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Increased myocardial short-range forces in a rodent model of diabetes reflect elevated content of β myosin heavy chain

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Abstract

Diastolic dysfunction is a clinically significant problem for patients with diabetes and often reflects increased ventricular stiffness. Attached cross-bridges contribute to myocardial stiffness and produce short-range forces, but it is not yet known whether these forces are altered in diabetes. In this study, we tested the hypothesis that cross-bridge-based short-range forces are increased in the streptozotocin (STZ) induced rat model of Type 1 diabetes. Chemically permeabilized myocardial preparations were obtained from 12 week old rats that had been injected with STZ or vehicle 4 weeks earlier, and activated in solutions with pCa (=$-\log_{10}[Ca^{2+}]$) values ranging from 9.0 to 4.5. The short-range forces elicited by controlled length changes were ~67% greater in the samples from the diabetic rats than in the control preparations. This change was mostly due to an increased elastic limit (the length change at the peak short-range force) as opposed to increased passive muscle stiffness. The STZ-induced increase in short-ranges forces is thus unlikely to reflect changes to titin and/or collagen filaments. Gel electrophoresis showed that STZ increased the relative expression of β myosin heavy chain. This molecular mechanism can explain the increased short-ranges forces observed in the diabetic tissue if β myosin molecules remain bound between the filaments for longer durations than α molecules during imposed movements. These results suggest that interventions that decrease myosin attachment times may be useful treatments for diastolic dysfunction associated with diabetes.

Keywords

diastole; biomechanics; myocardial stiffness

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INTRODUCTION

Diabetes Mellitus is an increasingly common cardiovascular risk factor. The American Heart Association estimates that nearly 12% of the adult population in the US have diabetes, with an additional 38% having pre-diabetes (an elevated fasting blood glucose concentration) [1]. A major consequence of diabetes is the onset of diabetic cardiomyopathy [2]. Clinical studies have shown that this condition can hinder systolic pump function [3], but the earliest known sign of diabetic cardiomyopathy in humans is diastolic dysfunction [4].

The streptozotocin (STZ) induced diabetes model utilizes the STZ toxin to damage pancreatic β-cells [5] and cause hyperglycemia [6]. This model strongly mimics type 1 diabetes (which accounts for 5-10% of diabetes in humans [1]), but it also reproduces some of the metabolic and phenotypic changes that occur in type 2 diabetes [6]. For example, the STZ model is known to exhibit diastolic dysfunction [6, 7] and altered expression of sarcomeric proteins, including a myosin isoform shift towards increased β content that slows cross-bridge kinetics [8].

Although the alteration in cross-bridge kinetics undoubtedly influences systolic function it could also impact diastolic function. Recently, it has been shown that cross-bridges contribute to passive tension in permeablized cardiac fibers at low calcium (pCa 9.0, where $pCa=-\log_{10}[Ca^{2+}]$) and in resting intact cardiomyocytes[9, 10]{Syme, 2013 #3755}. We hypothesized that in diabetic rats, the increased content of β myosin heavy chain molecules would prolong the attached lifetimes of these cross-bridges and increase myocardial short-range forces [11]. This was tested by assessing the dynamic mechanical properties of chemically permeablized myocardial samples isolated from control rats and rats that had been injected with STZ 4 weeks prior to sacrifice. Since women with diabetes are 2.5 times more likely to develop heart failure than men with diabetes [12], we also tested rats of both sexes.

The main result was that STZ treatment increased short-range forces by 67%. Since short-range forces are present even at very low levels of activation [13], this result implies that changes in cross-bridge properties may contribute to the diastolic dysfunction associated with diabetes. In the rats studied in this work, the dominant molecular mechanism is the isoform shift towards the slower cycling β isoform of myosin heavy chain. Only one mechanical parameter was different by sex, but differential phosphorylation of myosin light chain 2 and troponin T may contribute to the sex dimorphism in the development of diabetic heart failure.

METHODS

Animal model

8 week old Sprague-Dawley rats of both sexes were purchased from Harlan Laboratories (Indianapolis, IN). All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

The study used 4 experimental groups - female control (F-CTRL), female streptozotocin-injected (F-STZ), male control (M-CTRL) and male streptozotocin-injected (M-STZ) – with each group initially containing 7 animals. The 14 animals in the treatment groups were injected once with streptozotocin (60 mg kg^{-1} in citrate buffer, pH 4.5, tail-vein injection) and developed hyperglycemia (blood glucose concentrations > 10 mM) within 3 days. One M-STZ rat died during recovery from the inhalation anesthesia required for the tail-vein injection. The control animals were injected with an equivalent volume of the citrate buffer vehicle. The oestrous phase of the female rats was not studied.

Blood glucose concentrations were determined periodically during the conditioning period by analyzing blood samples collected from tail vein pricks using a commercial glucometer (Precision Xtra, Abbott Laboratories, Bedford, MA; maximum measureable level was 27 mM). All animals were weighed and euthanized 4 weeks after their initial injection by exsanguination under anesthesia.

Experiments with chemically permeabilized myocardial samples

Chemically permeabilized multicellular cardiac preparations (typically ~600 μm long and ~55 μm in diameter) were obtained by mechanical disruption of ventricular samples as previously described [14]. Individual preparations were then attached between a force transducer (403B, Aurora Scientific, Aurora, Canada, frequency response 600 Hz) and a motor (312B, Aurora, step-time ~0.6 ms) by crimping their ends into metal troughs with overlays of monofilament (see Fig 1B of [15]) and stretched in relaxing solution to a sarcomere length of ~2.25 μm. During the experiments, preparations were immersed in solutions with pCa values ranging from 9.0 (1 nM free Ca^{2+}) to 4.5 (32 μm free Ca^{2+}). Once tension had reached steady-state in each solution, the preparation was subjected to three triangular length changes (0.04 ± 0.12 l_0 s^{-1}, where l_0 is the initial length of the preparation) followed by a large shortening/re-stretch maneuver (0.2 l_0, 20 ms duration) to assess tension recovery (Fig 1). Preparations were also evaluated for their force-pCa relationship [14].

Mechanical records were analyzed in MATLAB (MathWorks, Natick, MA) using custom-written routines. k_{tr}, the rate of tension recovery following the rapid shortening/re-stretch maneuver, was calculated by fitting a single exponential function to the recovery time-course. Relative tension was defined as the steady-state force (P_{ss}, Fig 2B) divided by the corresponding value measured for the preparation in maximally-activating pCa 4.5 solution. The short-range force (SRF). elastic limit (EL) and the relative force at the end of the shortening movement (P_{min}/P_{ss}) (Fig 2B) were calculated using an algorithm that has been previously described by our group [14].

Gel electrophoresis

Myosin Heavy Chain—The relative content of α- and β-myosin heavy chain in the individual preparations that were used for mechanical experiments was determined using resolving gels that were 60 × 80 × 0.75 mm and which contained 7% Acrylamide (50:1 with bis-Acrylamide) and 35% glycerol. These gels were run at a constant current of 3.8 mA for ~20 hours at 4°C. Myosin heavy chain molecules were extracted from the individual preparations using a buffer containing 100 mM KCl, 100 mM KH_2PO_4, 50 mM K_2HPO_4, 10

mM EDTA, 10 mM $Na_4P_2O_7.10H_2O$, 4 mM β-Mercaptoethanol and 5% (v/v) Triton X-100 (pH 6.5, 4-8°C, 10 μl volume, 24 hours). Samples were prepared for loading on gels by mixing with double the volume of Laemmli sample buffer and heating to 95°C for 4 minutes. Gels were stained using a commercial kit (Silver Stain Plus, Bio-Rad, Hercules, CA) and scanned using a conventional office device (V500 Photo, Epson, Long Beach, CA). The relative content of each isoform was determined by fitting the densitometry profiles with asymmetric Gaussian functions using GelBandFitter software previously developed by our laboratory [16].

Tissue samples for other proteins—Gels to analyze relative titin isoform content and potential changes in the phosphorylation of selected sarcomeric proteins were run using samples prepared from through-wall ventricular tissue segments that were cut from the animal as soon as it was euthanized. These segments were immediately frozen in liquid N_2 and subsequently stored at −80°C. Gel samples were prepared from the segments by thawing and homogenizing small pieces of tissue in a sample buffer (30:1 v/w buffer to muscle tissue) containing 8 M Urea, 2 M Thiourea, 3% SDS w/v, 75 mM DTT, 0.03% Bromophenol blue, and 0.05 M Tris-HCl, pH 6.8. Once homogenization was complete the samples were boiled at 95°C for 3 minutes and then cooled on ice. Samples for titin gels were mixed with an additional 25% v/v glycerol before loading [17].

Titin—Titin isoforms were resolved using SDS-vertical agrose gel electrophoresis (SDS-VAGE) on 160 × 160 × 1 mm gel plates [17]. These contained 1.2% w/v Sea Kem Gold Agarose, 30% v/v Glycerol, 0.05 M Tris-base, 0.384 M Glycine, and 0.1% w/v SDS and were precooled to 6°C. The lower and upper chamber buffers contained 0.05 M Tris-base, 0.384 M Glycine, and 0.1 % SDS, with an additional 0.01 mM β-Mercaptoethanol in the upper chamber solution. Gels were run at constant voltage (220V) at 7°C for 6 hours and stained with SYPRO Ruby (Invitrogen, Carlsbad, CA). Gels were visualized with a Typhoon Trio+ Variable Mode scanner (GE Healthcare, Piscataway, NJ) and quantified with ImageQuant TL software (GE Healthcare).

Regulatory proteins—Samples were run on precast gels (Mini-Protean TGX 4-15%, 15 well combs, Bio-Rad) and stained with Pro-Q Diamond phosphoprotein gel stain (Invitrogen) according to the manufacturer's instructions with some minor modifications [18, 19]. Phosphorylation of myosin binding protein C (MyBP-C), myosin light chain 2 (MLC2), troponin I (TnI), and troponin T (TnT) was assessed by scanning the Pro-Q Diamond stained gel, and subsequently re-scanning the gel after it had been stained with SYPRO-Ruby to assess total protein content. The phosphorylation levels were quantified by integrating the densitometry profile (ImageQuant TL software) of the selected band in the Pro-Q Diamond image and dividing that result by the corresponding integral calculated for the SYPRO Ruby stained image. Relative phosphorylation levels for the different experimental samples were calculated by normalizing to the mean ratio calculated for the F-CTRL samples.

Statistics

Data relating to mechanical parameters (for example, isometric tension and short-range responses) were analyzed using linear mixed models (SAS 9.2, SAS Institute, Cary, NC). These procedures increased the statistical power of the analyses by nesting data obtained from each preparation that was analyzed within a random variable describing the animal. The categorical variables were sex and STZ treatment for 2 factor linear mixed models, and sex, STZ treatment and pCa for 3 factor linear mixed models. All tests assumed compound symmetry for the covariance structure. Only main effects and interaction terms were reported in this study.

Other types of data were analyzed using 2-way ANVOA (MATLAB, The Mathworks, Natick, MA) with sex and STZ treatment as the categorical variables. Only the main effects and interaction terms are reported.Stepwise linear regression was used to test which changes in isoform content and phosphorylation status were correlated with mechanical properties measured in pCa 4.5 solution. To determine the relationship to MHC, a simple linear regression was also performed between the myosin isoform ratio and the mechanical parameters. These tests were also performed in MATLAB and provide a way of testing which proteomic effects influence the observed mechanical effects.

Summary results were reported as mean ± SEM. p values less than 0.05 were considered statistically significant.

RESULTS

At the start of the study, blood glucose levels for the four groups of rats (F-CTRL, F-STZ, M-CTRL, MSTZ) ranged between 5 and 6 mM (Table 1). One week after STZ injection, blood glucose levels had risen to above 27 mM (the limit of our monitoring device) and remained elevated for the duration of the 4 week conditioning period. The glucose levels in the control animals did not change markedly during this time.

When the animals were euthanized, the hearts of the F-STZ and M-STZ animals weighed less than the hearts isolated from the control rats (Table 2). However, the M-STZ and F-STZ rats lost a proportionally greater amount of body weight than the control animals so that the heart weight to body weight ratio was significantly greater (2-way ANOVA, $p<0.004$) in the animals that had been injected with STZ. Interestingly, there was a statistical interaction ($p<0.046$) between the STZ status and sex showing that the M-STZ rats had greater heart-weight to body-weight ratios than the F-STZ rats at the end of the study.

Fig 1 shows force records that illustrate the mechanical properties of myocardial preparations isolated from the four experimental groups when immersed in solutions with pCa values of 9.0, 5.8, 5.7, 5.4 and 4.5. Each displayed record is the average of at least 17 experimental traces obtained using different preparations isolated from the 6 or 7 hearts in each experimental group. Recordings made at additional pCa levels were omitted from this figure for clarity. However, summary data calculated from these additional recordings are included in other figures (for example, Fig 2C) later in this work.

Isometric force values in maximally activating pCa 4.5 solution were not markedly different for the experimental groups ($p>0.05$ for STZ, sex and interaction, Linear Mixed Model, Table 3). Neither were the pCa_{50} values nor the Hill coefficients ($p>0.05$ for STZ, sex and interaction, Linear Mixed Model, Table 3). There were, however, marked differences in the force responses to the stretch-release cycles. For example, the short-range force responses were much more prominent in the preparations isolated from the STZ treated animals (F-STZ and M-STZ groups, Fig 1 panels B and D) than in the preparations from the control rats (F-CTRL and M-CTRL, panels A and C). This initial, qualitative, observation implies that the streptozotocin injection had a substantial effect on the dynamic mechanical properties of the myocardial tissue in both sexes even though it did not impact the steady-state tension-pCa relationships.

Fig 2A shows the average force responses to the first stretch-release cycle measured in maximally activating pCa 4.5 solution for the F-CTRL and F-STZ groups. Similar effects were seen with the male rats. The short-range forces were greater in the STZ preparations than in the CTRL samples and force also dropped to a lower level during the shortening phase of the movement. Recordings made at different levels of Ca^{2+} activation were objectively analyzed using the algorithm described in Figure 2 of Mitov *et al*. [14]. Fig 2C, D and E in the present work show grouped data (mean ± SEM for values calculated from the individual recordings) for the relative short-range force, (SRF/P_{ss}, Panel C), the elastic limit (EL, Panel D) and the relative force at the end of the shortening motion (P_{min}/P_{ss}, Panel E), all plotted against the relative isometric tension.

Statistical analysis showed that the relative short-range force was elevated by ~50% in the STZ treated animals (Fig 2C, $p<0.001$, Linear Mixed Model, Table 4). We also observed a statistically significant effect related to the activating pCa. However the magnitude of the pCa effect was relatively small (~10%) compared to the effect of STZ and the physiological significance is unclear. Note that when used with >17 preparations from 4 groups at each pCa, the linear mixed model has a high statistical power and can therefore assign low p values to relatively small changes. The elastic limit was also increased by ~50% in the STZ treated animals (Fig 2D, $p<0.001$, Linear Mixed Model, Table 4) and exhibited smaller variations (~25%) associated with pCa. The final dynamic property measured in this work, the relative tension at the end of the first shortening movement, varied significantly with STZ, sex and pCa (P_{min}/P_{ss}, Fig 2E Table 4). Again, the magnitude of the change (~25%) was greatest for the STZ factor.

Fig 3 shows that k_{tr} values, a standard measure of the cross-bridge cycling rate in isometric muscle, were depressed in tissue from the diabetic animals at high levels of activation. STZ treatment and activating pCa were both statistically significant factors in the linear mixed model (Table 4). It is also interesting to note that differences between the CTRL and the STZ values were much larger at relative tension values above ~0.5 than they were at lower relative tension values.

Consistent with previous studies [20, 21], the present experiments demonstrated that isometric force and passive stiffness values measured in pCa 9.0 solutions with minimal free Ca^{2+} did not differ between the CTRL and STZ groups at physiological sarcomere lengths

(p>0.05 for STZ, sex and interaction, Linear Mixed Model, Table 3). SDS-VAGE gels [17] did however show a small but statistically significant reduction in the proportion of titin found as the 'stiff' N2B isoform (molecular weight ~2.97 MDa) in the STZ samples (2 way ANOVA, p<0.008, STZ). The percentage of titin expressed as the larger N2BA isoform, N2BA-A1 (~3.39 MDa) was increased in the STZ animals while the relative content of the smaller N2BA-A2 isoform (~3.22 MDa) was decreased (Table 5) [22]. Myocardial collagen content was not measured in this work but others [21] have shown that it could be increased by streptozotocin injection at 6 weeks.

Analytical electrophoresis performed using the individual samples that were used for the mechanical experiments showed that the relative content of β-myosin heavy chain switched from 30 ± 4% and 35±2% in the F-CTRL and M-CTRL groups to 100% and 99±1% in the F-STZ and M-STZ groups (Table 5). The relative phosphorylation of selected regulatory proteins (MyBP-C, MLC2, TnI and TnT) were evaluated using Pro-Q Diamond. TnT molecules exhibited higher relative phosphorylations in the male samples than in those from the the female rats (p=0.002, 2 Way ANOVA). MLC2 phosphorylation was depressed by STZ treatment but the effect was much larger in females than in males (STZ, p=0.003, Sex, p<0.001, Interaction, p<0.001, 2 Way ANOVA, Table 5). Sample gels are shown in Supplemental Fig. S1. No other statistically signifcant changes in protein phosphorylation were detected.

Finally, stepwise multiple linear regression tests were performed to determine which of the relative isoform contents and/or relative phosphorylation levels were linearly related to the functional properties measured in pCa 4.5 solution. Summary results from these tests are shown in Table 6. The main finding was that the proportion of myosin expressed as β-myosin heavy chain was the most significant predictor of relative short-range force (SRF/P_{ss}), elastic limit (EL), and relative force at the end of the shortening motion (P_{min}/P_{ss}), and k_{tr}. The proportion of titin expressed as the N2B isoform was also statistically linked to the relative short-range force values but this effect was modest (p=0.013) compared to the statistical strength of the myosin effects (p values for each short-range parameter all less than 1×10^{-3}). Simple linear regression (Supplemental Fig. S2) suggests that these mechanical properties are also directly related to myosin isoform expression, and in humans where only ~7% of α-MHC is expressed, the mechanical properties change by ~5%.

DISCUSSION

Diabetic cardiomyopathy is becoming a very important clinical problem [1, 2] but the molecular mechanisms that underpin the associated ventricular dysfunction are not yet clear [23]. The earliest sign of the cardiomyopathy is diastolic dysfunction [4], which manifests as reduced early rapid ventricular filling. Since filling requires stretching myocardium, this suggests that the tissue's dynamic mechanical properties (those properties that are revealed during imposed length changes) might be affected earlier after the onset of diabetes than properties, such as tension-pCa curves, that are measured under near isometric conditions [24].

This study used the streptozotocin (STZ) induced rat model of type 1 diabetes that is known to exhibit diastolic dysfunction after 4 weeks[6, 7]. The novel finding of this work was that STZ treatment increased short-range forces by ~67% (Fig 2C). This effect was not due to an increase in the passive stiffness of the muscle but rather due to an increased elastic limit (Fig 2D). Put simply, the preparations from the diabetic animals could be stretched further before the force response peaked during the imposed movement. Previous work [25, 26] had shown that cross-bridges cycle more slowly under isometric conditions in myocardium isolated from diabetic rats than in samples from non-diabetic controls. The present results show that this effect persists in moving myocardium as well. This is significant because it implies that the myosin heads do not detach as soon as they are stretched to a certain length. Moreover, the present results suggest that the cross-bridge detachment in diabetic myocardium is slowed during both lengthening *and* shortening motions. The former effect is supported by the increased elastic limit in the diabetic preparations (cross-bridges are pulled farther before they detach during lengthening). The latter effect is implied by the decreased P_{min}/P_{ss} values in the STZ samples (Fig 2E) which can be explained if myosin heads are pulled further before they detach during shortening motions in diabetic tissue than in control samples.

Molecular mechanisms

The most obvious explanation for the STZ-induced increase in short-range forces is the shift in myosin heavy chain expression. Similar to previous studies [27, 28], our data (Table 5) show that the relative content of the slowly cycling β isoform increased from ~25% in the CTRL samples to 100% in the STZ preparations. This could explain the increased short-range forces because the slower cycling β myosin heavy chain molecules have recently been shown to have longer attached lifetimes [29, 30]. They will thus produce larger elastic limits and greater short-range forces. Since there is clear evidence that myosin heads remain attached during diastole[9, 10]{Syme, 2013 #3755}, this isoform shift could contribute to diabetes-induced diastolic dysfunction.

The contributions of other changes in isoform content and posttranslational status to the alteration in short-range forces are less clear. When the mechanical data obtained in pCa 4.5 solution were analyzed using stepwise linear regression (Table 6), the only significant effect not linked to β myosin heavy chain was a correlation between the proportion of titin expressed as the N2B isoform and the relative short range force. Although titin-actin interactions and Ig domain unfolding are known to influence passive mechanical properties [31], the relationship to short-range forces is a new and potentially important finding. One possible interpretation is that titin influences actin-myosin interactions, perhaps by altering thick filament compliance [32]. We also note that total STZ treatment did not change passive stiffness (Table 3). Although we did not measure collagen content in this work, Bupha-Intr *et al.* [21] have shown that it is increased 6 weeks after STZ injection in this animal model, potentially compensating for the reduced titin stiffness. These data support the finding that the elastic limit rather than passive stiffness drives the changes in short range force.

While not the focus of this study, no change in calcium sensitivity was observed. This is potentially due to the change in myosin isoforms[33] in combination with (or compensation

for) the change in phosphorylation[34-36] that we observed(Table 5). More detailed analysis of these factors and troponin T isoform expression[35] are required before additional conclusions can be made about calcium sensitivity in this model.

Relevance to human disease

At this point, the most conservative way to interpret the current data is to attribute most of the STZ-induced changes in dynamic mechanical properties to the large increase in the relative content of the slower cycling β myosin heavy chain isoform. Healthy humans are reported to express ~93% of their ventricular myosin as the β isoform [37, 38] (although some studies suggest that humans express little or no α-MHC[39]). We believe that even a small shift in myosin heavy chain may still be relevant to cardiac dysfunction in humans with diabetes. Our data shows that the short-range force and mechanical properties are primarily controlled by MHC isoform expression (Table 6). Further, simple linear regression reveals that a ~5% change in the mechanical properties of short-range force and elastic limit that related to cross-bridge forces would be found in humans (Supplemental Fig. S2). These small changes have a potentially large impact because the force generated by a single cross-bridge is similar to the passive elastic of a molecule of titin[40, 41] and may thus impact diastolic function[6, 7]. Furthermore, diabetic cardiomyopathy is a chronic condition that patients often live with for many years. The relatively small reduction in myocardial performance associated with a shift from 93% to 100% β myosin heavy chain might, in the long term, leave patients more vulnerable to cardiac remodeling and associated heart failure.

It is also possible to speculate that since humans experience smaller shifts in myosin heavy chain expression than rodents, posttranslational changes to sarcomeric proteins have a proportionally bigger influence on dynamic mechanical properties. Bearing this in mind, it is interesting to note that women with diabetes are more likely to develop heart failure than men with diabetes [42]. Our data (Table 5) show that there was a statistical interaction between sex and STZ for MLC2 phosphorylation, but that short-range forces for preparations from male and female rats were not different. The F-STZ samples exhibited lower relative phosphorylation levels than the F-CTRL samples but similar values to those measured for the M-STZ preparations. This could potentially indicate the loss of a “protective sex effect” in females with diabetes. Similarly, one could imagine that the lower relative TnT phosphorylation levels observed in the female samples (Table 5) detrimentally affects myocardial performance. Finally, it is possible that the increased risk of heart failure in females with diabetes reflects a decreased heart weight to body weight ratio similar to that shown in Table 2.

Summary

In conclusion, this study shows that dynamic short-range forces are larger in myocardium from diabetic rats than in control rats. The effect is represents increased content of β myosin heavy chain in the rats treated with STZ. These observations are worth exploring in future studies, perhaps with human samples, given the clinical significance of diabetic cardiomyopathy and the current lack of useful treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Diabetes is associated with diastolic dysfunction.
- We studied myocardial stiffness in a rat model of diabetes.
- Short-range force, a cross-bridge based property, is increased in diabetic hearts.
- An increase in β myosin heavy chain isoform expression is the likely mechanism.

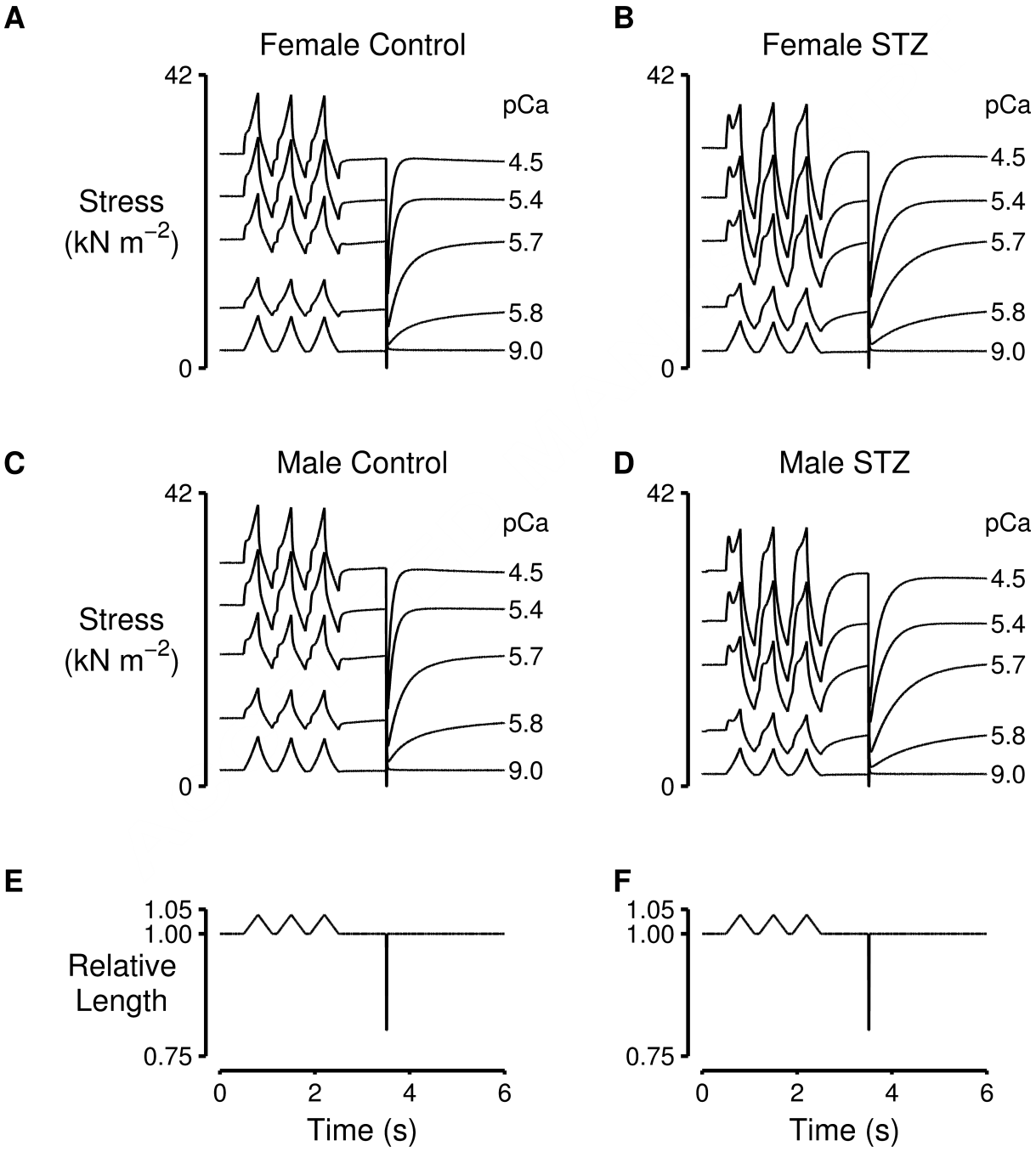

Figure 1.

Example experimental records. The top 4 panels (A, B, C and D) show the average of the force traces recorded for each experimental group for 5 selected pCa solutions. Each of the plotted traces is the average of trials performed with at least 17 different preparations from at least 6 animals. The relative length change (E and F) was the same for every trial and is shown in schematic form in the bottom panels.

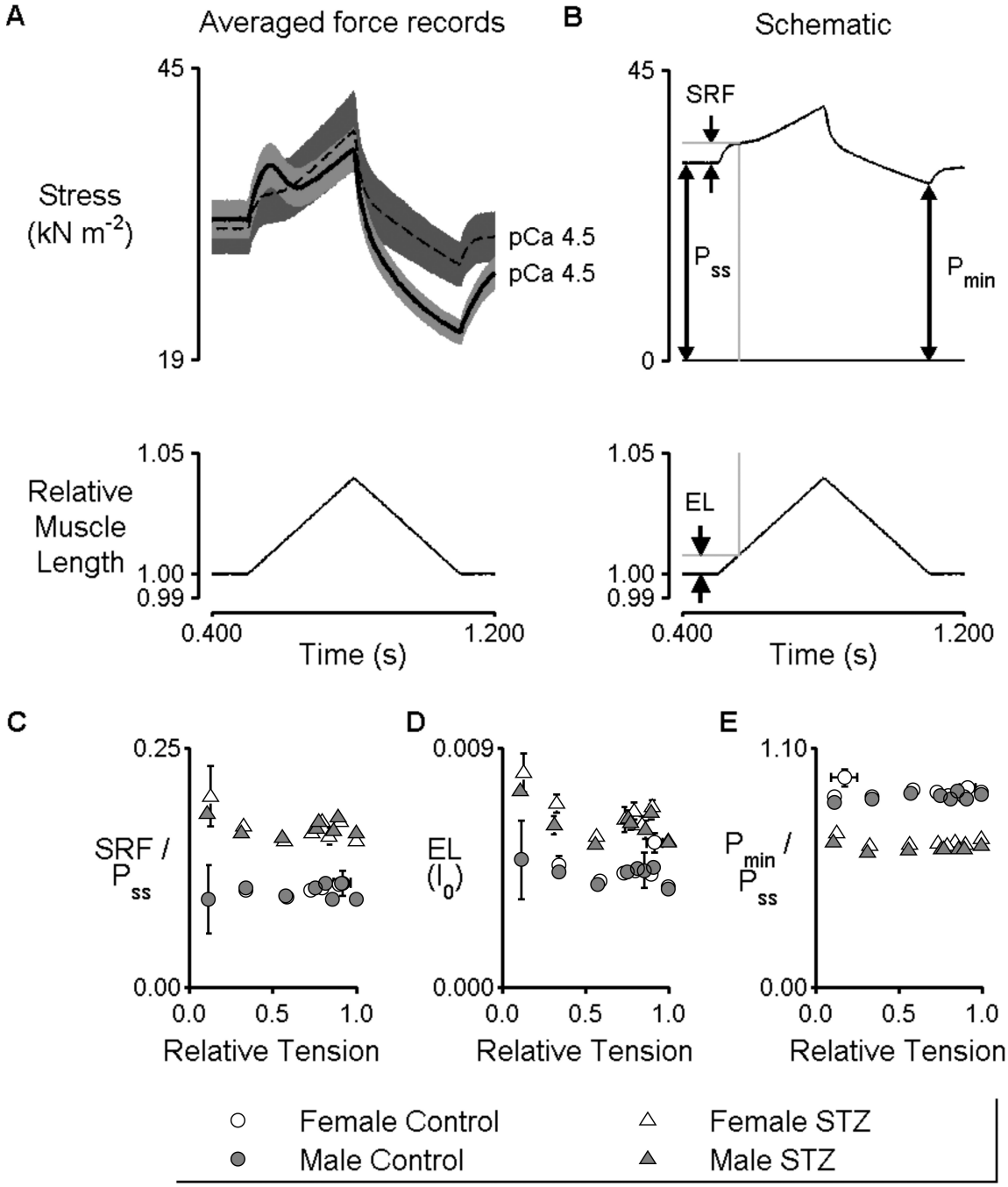

Figure 2.

Short-range properties. A) Enlarged version of the averaged force records obtained in pCa 4.5 solution for the first stretch-shortening cycle for the F-CTRL (dashed line) and F-STZ (thick black line) groups. The records are replotted from the top traces in Fig 1A,B. The gray regions around each record show the standard error of the force measurements obtained with the different preparations (n≥17 myocardial preparations for each experimental group, F-CTRL: dark gray; F-STZ: light gray). B) Schematic shows P_{ss} (steady-state force), SRF (short-range force), P_{min} (the force value at the end of the shortening movement) and EL

(the elastic limit, the length change at the short-range force). C-E) Symbols show the mean ± SEM of data values measured in different pCa solutions for (C) the relative short-range force (SRF/P_{ss}), (D) the elastic limit and (E) the relative force at the end of the shortening movement (P_{min}/P_{ss}) for the 4 experimental groups plotted against relative isometric tension. The relative tension in pCa 4.5 solution was defined as 1. See Table 4 for statistical comparisons.

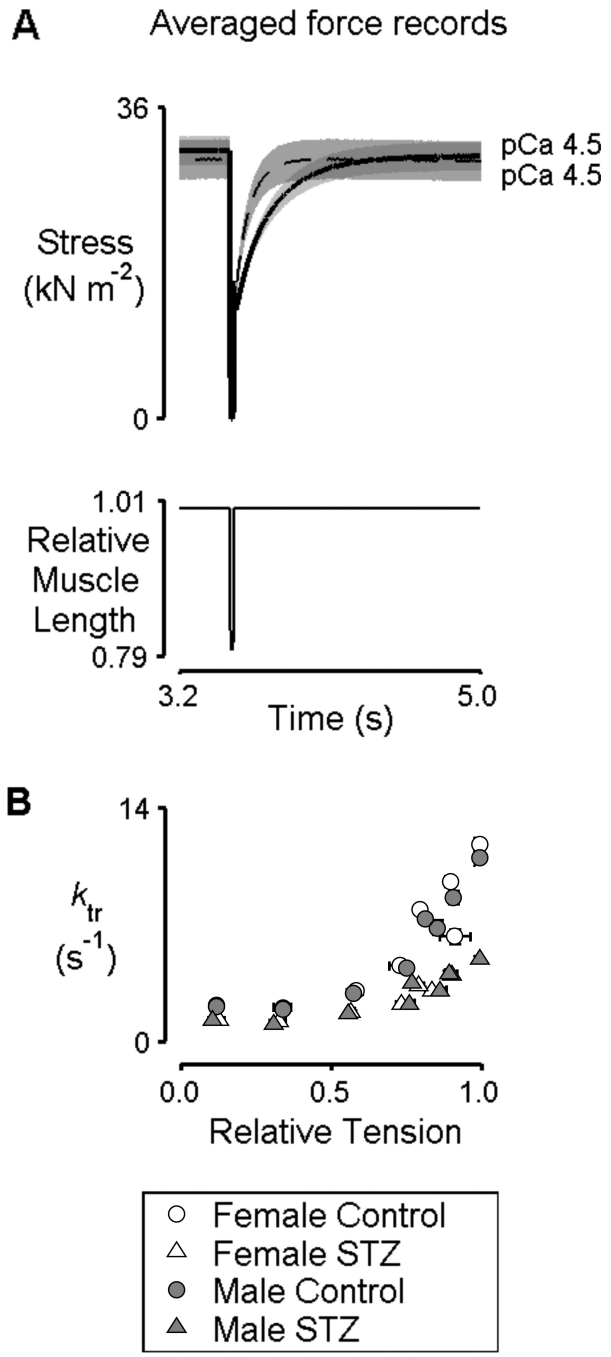

Figure 3.

Tension recovery kinetics. A) Enlarged version of the averaged force records obtained in pCa 4.5 solution for the rapid shortening/re-stretch maneuver for the F-CTRL (dashed line) and F-STZ (thick black line) experimental groups. The records are replotted from the top two panels in Fig 1. The gray regions around each record show the standard error of the force measurements obtained with the different preparations (n≥17 myocardial preparations for each experimental group, F-CTRL: dark gray; F-STZ: light gray). B) Symbols show the mean ± SEM of data values measured in different pCa solutions for the rates of tension

recovery (k_{tr}) for the different experimental groups plotted against relative isometric tension. The relative tension in pCa 4.5 solution was defined as 1. See Table 4 for statistical comparisons.

Table 1

Blood glucose concentrations.

Experimental Group			Blood glucose concentration (mM)				
				Weeks after injection			
			Initial	1	2	3	4
Control	Female	n=7	5.7 ± 0.2	4.5 ± 0.1	5.4 ± 0.1	5.4 ± 0.2	5.4 ± 0.2
	Male	n=7	5.1 ± 0.2	4.8 ± 0.3	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.2
STZ	Female	n=7	5.6 ± 0.2	>27	>27	>27	>27
	Male	n=6	6.0 ± 0.2	>27	>27	>27	>27

STZ: streptozotocin injected. Data mean±SEM.

Table 2

Physical parameters at time of sacrifice.

	Control		STZ		2 Way ANOVA p-value		
					Independent Factor		Interaction
	Female	Male	Female	Male	STZ	Sex	STZ × Sex
Body Weight (g)	286 ± 4	383 ± 10	248 ± 5	280 ± 8	<0.001	<0.001	<0.001
Heart weight (mg)	880 ± 14	1107 ± 50	789 ± 15	952 ± 40	0.001	<0.001	0.357
Heart weight/body weight (mg/g)	3.08 ± 0.08	2.89 ± 0.12	3.19 ± 0.09	3.40 ± 0.09	0.004	0.92	0.046

Data mean±SEM.

Table 3

Mechanical properties of passive and isometrically contracting chemically permeabilized myocardial preparations. Measurements were performed on 2 or 3 preparations from each of 6 or 7 hearts per experimental group.

	Control		STZ		Linear Mixed Model p-value		
					Independent Factor		Interaction
	Female	Male	Female	Male	STZ	Sex	STZ × Sex
Passive							
P_{ss} in pCa 9.0 (kN m^{-2})	2.53 ± 0.53	2.31 ± 3.89	2.46 ± 0.81	1.76 ± 026	0.460	0.279	0.552
Passive Stiffness (kN m^{-2})	75.8 ± 26.4	77.6 ± 21.7	94.3 ± 17.4	64.2 ± 14.3	0.908	0.466	0.403
Active							
P_{ss} in pCa 4.5 (kN m^{-2})	30.4 ± 2.3	31.6 ± 1.9	31.6 ± 2.0	31.0 ± 2.3	0.933	0.872	0.629
Hill coefficient	4.04 ± 0.15	3.95 ± 0.20	3.70 ± 0.16	3.64 ± 0.23	0.087	0.943	0.588
pCa_{50}	5.69 ± 0.01	5.69 ± 0.01	5.68 ± 0.01	5.68 ± 0.01	0.538	0.570	0.761

P_{SS}: steady state force; pCa: relative calcium concentration ($-\log_{10}[Ca^{2+}]$); pCa_{50}: calcium concentration generating half-maximal force, a measure of calcium sensitivity. Data mean±SEM.

Table 4

Significance of dynamic parameters (Figs 2 and 3) by a Linear Mixed Model.

	Linear Mixed Model p-value						
	Independent Factor			Interaction			
	STZ	Sex	pCa	STZ × Sex	STZ × pCa	pCa × Sex	STZ × Sex × pCa
SRF/P_{ss}	<0.001	0.665	<0.001	0.650	0.301	0.862	0.806
EL	<0.001	0.442	<0.001	0.764	0.199	0.848	0.915
P_{min}/P_{ss}	<0.001	0.048	<0.001	0.505	<0.001	0.891	0.997
k_{tr}	<0.001	0.336	<0.001	0.409	<0.001	0.510	0.185

SRF/P_{SS}:normalized short-range force; EL: elastic limit of bound myosin heads; P_{min}/P_{SS}; the relative force at the end of the shortening movement; k_{tr}: rate of force redevelopment upon re-stretch.

Table 5

Expression and phosphorylation of sarcomeric proteins.

	Control		STZ		2 Way ANOVA p-value		
					Independent Factor		Interaction
	Female	Male	Female	Male	STZ	Sex	STZ × Sex
Titin							
% N2BA-A1	1.3 ± 0.2	1.2 ± 0.2	5.1 ± 0.5	5.4 ± 0.5	<0.001	0.750	0.616
% N2BA-A2	5.7 ± 0.5	5.6 ± 0.3	3.2 ± 0.4	3.4 ± 0.5	<0.001	0.931	0.0628
% N2B	93.0 ± 0.6	93.2 ± 0.3	91.7 ± 0.5	91.1 ± 0.8	0.008	0.786	0.896
Myosin							
% β-myosin heavy chain	21.9 ± 5.2	24.4 ± 10.0	100 ± 0.0	98.71 ± 2.3	<0.001	0.191	0.055
Regulatory Protein Phosphorylation (relative to F-CTRL)							
pMyBP-C	1.00 ± 0.07	0.82 ± 0.08	0.88 ± 0.05	0.86 ± 0.05	0.526	0.125	0.279
pMLC2	1.00 ± 0.08	0.47 ± 0.04	0.60 ± 0.03	0.49 ± 0.05	0.003	<0.001	<0.001
pTnI	1.00 ± 0.12	1.05 ± 0.16	0.82 ± 0.07	0.89 ± 0.07	0.156	0.628	0.937
pTnT	1.00 ± 0.12	1.53 ± 0.11	1.31 ± 0.15	1.66 ± 0.05	0.108	0.002	0.388

Data mean±SEM.

Table 6

Stepwise regression analysis.

Parameter	R^2	p-value for multiple linear regression	p-values for individual variables						
			Proportion of myosin as	Proportion of titin as		Phosphorylation relative to F-CTRL values of			
			β-myosin heavy chain	N2B isoform	N2BA1 isoform	MyBP-C	MLC2	TnI	TnT
SRF/P_{ss}	0.927	2.0×10^{-7}	2.9×10^{-10}	0.013	0.342	0.382	0.433	0.767	0.685
EL	0.798	3.2×10^{-7}	3.0×10^{-4}	0.188	0.401	0.655	0.090	0.355	0.910
P_{min}/P_{ss}	0.945	2.5×10^{-8}	8.9×10^{-14}	0.148	0.588	0.187	0.533	0.668	0.685
k_{tr}	0.927	1.8×10^{-7}	1.0×10^{-12}	0.878	0.611	0.217	0.994	0.777	0.863