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EDITORIAL COMMENT

Determinants of Passive Myocardial Stiffness Along the Spectrum of Aortic Stenosis*

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In their study in this issue of *JACC: Basic to Translational Science*, Gotzmann et al. (1) report alterations in determinants of passive myocardial stiffness in patients with aortic stenosis (AS) undergoing valve replacement.

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Changes in the relaxation and filling properties of the left ventricle (LV) in cardiac hypertrophy and heart failure (HF) have been recognized for decades (2,3). Some of these include alterations in the speed and/or extent of myofilament deactivation. Thus, in patients with heart failure with reduced ejection fraction (HFrEF), abnormalities in both Na-Ca handling (4) and acto-myosin kinetics (5) lead to slowed and/or incomplete relaxation. Impaired myofilament deactivation due to abnormal Na-Ca handling and slowed acto-myosin kinetics has also been reported in patients with hypertension (HTN), concentric LV remodeling, and heart failure with preserved ejection fraction (HFpEF) (6,7).

Altered passive filling properties of the myocardium are also prominent in cardiac hypertrophy and HF. These are determined by the combined effects of collagen and titin, the giant myofilament spring protein that accounts for cardiomyocyte stiffness over the physiological sarcomere length range (8,9). Titin's contribution can be modulated by isoform variation and phosphorylation (10,11). The splice factor RBM20 is responsible for generation of 2 isoforms: the larger, more compliant N2BA, and the smaller N2B isoform (12). Titin phosphorylation occurs at multiple sites in its N2B and PEVK segments (10,11). Protein kinase (PK) A and PKG are active at the same sites; PKC-α is active at different sites. PKA/PKG reduces and PKC-α phosphorylation increases titin stiffness. Phosphorylation can rapidly alter titin and myocardial passive stiffness, for example, during exercise. Other kinases that phosphorylate titin (e.g., ERK2) and other post-translational modifications (e.g., disulfide bond formation) have been reported (10,11). These may be significant in human disease and are an emerging research focus. Recently, aggregation of titin was shown to contribute to elevated cardiomyocyte stiffness in AS and HFrEF (13).

Changes in determinants of passive stiffness have been reported in nonischemic dilated cardiomyopathy (HFrEF). Collagen content is increased (14,15). Titin undergoes a shift to the N2BA isoform that decreases its stiffness (14-16), but a concomitant decrease in N2B phosphorylation (presumably PKA/PKG sites) serves to increase stiffness. Passive myocardial stiffness is decreased in HFrEF (15), which implies that the titin isoform shift is the single most important change.

The syndrome of HFpEF is the most relevant clinical scenario in relation to AS. Pressure overload (HTN) is present in the vast majority of patients, and as in AS, concentric remodeling is present in most (17). In HFpEF, comorbidities (type 2 diabetes, obesity, obstructive sleep apnea, and chronic kidney disease) almost always contribute to impaired LV filling properties (18). Although similar comorbidities are common, AS patients have a purer form of pressure

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overload. Several groups have studied passive myocardial stiffness and its determinants in HFpEF using tissue obtained by epicardial biopsy during cardiac surgery or endomyocardial biopsy at time of cardiac catheterization (9,19-21). Although there is good reason to believe that passive stiffness is increased in HFpEF, its measurement is challenging and requires properly oriented, adequately sized muscle strips. We reported passive stiffness in strips obtained from intraoperative biopsies in patients with normal EF and a history of HTN undergoing coronary bypass surgery (CBG) with and without HFpEF compared with control subjects without HTN undergoing CBG (9). Passive stiffness was markedly increased in HFpEF, but was similar in control subjects and HTN without HFpEF. Similar to Kasner et al. (21), we reported increased collagen content and cross linking in HFpEF.

Paulus et al. (18-20,22) pioneered investigations of titin in HFpEF patients. They reported increased resting tension in cardiomyocytes (endomyocardial biopsies) and decreased phosphorylation of PKA/PKG sites. This was reversed by PKG treatment and associated with reduced PKG activity. They also reported a decrease in the N2BA isoform, presumably outweighed by phosphorylation changes. This was the first demonstration of a role for titin in HFpEF. In our study (9), we did not detect an isoform shift, perhaps because our patients had less advanced disease. We confirmed reduced PKA/PKG site phosphorylation in HFpEF and also documented increased phosphorylation of a PKC-α site. All of these phosphorylation changes increase cardiomyocyte and myocardial stiffness.

Collagen content/fibrosis is increased in AS (23), and it is believed that passive myocardial stiffness is increased (although it has never been measured). However, relatively little is known about titin. Moreover, there is an ongoing debate about the significance of low flow-low gradient AS with respect to whether this indicates severe AS, less-severe AS with impaired contractile function, or measurement inaccuracies. In their report, Gotzmann et al. (1) fill a major knowledge gap by quantifying fibrosis and titin characteristics in endomyocardial biopsy tissue from 3 categories of AS patients, "classical" (high gradient, normal flow, normal LVEF), "paradoxical" aortic stenosis (PAS) (low gradient, low flow, normal LVEF), and reduced EF. Control measurements were made in tissue from brain-dead patients. Fibrosis was increased and similar in all AS groups. A shift toward a higher proportion of N2BA titin was found, serving to reduce passive stiffness. This appeared to be greatest in PAS, but the observation is limited by the relatively small number of patients. The investigators reported a decrease in total titin phosphorylation, which did not appear to differ by AS group. Phosphorylation of a PKA/PKG site (S4185) was significantly reduced in AS patients when taken together. This appeared to be similar across groups; possibly due to small numbers, it was not statistically significant in any single group. This change would be expected to counteract the isoform shift and increase stiffness. There was no change in a PKC-α site phosphorylation. Thus, with respect to determinants of passive stiffness, patients with AS were qualitatively similar to HFpEF patients with regard to fibrosis and titin PKA/PKG site phosphorylation, but not PKC-α site phosphorylation. Because titin isoform results in HFpEF have been inconsistent (9,19), it is unclear whether AS patients resemble HFpEF in this regard. The results in general indicate that patients with PAS are not fundamentally different from other AS patients with regard to determinants of stiffness.

Gotzmann et al. (1) are to be congratulated for conducting these demanding studies. It is critical to understand how and why myocardial function is altered in patients with disease. Animal models are valuable, especially for elucidating mechanisms, but may not reliably reproduce human disease.

There are limitations to this study that should be noted. Passive stiffness was not measured. Because the observed titin isoform shifts should reduce stiffness, it is possible, albeit unlikely, that passive stiffness is not in fact increased in AS. Measurements of cardiomyocyte resting tension also were not performed. As a result, the net effect of titin alterations is unknown. The intriguing recent finding of titin aggregation (13) was also not assessed.

Research employing human myocardial tissue has some inherent limitations. Numbers of patents are often limited and "noise" in the data is often relatively high. The method of obtaining tissue has consequences. Endomyocardial biopsies are small ($<\sim$5 mg) and not suitable for myocardial mechanics studies (e.g., passive stiffness), where undamaged linear strips are preferred. The small volume of tissue can also limit other measurements of interest. The intraoperative biopsies we have employed (9) are larger (25 to 50 mg) and can be sculpted into linear strips, allowing various mechanical measurements. However, these biopsies are limited to patients undergoing cardiac surgery. Thus, as discussed, we have studied coronary bypass surgery patients (9). As pointed out by Gotzmann et al. (1), human biopsy methods are subject to sampling error because it is usually only possible to biopsy 1 site. Last, identifying suitable control subjects can be problematic. Brain-dead patients, as used by Gotzmann et al. (1),

have often been employed. The amount of tissue available is large, and it is usually possible to be confident that they do not have cardiac dysfunction. However, these patients are subject to high stress and have often received cardioactive drugs, for example, pressors. The control coronary bypass surgery patients we have used are difficult to come by and obviously have underlying coronary artery disease.

In summary, Gotzmann et al. (1) have provided important new information in regard to changes in the determinants of passive stiffness in patients with AS. Patients with PAS do not appear to differ fundamentally from other AS patients. Whereas studies using human myocardium may have limitations compared with more mechanistically rigorous animal experiments, there is no substitute for carefully conducted research such as this in patients with heart disease.

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REFERENCES

1. Gotzmann M, Grabbe S, Schöne D, et al. Alterations in titin properties and myocardial fibrosis correlate with clinical phenotypes in hemodynamic subgroups of severe aortic stenosis. J Am Coll Cardiol Basic Trans Science 2018;3:335–46.

2. Lewis GA, Schelbert EB, Williams SG, et al. Biological phenotypes of heart failure with preserved ejection fraction. J Am Coll Cardiol 2017;70:2186–200.

3. Weintraub RG, Semsarian C, MacDonald P. Dilated cardiomyopathy. Lancet 2017;390:400–14.

4. Hasenfuss G, Schillinger W, Lehnart SE, et al. Relationship between Na+-Ca2+-exchanger protein levels and diastolic function of failing human myocardium. Circulation 1999;99:641–8.

5. Hasenfuss G, Mulieria LA, Leavitt BJ, et al. Contractile protein function in failing and nonfailing human myocardium. Basic Res Cardiol 1992;87 Suppl 1:107–16.

6. Runte KE, Bell SP, Selby DE, et al. Relaxation and the role of calcium in isolated contracting myocardium from patients with hypertensive heart disease and heart failure with preserved ejection fraction. Circ Heart Fail 2017;10. pii:e004311.

7. Donaldson C, Palmer BM, Zile M, et al. Myosin cross-bridge dynamics in patients with hypertension and concentric left ventricular remodeling. Circ Heart Fail 2012;5:803–11.

8. Granzier HL, Irving TC. Passive tension in cardiac muscle: contribution of collagen, titin, microtubules, and intermediate filaments. Biophys J 1995;68:1027–44.

9. Zile MR, Baicu CF, Ikonomidis JS, et al. Myocardial stiffness in patients with heart failure and a preserved ejection fraction: contributions of collagen and titin. Circulation 2015;131:1247–59.

10. LeWinter MM, Granzier HL. Titin is a major human disease gene. Circulation 2013;127:938–44.

11. Linke WA, Hamdani N. Gigantic business: titin properties and function through thick and thin. Circ Res 2014;114:1052–68.

12. Guo W, Sun M. RBM20, a potential target for treatment of cardiomyopathy via titin isoform switching. Biophys Rev 2018;10:15–25.

13. Franssen C, Kole J, Musters R, Hamdani N, Paulus WJ. α-β crystallin reverses high diastolic stiffness of failing human cardiomyocytes. Circ Heart Fail 2017;10:e003626.

14. Makarenko I, Opitz CA, Leake MC, et al. Passive stiffness changes caused by upregulation of compliant titin isoforms in human dilated cardiomyopathy hearts. Circ Res 2004;95:708–16.

15. Nagueh SF, Shah G, Wu Y, et al. Altered titin expression, myocardial stiffness, and left ventricular function in patients with dilated cardiomyopathy. Circulation 2004;110:155–62.

16. Borbély A, Falcao-Pires I, van Heerebeek L, et al. Hypophosphorylation of the stiff N2B titin isoform raises cardiomyocyte resting tension in failing human myocardium. Circ Res 2009;104:780–6.

17. Zile MR, Gottdiener JS, Hetzel SJ, et al., for the I-PRESERVE Investigators. Prevalence and significance of alterations in cardiac structure and function in patients with heart failure and a preserved ejection fraction. Circulation 2011;124:2491–501.

18. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. J Am Coll Cardiol 2013;62:263–71.

19. Borbely A, van der Velden J, Papp Z, et al. Cardiomyocyte stiffness in diastolic heart failure. Circulation 2005;111:774–81.

20. Borbely A, Falcao-Pires I, van Heerebeek L, et al. Hypophosphorylation of the stiff N2B titin isoform raises cardiomyocyte resting tension in failing human myocardium. Circ Res 2009;104:780–6.

21. Kasner M, Westermann D, Lopez B, et al. Diastolic tissue Doppler indexes correlate with the degree of collagen expression and cross-linking in heart failure and normal ejection fraction. J Am Coll Cardiol 2011;57:977–85.

22. van Heerebeek L, Hamdani N, Falcao-Pires I, et al. Low myocardial protein kinase G activity in heart failure with preserved ejection fraction. Circulation 2012;126:830–9.

23. Krayenbuehl HP, Hess OM, Monrad ES, Schneider J, Mall G, Turina M. Left ventricular myocardial structure in aortic valve disease before, intermediate, and late after aortic valve replacement. Circulation 1989;79:744–55.

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