations with incident HF among women.[33,34] Women and White adults also have higher risk of HF with preserved versus reduced ejection fraction.[35,36] Systemic inflammation and microvascular dysfunction are thought to be central pathways leading to HF in those with obesity, particularly for HF with preserved ejection fraction.[37] Although we were unable to examine HF subtypes in this analysis, it is possible that the demographic differences described here reflect differences in the pathogenesis of HF across demographic subgroups. In fact, some studies have shown that circulating galectin-3 is associated with progressive diastolic dysfunction and HF with preserved ejection fraction.[38] Future studies are needed to further understand the pathophysiologic and clinical implications of demographic differences in the association between obesity and galectin-3.

Our findings have several clinical implications. There is growing emphasis on targeting individuals in the preclinical stages of HF to prevent progression to

Table 3. Odds Ratios and 95% CI* for the Association of Categories of BMI With Elevated Galectin-3 (≥75th Sex-Specific Percentile) Stratified by Age, Sex, and Race

Subgroup	Normal weight (BMI 18.5 to <25)	Overweight (BMI 25 to <30)	Obesity (BMI 30 to <35)	Severe obesity (BMI ≥35)	*P* interaction
<65 y N=5389 (n=1104 with elevated galectin-3)	Reference (1)	1.09 (0.88–1.35)	1.27† (1.01–1.61)	2.65† (2.04–3.44)	0.08
≥65 y N=3298 (n=1084 with elevated galectin-3)	Reference (1)	1.07 (0.86–1.34)	1.44† (1.12–1.86)	1.77† (1.28–2.45)	
Men N=3609 (n=903 with elevated galectin-3)	Reference (1)	0.81 (0.64–1.03)	0.97 (0.75–1.27)	1.32 (0.90–1.92)	<0.001
Women N=5078 (n=1285 with elevated galectin-3)	Reference (1)	1.30† (1.06–1.60)	1.65† (1.32–2.08)	3.00† (2.34–3.85)	
White race N=6828 (n=1613 with elevated galectin-3)	Reference (1)	1.18 (1.00–1.41)	1.47† (1.20–1.79)	2.57† (2.01–3.28)	0.04
Black race N=1859 (n=575 with elevated galectin-3)	Reference (1)	0.70 (0.49–1.00)	0.88 (0.60–1.28)	1.50† (1.01–2.22)	

ARIC indicates Atherosclerosis Risk in Communities; and BMI, body mass index.
*Adjusted for: age, sex, and race-ARIC center, except where stratified, as well as smoking status, alcohol intake, systolic blood pressure, use of antihypertensive medication, total cholesterol, high-density lipoprotein-cholesterol, triglycerides, diabetes, estimated glomerular filtration rate, and rs4644.
†$P<0.05$.

Table 4. Hazard Ratios and 95% CI* for the Association of Quartiles of Galectin-3 With Incident Heart Failure After Visit 4, Stratified by BMI

Subgroup	Galectin-3 Q1	Galectin-3 Q2	Galectin-3 Q3	Galectin-3 Q4	*P* interaction
Overall	Reference (1)	1.11 (0.94–1.30)	1.26† (1.07–1.49)	1.68† (1.41–2.00)	…
Normal weight (BMI 18.5 to <25)	Reference (1)	1.60† (1.13–2.26)	1.53† (1.08–2.19)	2.49† (1.76–3.52)	0.16
Overweight (BMI 25 to <30)	Reference (1)	0.95 (0.74–1.21)	1.19 (0.93–1.52)	1.36† (1.06–1.75)	
Obesity (BMI 30 to <35)	Reference (1)	1.00 (0.74–1.36)	1.06 (0.79–1.44)	1.33 (0.99–1.77)	
Severe obesity (BMI ≥35)	Reference (1)	0.98 (0.66–1.46)	1.13 (0.77–1.65)	1.50† (1.05–2.15)	

ARIC indicates Atherosclerosis Risk in Communities; and BMI, body mass index.

*Adjusted for age, sex, race-ARIC center, smoking status, alcohol intake, systolic blood pressure, use of antihypertensive medication, total cholesterol, high-density lipoprotein-cholesterol, triglycerides, diabetes, estimated glomerular filtration rate, and rs4644.

†$P<0.05$.

overt clinical disease. The strong association between obesity and elevated galectin-3 in an ambulatory population highlights the significant degree of subclinical myocardial inflammation and fibrosis related to excess weight. Because the combination of severe obesity and elevated galectin-3 was linked to markedly increased HF risk, these measures have potential to identify those who could particularly benefit from aggressive preventive strategies. Although marked weight loss among those with obesity is likely linked to lower HF risk, achieving and maintaining weight loss is often elusive for many. Beyond serving as a dynamic indicator of HF risk, galectin-3 could even be examined as a future pharmacologic target for HF prevention in the high-risk population of individuals with obesity, where therapeutic strategies beyond weight loss are largely undefined.

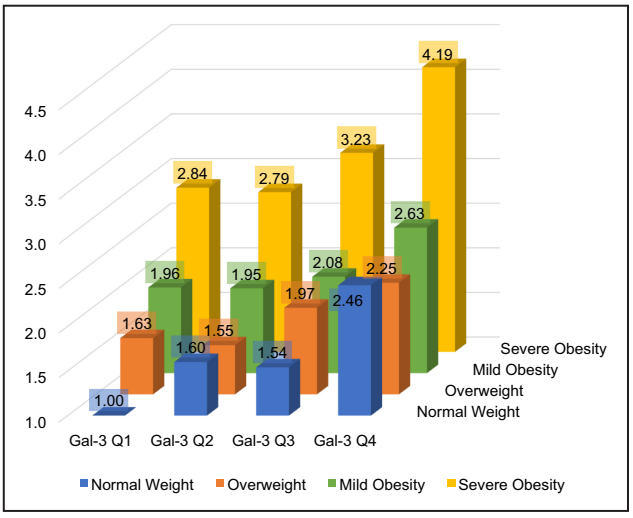

Figure 3. Combined associations of obesity and galectin-3 with incident HF.
Hazard ratios adjusted for age, sex, race-ARIC center, smoking status, alcohol use, hypertension, total cholesterol, HDL-c, triglycerides, diabetes, eGFR, and rs4644. *P* value for interaction between quartiles of galectin-3 and BMI categories on the outcome of incident HF, from likelihood ratio test, is 0.16. ARIC indicates Atherosclerosis Risk in Communities; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL-c, high-density lipoprotein cholesterol; and HF, heart failure.

Limitations of this study include its observational nature with possible residual confounding. We unfortunately did not have the data available to assess the associations of BMI and galectin-3 with the HF subtypes of HF with preserved versus reduced ejection fraction. Additionally, HF cases in ARIC were defined based on records of hospitalization and *ICD* codes, with potential for misclassification. Milder cases of HF diagnosed in the outpatient setting were not captured, likely leading to an underestimation of HF events. Nonetheless, this study has many strengths including the use of a broadly representative community-based sample with robust risk factor characterization and extended follow-up for HF events. The inclusion of a diverse cohort allowed us to identify important demographic differences in the association between obesity and elevated galectin-3. Furthermore, in the present study, genotyping allowed for adjustments for rs4644, likely improving the accuracy of the results.

In summary, we found that higher BMI is independently linked to elevated galectin-3, a marker of inflammation and fibrosis. The associations of obesity and galectin-3 varied according to sex and race, with possible implications for differences in the myocardial effects of obesity across these subgroups. Furthermore, the combination of severe obesity and elevated galectin-3, compared with those with normal weight and with low galectin-3, identifies a subgroup at particularly high risk of future HF who may benefit from aggressive preventive measures.

ARTICLE INFORMATION

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Disclosures

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Supplemental Material

Tables S1–S7

REFERENCES

1. Kenchaiah S, Evans JC, Levy D, Wilson PW, Benjamin EJ, Larson MG, Kannel WB, Vasan RS. Obesity and the risk of heart failure. *N Engl J Med*. 2002;347:305–313. doi: 10.1056/NEJMoa020245
2. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011–2012. *JAMA*. 2014;311:806–814. doi: 10.1001/jama.2014.732
3. Ndumele CE, Matsushita K, Lazo M, Bello N, Blumenthal RS, Gerstenblith G, Nambi V, Ballantyne CM, Solomon SD, Selvin E, et al. Obesity and subtypes of incident cardiovascular disease. *J Am Heart Assoc*. 2016;5:e003921. doi: 10.1161/JAHA.116.003921
4. Ebong IA, Goff DC Jr, Rodriguez CJ, Chen H, Bertoni AG. Mechanisms of heart failure in obesity. *Obes Res Clin Pract*. 2014;8:e540–e548. doi: 10.1016/j.orcp.2013.12.005
5. McGavock JM, Victor RG, Unger RH, Szczepaniak LS; American College of P, the American Physiological S. Adiposity of the heart, revisited. *Ann Intern Med*. 2006;144:517–524. doi: 10.7326/0003-4819-144-7-200604040-00011
6. Alpert MA, Lavie CJ, Agrawal H, Aggarwal KB, Kumar SA. Obesity and heart failure: epidemiology, pathophysiology, clinical manifestations, and management. *Transl Res*. 2014;164:345–356. doi: 10.1016/j.trsl.2014.04.010
7. Chow SL, Maisel AS, Anand I, Bozkurt B, de Boer RA, Felker GM, Fonarow GC, Greenberg B, Januzzi JL, Kiernan MS, et al. Role of biomarkers for the prevention, assessment, and management of heart failure: a scientific statement from the American Heart Association. *Circulation*. 2017;135:e1054–e1091. doi: 10.1161/CIR.0000000000000490
8. Schmitter D, Cotter G, Voors AA. Clinical use of novel biomarkers in heart failure: towards personalized medicine. *Heart Fail Rev*. 2014;19:369–381. doi: 10.1007/s10741-013-9396-5
9. de Boer RA, Voors AA, Muntendam P, van Gilst WH, van Veldhuisen DJ. Galectin-3: a novel mediator of heart failure development and progression. *Eur J Heart Fail*. 2009;11:811–817. doi: 10.1093/eurjhf/hfp097
10. Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JPM, Schroen B, André S, Crijns HJGM, Gabius H-J, Maessen J, et al. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation*. 2004;110:3121–3128. doi: 10.1161/01.CIR.0000147181.65298.4D
11. de Boer RA, Yu L, van Veldhuisen DJ. Galectin-3 in cardiac remodeling and heart failure. *Curr Heart Fail Rep*. 2010;7:1–8. doi: 10.1007/s11897-010-0004-x
12. Yu L, Ruifrok WPT, Meissner M, Bos EM, van Goor H, Sanjabi B, van der Harst P, Pitt B, Goldstein IJ, Koerts JA, et al. Genetic and pharmacological inhibition of galectin-3 prevents cardiac remodeling by interfering with myocardial fibrogenesis. *Circ Heart Fail*. 2013;6:107–117. doi: 10.1161/CIRCHEARTFAILURE.112.971168
13. Shah RV, Chen-Tournoux AA, Picard MH, van Kimmenade RR, Januzzi JL. Galectin-3, cardiac structure and function, and long-term mortality in patients with acutely decompensated heart failure. *Eur J Heart Fail*. 2010;12:826–832. doi: 10.1093/eurjhf/hfq091
14. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, Larson MG, Levy D. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *J Am Coll Cardiol*. 2012;60:1249–1256. doi: 10.1016/j.jacc.2012.04.053
15. de Boer RA, Lok DJA, Jaarsma T, van der Meer P, Voors AA, Hillege HL, van Veldhuisen DJ. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. *Ann Med*. 2011;43:60–68. doi: 10.3109/07853890.2010.538080
16. Lok DJA, Van Der Meer P, de la Porte P-A, Lipsic E, Van Wijngaarden J, Hillege HL, van Veldhuisen DJ. Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data from the DEAL-HF study. *Clin Res Cardiol*. 2010;99:323–328. doi: 10.1007/s00392-010-0125-y
17. Wright JD, Folsom AR, Coresh J, Sharrett AR, Couper D, Wagenknecht LE, Mosley TH, Ballantyne CM, Boerwinkle EA, Rosamond WD, et al. The ARIC (Atherosclerosis Risk in Communities) study: JACC focus seminar 3/8. *J Am Coll Cardiol*. 2021;77:2939–2959. doi: 10.1016/j.jacc.2021.04.035
18. Liu X, Wang Y, Wang C, Shi C, Cheng C, Chen J, Ma H, Lv L, Li L, Lou T, et al. A new equation to estimate glomerular filtration rate in Chinese elderly population. *PLoS One*. 2013;8:e79675. doi: 10.1371/journal.pone.0079675
19. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367:20–29. doi: 10.1056/NEJMoa1114248
20. Parrinello CM, Lutsey PL, Ballantyne CM, Folsom AR, Pankow JS, Selvin E. Six-year change in high-sensitivity C-reactive protein and risk of diabetes, cardiovascular disease, and mortality. *Am Heart J*. 2015;170:380–389. doi: 10.1016/j.ahj.2015.04.017
21. Saunders JT, Nambi V, de Lemos JA, Chambless LE, Virani SS, Boerwinkle E, Hoogeveen RC, Liu X, Astor BC, Mosley TH, et al. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the Atherosclerosis Risk in Communities Study. *Circulation*. 2011;123:1367–1376. doi: 10.1161/CIRCULATIONAHA.110.005264
22. Fashanu OE, Heckbert SR, Aguilar D, Jensen PN, Ballantyne CM, Basu S, Hoogeveen RC, deFilippi C, Cushman M, Folsom AR, et al. Galectin-3 and venous thromboembolism incidence: the Atherosclerosis Risk in Communities (ARIC) Study. *Res Pract Thromb Haemost*. 2017;1:223–230. doi: 10.1002/rth2.12038
23. de Boer RA, Verweij N, van Veldhuisen DJ, Westra H-J, Bakker SJL, Gansevoort RT, Muller Kobold AC, van Gilst WH, Franke L, Leach IM, et al. A genome-wide association study of circulating galectin-3. *PLoS One*. 2012;7:e47385. doi: 10.1371/journal.pone.0047385
24. Aguilar D, Sun C, Hoogeveen RC, Nambi V, Selvin E, Matsushita K, Saeed A, McEvoy JW, Shah AM, Solomon SD, et al. Levels and change in galectin-3 and association with cardiovascular events: the ARIC Study. *J Am Heart Assoc*. 2020;9:e015405. doi: 10.1161/JAHA.119.015405
25. Grove ML, Yu B, Cochran BJ, Haritunians T, Bis JC, Taylor KD, Hansen M, Borecki IB, Cupples LA, Fornage M, et al. Best practices and joint calling of the HumanExome BeadChip: the CHARGE Consortium. *PLoS One*. 2013;8:e68095. doi: 10.1371/journal.pone.0068095
26. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94:496–509. doi: 10.1080/01621459.1999.10474144

27. Grandin EW, Jarolim P, Murphy SA, Ritterova L, Cannon CP, Braunwald E, Morrow DA. Galectin-3 and the development of heart failure after acute coronary syndrome: pilot experience from PROVE IT-TIMI 22. *Clin Chem*. 2012;58:267–273. doi: 10.1373/clinchem.2011.174359
28. Ghorbani A, Bhambhani V, Christenson RH, Meijers WC, de Boer RA, Levy D, Larson MG, Ho JE. Longitudinal change in galectin-3 and incident cardiovascular outcomes. *J Am Coll Cardiol*. 2018;72:3246–3254. doi: 10.1016/j.jacc.2018.09.076
29. Papaspyridonos M, McNeill E, de Bono JP, Smith A, Burnand KG, Channon KM, Greaves DR. Galectin-3 is an amplifier of inflammation in atherosclerotic plaque progression through macrophage activation and monocyte chemoattraction. *Arterioscler Thromb Vasc Biol*. 2008;28:433–440. doi: 10.1161/ATVBAHA.107.159160
30. Martínez-Martínez E, López-Ándres N, Jurado-López R, Rousseau E, Bartolomé MV, Fernández-Celis A, Rossignol P, Islas F, Antequera A, Prieto S, et al. Galectin-3 participates in cardiovascular remodeling associated with obesity. *Hypertension*. 2015;66:961–969. doi: 10.1161/HYPERTENSIONAHA.115.06032
31. Malkina A, Katz R, Shlipak MG, Ix JH, de Boer IH, Sarnak MJ, Allison M, Kramer HJ, Lin J, Siscovick D, Peralta CA. Association of obesity and kidney function decline among non-diabetic adults with eGFR > 60 ml/min/1.73m(2): results from the Multi-Ethnic Study of Atherosclerosis (MESA). *Open J Endocr Metab Dis*. 2013;3:103–112. doi: 10.4236/ojemd.2013.32016
32. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, Crews DC, Doria A, Estrella MM, Froissart M, et al. New creatinine- and cystatin C-based equations to estimate GFR without race. *N Engl J Med*. 2021;385:1737–1749. doi: 10.1056/NEJMoa2102953
33. Lam CSP, Arnott C, Beale AL, Chandramouli C, Hilfiker-Kleiner D, Kaye DM, Ky B, Santema BT, Sliwa K, Voors AA, et al. Sex differences in heart failure. *Eur Heart J*. 2019;40:3859–3868c. doi: 10.1093/eurheartj/ehz835
34. Sillars A, Ho FK, Pell GP, Gill JMR, Sattar N, Gray S, Celis-Morales C. Sex differences in the association of risk factors for heart failure incidence and mortality. *Heart*. 2020;106:203–212. doi: 10.1136/heartjnl-2019-314878
35. Chang PP, Wruck LM, Shahar E, Rossi JS, Loehr LR, Russell SD, Agarwal SK, Konety SH, Rodriguez CJ, Rosamond WD, et al. Trends in hospitalizations and survival of acute decompensated heart failure in four US communities (2005–2014): ARIC study community surveillance. *Circulation*. 2018;138:12–24. doi: 10.1161/CIRCULATIONAHA.117.027551
36. Pandey A, Omar W, Ayers C, LaMonte M, Klein L, Allen NB, Kuller LH, Greenland P, Eaton CB, Gottdiener JS, et al. Sex and race differences in lifetime risk of heart failure with preserved ejection fraction and heart failure with reduced ejection fraction. *Circulation*. 2018;137:1814–1823. doi: 10.1161/CIRCULATIONAHA.117.031622
37. Sabbah MS, Fayyaz AU, de Denus S, Felker GM, Borlaug BA, Dasari S, Carter RE, Redfield MM. Obese-inflammatory phenotypes in heart failure with preserved ejection fraction. *Circ Heart Fail*. 2020;13:e006414. doi: 10.1161/CIRCHEARTFAILURE.119.006414
38. Ansari U, Behnes M, Hoffmann J, Natale M, Fastner C, El-Battrawy I, Rusnak J, Kim S-H, Lang S, Hoffmann U, et al. Galectin-3 reflects the echocardiographic grades of left ventricular diastolic dysfunction. *Ann Lab Med*. 2018;38:306–315. doi: 10.3343/alm.2018.38.4.306

SUPPLEMENTAL MATERIAL

Table S1. Baseline Characteristics of the Participants Excluded from the Study for not Having Information on Gal-3 Compared to Those Included.

Variable*	**Without Gal-3 (n=735)**	**With Gal-3 (n=8,687)**
Age, years	62.7 (5.7)	62.6 (5.6)
Female sex	398 (54.1%)	5,078 (58.5%)
Black race	157 (21.4%)	1,859 (21.4%)
Current smoker	440 (59.9%)	4,934 (56.8%)
Current alcohol use	374 (50.9%)	4,409 (50.8%)
SBP, mmHg	128.8 (19.4)	127.0 (18.7)
Anti-hypertensive medication use	276 (37.6%)	3,367 (38.8%)
Diabetes mellitus	120 (16.3%)	1,281 (14.7%)
Waist circumference, cm	101.6 (13.6)	101.7 (14.2)
Total cholesterol, mg/dl	204.9 (38.6)	201.7 (36.1)
HDL-c, mg/dl	49.4 (16.2)	50.7 (16.5)
Triglycerides, mg/dl†	123.0 (89.0-171.0)	121.0 (88.0-172.0)
eGFR*	88.7 (75.9-96.0)	89.5 (77.1-96.3)
BMI, kg/m^2	28.7 (5.3)	28.7 (5.4)
rs4644 - AA	116 (15.8%)	1,305 (15.0%)
rs4644 - AC	301 (41.0%)	4,052 (46.6%)
rs4644 - CC	318 (43.3%)	3,330 (38.3%)

Prevalent Atrial Fibrillation	7 (1.0%)	124 (1.4%)
Insulin Use	23 (3.1%)	259 (3.0%)

* Continuous variables are presented as means (standard deviation) unless indicated; categorial variables as number (proportion)

† Presented as median (interquartile range)

‡ eGFR = estimated glomerular filtration rate; Gal-3 = galectin 3; HDL-c = high-density lipoprotein cholesterol; SBP = systolic blood pressure

Table S2. Baseline (Visit 4) Characteristics of the Study Population Stratified by Quartiles of Gal-3

Variable*	Gal-3 Q1	Gal-3 Q2	Gal-3 Q3	Gal-3 Q4	p value
Age, years	61.3 (5.4)	62.1 (5.5)	62.7 (5.5)	64.2 (5.7)	<0.001
Female sex	1220 (58.0%)	1317 (59.2%)	1256 (57.9%)	1285 (58.7%)	0.80
Black race	371 (17.6%)	420 (18.9%)	493 (22.7%)	575 (26.3%)	<0.001
Current smoker	296 (14.1%)	313 (14.1%)	308 (14.2%)	323 (14.8%)	0.23
Current alcohol use	1150 (54.7%)	1213 (54.5%)	1109 (51.1%)	937 (42.8%)	<0.001
SBP, mmHg	125.3 (18.1)	126.6 (18.4)	127.0 (18.1)	129.3 (19.7)	<0.001
Anti-hypertensive medication use	632 (30.1%)	726 (32.6%)	838 (38.6%)	1171 (53.5%)	<0.001
Diabetes mellitus	277 (13.2%)	281 (12.6%)	326 (15.0%)	397 (18.1%)	<0.001
Waist circumference, cm	99.4 (13.2)	100.6 (13.7)	102.1 (14.3)	104.5 (15.0)	<0.001
Total cholesterol, mg/dl	200.8 (34.6)	202.0 (36.5)	201.1 (35.3)	202.8 (37.9)	0.27
HDL-c, mg/dl	51.7 (16.5)	51.2 (16.7)	50.6 (16.4)	49.4 (16.3)	<0.001
Triglycerides, mg/dl†	116.0 (83.0-163.0)	119.0 (86.0-169.0)	120.0 (89.0-170.0)	131.0 (94.0-186.0)	<0.001
eGFR*	92.6 (82.7-98.7)	90.8 (79.7-97.7)	89.1 (77.2-95.5)	82.8 (68.6-92.9)	<0.001
BMI, kg/m^2	27.9 (4.8)	28.4 (5.1)	28.8 (5.5)	29.8 (6.0)	<0.001
BMI ≥35 (%)	166 (7.9%)	228 (10.2%)	260 (12.0%)	378 (17.3%)	<0.001
rs4644 - AA	893 (42.5%)	276 (12.4%)	98 (4.5%)	38 (1.7%)	<0.001
rs4644 - AC	1022 (48.6%)	1380 (62.0%)	1014 (46.7%)	636 (29.1%)	

rs4644 - CC	188 (8.9%)	570 (25.6%)	1058 (48.8%)	1514 (69.2%)	
Prevalent Atrial Fibrillation	26 (1.2%)	22 (1.0%)	25 (1.2%)	51 (2.3%)	<0.001
Insulin Use	55 (2.6%)	46 (2.1%)	69 (3.2%)	89 (4.1%)	<0.001

* Continuous variables are presented as means (standard deviation) unless indicated; categorial variables as number (proportion)

† Presented as median (interquartile range)

‡ eGFR = estimated glomerular filtration rate; Gal-3 = galectin 3; HDL-c = high-density lipoprotein cholesterol; SBP = systolic blood pressure

§ Quartile of Gal-3: Males Q1 = 6-11 ng/mL, Females Q1 = 4-13 ng/mL; Males Q2 = 11-13 ng/mL, Females Q2 = 13-15 ng/mL; Males Q3 = 13-15 ng/mL, Females Q3 = 15-18 ng/mL; Males Q4 = 15-103 ng/mL, Females Q4 = 18-114 ng/mL

Table S3: Odds Ratios and 95% CI† for the Association of Quartiles of Waist Circumference with Elevated Galectin-3, Overall and Stratified by Age, Sex, and Race

Subgroup	WC Q1 (51-91 cm)	WC Q2 (92-100cm)	WC Q3 (101-109 cm)	WC Q4 (110-169 cm)	P Interaction
Overall N= 8687 (n=2188 with elevated gal-3)	Reference (1)	1.09 (0.92-1.30)	1.25‡ (1.04-1.49)	1.65‡ (1.38-1.98)	-
<65 years N= 5389 (n=1104 with elevated gal-3)	Reference (1)	1.17 (0.93-1.48)	1.15 (0.91-1.46)	1.77‡ (1.42-2.22)	0.13
≥65 years N= 3298 (n=1084 with elevated gal-3)	Reference (1)	1.00 (0.77-1.29)	1.36‡ (1.04-1.76)	1.49‡ (1.14-1.94)	
Men N= 3609 (n=903 with elevated gal-3)	Reference (1)	1.02 (0.76-1.37)	1.04 (0.77-1.40)	1.26 (0.92-1.71)	0.06
Women N= 5078 (n=1285 with elevated gal-3)	Reference (1)	1.06 (0.85-1.32)	1.36‡ (1.08-1.71)	1.88‡ (1.53-2.33)	
White N= 6828 (n=1613 with elevated gal-3)	Reference (1)	1.24‡ (1.02-1.51)	1.41‡ (1.14-1.73)	1.86‡ (1.51-2.28)	0.03
Black N= 1859 (n=575 with elevated gal-3)	Reference (1)	0.72 (0.50-1.02)	0.85 (0.59-1.21)	1.14 (0.82-1.60)	

* Abbreviation: WC = waist circumference

† Adjusted for: age, sex, race-center, smoking status, alcohol intake, systolic blood pressure, use of anti-hypertensive medication, total cholesterol, HDL-cholesterol, triglycerides, diabetes mellitus, eGFR, and rs4644.

‡ $p<0.05$

Table S4. Odds Ratios for the Association of Categories of BMI with Elevated Galectin-3 (≥75th Sex-Specific Percentile), Adjusted for eGFR based on Serum Creatinine and Cystatin C

Adjustment Model	Normal Weight (BMI 18.5-<25)	Overweight (BMI 25-<30)	Obesity (BMI 30-<35)	Severe Obesity (BMI ≥35)
Model 2a*	Reference (1)	1.03 (0.88-1.21)	1.18 (0.98-1.42)	1.80‡ (1.45-2.23)
Model 3a†	Reference (1)	0.94 (0.79-1.10)	1.02 (0.84-1.23)	1.39‡ (1.10-1.74)

* Model 2a: Adjusted for age, sex, race-center, smoking status, alcohol intake, rs4644, systolic blood pressure, use of anti-hypertensive medication, total cholesterol, HDL-cholesterol, triglycerides, diabetes mellitus and eGFR based on serum creatinine and cystatin C.

† Model 3a: Model 2a variables plus hsCRP, NTproBNP, and hs-cTnT.

‡ $p<0.05$

Table S5. Hazard Ratios and 95% CI for the Association of Quartiles of Gal-3 with Incident HF After Visit 4, Stratified by BMI

Subgroup	Gal-3 Q1	Gal-3 Q2	Gal-3 Q3	Gal-3 Q4	P Interaction
Model 2*					
Obesity absent (BMI 18.5-<30 kg/m2)	Reference (1)	1.15 (0.94-1.40)	1.31# (1.06-1.61)	1.71# (1.38-2.11)	0.79
Obesity present (BMI ≥30 kg/m2)	Reference (1)	1.02 (0.80-1.30)	1.14 (0.90-1.45)	1.49# (1.18-1.88)	
Model 2a†					
Obesity absent (BMI 18.5-<30 kg/m2)	Reference (1)	1.10 (0.90-1.34)	1.21 (0.98-1.49)	1.50# (1.21-1.87)	0.73
Obesity present (BMI ≥30 kg/m2)	Reference (1)	0.97 (0.76-1.24)	1.06 (0.83-1.34)	1.28# (1.01-1.63)	
Model 3‡					
Obesity absent (BMI 18.5-<30 kg/m2)	Reference (1)	1.10 (0.90-1.35)	1.16 (0.94-1.43)	1.37# (1.10-1.70)	0.82
Obesity present (BMI ≥30 kg/m2)	Reference (1)	0.95 (0.75-1.22)	1.04 (0.82-1.33)	1.28# (1.01-1.62)	
Model 4§					

Obesity absent (BMI 18.5-<30 kg/m2)	Reference (1)	1.11 (0.91-1.36)	1.18 (0.96-1.45)	1.38# (1.11-1.72)	0.78
Obesity present (BMI ≥30 kg/m2)	Reference (1)	0.96 (0.75-1.23)	1.03 (0.81-1.31)	1.27 (1.00-1.62)	
Model 5‖					
Obesity absent (BMI 18.5-<30 kg/m2)	Reference (1)	1.17 (0.95-1.43)	1.16 (0.94-1.42)	1.39# (1.12-1.73)	0.68
Obesity present (BMI ≥30 kg/m2)	Reference (1)	0.97 (0.76-1.24)	1.07 (0.84-1.36)	1.31# (1.03-1.66)	

* Model 2: adjusted for age, sex, race-center, smoking status, alcohol intake, systolic blood pressure, use of anti-hypertensive medication, total cholesterol, HDL-cholesterol, triglycerides, diabetes mellitus, eGFR, and rs4644

† Model 2a: adjusted for age, sex, race-center, smoking status, alcohol intake, systolic blood pressure, use of anti-hypertensive medication, total cholesterol, HDL-cholesterol, triglycerides, diabetes mellitus, eGFR based on serum creatinine and cystatin C, and rs4644

‡ Model 3: Model 2 variables plus hsCRP, NTproBNP, and hs-cTnT.

§ Model 4: Model 3 variables plus prevalent atrial fibrillation and insulin use.

|| Model 5: Model 4 variables plus time-varying coronary heart disease.

# $p<0.05$

Table S6. Hazard Ratios and 95% CI* for the Association of Quartiles of Gal-3 with Incident HF After Visit 4, Stratified by BMI, considering the competing risk of death

Subgroup	**Gal-3 Q1**	**Gal-3 Q2**	**Gal-3 Q3**	**Gal-3 Q4**
Obesity absent (BMI 18.5-<30 kg/m2)	Reference (1)	1.13 (0.93-1.38)	1.25† (1.01-1.54)	1.45† (1.17-1.80)
Obesity present (BMI ≥30 kg/m2)	Reference (1)	1.03 (0.80-1.31)	1.08 (0.84-1.37)	1.34† (1.05-1.70)

* Adjusted for age, sex, race-center, smoking status, alcohol intake, systolic blood pressure, use of anti-hypertensive medication, total cholesterol, HDL-cholesterol, triglycerides, diabetes mellitus, eGFR, and rs4644

† $p<0.05$

Table S7. Incidence Rates[†], Hazard Ratios and 95% CI[‡] for the Association of Cross-Categories of Obesity and Galectin-3 with Incident HF

Subgroup		Gal-3 Q1	Gal-3 Q2	Gal-3 Q3	Gal-3 Q4
Normal Weight (BMI 18.5-24.9)	IR (95% CI)	4.8 (3.6-6.3)	7.8 (6.2-9.6)	8.4 (6.7-10.4)	14.9 (12.2-18.1)
	HR (95% CI)	Reference (1)	1.60[§] (1.13-2.26)	1.54[§] (1.08-2.20)	2.46[§] (1.74-3.48)
Overweight (BMI 25-29.9)	IR (95% CI)	8.5 (7.2-10.1)	8.7 (7.3-10.3)	10.6 (9.0-12.4)	15.0 (12.9-17.3)
	HR (95% CI)	1.63[§] (1.19-2.25)	1.55[§] (1.12-2.14)	1.97[§] (1.43-2.72)	2.25[§] (1.63-3.11)
Obesity (BMI 30-34.9)	IR (95% CI)	11.0 (8.8-13.6)	10.9 (8.7-13.4)	11.9 (9.7-14.5)	18.0 (15.2-21.1)
	HR (95% CI)	1.96[§] (1.38-2.77)	1.95[§] (1.38-2.76)	2.08[§] (1.47-2.94)	2.63[§] (1.88-3.68)
Severe Obesity (BMI ≥35)	IR (95% CI)	15.7 (11.4-21.2)	15.2 (11.6-19.7)	18.7 (14.8-23.4)	24.3 (20.4-28.7)
	HR (95% CI)	2.84[§] (1.89-4.28)	2.79[§] (1.91-4.08)	3.23[§] (2.25-4.64)	4.19[§] (2.98-5.88)

* Abbreviations: BMI = body mass index, CI = confidence interval, Gal-3 = galectin-3, HF = heart failure, HR = hazard ratio, IR = incidence rate, Q = quartile

† IR per 1,000 person-years

‡ Adjusted for age, sex, race-center, smoking status, alcohol use, hypertension, total cholesterol, HDL-c, triglycerides, diabetes, eGFR, rs4644

§ $p<0.05$