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# Transcriptional and Posttranslational Modifications of Titin
## Implications for Diastole

Attila Borbély, Loek van Heerebeek, Walter J. Paulus

Myocardial diastolic stiffness has been variably attributed to extracellular matrix composition, cytoskeletal properties of cardiomyocytes, or residual diastolic cross-bridge cycling because of incomplete relaxation or cytosolic calcium removal.[1] Extracellular matrix and cardiomyocyte cytoskeleton are presumed to mediate chronic rises in myocardial diastolic stiffness, as occur during aging, pressure overload or heart failure, whereas residual diastolic cross-bridge cycling accounts for acute changes, as observed during ischemia, exercise, or pharmacological interventions. The elegant study by Krüger et al, published in this issue of *Circulation Research*, challenges this conceptual framework.[2] The study demonstrates that protein kinase (PK)G is capable of phosphorylating the giant cytoskeletal protein titin, as previously reported for PKA[3,4] and that phosphorylation by PKG or PKA of a serine residue within the N2B fragment of titin leads to an acute fall in cardiomyofibrillar stiffness. An acute effect produced by a cytoskeletal protein invalidates the concept of distinct mediators for chronic or acute changes in myocardial diastolic stiffness. From these and other recent observations it becomes evident that the cytoskeletal protein titin can alter myocardial diastolic stiffness, both acutely and chronically, through multiple mechanisms such as isoform shifts, phosphorylation by PKG or PKA, and titin–actin interaction at the Z-disc (Figure).

## Isoform Shifts of Titin

As a result of alternative splicing, human myocardium expresses both the stiff N2B and the compliant N2BA titin isoform. Higher expression of the compliant N2BA titin isoform has been observed in dilated cardiomyopathy with eccentric left ventricular (LV) remodeling (ie, low LV mass/volume ratio).[5,6] In these patients, myocardial N2BA/N2B titin isoform ratio correlated with peak oxygen consumption during exercise.[6] This correlation with exercise tolerance was explained by more N2BA and less N2B titin isoform improving diastolic LV distensibility and enhancing LV preload reserve.

The shift from the N2B to the N2BA titin isoform was therefore considered to be a compensatory mechanism correcting for a rise in myocardial stiffness induced by changed composition of the extracellular matrix. This hypothesis was corroborated by in vitro shifts in titin isoform expression observed in primary cardiomyocyte cultures prepared from embryonic rats.[7] In these cultures, the shift from the fetal compliant N2BA titin isoform to the adult stiff N2B titin isoform depended on matrix stiffness as it differed between flexible rubber membranes and rigid plastic surfaces.

Although the compensatory role of titin isoform shifts seems evident in eccentric LV remodeling, similar arguments are lacking for concentric LV remodeling (ie, high LV mass/volume ratio). Patients with diastolic heart failure and concentric LV remodeling had lower N2BA/N2B titin isoform ratios than patients with systolic heart failure and eccentric LV remodeling despite similar myocardial fibrosis.[8] Moreover, in spontaneously hypertensive rats with intense myocardial collagen deposition the N2BA/N2B ratio was lower than normal.[9] Hence, in concentric LV remodeling shifts in titin isoforms no longer seem to correct for elevated myocardial stiffness but seem to contribute to it. Stimuli other than extracellular matrix composition could therefore also be important for transcriptional modification of titin. This was indeed demonstrated in cardiomyocyte cultures,[7] in which 3,5,3′-triiodo-L-thyronine (T3) was a potent stimulus for shifting from the fetal N2BA to the adult N2B titin isoform. Regulation by T3 could also be relevant for failing myocardium because of the marked upregulation of T3 degrading deiodinase in cardiac hypertrophy and failure.[10,11]

## Phosphorylation of Titin

Previous studies showed administration of PKA to reduce resting tension of rat cardiomyocytes[3] and of rat cardiac myofibrils.[4] The fall in resting tension resulted from phosphorylation of the N2B fragment of titin.[3] The present study by Krüger et al[2] extended these observations as it identified the serine residue S469 situated within the N2B fragment of titin to be the site on the titin molecule responsible for the fall in resting tension following phosphorylation by either PKG or PKA. In contrast to PKA, which only phosphorylates titin at serine residue S469, PKG phosphorylates titin at multiple sites. An effect on myocardial passive stiffness by PKG resulted, however, only from phosphorylation of the serine residue S469. The distinct site, through which phosphorylation affects cardiomyocyte stiffness, allows for interactive effects between phosphorylation and isoform shifts and between phosphorylation and titin–actin overlap.

The phosphorylation-induced reduction of cardiomyocyte resting tension is titin isoform–dependent, with the largest

**Figure.** Titin alters cardiomyocyte stiffness through isoform shifts, phosphorylation, and titin–actin interaction. A, Sarcomeric structure with detailed view of I-band region of N2B titin isoform showing tandem immunoglobulin (Ig), N2B, and elastic PEVK segments. B through D, Shift from N2B to N2BA titin isoform (B) and phosphorylation by PKG or PKA at S469 (C) reduce stiffness of the elastic PEVK segment, whereas wider titin–actin overlap at the Z-disc possibly increases stiffness of the elastic PEVK segment (D).

effect observed in rat ventricular myocardium, which has an N2BA/N2B ratio of ≈0.1, and the smallest effect in bovine atrial myocardium, which has an N2BA/N2B ratio of ≈9.[12] These observations are consistent with the reported effects of PKA administration on resting tension of human cardiomyocytes isolated from LV biopsies of patients with systolic and diastolic heart failure.[8] In patients with systolic heart failure, who have a higher N2BA/N2B titin isoform ratio, adminis-

tration of PKA resulted in a smaller drop in cardiomyocyte resting tension than in patients with diastolic heart failure, who have a lower N2BA/N2B titin isoform ratio.

Patients with diastolic heart failure and type 2 diabetes mellitus had significantly higher cardiomyocyte resting tension and wider sarcomeric Z-discs than nondiabetic patients with diastolic heart failure.[13] Z-disc widening had also been observed previously in transgenic mice after nebulin or muscle LIM protein knockout. Z-disc widening could imply altered titin–actin interaction, which is an important determinant of cardiomyocyte resting tension. This was evident from treatment of cardiac myofibrils with gelsolin, which removed actin from the cardiomyocytes. Gelsolin treatment resulted in a fall in steady-state resting tension and dynamic resting tension transients[14,15] because less titin–actin interaction weakened the anchoring of titin in the Z-disc and reduced the internal force on the elastic segments of titin. Cardiomyocyte resting tension still responded to PKA after prior gelsolin treatment.[12] This indicated that phosphorylation of titin could compensate for cardiomyocyte stiffness changes induced both by titin isoform shifts and by titin–actin interaction at the Z-disc. Modified titin–actin interaction could derive not only from Z-disc widening but possibly also from oxidative myocardial damage, as suggested by the presence of nitrated titin and actin fragments in plasma of patients with Chagas heart disease.[16]

## Weakly Bound Crossbridges

Lower cardiomyocyte resting tension following administration of PKG or PKA could result not only from more phosphorylation of titin but also from less actin–myosin interaction of weakly bound crossbridges. An effect of weakly bound crossbridges on resting tension of normal cardiac myofibrils was ruled out in previous studies, which observed no effect on resting tension of 2,3-butadionemonoxime (BDM), an ATPase inhibitor that prevents actin–myosin interactions.[14] Increased myofilamentary calcium sensitivity observed in failing myocardium could, however, promote formation of weakly bound crossbridges, and, therefore, actin–myosin interaction could still be relevant for the high resting tension of failing cardiomyocytes.[8] This was confirmed in a study by Flagg et al, also reported in this issue of *Circulation Research*.[17] This study observed significant sarcomere lengthening after administration of BDM to cardiomyocytes derived from mice undergoing lipotoxic diabetic cardiomyopathy. Cardiomyocytes isolated from these cardiomyopathic hearts indeed had increased myofilamentary calcium sensitivity.

## "Adaptive Suspension"

Phosphorylation by PKG or PKA acutely adjusts the spring properties of titin and provides the cardiomyocyte with an "adaptive suspension." To improve insight into diastolic dysfunction of diabetic, hypertrophied and failing hearts, future studies should focus on this adaptive suspension and investigate titin phosphorylation deficits, relative phosphorylation of titin isoforms, and titin phosphorylation correcting for altered titin–actin interaction.

## Disclosures

None.

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