Progressive Myocardial Sheet Dysfunction from 3 to 16 months in Duchene Muscular Dystrophy Mice (mdx) Defined by Diffusion Tensor MRI (DTI)

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INTRODUCTION: Cardiomyocytes are organized in laminar sheet-like structure (aka, "sheets") that contribute to systolic wall thickening. Diffusion tensor magnetic resonance imaging (DTI) of water diffusion provides a non invasive measure of the sheet orientation and function in intact hearts. Our group has reported that the hearts of 16-month old *mdx* mice, which is a model of Duchene Muscular Dystrophy, exhibit regionally abnormal calcium transient kinetics and $[Ca^{2+}]$-dependent sheet dysfunction most prominent at the basal segment. In view of previous reports indicating that $[Ca^{2+}]_i$ mishandling in isolated *mdx* cardiomyocytes is age dependent, we hypothesized that sheet dysfunction in intact *mdx* hearts also might be age-dependent and also myocardial segment dependent

METHODS: Excised hearts from 16-mo old (n=5) and 3-mo old (n=4) *mdx* and age matched wildtype (WT, n=5, 5) were prepared for Langendorff perfusion, and sequentially arrested in diastole and then systole for DTI (Varian 11.7 T, b=800 s/mm^2, 12 directions). The diastolic arrest was achieved with St. Thomas' cardioplegic solution containing either normal $[Ca^{2+}]$ (1.2 mM, denoted as NC) or low $[Ca^{2+}]$ (0.078 mM, denoted with LC) to assess the regulatory effect of $[Ca^{2+}]$ on the absolute value of sheet angle ($|\beta|$), which served as an index of the state of contraction or relaxation of the sheet structure, since it is known to vary periodically throughout the heart cycle. The systolic arrest was achieved with barium. The $|\beta|$ was calculated from the angle between heart's radial axis and the 2nd large eigenvector of DTI (Figure A). MANOVA was used for statistical analysis.

RESULTS: In 16-mo old mice, systolic values for $|\beta|$ (not shown) were similar between WT and *mdx,* whereas diastolic values of $|\beta|$ in *mdx*-NC hearts were lower than for WT-NC hearts at the base but not at the apex, indicating a regional failure to fully relax myocardial sheet structures. Perfusion of the *mdx* heart with low $[Ca^{2+}]$ (*mdx*-LC) cardioplegic solution recovers the diastolic $|\beta|$ to normal values at both ages. The difference between *mdx*-NC and *mdx*-LC is 6˚±2˚ and 9˚±1˚ in 3- and 16-mo old mice, respectively (Figure), indicating that this $[Ca^{2+}]$-dependent sheet dysfunction is progressive (i.e. age dependent) and regionally manifest despite the ubiquitous dystrophin deficiency.

DISCUSSION & CONCLUSION: Ultra high field DTI delineated the micro-anatomical structure of *mdx* heart muscle and showed that $[Ca^{2+}]$ regulates the sheet function. Incomplete relaxation of *mdx* cardiomyocytes in diastole can be ameliorated by modulating extracellular $[Ca^{2+}]$ levels that are assumed to be in equilibrium with sarcoplasmic calcium in this preparation. Accordingly, diastolic dysfunction in *mdx* may be reversible in part with measures aimed at restoring normal calcium handling, which is a progressive pathophysiological feature of the dystrophin-deficient disease.

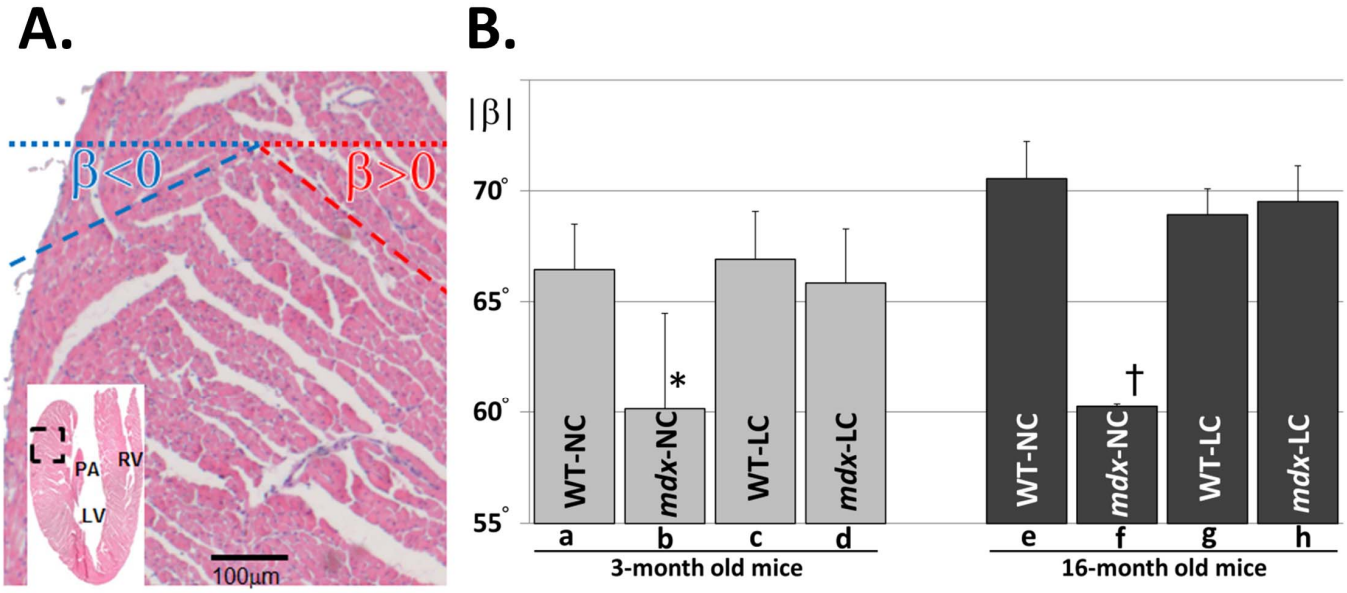

Figure: A. Photoacoustic imaging [Chi Zhang, J. Biomed. Opt. 17(6)] of mouse heart left ventricle free wall, showing the anatomical structure of sheet and the definition of sheet angle, β. FOV corresponds to the squared region in the inlet histology image. B. $|\beta|$ at base of diastolic arrested hearts. Compared to WT-NC and mdx-LC, *mdx*-NC has lowered $|\beta|$. 16-mo old *mdx*-NC (f compared to e and h) exhibits 50% more Ca-dependent difference than does 3-mo old *mdx*-NC (b compared to a and d). a. WT-NC (n=5); b. *mdx*-NC (n=4); c. WT-LC (n=5); d. *mdx*-LC (n=4); e. WT-NC (n=5); f. *mdx*-NC (n=5); g. WT-LC (n=5); h. *mdx*-LC (n=5). mean±SEM. *,p<0.05; †, p<0.001.