CORRESPONDENCE

Galectin-3, Cardiac Function, and Fibrosis

To the Editor-in-Chief:

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We read with interest the article by Frunza et al¹ in *The American Journal of Pathology*.

In this article, the authors studied the expression of galectin-3 (Gal-3) in the well-established transverse aortic constriction (TAC) mouse model. In wild-type (WT) mice, cardiac Gal-3 was shown to be markedly up-regulated, and the level of Gal-3 expression was associated with the severity of adverse cardiac remodeling.

Frunza et al¹ also studied mice deficient for Gal-3 (Gal-3 KO mice). Previously, Gal-3 KO mice have extensively been studied by our group and by others, and it has consistently been reported that Gal-3 KO mice are protected from fibrosis formation in response to profibrotic perturbations, including the heart,^{2–6} kidney,^{4,7–10} liver,¹¹ blood vessels,¹² and lung^{13,14} (Table 1). In contrast to these studies, Frunza et al¹ report that Gal-3 KO mice develop cardiac fibrosis; the reasons for this discrepant result warrant further study.

Furthermore, the authors report that Gal-3 has no role in the development of left ventricular (LV) dysfunction. However, when looking into their results on contractile function, we do not reach the same conclusion. Specifically, the results for LV function are reported as percentage change in left ventricular ejection fraction (LVEF). We assumed a normal LVEF in control mice of 80%. Looking at the results, we then calculated that after 28 days of TAC, LVEF in the Frunza study was decreased by approximately 8% in WT mice (from approximately 80% to approximately 74%), but only by approximately 1% in Gal-3 KO mice (from approximately 80% to approximately 79%). Furthermore, LVEF was decreased in (male) WT mice by approximately 18% (from approximately 80% to approximately 64%), whereas it was actually

improved by approximately 2% in (male) Gal-3 KO mice (from approximately 80% to approximately 82%). After 56 days of TAC, LVEF was decreased by approximately 10% in male WT mice (from approximately 80% to approximately 72%), whereas it was improved by 3% in male Gal-3 KO mice (from approximately 80% to approximately 83%). LV volumes were reported to be numerically larger in WT mice compared to Gal-3 KO mice. The results for females were mostly neutral, although chamber dilatation also appeared delayed in Gal-3 KO mice.

The authors interpret their findings on LV function as neutral because there were no statistically significant differences, and explain these disparate findings by pointing toward sex differences. But we do not agree that the data are disparate. The main issue is to know what the a priori hypothesis regarding LV remodeling was; in other words, what percentage change in LVEF was considered relevant by the authors? Differences in LVEF up to 20% are considered irrelevant by Frunza et al,¹ but from a clinical perspective, 10% or even a 5% change in LVEF is highly relevant, and the reported average outcomes unequivocally point toward a substantial improvement in LVEF in Gal-3 KO mice. It has been proposed that the effect size is the most important parameter to consider, not the *P* value (American Statistical Association, <https://www.amstat.org/newsroom/pressreleases/P-ValueStatement.pdf>, last accessed April 17, 2016). We have reported² exactly the same results regarding contractile function, so that these results by Frunza et al¹ and ours regarding myocardial contractile function after TAC are in fact completely compatible.

The preservation of LVEF in Gal-3 KO mice underlines the notion that Gal-3 may be an amenable factor in heart failure and myocardial fibrosis.¹⁵ But before embarking on

Supported by the Innovational Research Incentives Scheme program of the Netherlands Organization of Scientific Research VIDI grant 917.13.350 (R.A.d.B.) and the Netherlands Heart Foundation Dekker grant 2015 T034 (W.C.M.).

Disclosures: None declared.

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Table 1 Overview of Studies with Galectin-3 KO Mice

Study	Model	Main findings
Cardiac		
Yu et al ²	TAC (pressure overload)	Less fibrosis Preservation of LV function
Yu et al ²	Angiotensin II infusion	Less fibrosis Preservation of LV function
Pineda et al ³	Infection with <i>Trypanosoma cruzi</i> (Chagas)	No fibrotic content
Calvier et al ⁴	Aldosterone infusion	Resistant against aldosterone effects (remodeling)
Martinez-Martinez et al ⁵	Aldosterone infusion	Resistant to cardiac inflammation
Gonzalez et al ⁶	Myocardial infarction	Less fibrosis Larger infarct size
Renal		
Calvier et al ⁴	Aldosterone infusion	Less perivascular fibrosis
Fernandes Bertocchi et al ⁷	45 Minutes of renal pedicle occlusion	Less acute tubular necrosis More prominent tubular regeneration
Iacobini et al ⁸	Aging	Age-dependent increases in proteinuria, albuminuria, and glomerular sclerosis
Iacobini et al ⁹	N-carboxymethyllysine injection	Higher circulating and renal AGE levels Renal dysfunction and structural changes
Henderson et al ¹⁰	Unilateral ureteric obstruction	Comparable macrophage recruitment Less myofibroblast accumulation/activation Less fibrosis
Liver		
Henderson et al ¹¹	Carbon tetrachloride—induced liver injury	Less fibrosis
Lung		
Ge et al ¹³	Repetitive allergen challenge	Less remodeling of the airways with reduced mucus secretion, fibrosis, smooth muscle thickness, and angiogenesis
Mackinnon et al ¹⁴	Bleomycin-induced lung injury	Less lung fibrosis, reduced TGF- β —induced EMT, and myofibroblast activation
Vascular		
Calvier et al ¹²	Aldosterone infusion (6 hours and 3 weeks)	Less inflammation Less collagen type I deposition (Both short- and long-term)

AGE, advanced glycation end product; EMT, epithelial-mesenchymal transition; galectin-3 KO, mice deficient for galectin-3; LV, left ventricular; TAC, transverse aortic constriction; TGF- β , transforming growth factor- β .

further studies, future work is needed to describe the exact dynamics of Gal-3 expression. The results of Frunza et al¹ provide important novel data to this aim.

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<http://dx.doi.org/10.1016/j.ajpath.2016.05.002>

Authors' Reply

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We thank Meijers et al for their letter and for their interest in our article.¹ Our study tested the hypothesis that the β -galactoside binding animal lectin galectin-3 may play an important role in fibrosis, remodeling, and dysfunction of the pressure-overloaded heart. Using an established mouse model of transverse aortic constriction, we found that galectin-3 was markedly up-regulated in the pressure-overloaded myocardium and localized in macrophages, fibroblasts, and cardiomyocytes. Although cardiomyocyte-specific galectin-3 expression correlated with adverse remodeling and dysfunction, genetic disruption of galectin-3

had no effects on survival, systolic and diastolic dysfunction, or fibrosis, but it delayed the hypertrophic response.

Our findings are consistent with the growing body of evidence supporting the role of galectin-3 as a biomarker in clinical heart failure² and reveal a surprising localization of galectin-3 in a subset of cardiomyocytes. However, our observations are in conflict with the rigorous and well-documented studies by the Lopes-Andres and de Boer laboratories, which suggested that galectin-3 is critically involved in the pathogenesis of cardiac remodeling and fibrotic cardiomyopathy after pressure overload.^{3–5} The authors of the letter argue that the statistically nonsignificant 10% to 20% differences in ejection fraction between groups, observed in male mice, may be important from a clinical perspective. On that basis, they suggest that our findings may support the previously reported beneficial effects of galectin-3 loss in cardiac remodeling. Although we appreciate the comments, we disagree with this interpretation of our findings. Clearly, our data do not support a crucial pathophysiological role for galectin-3 in remodeling and dysfunction of the pressure-overloaded heart. Despite a large sample size (wild-type mice, $n = 50$; galectin-3 null, $n = 46$), galectin-3 null and wild-type mice had comparable survival curves after pressure overload and exhibited no statistically significant differences in end points associated with systolic and diastolic dysfunction. We also did not observe statistically significant effects of galectin-3 loss on myocardial fibrosis. In the absence of statistical significance, the null hypothesis cannot be rejected. Relatively modest and statistically nonsignificant differences in isolated specific end points between groups should not be overinterpreted and likely reflect the variability of responses in animal models.

Considering that all three studies used the same strain of galectin-3 knockout mice, the basis for the disparate findings remains unclear. Sex-specific effects of galectin-3 may be part of the explanation. Previously published studies focused exclusively on male mice,^{3,4} whereas our study examined both male and female animals. Age may also be a factor: in the study by Yu et al,³ young (6- to 10-week-old) mice were used, whereas we studied mature adult animals (2- to 4-month-old) in our experiments. Differences in the severity of pressure load between experimental protocols and in the experimental design may also account for the differences. Although Yu et al³ studied a single time point (28 days after transverse aortic constriction), we performed serial analysis at three different time points, ranging from 7 to 56 days. Considering the growing concerns regarding reproducibility of *in vivo* observations, it is crucial for investigators to report their own experience, especially when the findings contradict published literature. The combined experience from rigorous and well-documented studies from several independent groups paints a more realistic picture than any single study.

Do our findings have an impact on the growing interest⁶ on the potential role of galectin-3 as a therapeutic target? Although the absence of a role for galectin-3 in the pathogenesis of cardiac dysfunction and fibrosis observed in our

Supported by NIH grants R01 HL76246 and R01 HL85440.

Disclosures: None declared.

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