Editorial

Softening the Stressed Giant Titin in Diabetes Mellitus

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Abnormal left ventricular (LV) relaxation and increased diastolic stiffness are important features of heart failure with preserved ejection fraction (HFpEF); the 2 major contributors to LV diastolic function are the cardiomyocytes and the extracellular matrix.[1] Cardiomyocytes govern LV relaxation and are a determinant of LV chamber distensibility. Increased cardiomyocyte stiffness can be caused by sarcomeric myofilaments; the focus of the work by Hopf et al[2] is on the role of the myofilament titin, a major contributor to diastolic stiffening in HFpEF.[3,4] Approximately 1/3 of HFpEF patients have diabetes mellitus (DM), and Hopf et al[2] provide important insights in the insulin-related signaling pathways that modify titin's stiffness in HFpEF patients with DM.

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The extensible I-band region of titin comprises (1) tandem immunoglobulins segments, (2) the PEVK (proline-glutamic acid-valine-lysine-rich element), and (3) the N2B-Us (a unique sequence [Us] that is part of the N2B element).[5] Titin's stiffness can be modulated through differential mRNA splicing that gives rise to stiff and compliant titin isoforms.[6] Upregulation of compliant isoforms occurs in pathological conditions, presumably to counteract fibrosis.[7] Upregulating compliant titin can be experimentally accomplished by targeting RBM20 (RNA-binding motif protein 20), and this normalizes titin's stiffness in models with diastolic dysfunction.[8,9] Posttranslational modification of titin also alters titin's stiffness.[10] S4010 and S4099 in N2B-Us can be phosphorylated, resulting in a reduction of titin stiffness, whereas phosphorylation of the S11878 and S12022, located in the PEVK segment, results in increased stiffness.

Previous studies have shown that increased cardiomyocyte stiffness in DM is a consequence of deranged titin phosphorylation, but the pathways connecting insulin deficiency to altered titin phosphorylation have not been established. Hopf et al[2] shed light on the signaling pathways involved and, importantly, provide potential therapeutic targets for treating DM-associated HFpEF. Atrial biopsies were collected and cardiomyocytes were isolated and studied. Myocytes from diabetic patients have increased passive stress compared with nondiabetic counterparts, but unusual is that the passive stress-sarcomere length curves are vertically displaced without a change in slope (stiffness). The increased passive stress was accompanied by a phosphorylation deficit at S4099 in the N2B-Us, because of reduced PKG (protein kinase G) activity, and increased phosphorylation at S11878 in the PEVK, because of increased PKCα (protein kinase Cα) activity. It is important to highlight that the diabetic patients were on medications (insulin, metformin, and β-blockers) that might have altered their phosphorylation status.

The reduced S4099 and increased S11878 phosphorylation levels are both predicted to increase titin stiffness, although their individual effects were not directly determined. Additionally, the compliant N2BA titin isoform was found to be upregulated, suggesting a compensatory mechanism to counteract the effect of increased stiffness because of deranged phosphorylation. It is possible that the combined effects of all changes underlie the upward shift of the passive stress-sarcomere length relation without a change in slope. Although it seems likely that these studies on atrial cells extrapolate to the LV, future work should test whether this is indeed the case.

To study the mechanistic basis of their findings and explore therapeutic approaches for normalizing titin's stiffness, Hopf et al[2] studied embryonic rat cardiomyocytes in culture. This revealed that insulin mediates signal transduction through activation of (1) PI3K (phosphoinositide 3-kinase), which in turn activates PKG, resulting in phosphorylation at titin S4099, (2) ERK1/2 (extracellular signal-regulated kinase 1/2), resulting in phosphorylation at S4010, and (3) PKCα, resulting in phosphorylation at S11878. These findings can explain why S4099 is hypophosphorylated in DM patients but not why S11878 is hyperphosphorylated and S4010 is unaffected in patients.

Additionally, the antidiabetic drug metformin induces in cultured embryonic rat cardiomyocytes titin phosphorylation through the same signaling pathways as insulin. Interestingly, in adult rat cardiomyocytes, metformin only increases phosphorylation at S4010, highlighting that care has to be taken when extrapolating results from embryonic cells. Although phosphorylation of S4010 was not altered in DM patients, it is worth noting that by virtue of the increased phosphorylation of S4010 in adult cells and the expected ensuing reduction in passive stress, metformin might have a beneficial effect on diastolic function in HFpEF patients.

Hopf et al[2] also studied NRG-1 (Neuregulin-1), a member of the epidermal growth factor family that had previously been reported to improve cardiac function and reverse cardiac remodeling in a rat model of diabetic cardiomyopathy.[11] The authors found that in adult rat cardiomyocytes, NRG-1 increases titin phosphorylation at S4010 and lowers S11878 phosphorylation in rat myocytes, and if a similar effect occurs in DM patients, it would act toward correcting the phosphorylation

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status and lower diastolic stiffness. Thus, NRG-1 holds promise as a therapeutic that lowers passive stiffness in DM.

To test the therapeutic efficacy of NRG-1, 2 diabetic animal models were studied: (1) the Streptozotocin-treated ApoE$^{-/-}$ mice, a model of type 1 DM, and (2) the diabetic ZSF-1 rats, a model of type 2 DM. Streptozotocin-treated ApoE$^{-/-}$ mice were used to establish long-term effects and diabetic ZSF-1 rats for acute effects of NRG-1. The Streptozotocin-treated ApoE$^{-/-}$ mice had phosphorylation deficits at S4010 and S4099 and increased phosphorylation at S11878, mimicking the deranged titin phosphorylation of diabetic patients. Cardiomyocyte passive stress was elevated in Streptozotocin-treated ApoE$^{-/-}$ mice but was normalized in the group of animals that were treated with insulin or NRG-1 for 14 weeks. Insulin increased the phosphorylation at S4010, and NRG-1 increased phosphorylation at S4010 and S4099 and reduced phosphorylation at S11878, reversing the deranged titin phosphorylation from insulin deficiency. A concern is that Streptozotocin-treated ApoE$^{-/-}$ mice do not develop LV chamber stiffening, despite the significant increases in passive stress of the cardiac myocytes. This warrants studies of both cells and extracellular matrix stiffness[4,12] to determine whether one compensates for the other.

The diabetic ZSF-1 rats did develop elevated LV chamber stiffness, observed as a steeper end-diastolic pressure-volume relation. Both the end-diastolic pressure volume relation steepness and the LV end-diastolic pressure showed a trend toward reduction after administering of NRG-1; however, this was not significant and there was no change in titin phosphorylation.

The differences in the NRG-1 response of the 2 diabetic animal models might be partly explained by the distinct pathophysiology of diastolic dysfunction in type 1 and type 2 DM. In addition, the phosphorylation status of titin after insulin or NRG-1 treatment in cultured cardiomyocytes differs from that in the animal models, emphasizing the importance of performing studies on cells that are continuously mechanically loaded/unloaded and calcium activated/relaxed, processes that are likely to profoundly alter signaling pathways, relative to quiescent cells in culture. Despite several concerns, inherent in cell culture and animal work, results of the Hopf study overall support that NRG-1 can ameliorate diastolic dysfunction in DM and HFpEF, and follow-up work is warranted. Such work should include whether the NRG-1-induced hypertrophy found in cell culture promotes hypertrophy in the diabetic heart, as this would curtail its usefulness.

The above-noted limitations and discrepancies might indicate that unexamined phosphorylation sites exist in titin's spring region. This notion is supported by the unexpected large passive stress effects, such as the passive stress of myocytes from DM mice treated with NRG-1 that is >2-fold less than that of untreated mice. Such large effects are surprising considering the changes in phosphorylation of 3 residues only. Thus, the full spectrum of all phosphoresidues needs to be established. Other types of posttranslational modifications might be relevant as well. For example, internal disulfide bonds can increase titin-based stiffness,[13] and glutathionylation can lower titin stiffness.[14] Arginylation might play a role as well.[15] Impaired PKG signaling and increased PKCα activity were also reported as characteristic features of HFpEF regardless of DM[4]; however, myocytes of diabetic hearts were found to be stiffer than those of nondiabetic HFpEF[16] patients, further supporting that additional pathomechanisms await discovery.

More research on titin's spring region is required. A multiprong research approach focused on both post-transcriptional (RBM20) and posttranslational mechanisms will increase the chance that effective therapeutics will be obtained. Additionally, the detailed structural changes in titin's spring region caused by phosphorylation need to be studied, providing a basis for selecting ideal drugable targets and aiding future drug screening approaches. Multiple HFpEF subtypes are likely to exist, each with a unique pathophysiology and each requiring a tailored therapeutic approach. A broad and basic understanding of the biophysical changes in titin induced by posttranscriptional and posttranslational mechanisms is needed.

In summary, the Hopf et al[2] found increased passive stress of cardiomyocytes in diabetic patients, which is likely because of impaired PKG signaling and increased PKCα activity, resulting in deranged titin phosphorylation and increased stiffness. Many discrepancies and unresolved issues remain. Nevertheless, the in vitro studies performed by Hopf et al[2] on neonatal and adult rat cardiomyocytes and the studies on animal models provide mechanistic insights in the signaling pathways that alter titin's stiffness in DM and support that NRG-1 could be useful for normalizing titin's stiffness in DM. Considering titin's complexity and wide involvement in diastolic stiffening in the broad-spectrum phenotypes of HFpEF, titin's molecular biophysical and biochemical features and its potential as a therapeutic target warrant increased research focus.

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Disclosures

None.

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