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

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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 (ktr) 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.

1. 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 maintenance [1]. Heart failure constitutes, together with respiratory failure, the main leading cause of mortality in patients with muscular dystrophy [2]. 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 efficiency [8]. Myofilament contractile heterogeneity is also observed in the heart of healthy Golden Retriever dogs [3]. Similar to rodent models of heart failure, the gradient of transmural contractility is altered in Golden Retriever

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muscular dystrophy (GRMD) dogs [3]. The GRMD mimics more closely the human disease of DMD compared to other existing mammalian models of dystrophin deficiency [9]. 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 content [3].

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 mechanism [12]. Increased Ca^{2+} 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.

2. Methods

An expanded Methods section is available in the Data Supplement.

2.1. 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 ≤ 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 2-5 MHz phased-array transducers (GE, Waukesha, WI) as previously described [3]. 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.

2.1.1. 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 ($[Ca^{2+}]$ = 1.3 μmol/L) or at maximal calcium activation ($[Ca^{2+}]$ = 32 μmol/L) [5]. For each cardiomyocyte, at a given SL, the above protocol was repeated 3 times and the average k_{tr} was estimated.

2.1.2. Myofilament lattice spacing

Permeabilized LV myocardium was prepared according to previous reports [13]. Frozen LV was rapidly thawed, homogenized in ice-cold relaxing solution using Polytron homogenizer (3 s, 1000 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 previously [15]. The sample to detector distance for the small-angle X-ray diffraction camera was ~3 m and the focal spot size was about 150 × 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 × 160 mm. Exposure times were ~1 s with an incident X-ray flux of ~1 × 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.

2.1.3. 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).

3. Results

3.1. 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 (Fig. 1).

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 (Fig. 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 (Fig. 2B). 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 (Fig. 2C) 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.

3.2. 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).

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