Diminished Ca^{2+} Sensitivity of Skinned Cardiac Muscle Contractility Coincident With Troponin T–Band Shifts in the Diabetic Rat

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Abstract We have measured the apparent Ca^{2+} sensitivities of force development in skinned cardiac trabeculae at different sarcomere lengths together with shifts in troponin (Tn) T subunits on specimens from the same hearts and drawn insights into the pathogenesis of myocardial dysfunction in the diabetic rat. The Ca^{2+}-force relations were measured at a long (2.4-μm) and a short (1.9-μm) sarcomere length. In disease, compared with the control condition, the apparent Ca^{2+} sensitivity was greatly diminished at a sarcomere length of 1.9 μm but not affected at all at the long length (2.4 μm). We also examined the alterations in contractile regulatory proteins TnT and TnI by both sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blots. The TnI band was largely unperturbed, but major changes were discerned in TnT. The normal rat heart indicated two major bands (TnT1 and TnT2) and a faint third band (TnT3); in the diabetic rat heart, there was a significant shift in intensity from TnT1 to TnT3. Since myosin isozyme shifts also accompany diabetes in the rat, we used a prototypical hypothyroid rat as well to evaluate the myosin influence in the length-induced effects on Ca^{2+} sensitivity. Myosin shifts during hypothyroidism were unaccompanied by significant changes in TnT, and there were also no length-dependent modifications in Ca^{2+} sensitivity. The findings raise the possibility that diabetic Ca^{2+}-sensitivity changes in the myocardium are coupled with TnT alterations. A plausible explanation is offered whereby these TnT alterations modify the length dependence of Ca^{2+} sensitivity. (***Circ Res.*** **1995;76: 600-606.)**

Key Words • troponin T • diabetes • heart failure • hypothyroidism • Ca^{2+}

Heart failure is associated with manifest loss of myocardial contractility and energy deprivation, as well as apoptosis or cell loss,[1,2] but major gaps remain in defining the underlying molecular abnormalities in the contractile proteins. Recent genetic studies of human familial hypertrophic cardiomyopathy have indicated several point mutations in myosin, as well as in the regulatory proteins (tropomyosin and troponin [Tn] T) of the sarcomere.[3,4] Interestingly, alterations in TnT were noted earlier by Anderson et al[5] by the analysis of the protein bands as well. Furthermore, we found significant modifications in TnT, and possibly also in TnI accompanying hypertrophy and heart failure in aortic-banded guinea pigs.[6] Functionally, myosin cross-bridge kinetics are also modified in the myopathic heart tissue,[7] and the corresponding changes are mimicked in the motility assays of isolated myosin with similar point mutations,[8,9] but whether there are specific functional deficiencies relative to alterations in regulatory proteins on the thin filament has proved elusive in both human and animal tissue studies.

Thus, whereas the apparent Ca^{2+} sensitivity for force development in skinned cardiac muscles, a canonical functional probe of the intrinsic myocardial regulatory complex, was modified in some studies of the defective hearts[10,11] and compared with normal tissue, myopathic cardiac muscle was more responsive to a variety of Ca^{2+} sensitizers (eg, caffeine and DPI 201-106)[12,13]; in other instances, Ca^{2+} sensitivity appeared completely unaffected.[14,15] Of the three subunits of cardiac Tn (ie, TnC, TnI, and TnT), TnC is firmly invariant.[16] However, TnI and especially TnT are highly labile (reviewed in Reference 17) and are likely candidates for producing contractile adaptations in heart disease. Therefore, to reexplore the functional consequences of alterations in regulatory proteins, in the present study we have pursued contractile studies on cardiac skinned trabeculae coincidently with measurements of TnI- and TnT-band shifts accompanying heart abnormalities.

The diabetic rat was selected as the primary investigative implement because of its ready familiarity and simplified experimental manipulability.[18,19] The manifold cardiac abnormalities accompanying experimental diabetes are well documented, eg, the decreased sarcoplasmic reticular activity, altered energetics, and abnormal papillary muscle contractility (diminished unloaded shortening velocity as well as actomyosin ATPase) due to shifts in the expression of myosin isozymes from V1 predominance to V3.[20] Moreover, the model was preferable for the present pathobiophysical studies, because previous contractile diabetic abnormalities occurred without discernible histological deformities in cardiac muscle.[20] The understanding of cardiac abnormalities accompanying diabetes is of high clinical relevance as well, since, interestingly, according to the Framingham epidemiological study, diabetes mellitus manifests a greatly increased risk of human congestive heart failure even in the absence of coronary atherosclerosis.[21] Comparative investigations of the contractile dysfunctions were also made on the hypothyroid rat.

The studies of Ca^{2+} sensitivity for force development in skinned cardiotrabeculae were made at two sarcomere lengths (2.4 and 1.9 μm). In the normal tissue, Ca^{2+} sensitivity is greatly reduced at a sarcomere length

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of 1.9 compared with 2.4 μm—a fundamental property also manifesting molecular aspects of Starling's basic law of the heart.[22,23] Thus, by comparing the contractile properties at these sarcomere lengths and correlating the contractile changes with possible shifts in the regulatory subunits, we sought to explore the molecular basis of cardiomyopathy and also to draw specific insights into the pathways for interventions in the Starling mechanism.

Materials and Methods

Diabetic Rat Model

Female Wistar rats (6 to 8 weeks in age, 200 to 220 g body weight) were made diabetic with an intravenous injection of 6 mg/100 g of streptozotocin, as previously described.[18,19] The rats were used no earlier than 3 months, and usually within 6 months, after the streptozotocin injection, when blood glucose levels were in the 400- to 700-mg/100 mL range. The control glucose in the rat is in the 150- to 200-mg/100 mL range. Thus, the diabetic maladaptation in the rats was relatively well developed and stable.

Hypothyroid Rat Model

Six- to 8-week-old male rats were given 0.05% 6-*n*-propyl-2-thiouracil (PTU, Sigma Chemical Co) in the drinking water for the next 5 weeks. The growth rates of PTU-treated rats were characteristically[24] retarded compared with paired control rats, as evidenced by the total body weight, which was 43±4% less (n=9). Also, as is further characteristic of hypothyroidism, the mean heart rates of the PTU-treated rats were lower (control rats, 368±14 beats per minute; PTU-treated rats, 284±7 beats per minute). Moreover, the thyroid weights of the PTU-treated rats were greatly increased (control rats, 5.7±0.7 mg/100 g body wt; PTU-treated rats, 43.8±3.1 mg/100 g body wt).

Skinned Trabeculae From the Right Ventricle

After the animals were killed, the hearts were instantly removed and rinsed in Kreb's solution (mmol/L: KCl 5, NaCl 121, $NaHCO_3$ 22.5, sodium phosphate 1.5, $MgCl_2$ 1.2, and $CaCl_2$ 0.7, with 5% glucose freshly added) that was vigorously bubbled with 5% CO_2 and 95% O_2. Each heart was then transferred to a chamber containing relaxing solution (see below) with 1% Triton X-100 at 4°C for initial skinning. At this stage, under the dissecting microscope (×10 to ×40, Zeiss M3A), the right ventricular chamber was cut open to expose the endocardial trabeculae. After 30 minutes in the Triton solution (4°C), fresh Triton-free relaxing solution was replaced with several changes to thoroughly wash out the detergent.

The dissection of trabeculae and their attachment to transducers were as described previously.[25] The detergent-skinned trabeculae for the experiments were visually inspected under the compound microscope (×250) for overall uniformity of the striations along the entire length. Usually, over half of the specimens were found unsuitable and rejected at this stage.

After attachment, the sarcomere length of the specimen was adjusted to 2.4 or 1.9 μm, as desired, by using laser diffraction. The longer sarcomere length was the extreme to which myocardium could be stretched reproducibly without permanent impairment, and the shorter length of 1.9 μm was chosen to minimize possible complications arising from double overlap of thin filaments. Also, the integrated laser diffraction pattern of the trabeculum was monitored at the end of each activation throughout the experiment to further ascertain the viability of the tissue specimen. In accepted preparations, the pattern remained sharp, whereas a progressively diffused and widening pattern was the indication of deteriorating sarcomere length homogeneity, and these preparations were rejected. Progressive decline in force and the broadening of laser pattern were generally coupled, and the experiment was promptly discontinued when force decline exceeded 20%.

The various experimental solutions used in the present study on the skinned fiber were as follows: relaxing solution (150 mmol/L potassium propionate, 5 mmol/L ATP, 5 mmol/L EGTA, 15 mmol/L phosphocreatine, 400 U/mL creatine phosphokinase, 6 mmol/L $MgCl_2$, and 20 mmol/L imidazole, pH 7.0; modified from that described in Reference 26) and activating solution (same as above except that EGTA was replaced with equivalent amounts of Ca^{2+}-EGTA and free Mg^{2+} was kept at 1 mmol/L. The ionic strength of the solution was maintained at ≈180 mmol/L). All activations were made at 20°C. In experiments with dextran, the appropriate amount was included in relaxing and activating solutions.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblots

Protein electrophoresis of the ventricular strips was carried out on 8% polyacrylamide discontinuous Laemmli gels (16×20 cm) by use of the BioRad Protean II slab cell. Tissue samples (3-mm-long and 150-μm-thick trabeculae) in 40-μL sodium dodecyl sulfate (SDS) sample buffer[25] were vigorously dissolved by use of microtip ultrasonication (Bronson Sonifier, model 200). The sample buffer was kept in an ice bath preceding and during sonication to prevent overheating. The gels were run in pairs, with the sample (40 μL) being divided evenly. After the run, one gel of the pair was silver-stained (see below) for estimation of the relative contents in the various bands; the second member of the gel pair was used for the immunoblots.

For protein quantification, silver-stained lanes were scanned with a laser densitometer (100-μm laser beam, UltroScan XL, LKB Pharmacia). The TnT and TnI intensities were normalized to the myosin light chain 1 (LC1) band in the same gel lane. The identifications of the pertinent bands were cross-checked with immunoblots (see below), wherever appropriate.

For Western blots, the paired unstained gel was transferred to a nitrocellulose membrane (0.45-μm pore size, Schleicher & Schuell Co) by use of the BioRad Trans Blot cell. The membrane was probed by use of the ECL kit (RPN 2109, Amersham). Dilutions of 1:1000 for TnT antibody and 1:2000 for TnI antibody were used. The same membrane could be probed sequentially with each antibody, which facilitated comparisons between various probes on the same tissue sample.

The two antibodies used in the present study were monoclonal anti-rabbit skeletal TnT (JLT-12), purchased from Sigma, and a cardiac TnI monoclonal, a gift of Dr Schiaffino, Padova, Italy.

Data Analysis

The Hill equation was used to fit the pCa-force relations as previously described.[25] The pCa values for half-maximal tension response (pCa_{50}) were taken as the apparent Ca^{2+} sensitivities under the applicable test conditions. All data are given as mean±SEM, wherever appropriate. To test for the significance, Student's *t* test was used, and values of $P<.05$ were noted as significant.

Results

pCa-Force Relations

pCa-Force Relations at Different Sarcomere Lengths in Diabetic Rats

Table 1 compares the maximal tension development of normal and diabetic skinned trabeculae (with activation at a pCa value of 4 [pCa4]) at lengths of 2.4 and 1.9 μm. The absolute tension values (maximal activation with pCa4) and the tension ratios (pCa4 tension at 1.9 μm/pCa4 tension at 2.4 μm) were similar for the normal and diabetic specimens. The short-to-long length–tension ratios were 0.69 in both instances, which is the expected response on the basis of known length-tension relations in skeletal muscle (see Reference 27).

Fig 1 compares the typical pCa-tension relations of normal and diabetic specimens. At 2.4 μm, the pCa-tension curves are indistinguishable (Fig 1, left). At 1.9

TABLE 1. Comparison of Maximal Tension of the Trabeculae at Two Sarcomere Lengths

Source of Trabeculae	Width of Trabeculae, μm	Developed Tension, kN/m²		Tension Ratio (1.9 μm/2.4 μm)
		2.4-μm SL	1.9-μm SL	
Control rat (n=14)	113±12	40±2	28±2	0.69±0.03
Diabetic rat (n=12)	95±10	39±2	27±2	0.69±0.03

SL indicates sarcomere length. Values are mean±SEM.

μm (Fig 1, right), contrastingly, there is a major shift: the pCa-tension curve corresponding to the diseased tissue is shifted to the right, indicating diminished Ca^{2+} sensitivity. Such findings on all 26 specimens—14 control and 12 diabetic—are listed in Table 2. The results indicate that at 2.4 μm the average values of the pKs for the pCa-tension relations are reproducibly similar for normal and diabetic specimens. However, the rightward shift (ie, the last column marked ΔpK in Table 2) for a length change of 2.4 to 1.9 μm is nearly twofold greater in the diabetic tissue (0.33 pCa unit) than in the control tissue (0.18 pCa unit). The net extra ΔΔpK shift (ie, 0.33−0.18=0.15 pCa unit) represents a highly significant effect of the disease in such measurements and should reflect a disability of the diabetic heart to respond to sarcoplasmic Ca^{2+}.

Several independent measures were included in the present experiments to further substantiate the validity of these results. First, the pCa-tension measurements at the two sarcomere lengths were randomized; ie, in about half of the fibers from diabetic and normal hearts, contractility was evaluated first at the long length then at the short length; in others, the sequence was reversed. Second, the complete cycles—long to short to long or the reverse sequence, as appropriate—were used routinely. And, finally, the maximal tension response at 2.4 μm (at pCa4) was measured at the end of the experiment to verify the functional integrity: force level at the end of the experiment was within 20% of the starting level; otherwise the experiment was not counted.

pCa-Force Relations in Hypothyroid Rats

To help differentiate the relative contributions of the myosin isozyme shifts from those of other proteins, we made additional studies on especially developed PTU-treated hypothyroid rats in which the myosin composition was altered.[28] The measurements of length dependence of Ca^{2+} sensitivity in these rats are described in Fig 2B.

The determinations of pCa-force relations were made as above at sarcomere lengths of 2.4 and 1.9 μm on paired animals (euthyroid and PTU treated). The difference between the pCa_{50} values at these lengths was derived for each animal. The mean Ca^{2+}-sensitivity changes noted in Fig 2B (ΔpK=0.16 pCa unit±0.01 for five determinations in control rats; ΔpK=0.13 pCa unit±0.01 for four determinations in hypothyroid rats) indicate a small insignificant (by *t* test) decrease in the length dependence in the PTU-treated rat. In contrast, in the diabetic rat (Fig 2A), there was a twofold increase in length dependence. The inevitable conclusion would be that the myocardial Ca^{2+}-sensitivity changes in diabetes are unrelated to the accompanying myosin isozyme shifts.

SDS–Polyacrylamide Gel Electrophoresis and Immunoblots for TnT and TnI

The altered Ca^{2+} sensitivity at 1.9 μm in cardiomyopathy accompanying fully developed diabetes suggested the possibility of modifications in regulatory proteins, especially cardiac Tn comprising TnC, TnI, and TnT. Since cardiac TnC expression in the heart is highly conserved, alterations of TnT and TnI are the other interesting possibilities for changes caused by diabetes. To investigate these, we have made use of two specific antibodies to TnT and TnI to conduct studies of the Western blots on the cardiac tissue. The samples for the gels were processed from hearts whose trabeculae were used for pathophysiological measurements. The typical results are shown in Fig 3.

Fig 3A indicates an 8% polyacrylamide silver-stained gel; and Fig 3B, the corresponding Western blots (on right ventricular strips of the rat heart). The immunoblot was labeled sequentially with TnT and TnI antibodies.

Three TnT bands were identified in immunoblots, and the corresponding bands were also resolved in the silver-stained gels. In the normal rat, two bands, so-called TnT1 and TnT2, were found to be dominant. The third band, TnT3, was of relatively minor intensity. In myopathic tissue, three similar bands were visible, but the TnT3 band was much stronger. The quantitative laser-densitometric analyses made on silver-stained bands on multiple (14 total) ventricular strips are summarized in Fig 4. For data interpretation, it must be noted, however, that the protein and biophysical data were each obtained from different preparations of the same heart.

The bars in Fig 4 corresponding to three rat TnT bands, presented as the fractions of total TnT (TnT1+TnT2+TnT3) in each sample, indicate that the increase in TnT3 intensity is

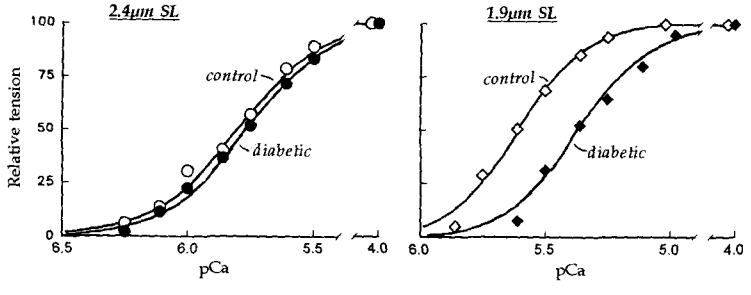

FIG 1. Graphs showing pCa-tension relations at sarcomere lengths (SLs) of 2.4 and 1.9 μm for trabeculae from normal and diabetic rats.

TABLE 2. Sensitivity of Trabeculae From Control and Diabetic Rats at Sarcomere Lengths of 2.4 and 1.9 μm

Experiment	pK		ΔpK
	2.4 μm	1.9 μm	
Control rats			
A19C3	5.76	5.60	0.16
A20C1	5.90	5.76	0.14
A20C2	5.89	5.73	0.16
B12C1	5.68	5.47	0.21
B14C1	5.82	5.58	0.19
B14C2	5.81	5.59	0.22
B14C4	5.65	5.5	0.15
B15C1	5.65	5.51	0.14
B16C1	5.60	5.39	0.21
B16C2	5.55	5.38	0.17
16C23	5.85	5.67	0.18
17C14	5.77	5.59	0.18
X4C1	5.65	5.45	0.20
X4C2	5.67	5.48	0.19
Mean±SEM	5.73±0.03	5.55±0.03	0.18±0.01
Diabetic rats			
A7D1	5.89	5.62	0.27
A7D2	5.70	5.44	0.26
A7D3	5.68	5.21	0.47
A7D4	5.84	5.52	0.32
B3D1	5.78	5.48	0.30
B3D2	5.77	5.36	0.41
B7D2	5.60	5.30	0.30
B7D3	5.76	5.44	0.32
A7D5	5.81	5.49	0.32
B7D	5.86	5.39	0.47
X5D1	5.88	5.58	0.30
X2D1	5.66	5.40	0.26
Mean±SEM	5.77±0.03	5.44±0.03	0.33±0.02

evidently the result of a shift in mass from the TnT1 band. There is also observed a small drop in TnT2.

For silver-stained gels, we also estimated the relative amount of the total TnT1+TnT2+TnT3 normalized to LC1 in the same lane. The TnT/LC1 values summarized in Table 3 indicate nearly identical amounts for the control and diabetic samples. This is taken as an indication that intensity loss from TnT1 and TnT2 bands in Fig 3 accounted for the increased intensity of TnT3.

The inset in Fig 4 depicts the results of TnT shifts on trabeculae isolated from control and diabetic right ventricular samples. The TnT1 to TnT3 alteration is similar to that in the main figure.

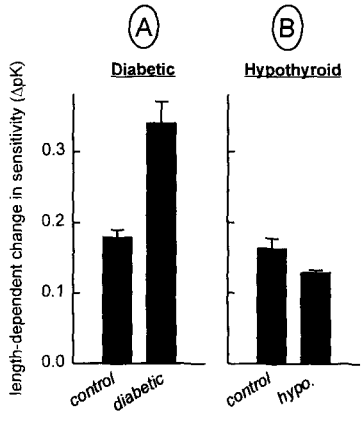

FIG 2. Bar graphs comparing length-dependent shifts in Ca^{2+} sensitivity in diabetic rats (A) and 6-*n*-propyl-2-thiouracil (PTU)–treated hypothyroid (hypo.) rats (B). The results in panel A are the means of the values listed in Table 2. The results in panel B are from a total of nine fibers from different rats.

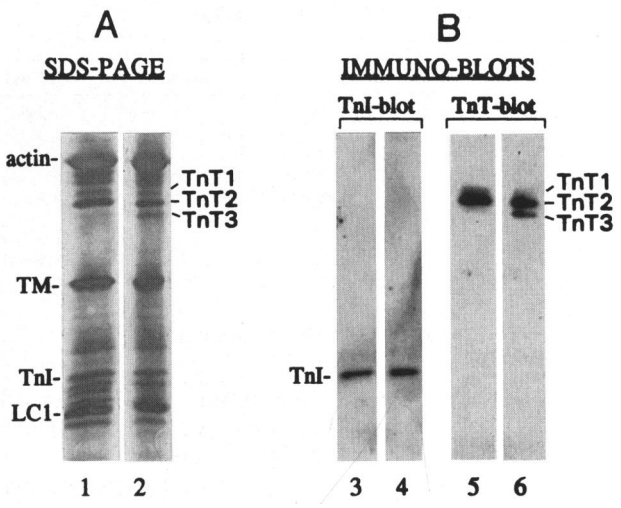

FIG 3. Typical 8% polyacrylamide gel runs (A) and immunoblots (B). Lanes 1, 3, and 5 are samples from control rats; lanes 2, 4, and 6 are samples from diabetic rats. SDS indicates sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; TM, tropomyosin; and Tn, troponin.

Additionally, the analysis of PTU-treated hypothyroid specimens indicated no significant shifts in TnT (Table 4). This finding is also in agreement with the published results involving another hypothyroid model.[17] The TnI bands were not analyzed, especially since a previous study indicated little change in this subunit in the adult rat.[24]

TnI Analysis in Diabetic Tissue

For TnI, we used a monoclonal antibody[29] for immunoblot analysis. This antibody stained a single TnI band in both normal and diabetic rats (lanes 3 and 4 in Fig 3B). The analysis of total mass of TnI (normalized to

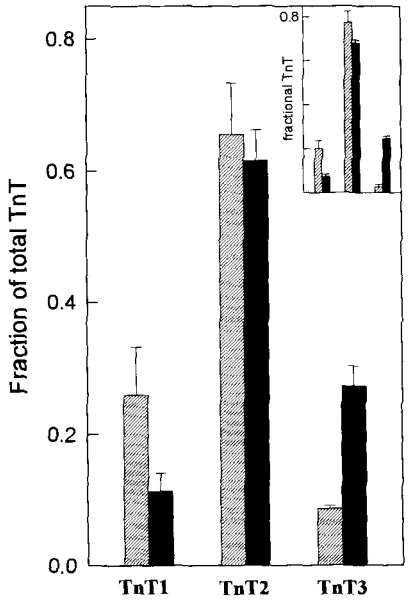

FIG 4. Bar graphs showing intensity distributions of the various troponin (Tn) T bands. The shaded bars represent tissue from control rats, and the black bars represent tissue from diabetic rats. The results are means of 14 cardiac strips each. The inset indicates the corresponding TnT shifts for five control and nine diabetic trabeculae from the right ventricles of different rats.

TABLE 3. Protein Quantification From Silver-Stained Gels

Protein Subunit	Control	Diabetic	t Test
TnT/LC1	0.45±0.05 (5)	0.41±0.05 (9)	NS
TnI/LC1	0.48±0.05 (4)	0.47±0.02 (4)	NS

Tn indicates troponin; LC1, myosin light chain 1. Values are mean±SEM. Numbers in parentheses indicate the number of samples.

LC1) further indicated that the amount of TnI was well conserved in diabetes (Table 3).

Radial Compressions of the Trabeculae With Dextran

To further explore the mechanism of length-dependent (1.9 to 2.4 μm) alterations in apparent Ca^{2+} sensitivity in diabetic specimens, the study was also made with dextran T500. On stretching, the trabeculae become radially compressed; therefore, it seemed worthwhile to investigate at 1.9 μm whether the equivalent radial compression caused with dextran would produce a differential in Ca^{2+} sensitivity comparable to the sensitivity change following stretch from 1.9 to 2.4 μm. The results are depicted in Fig 5.

The bar graphs at the top of Fig 5 show the relative compressions: the first set of bars (Fig 5A) shows that the width at 2.4 μm was 8% less than the width at 1.9 μm for control trabeculae and 7% less than the width at 1.9 μm for diabetic trabeculae. This difference (8% versus 7%) between the effects on normal and diabetic specimens was not significant. The second set of bars in the upper panel (Fig 5B) shows that in 2.5% dextran, the widths at 1.9 μm were similarly reduced by 9% and 8% relative to the noncompressed values for the normal and diabetic specimens, respectively.

The bar graphs at the bottom of Fig 5 compare the derived Ca^{2+}-sensitivity effects of length change (1.9 to 2.4 μm) in the absence of dextran (Fig 5C) and the Ca^{2+}-sensitivity change induced by 2.5% dextran (at 1.9 μm, Fig 5D) on typical specimens from control and diabetic rats. The dextran-free ΔpK with a length change of 1.9 to 2.4 μm was 0.19 pCa unit for control and 0.30 pCa unit for the diabetic specimen (see also Fig 1 and Fig 2A). In contrast, dextran-induced compression increased the pKs by 0.05 and 0.06 pCa units for control and diabetic tissues, respectively. Thus, compression per se contributes negligibly to the differential in the length effect on Ca^{2+} sensitivity between normal and diabetic tissues. Similarly, no significant difference between control and diabetic Ca^{2+} sensitivities was observed in 5% dextran (data not shown).

The study of the effect of dextran was limited to 1.9 μm, since Ca^{2+} sensitivities at 2.4 μm were indistinguishable between normal and diabetic specimens, and further compression with dextran was therefore outside the range of current interest.

TABLE 4. Troponin T Isoform Distribution in Hypothyroid Hearts

Source of Trabeculae	% of Total TnT		
	TnT1	TnT2	TnT3
Control rat	20.1±1.3	70.3±1.9	9.6±2.0
Hypothyroid rat	17.0±1.4*	74.3±1.4*	8.7±1.0*

Tn indicates troponin. Values are mean±SEM.
*Difference from the respective control isoform is not significant by Student's *t* test.

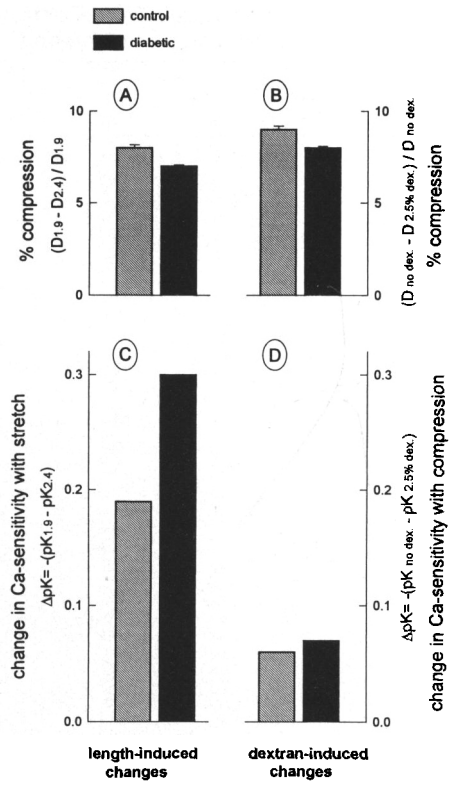

FIG 5. Bar graphs showing the effect of radial compression on pCa-force relations. A and B, Percent compressions of the trabecula on four preparations with length change from 1.9 to 2.4 μm (A) and percent compression with 2.5% dextran (B). C and D, Increase in Ca^{2+} sensitivity with length change (C) and with 2.5% dextran (D) on a typical set of cardiotrabeculae from a normal and diabetic rat. Similar results were observed in another set. On the ordinates, D indicates width; 1.9 and 2.4, lengths of 1.9 and 2.4 μm, respectively; and dex., dextran.

Discussion

The present studies on permeabilized cardiotrabeculae have defined specific length-dependent shifts in Ca^{2+} regulation of force development in streptozotocin-induced diabetes in the rat. The studies of these mechanical properties were made on skinned specimens at sarcomere lengths of 2.4 and 1.9 μm. The apparent Ca^{2+} sensitivity was markedly perturbed in diabetic rats at the short sarcomere length, even though there was no significant effect on the Ca^{2+}-force relation at the long length. Coincident with this pattern of length-dependent Ca^{2+}-sensitivity alteration, we also observed the interconversion of mass between two of the three TnT bands (Figs 3 and 4). The increased sarcomere length dependence of Ca^{2+} sensitivity normally corresponds to a steeper length-tension curve for partial activation.[22,23] Moreover, the length-tension curve in cardiac muscle has direct correspondence with the Starling curve in the heart pressure-volume relation.[30] Manifest diminishment in Ca^{2+} sensitivity at a sarcomere length of 1.9 μm in diabetic rats indicates that the heart length-tension curve is steeper, which would imply that there is also an associated loss of myocardial reserve in the Starling mechanism and that cardiac performance is correspondingly impaired. Furthermore, we explore below the possibility that the molecular mechanisms underlying the diabetic pathophysiological changes are tightly coupled with TnT alterations.

Because myosin isozyme modifications occur in diabetes in the rat, we further investigated whether these

myosin shifts were also coupled to changes in Ca^{2+} sensitivity. This was investigated on the PTU-treated hypothyroid rat (nondiabetic), which is a prototypical model for the conversion of myosin from fast to slow types (putative V1 to V3 conversion). In the diabetic rat, the normally predominant V1 myosin isoform pattern (71% V1, 18.6% V2, and 10.9% V3) is converted to V3 dominance (12% V1, 17% V2, and 71% V3).[20] In the adult nondiabetic hypothyroid rat, V3 also predominates,[24] but in this instance, there are no concomitant alterations in TnT (present findings and Reference 17). Presently, we show that length-dependent Ca^{2+} sensitivity for force was also relatively unaffected with hypothyroidism in PTU-treated rats (Fig 2B), discounting a significant contribution from myosin shifts to length-dependent Ca^{2+}-sensitivity alterations in diabetic cardiomyopathy. The present findings also exclude the possibility that radial compressions accompanying sarcomere length changes transduce the length-dependent contractilities in diabetes. On the other hand, since TnT is an integral component of the Ca^{2+} switch for contractility, the possibility is worth considering that TnT alterations in diabetes are related to the effects on Ca^{2+} sensitivity at a sarcomere length of 1.9 μm.

Convertible TnT in the Regulatory Protein Complex in Myocardium

Whether the multiplicity in rat TnT bands is a manifestation of disparate isoforms or whether it originates from chemical modifications of the original molecule (eg, phosphorylation, dephosphorylation, etc; see Reference 31), the available evidence in the literature would seem to favor distinct isoform shifts as the cause for evident band shifts. Inordinate propensity for alternate splicing potential in the cardiac TnT gene (as well as in the skeletal gene) is well documented: Jin et al[32] have provided direct evidence that cardiac exons 4 and 12 are being alternately spliced in the rat. Additionally, exon 3 is multiply spliced in the rabbit heart,[33] yielding 12 TnT (mRNA) myocardial isoforms. Strikingly, in skeletal muscle, there are potentially 64 or possibly 128 TnT variants involving multiple combinations of exons 4 through 8, as well as alternative exons 16 and 17.[34,35] Presently, we recognize three protein bands of TnT in the rat, of which only the middle band is relatively unperturbed with disease and the intensity shift is predominantly from TnT1 (low mobility) to TnT3 (highest mobility band). This shift is striking—58% of the TnT1 intensity is converted into TnT3 conformation—even though the net shift is ≈15% of the total TnT (ie, TnT1+TnT2+TnT3). Although these studies in fully developed diabetes are suggestive of correlations between TnT shifts and contractile alterations at the short length, future investigations at earlier stages of the disease are needed for critical tests of these couplings. Another possible source for the heterogeneity among samples tested is that mechanical shifts and TnT alterations were evaluated in separate samples, albeit from the same heart.

There is previous evidence that normal TnT isoforms influence Ca^{2+} sensitivity. For instance, Tobacman and Lee[36] have shown that reconstituted thin filaments containing one or the other of two purified bovine cardiac TnT isoforms modify in vitro Ca^{2+}-regulated actomyosin ATPase activity. Moreover, the relative amount of cardiac TnT2 in the newborn rabbit is found to be positively correlated with Ca^{2+} sensitivity in myofilaments.[37] In explaining the observed myocardial length dependence in disease, it would be helpful in the future to know whether the various sarcomeric cardiac TnT species were uniformly distributed along the thin filament or whether each species was clustered in defined regions.

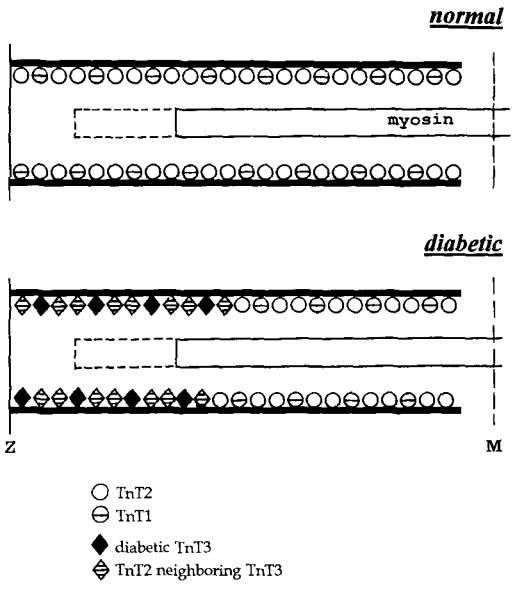

FIG 6. A normal and a diabetic half-sarcomere (modified from Reference 38). The full-length myosin filament indicates the overlap at 2.4 μm, and the dashed extension indicates the increased overlap at 1.9 μm. The number of troponin (Tn) T units per half thin filament are taken as 24, according to Ohtsuki.[41] The Z line and M line are marked.

Vectorial Modification of TnT in Cardiomyopathy: A Plausible Paradigm

A surprising (and novel) aspect of our findings was that the change in myocardial Ca^{2+} sensitivity was so distinctly length dependent. Because of TnT isoformic multiplicity and because of the shifts in the relative amounts of the various isoforms in diabetic cardiomyopathy, we consider the possibility that the TnT shifts are mechanistically correlated with the mechanical alterations. One explanation envisages that the modified TnT may be replaced preferentially on part of the thin filament closest to the Z line (Fig 6). Accordingly, corresponding to the actomyosin overlap at the long length (2.4 μm), the unmodified regulatory complex will retain the control of crossbridge interaction, but at the shorter length, the newly recruited thin-filament overlap region will include altered cardiac TnT units modifying the property of their paired TnCs. Moreover, for the modified region of the thin filament to elicit lower Ca^{2+} affinity, it is imperative that within the thin-thick filament overlap domain there must also exist significant nearest-neighboring interaction between the adjacent TnT units regardless of isoform identity (see Fig 6).

Alternatively, if new TnT molecules were randomly dispersed along the entire thin filament, the explanation for the length dependence of Ca^{2+} sensitivity would require that the modified TnT species also appropriately reset the intrinsic property of the thin filament (see Reference 23). Thus, for either of these processes to operate in diabetes, there are implications for the overall length-sensing mechanism in myocardium. We have previously indicated that TnC itself is a length sensor,[3]

and this possibility is under critical scrutiny with genetically mutated TnC isoforms.[39] The present considerations would indicate, moreover, that such TnC function could be modulated by TnT. Therefore, the particular TnT isotype may also modify the performance of the Starling mechanism in the heart. When normal mixtures of the TnT subunits are used, the extension of the TnT-exchange protocols used on skinned skeletal fibers[40] to the myopathic specimens should yield important insights in future studies.

Note added in proof. In a recent report by McDonald et al[42] testing the length-sensing role of TnC in Parmacek-Leiden-Field transgenic mice, evidence indicated that ectopically expressed sTnC in the heart left the sarcomere length–dependence of Ca^{2+} sensitivity relatively unaffected. In light of present results (see also Ding et al[43]), the possibility that other regulatory subunits are modified with TnC in the transgenic mouse is worth considering.

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