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Altered myofilament structure and function in dogs with Duchenne muscular dystrophy cardiomyopathy

Ait Mou *et al*, Myofilament structure - function in DMD

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Abstract

Aim: Duchenne Muscular Dystrophy (DMD) is associated with progressive depressed left ventricular (LV) function. However, DMD effects on myofilament structure and function are poorly understood. Golden Retriever Muscular Dystrophy (GRMD) is a dog model of DMD recapitulating the human form of DMD.

Objective: The objective of this study is to evaluate myofilament structure and function alterations in GRMD model with spontaneous cardiac failure.

Methods and results: We have employed synchrotron x-rays diffraction to evaluate myofilament lattice spacing at various sarcomere lengths (SL) on permeabilized LV myocardium. We found a negative correlation between SL and lattice spacing in both sub-epicardium (EPI) and sub-endocardium (ENDO) LV layers in control dog hearts. In the ENDO of GRMD hearts this correlation is steeper due to higher lattice spacing at short SL (1.9 μm). Furthermore, cross-bridge cycling indexed by the kinetics of tension redevelopment (k_{tr}) was faster in ENDO GRMD myofilaments at short SL. We measured post-translational modifications of key regulatory contractile proteins. S-glutathionylation of cardiac Myosin Binding Protein-C (cMyBP-C) was unchanged and PKA dependent phosphorylation of the cMyBP-C was significantly reduced in GRMD ENDO tissue and more modestly in EPI tissue.

Conclusions: We found a gradient of contractility in control dogs' myocardium that spreads across the LV wall, negatively correlated with myofilament lattice spacing. Chronic stress induced by dystrophin deficiency leads to heart failure that is tightly associated with regional structural changes indexed by increased myofilament lattice spacing, reduced phosphorylation of regulatory proteins and altered myofilament contractile properties in GRMD dogs.

Keywords: Heart Failure; Left Ventricular failure; Length Dependent Activation, Myofilament Properties; Contractile Proteins; Transmural Heterogeneity; Muscular Dystrophy; Hypertrophic Cardiomyopathy

Introduction

Duchenne Muscular Dystrophy (DMD) is an X-linked genetic mutation and lethal muscular disorder. This pathology is characterized by loss of dystrophin protein, a protein involved in striated muscle structure and function maintenance¹. Heart failure constitutes, together with respiratory failure, the main leading cause of mortality in patients with muscular dystrophy². Only a few studies have examined the impact of DMD on cardiac contractility^{3,4}. Thus, the cellular molecular mechanisms underlying this myopathy-linked myocardial contractile disorder are poorly understood and yet to be discovered.

In most species, the left ventricle (LV) is characterized by a gradient of contractility across its free wall. The inner LV layer (Endocardium, ENDO) develops higher passive and active contractile properties, when compared to the outer LV layer (Epicardium, EPI)⁵⁻⁸. This gradient of contractility is significantly reduced in ischemic heart failure, caused, mainly, by failure in the ENDO layer contractile efficiency⁸. Myofilament contractile heterogeneity is also observed in the heart of healthy Golden Retriever dogs³. Similar to rodent models of heart failure, the gradient of transmural contractility is altered in Golden Retriever muscular dystrophy (GRMD) dogs³. The GRMD mimics more closely the human disease of DMD compared to other existing mammalian models of dystrophin deficiency⁹. A mutation in the dystrophin gene of GRMD dogs leads to dystrophic muscle lesions, inflammatory foci, progressive fibrosis and fatty infiltration, early locomotor impairment, and premature death due to respiratory or cardiac failure. Our previous report showed that GRMD dogs with heart failure display marked alterations in contractile properties as assessed from myocytes isolated from the ENDO layer. These alterations were correlated with abnormal sarcomeric protein phosphorylation and impaired e/nNOS content³.

In the present work, we investigated the effect of muscular dystrophy on sarcomere structure and its impact on contractile function. To this aim, we investigated the modulation of cardiac force production by length change, a phenomenon termed length-dependent activation (LDA)^{10,11}, in the GRMD model. LDA forms the cellular basis of the cardiac Frank-Starling mechanism¹². Increased Ca²⁺ sensitivity of the sarcomere upon stretch involves complex and dynamic mechanisms at the myofilament level that are still incompletely understood. We found a negative correlation between sarcomere length (SL) and lattice spacing both in EPI and ENDO non-failing wild-type dog myocardium. At short SL, muscular dystrophy induced an increased myofilament lattice spacing and decreased phosphorylation levels of regulatory contractile proteins that are associated with increased myofilament sensitivity and cross-bridge cycling kinetics. We hypothesized that increased myofilament lattice spacing yield to more optimal orientation of myosin heads resulting in improved cross-bridge kinetics.

Methods

An expanded Methods section is available in the Data Supplement.

Animal model

The animal care and the experimental protocol were in accordance with the Directive 2010/63/EU of the European Parliament and approved by the local animal ethical committee.

Cardiac tissue was obtained from one-year old GRMD dogs (CEDs, Mézilles, France) with a fractional shortening $\leq 30\%$ (n=4) and age-matched normal golden retriever dogs

of the same genetic background (n=4). Conventional echocardiography and 2D color tissue Doppler imaging (TDI) were performed with a Vivid 7 ultrasound unit equipped with 5-7.5 and 2-5MHz phased-array transducers (GE, Waukesha, WI) as previously described³. After sacrifice (pentobarbital, 100 mg/kg), cardiac tissues were collected from the sub-endocardium or sub-epicardium, frozen in liquid nitrogen and stored at -80°C until use.

Permeabilized cardiomyocyte mechanics:

Myofilament Ca^{2+} sensitivity and cross-bridge cycling kinetics as indexed by the exponential rate of tension redevelopment (k_{tr}) were measured in single permeabilized cardiomyocytes isolated from frozen dog LV myocardium as previously described^{3,6}. The Force-calcium relationship was studied at either 1.9 or 2.3 μm sarcomere length (SL)⁸. Kinetic of tension redevelopment (k_{tr}) was measured by mechanically disrupting force-generating cross-bridges induced by rapid release/restretch protocol at either sub-maximal activating solution ($[\text{Ca}^{2+}] = 1.3 \mu\text{mol/L}$) or at maximal calcium activation ($[\text{Ca}^{2+}] = 32 \mu\text{mol/L}$)⁵. For each cardiomyocyte, at a given SL, the above protocol was repeated 3 times and the average k_{tr} was estimated

Myofilament lattice spacing:

Permeabilized LV myocardium was prepared according to previous reports¹³. Frozen LV was rapidly thawed, homogenized in ice-cold relaxing solution using Polytron homogenizer (3s, 1,000 RPM; Power Gen 700D; Fisher Scientific), and permeabilized with 1% Triton X-100 containing relaxing solution overnight at 4°C.

X-ray diffraction experiments were conducted at the BioCAT undulator-based beamline 18ID at the Advanced Photon Source (Argonne National Labs, Lemont,¹⁴). The permeabilized muscle bundles (~3-5 mm) were selected based on their shape (i.e. well defined edges) and striation visibility. Lattice spacing was measured as described previously¹⁵. The sample to detector distance for the small-angle X-ray diffraction camera was ~3m and the focal spot size was about 150 x 50 μm in the detector plane. Diffraction images were collected on a high sensitivity CCD-based X-ray detector with 39 μm pixels and an active area of 80 x 160mm. Exposure times were ~1s with an incident X-ray flux of $\sim 1 \times 10^{12}$ photons/s. Lattice spacing ($d_{1,0}$) was evaluated from the diffraction pattern using Bragg's law. The ratio of the intensities of the 1,1 and 1,01 equatorial reflections (I_{11}, I_{10}) were measured from nonlinear least square fits to 1-dimensional projections of the integrated intensity along the equator. X-ray reflections were assumed to have a Gaussian peak shape with the widths constrained by those expected for a paracrystalline hexagonal lattice.^{16, 17} To evaluate the length effect on myofilament lattice spacing x-rays diffraction experiments were conducted on relaxed muscles (~1.9 μm SL) and following stretch to various SL.

Western blot analysis.

Myocardial protein expression was studied, as previously described.¹⁸ Solubilized proteins in non-reducing Laemmli buffer were separated using SDS-PAGE electrophoresis and were revealed overnight with primary antibodies (see Supplemental Table S1). Bands were revealed and quantified with the Odyssey system (LI-COR Biosciences, Lincoln, Nebraska)

Results:

Effect of sarcomere length on lattice spacing in golden retriever dog model:

To evaluate trans-mural contractile heterogeneity in non-failing dog hearts, myocardium samples were isolated from both EPI and ENDO left ventricular layers. Here we tested whether myofilament lattice spacing responds differently to stretch in ENDO and EPI tissues. We obtained clear X-ray patterns from our tissue sample showing sharp equatorial reflections (Figure 1).

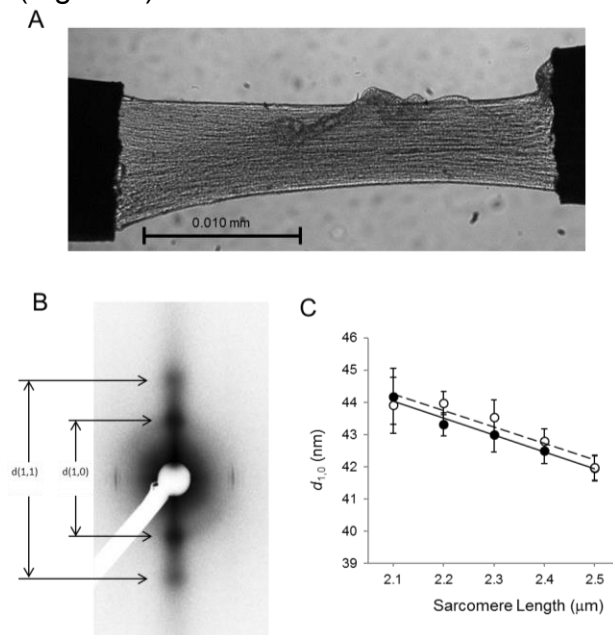


Figure 1: Transmural myofilament lattice spacing in myocardium from a dog animal model. A-B) Typical CCD image showing LV free wall permeabilized muscle strip (A) and equatorial diffraction pattern obtained from an ENDO dog myocardium (B). C) Myofilament lattice spacing ($d_{1,0}$) vs. SL relationships obtained in ENDO (open circle) and EPI (solid circle) control dog myocardium. (Data are expressed as mean \pm SEM, N=5)

As expected, spacing between thin and thick filament was reduced upon stretch. We found that $d_{1,0}$ decreased linearly with SL over the SL range from 2.1 to 2.5 μm in ENDO and EPI. The slope of the SL– $d_{1,0}$ relationship was similar in both myocardial tissue layers (Figure 1). To study the correlation between lattice spacing and LDA, we investigated sarcomere function in isolated myocytes from the same hearts by evaluating the myofilament Ca^{2+} sensitivity at short and long SL. Both ENDO and EPI cardiomyocytes showed a decreased EC_{50} at long SL indicating increased Ca^{2+} sensitivity (Figure 2, B). To estimate LDA, we computed the difference between EC_{50} at short (1.9 μm) and long (2.3 μm) SL (EC_{50}). This parameter is commonly employed to evaluate LDA. We found LDA to be higher in ENDO cardiomyocytes (Figure 2, C) as indexed by a significantly higher ΔEC_{50} in this region. These results indicate that in healthy dog myocardium, the higher length sensitivity of activation in the inner layer of the ventricle cannot be explained by a differential interfilament lattice spacing.

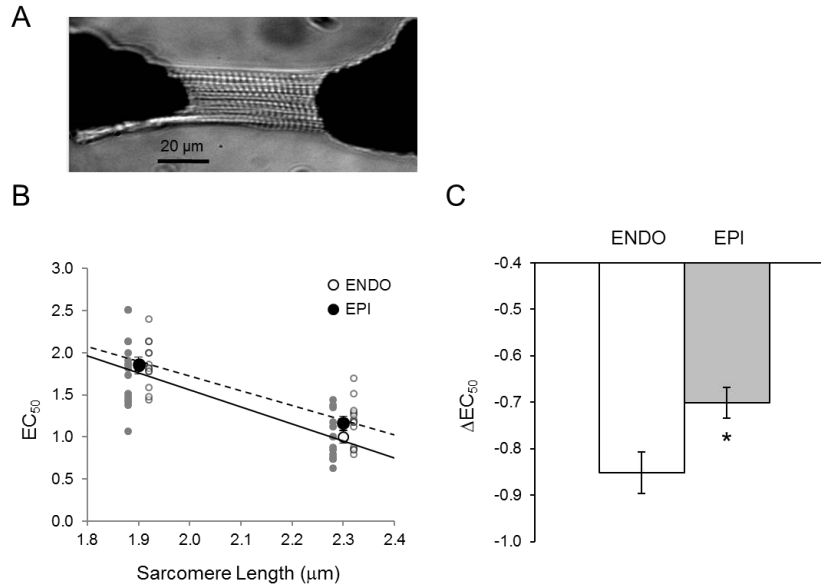


Figure 2: Transmural Length Dependent Activation (LDA) in permeabilized cardiomyocyte isolated from a dog heart. A) Typical CCD image showing a permeabilized dog myocyte. B) A typical negative relationship between $[Ca^{2+}]$ generating half of maximal active force (EC_{50}) and SL (1.9 and 2.3 μm) on EPI (open circle) and ENDO (solid circle) cardiomyocytes. Regression lines were obtained from the average of individual regression lines. C) LDA is evaluated by computing $\Delta EC_{50} = EC_{50}(2.3 \mu m SL) - EC_{50}(1.9 \mu m SL)$. (N=4 cells per animal, 4 animals per group). * vs. ENDO; $P < 0.05$.

Effect of DMD on cardiac myofilament Ca^{2+} sensitivity and lattice spacing:

To evaluate the severity of heart failure in GRMD, echocardiography measurement of LV function was performed. Our result showed significant reduction of the Fractional Shortening (FS, Table 1).

	Control	GRMD
Age (year)	1.1±0.1	1.0±0.0
Weight (kg)	26.8±2.5	16.8±0.6*
Heart rate (bpm)	101±12	160±21*
Fractional Shortening (FS) (%)	34.3±1.7	24.7±0.9*
LVDD (mm)	44.6±2.5	43.2±3.7
LVDD/BW	1.7±0.1	2.6±0.2*
N	4	4

Table 1: Echocardiographic data obtained from Control (non-failing) and GRMD (failing) dogs. Left Ventricular End Diastolic Diameter (LVDD); Body Weight (BW). Data are represented as mean±SEM (* vs. Control; $p < 0.05$).

We evaluated myofilament Ca^{2+} sensitivity and LDA on ENDO permeabilized cardiomyocytes isolated from both non-failing control dogs and GRMD with cardiac failure. Myofilament Ca^{2+} sensitivity was higher at short SL in ENDO GRMD myocytes compared with control dogs as indexed by a significant lower EC_{50} (Figure 3A).

Differences of myofilament Ca^{2+} sensitivity between non-failing and GRMD ENDO myocytes disappeared following stretch. As a result, LDA indexed by the average EC_{50} was lower in GRMD myocytes (Figure 3B). We did not find any significant difference in EPI cardiomyocytes contractile parameters (data not shown) as we previously reported¹⁹. To determine whether the changes in myofilament Ca^{2+} sensitivity in ENDO myocytes from failing hearts were associated with myofilament structure alteration, we performed small-angle X-ray diffraction experiments on permeabilized control dog and GRMD endocardial myocardium at short ($\sim 1.9 \mu\text{m}$) and long ($\sim 2.3 \mu\text{m}$) sarcomere length^{13, 15}. Figure 3C shows a typical CCD captured image of X-ray diffraction pattern. Pixel intensity was plotted and the 1,0 equatorial reflection derived lattice spacing estimated (Figure 3D). As observed with the first experiments (Figure 1), the lattice spacing reduces with stretch in both CTRL and GRMD myocardium (Figure 3D). Interestingly, at short SL, the interfilament lattice spacing was significantly higher in GRMD ENDO myocardium ($46.22 \pm 0.31 \text{nm}$) compared to control non-failing myocardium ($43.81 \pm 0.26 \text{nm}$). Stretch eliminates this difference by inducing an ENDO GRMD interfilament lattice spacing reduction to a value that matches the spacing obtained in control non-failing dog myocardium ($42.91 \pm 0.20 \text{nm}$ in CTRL vs. $42.90 \pm 0.46 \text{nm}$ in GRMD).

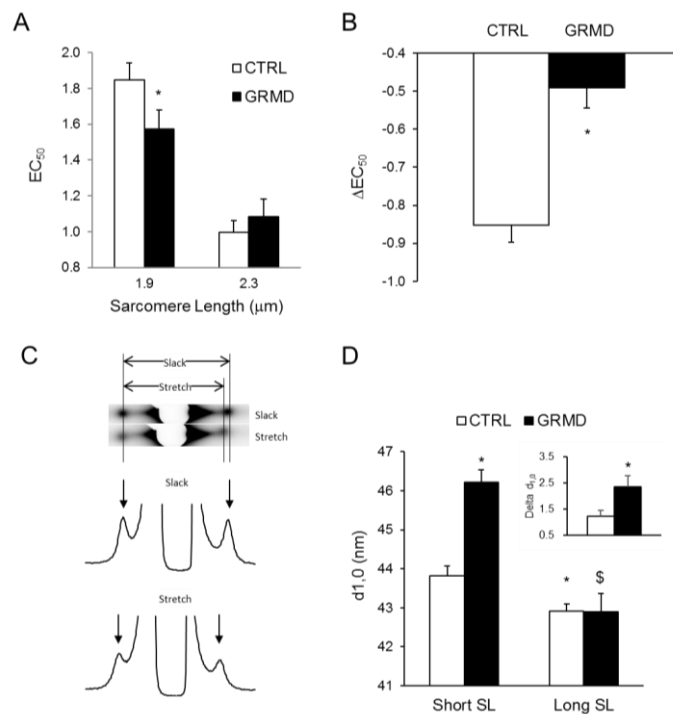


Figure 3: Effect of myopathy on myofilament properties of ENDO permeabilized cardiomyocytes.

Myofilament Ca^{2+} sensitivity was indexed by EC_{50} (A) and LDA (B) were evaluated on CTRL (open bars) and GRMD (solid bars) ENDO permeabilized cardiomyocytes at short ($1.9 \mu\text{m}$) and long ($2.3 \mu\text{m}$) SL. (N=4 cells per animal, 4 animals per group). * vs. CTRL; $P < 0.05$. C) Representative X-ray and intensity profile of permeabilized GRMD myocardium as recorded at slack length and after stretch ($+20\% L_0$). D) Average myofilament lattice spacing as measured on ENDO myocardium of CTRL (open bars) and GRMD (solid bars) at slack length (Short SL) and after $20\% L_0$ stretch (Long SL). (N=4 animals per group). * vs. CTRL short SL; \$ vs. GRMD short SL; $P < 0.05$.

The myopathy induced a myofilament lattice expansion at short length that exceeded the normal physiological range. In order to test if the interfilament spacing expansion was not due to the lack of dystrophin we also analyzed the myocardium dissected from the sub-epicardium. We did not find any significant difference between GRMD and CTRL EPI myocardium at both short SL ($43.61 \pm 0.48 \text{ nm}$ vs. $44.55 \pm 0.91 \text{ nm}$, respectively) and long SL ($42.47 \pm 0.36 \text{ nm}$ vs. $43.28 \pm 0.93 \text{ nm}$, respectively). Therefore, we conclude that the higher lattice spacing in ENDO GRMD myocardium may be involved in the increased myofilament Ca^{2+} sensitivity observed at short SL independently of dystrophin absence. Equatorial intensity ratios (I_{11} , I_{10}), under relaxed conditions were not significantly different under all conditions studies indicating that the relative degree of association of myosin heads with actin under relaxed conditions were similar in all samples. To further study the effect of lattice spacing on the myofilament contractile performance, we evaluated the cross-bridge cycling kinetics.

Myopathy in GRMD alters cross-bridge properties

We measured the kinetics of tension redevelopment (k_{tr}) in both non-failing and GRMD permeabilized ENDO cardiomyocytes (Figure 4). k_{tr} is usually used to estimate the rate of transition from weakly bound (non-force-generating) to strongly bound (force-generating) cross-bridges. Therefore, an estimation of cross-bridge cycling performance can be obtained. In ENDO cardiomyocytes from healthy dogs, k_{tr} obtained at sub-maximal Ca^{2+} activation tended to increase after stretch but did not reach significance (Figure 4 B). Interestingly, cross-bridge cycling redevelopment was accelerated in ENDO GRMD myocytes only at short SL as indicated by the higher k_{tr} (Figure 4B). No significant effect between groups was found on cross-bridges kinetics at maximal calcium activation, nor in EPI myocardium under all conditions (Figure 4B). Collectively, our results suggest that the structural changes that occurred in myocytes from GRMD dog with heart failure were associated with altered myofilament contractile properties.

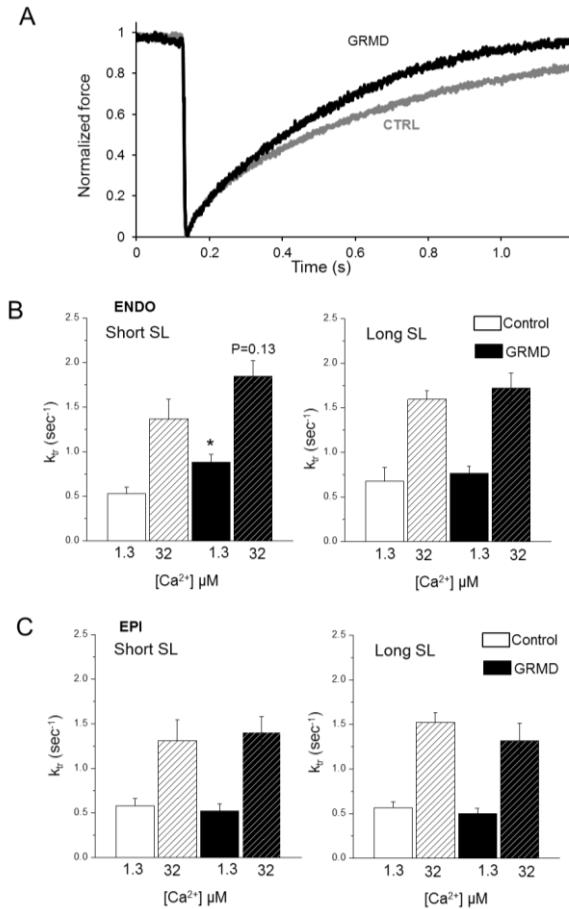


Figure 4: Effect of myopathy on the rate of tension redevelopment (k_{tr}). A) Raw tension recording obtained following rapid (2ms) 20% initial muscle length shortening of CTRL (gray trace) and GRMD (black trace) permeabilized cardiomyocyte. B-C) Average k_{tr} obtained from CTRL (white bars) and GRMD (gray bars) ENDO (B) and EPI (C) tissue at short (1.9 μ m) and long (2.3 μ m) SL at maximal [Ca²⁺] (hashed bars) and submaximal [Ca²⁺] (solid bars). (N=4 cells per animal, 4 animals per group) * vs. CTRL; P<0.05.

Intracellular signaling pathways:

To further understand the intracellular mechanisms involved in the GRMD myocytes contractile dysfunction, we measured post-translational modifications of key regulatory contractile proteins. We measured the PKA dependent phosphorylation of the cardiac Myosin Binding Protein-C (cMyBP-C) at Ser-284, which was significantly reduced in GRMD ENDO tissue and more modestly in EPI tissue (Figure 5A).

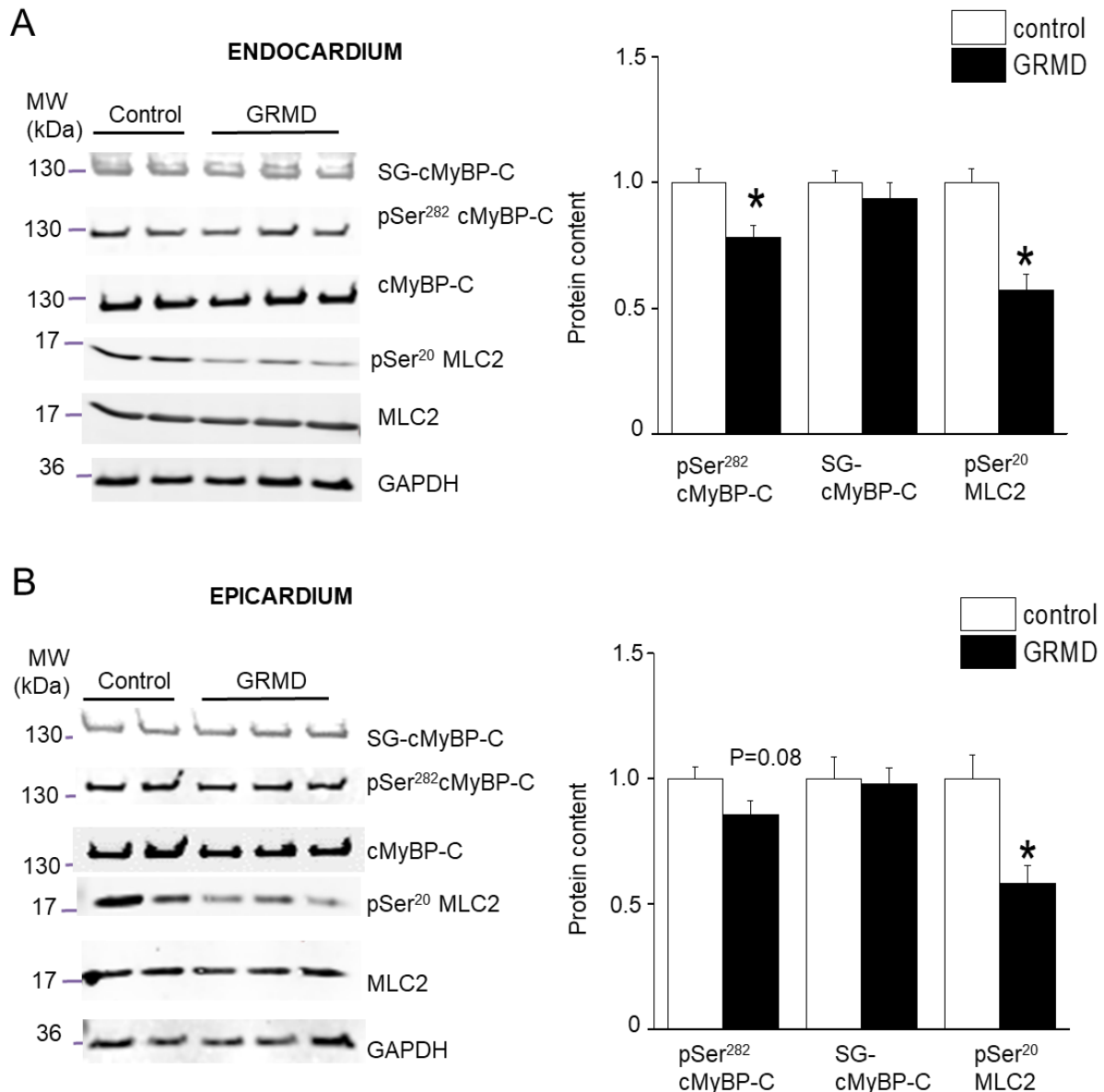


Figure 5: Effect of GRMD on thick filament post-transcription (phosphorylation and glutathionylation) modification. All data are shown for control (open bars) and GRMD (closed bars) ENDO (A) and EPI (B) myocardium. Typical Western blots (left panel) and averaged results (right panel) for cardiac Myosin Binding Protein-C (cMyBP-C) Ser-282 phosphorylation site, cMyBP-C S-glutathionylation, and Myosin Light Chain 2 (MLC2) phosphorylation at Ser20 site. All post-transcriptional modifications were normalized by the total protein content on the same band. (N=4 animals per group, in duplicate) * vs. CTRL; P<0.05.

We also measured the S-glutathionylation of cMyBP-C that has been shown *in vitro* to be activated in conditions of oxidative stress and to lead to increased myofilament Ca²⁺ sensitivity²⁰. The level of S-glutathionylated cMyBP-C was similar in both regions of control and GRMD hearts (Figure 5). Next, we studied the myosin light chain 2 (MLC2) whose phosphorylation increases myofilament Ca²⁺ sensitivity. The pSer20-MLC2 level decreased in both EPI and ENDO GRMD tissues to a similar extent (Figure 5). Troponin

I phosphorylation is an important modulatory process of cardiac myofilament properties²¹. Tnl phosphorylation at the Ser22/23 site decreased significantly in GRMD hearts and to a higher extent in ENDO tissues compared with EPI (Figure 6). The Tnl phosphorylation level at Thr143 site was not altered. Finally, the levels of the dephosphorylated form of cTnl were similar between regions and conditions. Altogether, the results indicate a profound remodeling of the post-translational modifications of all major regulatory contractile proteins investigated in GRMD hearts. cTnl and cMyBP-C exhibited regional alterations that may be responsible of the regional change in myofibrillar structure/function and the resulting lower LDA.

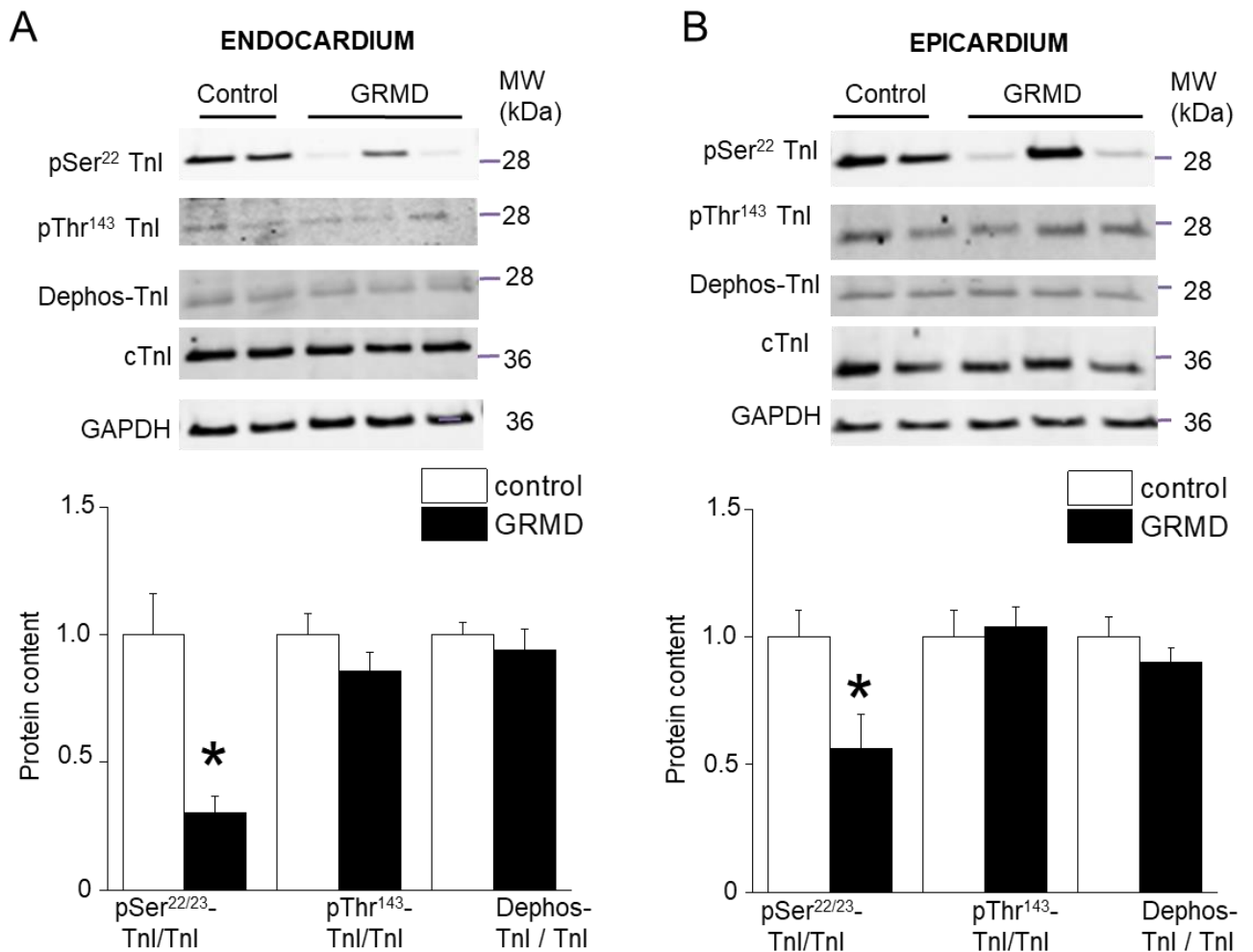


Figure 6: Effect of GRMD on the cardiac Troponin I (cTnl) phosphorylation and dephosphorylation status. All data are shown for control (open bars) and GRMD (closed bars) Endocardium (A) and Epicardium (B) myocardium. The top panels show a typical Western blot for phosphorylation at Ser22/23 and Thr143 and for de-phosphorylation (Dephos) status of the cTnl that have been normalized but the total cTnl content on the same band. The bottom panels show the respective averaged results. (N=4 animals per group, in duplicate) * vs. CTRL; P<0.05.

Discussion:

Here, we report the first observations on the contractile machinery structure and function of myocardium isolated from non-failing (CON) and spontaneous muscular dystrophy associated cardiomyopathy Golden Retriever (GRMD) dogs. In normal dog myocardium, the higher length sensitivity of activation observed in the inner layer of the ventricle cannot be explained by different interfilament lattice spacing. Chronic stress induced by the lack of dystrophin leads to heart failure that is tightly associated with regional structural changes, post-translational modifications of regulatory proteins and altered myofilament contractile properties in GRMD dogs.

Synchrotron X-rays diffraction has previously been employed to study myofilament structure-function relationships in different striated muscle types isolated from various species²². With stretch, sarcomere length clearly increases while interfilament lattice spacing decreases^{13, 22-24}. The effect of lattice spacing on LDA of cardiac myofilament has been assessed²⁴⁻²⁶. However, it is still not clear whether the lateral spacing between thin and thick filament directly regulates myofilament contractile performance, particularly following stretch. The reduction of lattice spacing has been first proposed to underlie LDA in the myocardium over the physiological ranges of SL¹⁵. This hypothesis has been refined a few years later by the same group showing that myofilament lattice spacing alone was not sufficient to explain all stretch-induced alterations of Ca²⁺ sensitivity in permeabilized myocardium²⁵. Most of these studies have been performed in rodents or insects. Rare are studies that investigated cardiac sarcomere structure-function in a larger animal model such as those with bovine hearts²⁴. Our present work is the first, to our knowledge, to evaluate the cardiac sarcomere structure-function both in healthy dogs and in dogs with heart failure. Here, we evaluated whether lattice spacing is involved in LDA and cross-bridge cycling kinetic alterations observed in dystrophic dog myocardium. In dogs, we found that lattice spacing decreases with stretch (*i.e.* increases in SL), which is consistent with the results obtained on other species^{13, 22-24}. Because of the large amount of tissue available, it was possible in the present work to study, for the first time, regional difference of structure-function across the LV wall. Our present work shows that in the canine animal model, this relationship is identical between the ENDO and EPI region across the LV free wall over the predefined SL range. Moreover, consistent with findings in rodent animal models¹¹, we found LDA to be significantly higher in the ENDO myocardium. Thus, under normal conditions, lattice spacing *per se* cannot explain the stretch-induced regional contractile heterogeneity. Several studies suggested that stretch and LDA could involve other structural changes than interfilament spacing. For instance, changes in myosin head orientation or position relative to the thin filaments can alter LDA without affecting interfilament lattice spacing^{27, 28}. Our measurements of the equatorial intensity ratio, I_{1,1}/I_{1,0}, showed no significant differences in all tissues studied, excluding differences in relative degree of myosin heads with actin as an explanation of our findings. LDA is certainly a complex phenomenon which regulation is not exclusively limited to environment dependency such as post-translational status of the contractile proteins, or titin-based passive tension that have been shown to mediate some structural changes with stretch^{24, 26} on the tested species. Moreover, we showed recently that LDA involves titin-based simultaneous structural rearrangements within both thin and thick filaments and not myofilament lattice spacing *per se*²⁹. In the present study, passive tension is lower in GRMD myocytes (5.1±0.3 mN/mm²) compared with control myocytes (7.7±0.7

mN/mm²). It is thus possible that the reduced LDA observed here involves a titin-based modulation of activation.

With heart failure, we observed at short SL several regional changes in interfilament lattice spacing, myofilament Ca²⁺ sensitivity and cross-bridge cycling kinetics. These changes might be interconnected. The preferential sub-endocardium alteration, observed here, has been described in several types of heart failure (i.e. Ischemic, Hypertrophic, Congenital) and different animal models (rodent, dog, pig, guinea-pig)^{6, 30, 31}. Several investigations have demonstrated in a normal heart and during disease, regional heterogeneity of the myocardial phenotype and function, including myocardial contractility and the electrophysiological properties of cardiomyocytes. During HF, regional changes in protein expression were reported such as higher atrial natriuretic peptide levels in basal endocardium in a canine model of LV hypertrophy³² or lower SERCA2a and PLB transmurial expression gradients in the LV free wall endocardium of failing human hearts^{33, 34}. A transmural gradient of oxidative mitochondrial activity was reported being lower in sub-endocardium associated with higher NO and ROS productions³⁵. Additionally, cardiac hypertrophy³⁵, ischemic heart failure¹⁸, aging and streptozotocin-induced diabetes³⁶ might be associated with a decreased transmural gradient in mitochondrial respiratory activity. Several reports from our laboratory and others indicate that the contractile machinery itself can be altered in other types of heart failure indicating that the sarcomere phenotype is not specific to DMD model. It is to note however, that the pattern of alteration differs between models. For instance, in the rodent animal model of ischemic heart failure, the gradient of contractility is altered because of a reduced Ca²⁺ sensitivity at long sarcomere length in the ENDO layer. Instead, in the present study, we observed that the lost gradient of contractility is mainly due to increased Ca²⁺ sensitivity a short sarcomere length in the ENDO layer. Nevertheless, both models show the ENDO layer to be specifically affected by various types of heart failure.

There is still debate on what should be the alteration of myofilament Ca²⁺ sensitivity during heart failure due to contradictory results published with human samples and animal models (see^{37, 38} for debates). Discrepancies may in part result from the duration of the studies (induction of cardiac disease in the course of weeks), compared to long-term studies in our studies (7–8 months duration). It is still discussed that heart failure may be associated with early increase in myofilament Ca²⁺ sensitivity that reverts to decreased Ca²⁺ sensitivity at end-stage heart failure, but this needs further study. Mutations in troponin and tropomyosin that cause familial DCM show a consistently lower Ca²⁺-sensitivity and this was proposed to account for inadequate contractility in familial DCM (see for review³⁷). Decreased myofilament Ca²⁺ sensitivity in patients suffering from type-2 diabetes mellitus compared to patients without diabetes has also been reported³⁹. There have been reports of increased myofilament Ca²⁺ sensitivity in animal models of cardiac disease, both large animal (dog, pig), and small rodents (mouse)³⁸. Increased myofilament Ca²⁺ sensitivity and reduced phosphorylation levels of regulatory proteins is a common feature reported in human heart failure in particular with patients treated with beta-blockers at a stage where β-adrenergic signaling is defective in failing heart with β-receptor desensitisation, blunted inotropic response and reduced cardiac reserve. Van der Velden et al showed, using human heart samples with different degrees of failure, a correlation between myofilament Ca²⁺ sensitivity and regulatory proteins phosphorylation

such as troponin I⁴⁰ and MLC-2⁴¹. A previous report showed in old hypertensive dogs by bilateral renal wrapping a model of HF with preserved ejection fraction, increased myofilament Ca²⁺ sensitivity and reduced phosphorylation levels of MyBP-C, TnI and MLC-2⁴², similar changes that observed in our study. Thus it appears that hypophosphorylation of sarcomeric proteins could be a general property in the transition to many HF pathologies but not all.

Considering that phosphorylation of either cardiac TnI or MyBP-C has a large and opposite impact on interfilament function⁴³, it is expected that they participate in the regional change of the myofilament structure and function observed in the GRMD dogs. Similar increases in myofilament Ca²⁺ sensitivity only at short SL have been reported in models of cMyBP-C truncation⁴⁴⁻⁴⁶ that was also associated with increased k_{tr}⁴⁷. Because k_{tr} encompasses both the rates of cross-bridge attachment (f) and detachment (g)⁴⁸, it is impossible from our experiments to determine which step is affected in GRMD dogs. The observed increase in Ca²⁺ sensitivity at short SL is likely arising from an increased number of cross-bridges interacting with the thin-filament⁴⁷ or may be due to an increase in the apparent Ca²⁺ binding affinity of TnC mediated by strongly-bound cross-bridges⁴⁹⁻⁵¹. The increased cross-bridge cycling kinetics observed in the present work may lead to increased myofilament Ca²⁺ sensitivity through a positive cooperative activation of strongly bound myosin heads on thin filament⁵². Moreover, k_{tr} acceleration observed in GRMD ENDO tissues could result from reduced contractile protein phosphorylation. This effect could be accentuated by the observed lattice spacing expansion. Similar results have been observed on insect permeabilized muscle fibers where cross-bridge attachment and detachment kinetics were faster with larger thick-to-thin interfilament lattice spacing⁵³. Hypophosphorylation of MLC2 is also expected to affect thin filament structure and Ca²⁺ sensitization⁵⁴. Interestingly, the D166V mutation in the MLC2 induces hypertrophic cardiomyopathy with reduced MLC2 phosphorylation, increased myofilament sensitivity⁵⁵ and larger lattice spacing⁵⁶.

From a therapeutic perspective, Taking into account regional alterations of cardiac contractility is certainly an important aspect for future treatment. The new ultrasound technique, speckle-tracking echocardiography (STE) assesses regional myocardial deformations. This technique has clearly shown benefits to detect early contractile defects compared with conventional echocardiography. Early measurement of longitudinal strain by STE revealed in a pig model of acute pressure overload systolic abnormalities upon mild aorta banding despite normal conventional indices of systolic function such as LV fractional shortening and LV dP/dtmax⁵⁷. Endocardial to epicardial velocity gradients measured by tissue Doppler imaging can also be used to identify early LV dysfunction before the ejection fraction is altered. Our results are in agreement with previous report showing that young GRMD dogs exhibited an important decrease in both systolic and diastolic endocardial velocities, whereas epicardial velocities and fractional shortening were not affected⁵⁸. Similarly, myocardial velocity gradient was also lost due to altered endocardial velocities and strains in other models of cardiac pathologies such as ischemic⁶, aortic valve stenosis^{30, 31} and hypertensive after abdominal aortic banding⁴⁵ often earlier before heart failure is detected. Treatments will have to target at least the altered layer with minimum impact on the preserved layers. Previous studies indicate that the

altered layer can be preferentially restored by treatments such as moderate exercise training⁶ or pharmacological calcium sensitizer⁵⁹ and bradykinin³. Although interesting, the molecular mechanism underlying this ENDO-specificity is yet to be unraveled.

Conclusion:

Our study showed transmural differences in sarcomere structure and function in dog myocardium with heart failure, mostly in the lower physiological length range. The increase in myofilament Ca²⁺ sensitivity observed in GRMD dogs may contribute to the impairment of relaxation during the early phase of diastole observed in this model. Altogether, our findings suggest that impaired LDA may contribute to depressed contractile function in human patients harboring in dystrophin and Duchenne Muscular Dystrophy.

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