Galectin-3 Participates in Cardiovascular Remodeling Associated With Obesity


Abstract—Remodeling, diastolic dysfunction, and arterial stiffness are some of the alterations through which obesity affects the cardiovascular system. Fibrosis and inflammation are important mechanisms underlying cardiovascular remodeling, although the precise promoters involved in these processes are still unclear. Galectin-3 (Gal-3) induces inflammation and fibrosis in the cardiovascular system. We have investigated the potential role of Gal-3 in cardiac damage in morbidly obese patients, and we have evaluated the protective effect of the Gal-3 inhibition in the occurrence of cardiovascular fibrosis and inflammation in an experimental model of obesity. Morbid obesity is associated with alterations in cardiac remodeling, mainly left ventricular hypertrophy and diastolic dysfunction. Obesity and hypertension are the main determinants of left ventricular hypertrophy. Insulin resistance, left ventricular hypertrophy, and circulating levels of C-reactive protein and Gal-3 are associated with a worsening of diastolic function in morbidly obese patients. Obesity upregulates Gal-3 production in the cardiovascular system in a normotensive animal model of diet-induced obesity by feeding for 6 weeks a high-fat diet (33.5% fat). Gal-3 inhibition with modified citrus pectin (100 mg/kg per day) reduced cardiovascular levels of Gal-3, total collagen, collagen I, transforming and connective growth factors, osteopontin, and monocyte chemoattractant protein-1 in the heart and aorta of obese animals without changes in body weight or blood pressure. In morbidly obese patients, Gal-3 levels are associated with diastolic dysfunction. In obese animals, Gal-3 blockade decreases cardiovascular fibrosis and inflammation. These data suggest that Gal-3 could be a novel therapeutic target in cardiac fibrosis and inflammation associated with obesity. (Hypertension. 2015;66:961-969. DOI: 10.1161/HYPERTENSIONAHA.115.06032.) • Online Data Supplement

Key Words: fibrosis • galectin-3 • inflammation • obesity • vascular stiffness

Obesity, especially morbid obesity, is associated with a high cardiovascular morbidity—mortality and is a major burden on the healthcare system because of its increased worldwide prevalence, which affects people of all age ranges.1 The concomitant presence of other comorbidities, such as hypertension or diabetes mellitus, which can also have an effect on the cardiovascular system, may make it difficult to establish the specific cardiovascular consequences of obesity. Cardiac remodeling and vascular remodeling are characterized by an initial inflammatory response that triggers extracellular matrix (ECM) deposition and fibrosis; these could ultimately promote diastolic dysfunction and arterial stiffness that are major alterations associated with obesity,2,3 and they can facilitate the development of heart failure (HF). The precise mechanisms contributing to fibrosis in the context of obesity have not been fully elucidated, although a complex variety of factors (humoral, hemodynamic, oxidative stress, inflammation, and adipokines) have been suggested. For this reason, understanding the molecular mechanisms involved in these processes could identify new biomarkers that may be considered to be targets for specific pharmacological interventions.

Galectin-3 (Gal-3) is a member of a β-galactoside–binding lectin family with a N-terminal domain and a C-terminal carbohydrate recognition domain. It is produced in many tissues, including myocardium and vessels.4,5 Gal-3 has emerged as a potential mediator of cardiac damage in different pathological situations6,7 through its ability to stimulate ECM deposition and by amplification of key proinflammatory molecules.8 In

*These authors contributed equally to this work.

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Correspondence to Victoria Cachofeiro, Departamento de Fisiología, Facultad de Medicina, Universidad Complutense, Madrid 28040, Spain. E-mail vcaro@ucm.es.

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addition, clinical studies have shown that increased levels of Gal-3 in patients with HF are associated with adverse long-term cardiovascular outcomes, and the combination of Gal-3 with the aminoterminal portion of pro–brain natriuretic peptide is the best predictor for prognosis in individuals with acute HF.\textsuperscript{9,10} Several inhibitors of Gal-3 have been described, such as the modified citrus pectin (MCP).\textsuperscript{11} MCP is a water soluble indigestible polysaccharide, which contains fragments that are recognized C-terminal carbohydrate recognition domains of Gal-3 with a high affinity and block the lectin’s activity.\textsuperscript{5} In the context of obesity, elevated circulating Gal-3 levels has been reported in obese patients.\textsuperscript{12,13} In addition, we have found a local increase of Gal-3 in the heart from diet-induced obesity and inflammation in a normotensive animal model of obesity.\textsuperscript{4} Moreover, our group has recently shown that Gal-3 plays an important role in aldosterone-induced cardiac and vascular remodeling.\textsuperscript{5,14} Although these observations suggest that Gal-3 may directly induce cardiac and vascular remodeling in situations with high aldosterone levels, no studies have investigated its role and the potential benefits of its inhibition in the cardiovascular alterations associated with obesity.

Therefore, our hypothesis is that Gal-3 could be a key factor in the cardiovascular remodeling associated with obesity by facilitating ECM production and a proinflammatory state. To address this hypothesis, we characterized circulating Gal-3 levels and the anatomofunctional alterations of the heart in morbidly obese patients, compared with nonobese volunteers. Also, we evaluated the protective effect of the Gal-3 pharmacological inhibition with the specific inhibitor of Gal-3 activity, MCP,\textsuperscript{15} and its involvement in the occurrence of cardiovascular fibrosis and inflammation in a normotensive animal model of obesity.

**Methods**

Detailed methods are available in the online-only Data Supplement.

**Study Population**

Morbidly obese patients who were referred to bariatric surgery were consecutively recruited from the Obesity Care Unit of Fuenlabrada University Hospital, Spain. The selection of the patients was performed by a multidisciplinary committee, which includes personnel from endocrinology, general and upper gastroenterology surgery, and internal medicine and cardiology services. Inclusion criteria were age ≥18 years and universally accepted indications for bariatric surgery: long-term obesity (>4 years), body mass index ≥25 kg/m\(^2\) despite other weight loss strategies, or body mass index ≥35 kg/m\(^2\) in the presence of obesity-related comorbidities (diabetes mellitus, obesity hypoventilation syndrome, obstructive sleep apnea syndrome, and hypertension). Exclusion criteria were age >60 years and unacceptable surgical risk because of concomitant comorbidities. Nonobese volunteers (body mass index, ≤25 kg/m\(^2\)) were recruited from staff of the hospital. The study protocol was approved by the ethics committee, and all participants signed the informed consent. This study was conducted in compliance with Good Clinical Practice Guidelines and the ethical principles stated in the Declaration of Helsinki.

**Animal Study**

Male Wistar rats of 150 g (Harlan Ibérica, Barcelona, Spain) received either a high-fat diet (HFD, 33.5% fat; Harlan Teklad number, TD.03307, MN; n=16) or a standard diet (3.5% fat; Harlan Teklad number, TD.2014, MN; n=16) for 6 weeks. Half of the animals of each group received the Gal-3 activity inhibitor, MCP (100 mg/kg per day), in the drinking water for the same period as previously described.\textsuperscript{14} The Animal Care and Use Committee of Universidad Complutense de Madrid approved all experimental procedures according to guidelines for ethical care of experimental animals of the European Community.

Body weight was measured once a week. Food and water intake were determined throughout the experimental period. Systolic blood pressure was estimated basally, at midstudy and at the end of the study through the use of a tail-cuff plethysmograph (Narco Bio-Systems) in unrestrained animals as previously reported.\textsuperscript{16}

**Statistical Analysis**

Continuous variables are expressed as mean±SD or median (interquartile interval) in the case of asymmetry. Categorical variables are expressed in absolute values and percentages. The differences between categorical variables were analyzed using the χ\(^2\) test. Normality of distributions was verified by means of the Kolmogorov–Smirnov test. In the case of continuous variables, differences between 2 groups were analyzed by either unpaired Student t test or Mann–Whitney U test as parametric and nonparametric tests, respectively. Specific differences between more groups were analyzed using Kruskall–Wallis test followed by Dunn test for non-normal distribution variables. For normal distribution variables, 1-way ANOVA was used followed by Tukey test. Either Pearson or Spearman correlation analysis was used to examine association among different variables according to whether they are normally distributed. The factors associated with the left ventricular (LV) hypertrophy were addressed by a logistic model, and their odds ratio and 95% confidence interval (CI) were calculated. To find the factors associated with diastolic function as E/e′ ratio, the β-correlation coefficients (slope or mean difference, along with their 95% CIs) were obtained using a linear regression model. In both cases, variables shown to have a statistical significance by univariate analysis and those clinically relevant were included to build the model. A value of P<0.05 was used as the cutoff value for defining statistical significance. Data analysis was performed using the statistical program SPSS version 22.0 (SPSS Inc, Chicago, IL).

**Results**

**Epidemiological Data, Metabolic Profile, and Cardiovascular Risk of Patients**

Table 1 shows the demographic and clinical characteristics of the participants. The mean age of both groups was similar, with 80.4% of the obese patients being women versus 59.1%.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Lean Subjects, n=66</th>
<th>Obese Subjects, n=56</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>41.4±5.1</td>
<td>41.1±9.7</td>
<td>0.843</td>
</tr>
<tr>
<td>Men/women, n</td>
<td>27/39</td>
<td>11/45</td>
<td>0.01</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>22.8±1.9</td>
<td>46.2±5.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>110.7±12.1</td>
<td>122±21.5</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>67.1±7.6</td>
<td>74.1±12.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>66.2±10.4</td>
<td>73.6±12.2</td>
<td>0.007</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>87.3±9.5</td>
<td>103.7±24.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting insulin, pg/mL</td>
<td>164.7±62.7</td>
<td>365.1±255.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HOMA index, AU</td>
<td>0.79±0.32</td>
<td>2.34±2.79</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>85.6±56.6</td>
<td>167.2±84.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>198.1±32.7</td>
<td>167.5±39.1</td>
<td>0.034</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comorbidities</th>
<th>Lean Subjects, n=6</th>
<th>Obese Subjects, n=56</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension, n (%)</td>
<td>4 (6)</td>
<td>25 (44.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>0</td>
<td>18 (32.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>6 (9)</td>
<td>20 (35.7)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD or n (%). AU indicates arbitrary unit; DBP, diastolic blood pressure; HOMA, homeostasis model assessment-insulin resistance; and SBP, systolic blood pressure.
Table 2. Echocardiographic Data of Lean Control Subjects and Morbidly Obese Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lean Subjects, n=66</th>
<th>Obese Subjects, n=56</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD, cm</td>
<td>4.57±0.46</td>
<td>5.04±0.53</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LVESD, cm</td>
<td>2.65±0.36</td>
<td>3.03±0.51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Interventricular septum</td>
<td>0.86±0.14</td>
<td>0.98±0.24</td>
<td>0.003</td>
</tr>
<tr>
<td>Relative wall thickness, cm</td>
<td>0.44±0.51</td>
<td>0.39±0.09</td>
<td>0.494</td>
</tr>
<tr>
<td>LV mass, g/m² (lean)</td>
<td>77.1±16.6</td>
<td>49.3±14.1</td>
<td>n.a.</td>
</tr>
<tr>
<td>E/e</td>
<td>1.49±0.48</td>
<td>1.26±0.38</td>
<td>0.008</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>4.40±1.83</td>
<td>9.68±3.77</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD or n (%). n.a. indicates not available; LA, left atrium; LV, left ventricle; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter.

in the lean group. The majority (84.6%) of obese patients had associated comorbidities with the most frequent being as follows: hypertension (44.6%), dyslipidemia (35.7%), and diabetes mellitus (32.1%). Only 17.8% of the lean subjects showed comorbidities, with diabetes mellitus (9%) and hypertension (6%) being the most frequent ones (Table 1). Drug treatment for hypertension included angiotensin converting enzyme inhibitors (or angiotensin II type 1 receptor antagonists), whereas all dyslipidemic patients were on statins.

Systolic and diastolic blood pressure levels and heart rate were higher in obese than in lean subjects (Table 1). With regard to the metabolic profile, fasting plasma levels of glucose, triglycerides, insulin, and in consequence homeostasis model assessment-insulin resistance (HOMA) index were higher in obese than in lean subjects (Table 1).

Echocardiography Findings in Patients

Structural Characteristics

Morbidly obese patients showed an increase in LV end-diastolic diameter, LV end-systolic diameter, and average thickness of the interventricular septum, but no differences were observed in relative wall thickness (Table 2). The average volume of the left atrium was higher in obese patients than in lean ones. Overall, mean values of indexed LV mass were within normal ranges in both groups.

Normal LV geometric pattern was observed in 50.0% of obese patients when compared with 76.9% observed in lean individuals (Table 2). The remaining 50.0% had some form of ventricular remodeling, with eccentric hypertrophy (which was present in 18% of patients) and concentric hypertrophy (which was present in 20% of patients) being the most prevalent.

Myocardial and Diastolic Function

LV ejection fraction was lower in obese patients than lean patients although inside normal values. No differences in shortening fraction, stroke volume, or cardiac output were observed between both groups (data not shown). E/A ratio was lower, and E/e ratio was higher in morbidly obese than in normoweight subjects (Table 2).

Circulating Markers in Plasma From Patients

As shown in Table 3, obese patients had higher serum levels of C-reactive protein (CRP), aminoterminal portion of pro–brain natriuretic peptide, and Gal-3 than lean subjects. However, type I C-terminal collagen propeptide and the ratio of matrix metalloproteinase 2/tissue inhibitor of metalloproteinase 2 complex values were lower in obese than in lean subjects. No changes were observed in neutrophil gelatinase-associated lipocalin levels between both groups (Table 3).

Determinants of LV Hypertrophy: Multivariate Logistic Analysis

After adjustment for epidemiological and clinical and metabolic characteristics (sex, HOMA index, obesity, hypertension, aminoterminal portion of pro–brain natriuretic peptide, CRP, Gal-3, C-terminal collagen propeptide, neutrophil gelatinase-associated lipocalin, and matrix metalloproteinase 2/tissue inhibitor of metalloproteinase 2 concentrations), the independent factors to predict LV hypertrophy were obesity (odds ratio, 10.87; 95% CI, 2.20–53.73; P=0.003) and hypertension (odds ratio, 2.85; 95% CI, 0.87–9.37; P=0.084).

Determinants of Diastolic Function: Linear Regression Analysis

The factors associated with diastolic function were defined by a linear regression analysis. The independent association of obesity was tested among other significant structural, functional, and metabolic covariates (hypertension, LV hypertrophy, HOMA index, aminoterminal portion of pro–brain natriuretic peptide, CRP, Gal-3, C-terminal collagen propeptide, neutrophil gelatinase-associated lipocalin, and matrix metalloproteinase 2/tissue inhibitor of metalloproteinase 2 concentrations). Independent predictors of E/e ratio were LV hypertrophy (mean difference, 1.75; 95% CI, 0.40–3.46; P=0.045), HOMA index (mean difference, 1.33; 95% CI, 0.43–2.23; P=0.004), CRP (mean difference, 0.741; 95% CI, 0.1–1.38; P=0.024), and Gal-3 (mean difference, 0.157; 95% CI, 0.015–0.300; P=0.031).

Effect of Gal-3 Activity Inhibition on Body Weight, Blood Pressure, and Cardiac Function in HFD-Fed Rats

To progress in the understanding of the role of Gal-3 in the cardiovascular changes associated with obesity, we characterized its involvement in rats through the pharmacological inhibition of Gal-3. For this purpose, we used a model of diet-induced obesity previously described in which no modifications in
blood pressure were observed\(^4\,17\) to avoid any potential confounding on cardiac fibrosis and inflammation.

General hemodynamic and cardiac parameters of rats are presented in Table 4. The HFD induced an increase in body weight that reached a significant difference from the second week. This difference was maintained until the end of the study (Table 4). MCP administration was unable to modify the effect of HFD on body weight (Table 4). Obese animals showed cardiac hypertrophy characterized by higher relative cardiac weight than control animals (Table 4), an effect that was not prevented with the pharmacological inhibition of Gal-3. MCP was also unable to modify the increase in cardiomyocyte cross-sectional area induced by HFD (Table 4). No differences were found in either echocardiographic parameters or systolic blood pressure in any of the groups studied (Table 4).

The expression of Gal-3 in the heart and aorta was investigated in diet-induced obese rats. HFD rats presented increased cardiac (Figure 1A) and vascular (Figure 1B) Gal-3 expression at mRNA and protein levels. The Gal-3 inhibitor MCP treatment abolished cardiac (Figure 1A) and aortic (Figure 1B) Gal-3 increase observed in obese animals at mRNA and protein levels.

**Inhibition of Gal-3 Blocks Myocardial Fibrosis, Inflammation, and Superoxide Anion Levels in HFD Rats**

HFD rats presented an increase in cardiac interstitial collagen when compared with control ones. This fibrosis observed in obese animals was prevented by MCP treatment (Figure 2A). These results on fibrosis were confirmed by the complementary analysis of cardiac mRNA and protein expression of ECM components. Diet-induced obesity enhanced mRNA and protein levels of collagen type I and the 2 profibrotic markers transforming growth factor-\(\beta\) and connective tissue growth factor (Figure 2B). Cardiac Gal-3 protein levels were correlated with collagen content (\(r=0.692;\ P=0.003\) showing the possible role of Gal-3 in cardiac fibrosis observed in obese rats.

In addition, HFD rats presented cardiac increase in 2 proinflammatory markers, such as osteopontin and chemokine (C-C motif) ligand 2 (CCL2), at mRNA and protein levels (Figure 2C). The pharmacological inhibition of Gal-3 blocked the increase in the inflammatory markers induced by HFD (Figure 2C). In addition, Gal-3 values were associated with osteopontin ones (\(r=0.621;\ P=0.003\) and CCL2 (\(r=0.649;\ P=0.007\)).

HFD rats presented an increase in the intensity of fluorescence induced by dihydroethidium, suggesting higher cardiac levels of \(O_2^-\) in the obese animals, an effect that was prevented with the pharmacological inhibition of Gal-3 (Figure S1A in the online-only Data Supplement).

**Inhibition of Gal-3 Blocks Aortic Fibrosis, Inflammation, and Superoxide Anion Levels in HFD Rats**

Diet-induced obese animals showed important changes in the aortic wall. HFD rats presented vascular collagen deposition (Figure 3A) and increased mRNA and protein levels of ECM components, such as collagen type I, transforming growth factor-\(\beta\), and connective tissue growth factor (Figure 3B). MCP treatment normalized all the fibrosis markers studied. As that occurs at cardiac level, aortic protein Gal-3 levels were correlated with collagen content (\(r=0.745;\ P<0.0084\)).

The aorta from the HFD group presented an increase at mRNA and protein levels of the inflammatory markers osteopontin and CCL2 (Figure 3C), whereas MCP-treated rats presented similar levels when compared with controls. Osteopontin and CCL2 levels were correlated with aortic Gal-3 levels (0.794; \(P=0.0007\) and 0.832; \(P<0.0002\), respectively).

Aorta from HFD rats presented an increase in aortic production of \(O_2^-\), an effect that was prevented by MCP treatment (Figure S1B).

**Discussion**

This study investigates the role of Gal-3 in the cardiovascular remodeling associated with obesity. Our data show that obesity upregulates Gal-3 in the cardiovascular system. The pharmacological inhibition of Gal-3 activity decreases fibrosis and inflammation at cardiac and vascular levels in normotensive obese rats without affecting body weight gain. Interestingly, morbidly obese patients show high circulating Gal-3 levels that were independent predictors of diastolic dysfunction. Thus, Gal-3 emerges as one of the factors involved in the cardiovascular damage associated with obesity.

Increased circulating Gal-3 levels have been reported in different conditions, such as HF and obesity that can be considered a stage A of HF\(^9\,10\,13\,18\,19\) Accordingly, we found that circulating Gal-3 levels are increased in morbidly obese patients. Interestingly, Gal-3 levels were an independent factor involved in diastolic function supporting a role of Gal-3 in obesity cardiomyopathy. Fibrosis is a common characteristic of the cardiac damage associated with obesity\(^4\,20\,21\) which facilitates the development of diastolic dysfunction. Therefore, Gal-3 could participate in the obese cardiomyopathy, facilitating the development of cardiac fibrosis and inflammatory state. However, other possible actions could be involved because in patients with HF of hypertensive origin, no correlation has been reported between myocardial or plasma Gal-3 with myocardial collagen and circulating biomarkers\(^19\). We have found

**Table 3. Circulating Levels of CRP, NT-proBNP, Galectin-3, Type I CICP, MMP2/TIMP2, and NGAL in Lean Control Subjects and Morbidly Obese Patients**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lean Subjects, n=86</th>
<th>Obese Subjects, n=56</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/dL</td>
<td>0.15±0.36</td>
<td>1.32±1.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL</td>
<td>7.7±7.63</td>
<td>15.78±15.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Galectin-3, ng/mL</td>
<td>10.2±2.8</td>
<td>16.8±6.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CICP, ng/mL</td>
<td>94.1±37.2</td>
<td>58.4±22.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MMP2/TIMP2, µg/mL</td>
<td>349.9±110.5</td>
<td>210.9±44.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NGAL, µg/mL</td>
<td>113.6±106.2</td>
<td>134.9±58.6</td>
<td>0.186</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. CICP indicates C-terminal collagen propeptide; CRP, C-reactive protein; NT-proBNP, aminoterminal portion of pro-brain natriuretic peptide; MMP2/TIMP2, matrix metalloproteinase 2/tissue inhibitor of metalloproteinase 2; and NGAL, neutrophil gelatinase-associated lipocalin.
a reduction in the matrix metalloproteinase 2/tissue inhibitor of metalloproteinase 2 complex levels in obese patients when compared with normoweight individuals, suggesting a minor ECM degradation. However, a reduction in C-terminal collagen propeptide levels was surprisingly observed in obese patients; a similar finding was found in patients with a less severe type of obesity in which an increase in collagen type III turnover was observed to be associated with diastolic dysfunction. Therefore, our results suggest that overall production of collagen is not hyperenhanced at the timing of the measurement in morbidly obese patients. This is not in disagreement with the existence of fibrosis that may have developed and established earlier in the course of the cardiac adaptations of this severe form of obesity. Furthermore, an established cardiac fibrosis could explain our previous findings of persistent diastolic dysfunction despite LV mass decrease in patients with bariatric surgery–induced weight loss.23

HOMA index, CRP levels, and LV hypertrophy are associated with a worsening of diastolic function in morbidly obese patients. This association between insulin resistance, low-grade inflammation, and LV hypertrophy has been previously reported not only in clinical but also in experimental studies.24–26 Neither obesity nor hypertension seems to be determinants of diastolic function in this study, as opposed to what was previously observed in patients with class I obesity,22 indicating that the degree of adiposity could matter.

In agreement with previous studies,22,27–30 our data show that both morbid obesity and hypertension are the 2 factors associated with LV hypertrophy—either concentric or eccentric—which is the main way of cardiac remodeling (38%) observed in these patients. In keeping with this, we have observed that the weight loss by bariatric surgery is accompanied by significant improvement in LV remodeling.23

To support the theory of a time-course dependent development of cardiac fibrosis, we studied the role of Gal-3 in an animal model of 6-week diet-induced obesity. Our results show a role of Gal-3 in the cardiac fibrosis associated with obesity because a correlation was found between cardiac Gal-3 levels and collagen content and inhibition of Gal-3 activity prevent the increase in cardiac fibrosis in the obese normotensive animals even in the presence of a body weight increment similar to that in the vehicle-treated animals. Moreover, obesity upregulates Gal-3 levels at both mRNA and protein levels in the cardiovascular system. A cardiac increase in Gal-3 has been previously reported not only in obese rats4 but also in mice after acute myocardial infarction13 or autoimmune myocarditis32 and experimental hyperaldosteronism that was accompanied by fibrosis. Blockage or knockout of Gal-3 prevents the development of cardiac fibrosis in response to aldosterone.14 Similarly, high Gal-3 levels were found in LV from patients

Table 4. Effect of the Inhibition of Galectin-3 Activity With MCP (100 mg/kg per day) on Body Weight, Relative Heart Weight, Echocardiographic Parameters, and SBP in Rats Fed a Standard Diet (CT) or a HFD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CT</th>
<th>HFD</th>
<th>HFD+MCP</th>
<th>MCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>320.4±21</td>
<td>372.7±21†</td>
<td>385.7±17†</td>
<td>328.4±21</td>
</tr>
<tr>
<td>HW/TL, g/cm</td>
<td>0.22±0.02</td>
<td>0.25±0.02*</td>
<td>0.25±0.03*</td>
<td>0.22±0.03</td>
</tr>
<tr>
<td>IVT, mm</td>
<td>1.44±0.15</td>
<td>1.51±0.13</td>
<td>1.51±0.13</td>
<td>1.42±0.17</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>1.66±0.24</td>
<td>1.55±0.14</td>
<td>1.64±0.20</td>
<td>1.64±0.18</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>5.91±0.80</td>
<td>6.44±0.22</td>
<td>6.05±0.29</td>
<td>6.03±0.27</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>2.70±0.41</td>
<td>3.13±0.39</td>
<td>2.99±0.31</td>
<td>3.03±0.33</td>
</tr>
<tr>
<td>EF, %</td>
<td>89.9±2.8</td>
<td>86.7±4.3</td>
<td>86.1±4.1</td>
<td>85.1±3.9</td>
</tr>
<tr>
<td>FS, %</td>
<td>55.4±3.9</td>
<td>51.4±3.8</td>
<td>50.5±5.4</td>
<td>49.2±5.1</td>
</tr>
<tr>
<td>MWFS, %</td>
<td>13.9±2.9</td>
<td>13.1±2.7</td>
<td>11.7±3.7</td>
<td>12.6±3.2</td>
</tr>
<tr>
<td>CCSA, μm²</td>
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<td>369.6±41.8*</td>
<td>368.6±25.7*</td>
<td>314.9±23.7</td>
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<tr>
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Data values represent mean±SD of 8 animals. CCSA indicates cardiomyocyte cross-sectional area; CT, standard diet; EDD, end-diastolic diameter; EF, ejection fraction; ESD, end-systolic diameter; FS, fractional shortening; HFD, high-fat diet; HW, heart weight; IVT, interventricular septum thickness; MCP, modified citrus pectin; MWFS, midwall fractional shortening; PWT, posterior wall thickness; SBP, systolic blood pressure; and TL, tibia length.

Figure 1. Cardiac and aortic expression of galectin-3 (Gal-3). Gal-3 mRNA and protein levels from rats fed a standard diet (CT) or a high-fat diet (HFD) and treated with vehicle or with the inhibitor of Gal-3 activity (modified citrus pectin [MCP], 100 mg/kg per day) in the heart (A) and in the aorta (B). Representative images of the immunohistochemical analysis for Gal-3 are presented (magnification, ×40). Histogram bars represent the means±SD of 6 to 8 animals in arbitrary units (A.U.) or as a percentage of staining normalized to hypoxanthine-guanine phosphoribosyltransferase or β-actin for cDNA and protein, respectively. *P<0.05 and †††P<0.001 vs control group. †P<0.001 vs HFD group.
with advanced HF, which were accompanied by fibrosis.\textsuperscript{19,33} In addition, the inhibition of Gal-3 activity normalized CCL2, osteopontin, and superoxide anion levels at both cardiac and vascular levels in obese animals supporting that Gal-3 could also participate in cardiovascular remodeling associated with obesity through its proinflammatory and prooxidants actions. Therefore, our results build and extend on the context of obesity, an initial stage of HF, previous observations in which Gal-3 is associated with cardiac fibrosis in different settings, including nonischemic dilated cardiomyopathy, hyperaldosteronism, HF, or acute myocardial infarction.\textsuperscript{34–37}

**Perspectives**

Gal-3, which is overexpressed in the cardiovascular system in obesity, emerges as a new molecule for regulating cardiovascular fibrosis and inflammation development associated with diet-induced obesity. This study highlights the relevance of Gal-3 in the cardiovascular alterations associated with obesity, a risk factor for and a direct cause of HF, and demonstrates the beneficial effects of Gal-3 blockade in this pathological context, representing a novel therapeutic target in cardiac fibrosis and inflammation.

**Study Limitations and Clinical Implications**

Some limitations of the present analysis deserve to be concisely mentioned. Whether circulating Gal-3 levels reflected the local levels at the heart is not well established because of the variety of results obtained in different clinical conditions (patients with preserved or reduced LV ejection fraction and different LV remodeling), limiting the interpretation of

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**Figure 2.** Pharmacological inhibition of galectin-3 (Gal-3) blocked cardiac fibrosis and inflammation in obese rats. Representative microphotographs of sections stained with picrosirius red and quantification of collagen volume fraction (CVF) in the heart (A) from a standard diet (CT) and high-fat diet (HFD) rats treated with vehicle or with the inhibitor of Gal-3 activity (modified citrus pectin [MCP], 100 mg/kg per day; magnification, ×40). Effects of MCP treatment on cardiac fibrosis markers collagen type I, transforming growth factor-β (TGF-β), and connective tissue growth factor (CTGF) at mRNA and protein levels (B) and on inflammatory markers osteopontin (OPN) and chemokine (C-C motif) ligand 2 (CCL2) at mRNA and protein levels (C). Histogram bars represent the means±SD of 6 to 8 animals in arbitrary units (A.U.) or as a percentage of staining normalized to hypoxanthine-guanine phosphoribosyltransferase or β-actin for cDNA and protein, respectively. *P<0.05, **P<0.01, and ***P<0.001 vs control group. †P<0.05 and †††P<0.001 vs HFD group.
the results. In experimental models, although a parallel increase has been found in cardiac and circulating levels of Galectin-3 (Gal-3) in mice with proinflammatory conditions, such as acute myocardial infarction or autoimmune myocarditis, the lack of specific methods for the measurement of circulating Gal-3 levels in rats made it difficult to confirm in the present conditions. We did not examine the markers of collagen turnover locally in the heart, which may ultimately demonstrate cardiac fibrosis in morbidly obese patients because serum levels are not heart specific. However, animal studies clearly demonstrated the presence of cardiac fibrosis and the efficacy of Gal-3 activity inhibition in the reduction of collagen content even in absence of body weight changes or hypertension.

Although most patients in both groups of the clinical study were women, the experimental study was performed only in male rats; however, considering the sex distribution of the patients in the clinical study did not affect the variable analysis. Therefore, further work on the demonstration of ECM in this subset of patients would help clarify the underlying mechanisms because an early development of cardiac fibrosis would have a prominent role in the approach of obesity-related heart damage.

Acknowledgments
We thank Encarna Muñoz-Ferrero and Blanca Martínez for their technical help and Anthony DeMarco for his help in editing the article.
Sources of Funding

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Disclosures

None.

References

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**Novelty and Significance**

**What Is New?**

- Circulating galectin-3 (Gal-3) levels that are upregulated in obese patients are an independent predictor of diastolic dysfunction.
- Gal-3 blockade protects against cardiac and vascular fibrosis and inflammation in an experimental model of diet-induced obesity.

**What Is Relevant?**

- Gal-3 could be a new biomarker of diastolic dysfunction in obese patients.
- This study provides a new pharmacological agent that attenuates cardiac and vascular fibrosis and inflammation in situations of obesity even in the absence of blood pressure or functional changes in the cardiovascular system.

**Summary**

This study demonstrates that Gal-3 levels are increased in obese patients and plays an important role in the cardiovascular remodeling associated with obesity and could have important consequences on cardiac function. The inhibition of Gal-3 activity decreased cardiac and vascular fibrosis and inflammation in normotensive obese animals.
Galectin-3 Participates in Cardiovascular Remodeling Associated With Obesity

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GALECTIN-3 PARTICIPATES IN CARDIOVASCULAR REMODELING ASSOCIATED WITH OBESITY

Ernesto Martínez-Martínez1*, Natalia López-Ándres1,2*, Raquel Jurado-López3, Elodie Rousseau2, Mará Visitación Bartolomé4, Amaya Fernández-Celis1, Patrick Rossignol2, Fabian Islas5, Alfonso Antequera6, Santiago Prieto7, María Luaces59 Victoria Cachofeiro3

*The first two authors contributed equally to this work
†The last two authors contributed equally to this study.

1 Cardiovascular Translational Research. Navarrabiomed (Fundación Miguel Servet). Pamplona. Spain;

2INSERM, Centre d’Investigations Cliniques- Plurithématique 1433, UMR 1116 Universitè de Lorraine, CHU de Nancy, and INI-CRCT (Cardiovascular and Renal Clinical Trialists), Nancy, France.

3Physiology Department, Facultad de Medicina, Universidad Complutense, Madrid, Spain. Instituto de Investigación Sanitaria Gregorio Marañón (IIISGM). Madrid. Spain.

4 Ophthalmology and otolaryngology Department. Facultad de Psicología, Universidad Complutense, Madrid, Spain


6 Upper Gastroenterology & Bariatric Surgery Department. Madrid, Spain.

7 Clinical Analysis Department. Hospital Universitario de Fuenlabrada. Madrid, Spain.

Corresponding Author: Natalia López-Andrés. Cardiovascular Translational Research. Navarrabiomed (Miguel Servet Foundation). C/Irunlarrea 3, 31008 Pamplona, Spain. Telephone: 34-848422359. Fax: 34-848422300 E-mail: natalia.lopez.andres@navarra.es

This file includes:

1 supplemental method
5 supplemental references
1 supplemental table
1 supplemental figure
METHODS

Study Population

Morbidly obese patients referred to bariatric surgery were consecutively recruited from the Obesity Care Unit of Fuenlabrada University Hospital, Spain. The selection of the patients was performed by Committee, which includes personnel from Endocrinology, general and upper gastroenterology surgery, internal medicine and cardiology services. Inclusion criteria: ≥ 18 years and universally accepted indications for bariatric surgery, long-term obesity (more than 4 years); body mass index (BMI) ≥ 40 despite other weight loss strategies or BMI ≥ 35 in the presence of obesity-related comorbidities (diabetes mellitus, obesity hypoventilation syndrome, obstructive sleep apnea syndrome, hypertension). Exclusion criteria were age > 60 years and unacceptable surgical risk due to concomitant comorbidities. Volunteers (BMI ≤ 25) were recruited from staff of the Hospital. The study protocol was approved by the Ethics Committee, and all participants signed the informed consent. The study was conducted in compliance with Good Clinical Practice guidelines and the ethical principles stated in the Declaration of Helsinki.

Anthropometric Measurements

All participants underwent physical examination which included anthropometric measurements, blood tests, 12-lead electrocardiogram (ECG), and transthoracic echocardiogram. The height and weight of patients were recorded when taking the echocardiogram, with the patients wearing light clothing without shoes. We used wall scales. After 10 min rest in a sitting position, a 12-lead ECG was taken and blood pressure was measured in the non-dominant arm. BMI was calculated according to the formula: weight (kg)/height squared (meters).

Echocardiogram

Echocardiography studies were performed using a commercially available unit Vivid I (GE Healthcare, Waukesha, WI, USA) equipped with a 2.5 MHz probe. Transthoracic echocardiography study was performed according to the recommendations of the European Society of Echocardiography. The mass of the left ventricle (LV) in grams was calculated using the Devereaux et al. formula:

\[
\text{Mass of LV (g)} = 0.8 \times 1.04 \times [(LVEDD+dPW)^3-LVEDD^3] \times 0.6
\]

where LVEDD is the LV end-diastolic diameter and dPW LV is the posterior wall thickness at end diastole.

The LV mass was indexed by the body surface area to the power of 2, in normoweight subjects and to the power of 2.7 in obese patients to minimize the interference of obesity in the estimate of ventricular mass. The relative parietal thickness (RPT) was calculated using the formula:

\[
RPT = (dIVS-dPW)/LVEDD
\]
where dIVS is the interventricular septal wall thickness in diastole.

An indexed value for LV mass $\geq 45$g/m$^2$ for both male and female normoweight subjects and $\geq 51$g/m$^2$ for both male and female obese patients, and RPT $\geq 0.45$ were considered to be defining values for concentric hypertrophy.

According to the RPT and the indexed mass for the LV, 4 LV geometric patterns were defined:

- 1. Normal: RPT < 0.45 and an indexed LV mass $< 51$g m$^{-2.7}$.
- 2. Concentric remodelling: RPT $\geq 0.45$ and an indexed LV mass $< 51$g m$^{-2.7}$.
- 3. Concentric hypertrophy: RPT $\geq 0.45$ and an indexed LV mass $\geq 51$g/m$^{2.7}$.
- 4. Eccentric hypertrophy: RPT $< 0.45$ and an indexed LV mass $\geq 51$g m$^{-2.7}$.

The volume of the left atrium (LA) was calculated according to validated formulas.$^{1,2}$ Doppler analysis was performed according to standard recommendations.$^{2}$ The average of the measurements for 3 consecutive cardiac cycles was calculated for each value. The assessment of echocardiographic measurements was performed by a single observer under masked conditions. All measurements were performed in a post-processing workstation EchoPAC® (GE Healthcare, Waukesha, WI, USA).

**Laboratory Tests**

Blood samples were obtained following the clinical protocol approved for bariatric surgery. Briefly, venous blood samples (20 mL) were collected after an overnight fast (>10 hours) between 7:00 a.m. and 9:00 a.m. into vacutainers Rapid Serum Tubes. Serum was separated from whole blood by centrifugation (20 minutes at 300 g) and stored at $-80^\circ$C until analysis. Routine hematologic and blood chemistry were performed with standard methods. C-reactive protein (CRP) serum levels were measured by EIA with highly sensitive latex based turbidimetric immunoassay on a Hitachi analyzer (Sigma Chemical). Serum levels of Pro-BNP (MilliplexMAP; Millipore Corporation), Gal-3 (BG Medicine), type I C-terminal collagen propeptide (CICP; Micrvue bone health) and the metalloproteinase 2 tissue inhibitor of metalloproteinases 2 (MMP-2/TIMP-2; Complex (R&D Systems) were measured with specific EIA according to the manufacturer’s protocols. All samples were run in duplicate and analyzed on the same day to minimize day-to-day variation.

**Animal study**

Male Wistar rats of 150g (Harlan Ibérica, Barcelona, Spain) received either a high-fat diet (HFD, 33.5% fat; Harlan Teklad #TD.03307, MN, USA; n=16) or a standard diet (3.5% fat; Harlan Teklad #TD.2014, MN, USA; n=16) for 6 weeks. Half of the animals of each group received the Gal-3 activity inhibitor, modified citrus pectin (MCP; 100 mg/kg/day) in the drinking water for the same period as previously described.$^{3}$ The Animal Care and Use Committee of Universidad Complutense de Madrid approved all experimental procedures according to guidelines for ethical care of experimental animals of the European Community. Body weight was measured once a week. Food and water intake were determined throughout the experimental period. Blood pressure (SBP) was estimated basally, at mid-study and end-of-study through use of a tail-cuff plethysmograph (Narco Bio-Systems) in unrestrained animals as previously reported.$^{4}$
Evaluation of cardiac structure and function

Cardiac structure and function were evaluated by transthoracic echocardiography with a Philips CX50 (Philips, Netherlands) connected to a L12-3 MHz linear transducer in rats anesthetized with isoflurane (2%). 2D-guided M-mode recordings made from short axis views in order to measure LV chamber dimensions, interventricular septum (IVT) and posterior wall thickness (PWT) were measured from the bidimensional parasternal long-axis view. The mean measurements from several consecutive beats were used for data analysis.

Left ventricular ejection fraction (LVEF) was calculated according to the Teicholz Formula: \( (\text{EDD}^3 \times 7) / (2.4 + \text{EDD}) \) and LV systolic chamber function (pump function) was determined from LV endocardial fractional shortening (FS) = \( (\text{EDD} - \text{ESD}) / \text{EDD} \times 100 \) and LV midwall fraction (MWFS) = \( (\text{EDD}/2 + \text{PWTd}/2) - (\text{ESD}/2 + \text{PWTs}/2) / (\text{EDD} + \text{PWTd}/2 + \text{IVT}/2) \), where EDD is end-diastolic diameter in left ventricle, ESD is end-systolic diameter in LV, PWTd is posterior wall thickness in diastole and PWTs is posterior wall thickness in systole.

Morphological and histological evaluation

Cardiac and aorta tissue samples were dehydrated, embedded in paraffin and cut in 4 \( \mu \text{m} \)-thick sections. Both sections were stained with picrosirius red by routine methods. The area of cardiac interstitial fibrosis was identified as the ratio of interstitial fibrosis or collagen deposition to the total tissue area after excluding the vessel area from the region of interest. Aorta fibrosis was only quantified in the media layer as the ratio of interstitial fibrosis or collagen deposition to the total media area. For each sample, 10 to 15 fields were analyzed with a 40X objective under transmitted light microscope (Leica DM 2000; Leica AG, Germany). Myocytes (60–80 per animal) with visible nucleus and intact cellular membranes were chosen for determination of cross-sectional area in cardiac sections stained with hematoxylin and eosin. Quantitative analysis was performed using an analysis system (Leica LAS 4.3; Leica AG, Germany). A single researcher unaware of the experimental groups performed the analysis.

Real-time PCR

Total RNA was extracted with Trizol Reagent (Euromedex) and purified using the RNeasy kit, according to the manufacturer’s instructions (Qiagen). First strand cDNA was synthesized according to the manufacturer’s instructions (Roche). Quantitative PCR analysis was then performed with SYBR green PCR technology (ABGene) (Table S1).

Relative quantification was achieved with MyiQ (Bio-rad) software according to the manufacturer's instructions. Data were normalized by HPRT levels and expressed as percentage relative to controls. All PCRs were performed at least in triplicate for each experimental condition.

Western Blot

Total proteins were prepared as previously described from either cardiac or aortic homogenates from obese and control rats. Proteins were separated by SDS-PAGED on
10 % polyacrylamide gels and transferred to Hybond-c Extra nitrocellulose membranes (Hybond-P; Amersham Biosciences, Piscataway, NJ). Membranes were probed with primary antibody for Gal-3 (Thermo Scientific, Rockford, IL; dilution 1/2000), collagen I (AbDSerotec, Oxford, UK; dilution 1/1000), fibronectin (Millipore, Temecula, CA, USA; dilution 1/500), transforming growth factor β (TGF-β; Abcnnple, Cambridge, UK; dilution 1/500), connective tissue growth factor (CTGF; Torrey Pines BiolabsInc, East Orange, NJ; dilution 1/1000), osteopontin (OPN; Santa Cruz, CA, USA; dilution 1/500), monocyte chemotactic protein-1 (CCL2; Santa Cruz, CA, USA; dilution 1/500) and α-tubulin (Sigma; dilution 1/1000) as a loading control. Signals were detected using the ECL system (Amersham Pharmacia Biotech). Results are expressed as an n-fold increase over the values of the control group in densitometric arbitrary units.

Detection of superoxide anion levels

Briefly, cardiac and aortic sections (14 μm) were equilibrated in Krebs-HEPES buffer (in mmol/L: NaCl 130, KCl 5.6, CaCl2 2, MgCl2 0.24, HEPES 8.3, glucose 11, pH 7.4). Fresh buffer containing DHE (5x10⁻³ mmol/L, 30 min, 37°C) was then added and viewed by either fluorescent laser scanning microscope (40X objective in a Leica DMI 3000 microscope) or inverted Leica TCS SP2 confocal laser scanning microscope with oil immersion lens (image size 379x379 μm). Quantitative analysis of O₂⁻ production was performed with image analyzer (LEICA Q550 IWB). Three sections per animal were quantified and averaged for each experimental condition. The mean fluorescence densities in the target region were analyzed. Results are expressed as an n-fold increase over the values of the control group in arbitrary units.

Statistical Analysis

Continuous variables are expressed as mean±standard deviation or median (interquartile interval) in case of asymmetry. Categorical variables are expressed in absolute values and percentages. The differences between categorical variables were analyzed using the chi-square test. Normality of distributions was verified by means of the Kolmogorov-Smirnov test. In the case of continuous variables, differences between two groups were analyzed by either unpaired Student's t-Test or Mann-Whitney as parametric and non-parametric tests, respectively. Specific differences between more groups were analyzed using Kruskall-Wallis followed by Dunn's test for non-normal distribution variables. For normal distribution variables, one-way analysis of variance was used followed by Tukey test. Either Pearson or Spearman correlation analysis was used to examine association among different variables according to whether they are or not normally distributed, respectively. The factors associated with the left ventricle (LV) hypertrophy were addressed by a logistic model and their OR and 95% confidence interval were calculated. In order to find the factors associated with diastolic function as E/e' ratio, the beta correlation coefficients (slope or mean difference, along with their 95% confidence intervals), were obtained using a linear regression model. In both cases, variables shown to have a statistical significance by univariate analysis and those clinically relevant were included to build the model. A value of P<.05 was used as the cut-off value for defining statistical significance. Data analysis was performed using the statistical program SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).
References


**SUPPLEMENTAL TABLES**

**Table S1: Primers used in rats in real time PCR analysis**

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Figure S1. Impact of Gal-3 inhibition on superoxide anion levels in heart and aorta from control and obese rats. Heart and aorta from rats fed a standard diet (CT) or a high fat diet (HFD) treated with vehicle or with the inhibitor of Gal-3 activity (Modified Citrus Pectin, MCP; 100 mg/Kg/day) were analyzed with either fluorescent laser scanning microscope (40X) or inverted Leica TCS SP2 confocal laser scanning microscope (image size 379x379 μm). Representative microphotographs labeled with DHE and quantification in heart (A) and aorta (B). Bar graphs represent the mean ± SD of 6-8 animals. ***p<0.001 vs. control group. †††p<0.001 vs. HFD group.