High myocardial diastolic stiffness has usually been attributed to excessive myocardial collagen deposition. Over the last decade, stiff cardiomyocytes were also identified as important contributors to high myocardial diastolic stiffness, especially in heart failure (HF) with preserved ejection fraction (HFPEF). Cardiomyocyte stiffness relates to elasticity of the giant cytoskeletal protein titin, which spans the sarcomere from the Z disk to the M line and functions as a bidirectional spring responsible for early diastolic recoil and late diastolic distensibility of cardiomyocytes. In HFPEF patients and in HFPEF animal models, the observed increase in cardiomyocyte stiffness was always accompanied by increased deposition of collagen; therefore, it remained unclear whether impaired elasticity of titin could be solely responsible for high myocardial diastolic stiffness and HFPEF. In this issue of Circulation, however, Chung et al provide compelling evidence for titin being the sole perpetrator in the diastolic dysfunction of an HFPEF mouse model. They generated mice with a deletion of nine immunoglobulin (Ig)-like domains from the proximal tandem Ig segment of the titin spring region (IG KO). This deletion extended the remaining titin spring segments and increased overall titin stiffness. Despite unaltered myocardial collagen content or composition, the IG KO mice developed HFPEF, evident from a reduced exercise tolerance, an enlarged left atrium, and a steeper LV end-diastolic pressure-volume relationship. The elegant study by Chung et al therefore clearly establishes myocardial titin to be able to sufficiently compromise diastolic LV function to induce HFPEF.

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The Adjustable Spring Properties of Titin

Titin, with a molecular mass of up to 3800 kDa, spans half-sarcomeres from the Z disk to the M band and contains a molecular spring segment, the I-band region, that supports early diastolic recoil and late diastolic resistance to stretch (Figure 1A). The I-band region has a complex structure comprising 3 extensible segments: tandem Ig-like domain regions with a proximal and a distal segment, a proline-glutamate-valine-lysine segment, and a unique sequence that is part of the N2B element (N2-Bus) and expressed only in cardiac titin. In the study by Chung et al, 9 Ig-like domains from the proximal tandem Ig segment were deleted. This shortened the overall length of titin and increased titin stiffness. Changes in titin stiffness have also been reported after isoform shifts or posttranslational modifications like phosphorylation or oxidation. The 2 main titin isoforms expressed in human (and other mammalian) hearts are N2B, which is the shorter and stiffer isoform, and N2BA, which is the longer and more compliant isoform. Higher expression of the N2BA titin isoform was reported in eccentrically remodeled hearts of ischemic or dilated cardiomyopathy. In contrast, only a minor shift toward the N2BA titin isoform was observed in concentrically remodeled hearts of HFPEF patients (Figure 1B). Titin also adjusts cardiomyocyte stiffness through posttranslational modifications like phosphorylation or oxidation (Figure 1C and 1D). Protein kinase A and protein kinase G phosphorylate titin at its N2B segment. This allows β-adrenergic stimulation, nitric oxide, or natriuretic peptides to acutely lower cardiomyocyte stiffness. In HFPEF, titin is hypophosphorylated, probably as a result of low myocardial protein kinase G activity induced by low nitric oxide bioavailability and high oxidative stress. Protein kinase Cα phosphorylates titin at its proline-glutamate-valine-lysine region. Protein kinase Cα administration raises the stiffness of cardiomyocytes isolated from normal myocardium but has no effect on cardiomyocytes retrieved from an animal model. Titin can also be phosphorylated by extracellular signal-regulated kinase-2 and at several sites by calcium calmodulin-dependent kinase II. Both extracellular signal-regulated kinase-2 and calcium calmodulin-dependent kinase II lower cardiomyocyte stiffness. The pathophysiological relevance of phosphorylation by protein kinase Cα, extracellular signal-regulated kinase-2, and calcium calmodulin-dependent kinase II for diastolic LV dysfunction in HFPEF remains to be elucidated. Finally, the stiffness of cardiomyocytes also rises in the presence of oxidative stress because of formation within the titin molecule of disulfide bridges, which reduce the contour length of the N2B segment (Figure 1D).

Interstitial Collagen: A Loose or a Tight Fit?

In diastolic LV dysfunction, cardiomyocyte stiffness and collagen deposition are often unequally involved. Resting tension of isolated cardiomyocytes stretched to 2.2-µm sarcomere length (Fpassive) and myocardial collagen volume fraction...
(CVF) were simultaneously assessed in several HF animal models\textsuperscript{5,15,16} and in patients with HFPEF, HF with reduced ejection fraction, and aortic stenosis.\textsuperscript{1,2,17} Titin IG KO mice\textsuperscript{6} and obese ZSF1 rats\textsuperscript{15} are at one end of the spectrum with a 2.0-times increase in $F_{\text{passive}}$ and no change in CVF. Patients with aortic stenosis are at the other end of the spectrum with no change in $F_{\text{passive}}$ and a 2.4-times increase in CVF.\textsuperscript{17} HFPEF patients occupy an intermediate position with a 2.0-times increase in $F_{\text{passive}}$ and a 2.3-times increase in CVF, whereas patients with HF with reduced ejection fraction come close to aortic stenosis patients, presenting with a 1.5-times increase in $F_{\text{passive}}$ and a 2.6-times increase in CVF.

Apart from unequal involvement in different HF phenotypes, cardiomyocyte stiffness and collagen deposition can also vary widely within a single HF phenotype. When HFPEF patients were classified according to myocardial CVF (low, $<5\%$; intermediate, $5\%$–$10\%$; high, $>10\%$), they were equally distributed over the 3 classes of CVF.\textsuperscript{1} Furthermore, LV end-diastolic pressure, circumferential LV end-diastolic wall stress, and radial myocardial stiffness modulus were similarly

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Adjustable spring properties of titin. A, Structural components and phosphorylation sites of N2BA titin. The orange box shows the Ig domain deleted in the study of Chung et al.\textsuperscript{6} B through D, Modulation of cardiomyocyte stiffness (passive-length tension relation) by titin isoform shift (B), by titin phosphorylation (C), and by oxidation (D). CaMKII indicates calcium calmodulin dependent kinase II; ERK2, extracellular signal-regulated kinase-2; N2-Bus, unique sequence; P, phosphorylation site; PKA, protein kinase A; PKC\textalpha, protein kinase C\textalpha; PKG, protein kinase G; and S, sulfide.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Titin- and matrix-based components of the myocardial passive-length tension relation. Myocardial passive-length tension relation and its components in normal myocardium (A), in the presence of a stiffer titin (B), and in the presence of a stiffer matrix (C). $F_{\text{pressure}}$ indicates the resting tension of isolated cardiomyocytes.}
\end{figure}
The very low cGMP content does not seem to be substantially improved by blocking cGMP breakdown, as evident from the Evaluating the Effectiveness of Sildenafil at Improving Health Outcomes and Exercise Ability in People With Diastolic Heart Failure (RELAX) trial, in which sildenafil failed to significantly raise plasma cGMP levels or to ameliorate diastolic LV dysfunction. Restoring myocardial cGMP content probably requires the supply of cGMP to be boosted either by increasing myocardial nitric oxide bioavailability or by increasing natriuretic peptide signaling. The former can be achieved by stringent control of metabolic comorbidities; the latter, by the novel angiotensin receptor neprilysin inhibitor LCZ696, which inhibits the breakdown of natriuretic peptides and was recently shown in a phase II study to improve diastolic LV dysfunction of HFPEF patients. Finally, as insight into post-translational modifications of titin progresses, other new therapeutic in-roads will appear that reduce stiffness of titin and improve diastolic LV dysfunction in HFPEF.

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None.

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Myocardial Titin and Collagen in Cardiac Diastolic Dysfunction: Partners in Crime
Nazha Hamdani and Walter J. Paulus

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