Considerations for Cardiac Gene Therapy in Duchenne/Becker Muscular Dystrophy

H. Lee Sweeney, Ph.D.
Director, UF Myology Institute
Department of Pharmacology & Therapeutics
College of Medicine
University of Florida

MYOLOGY Institute

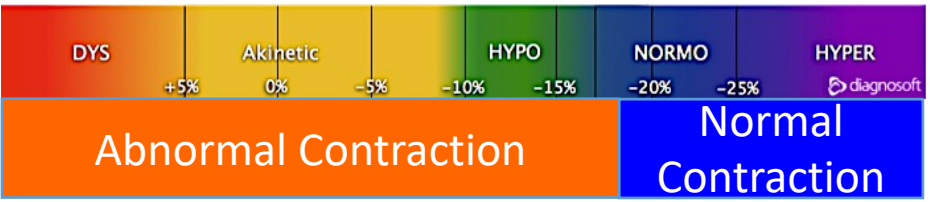
UF | UNIVERSITY of FLORIDA

Dilated Cardiomyopathy (DCM) Associated with DMD

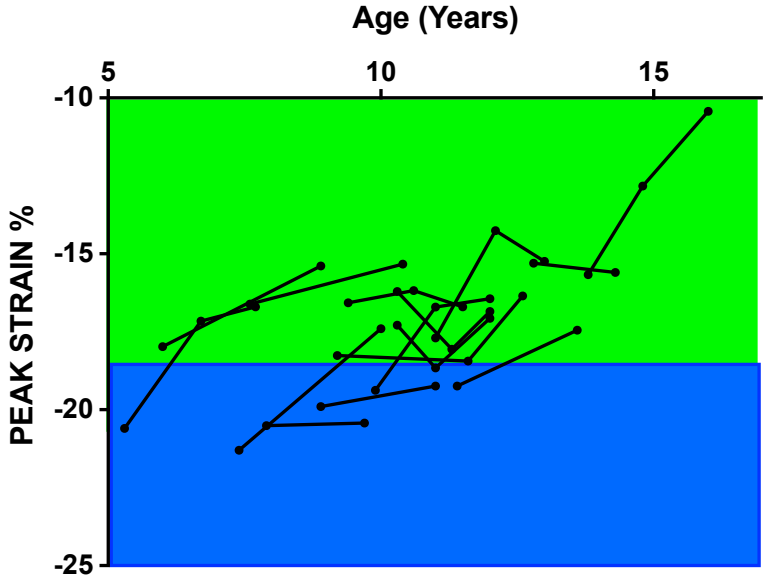
The human disease

DMD Cardiac Disease

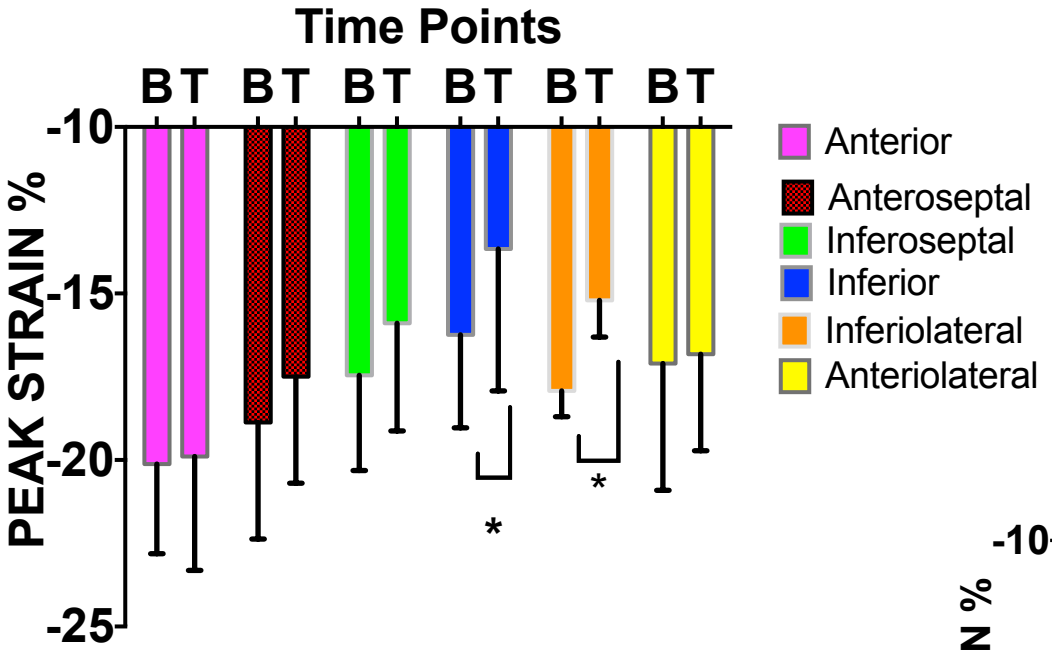
Global LV Strain



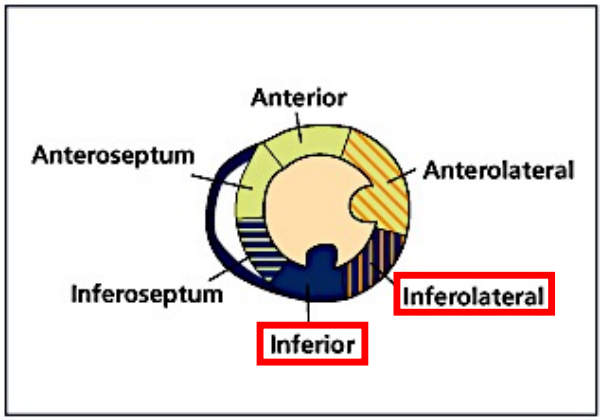
http://diagnosoft.com/strain/clinical-value-of-strain

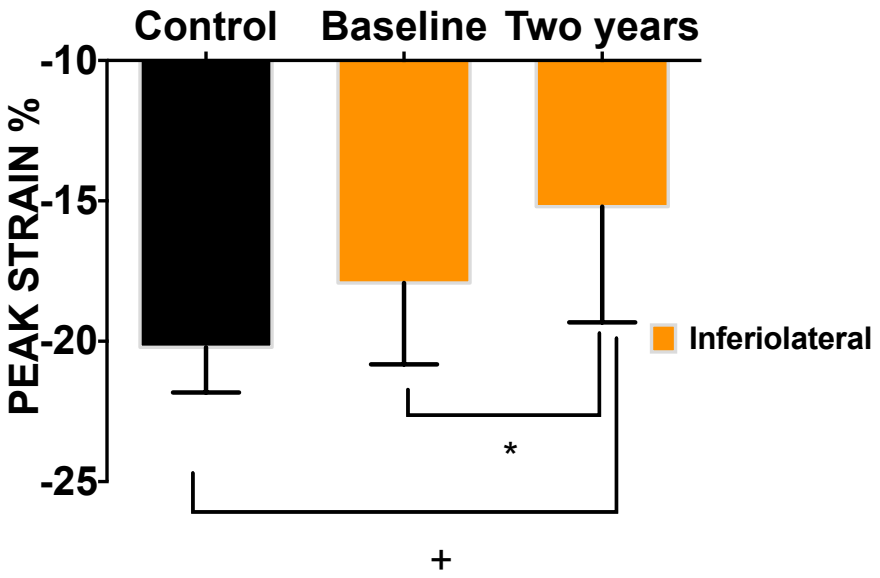


Inferior and Inferoseptal Region Showed the most Significant Decline in Strain

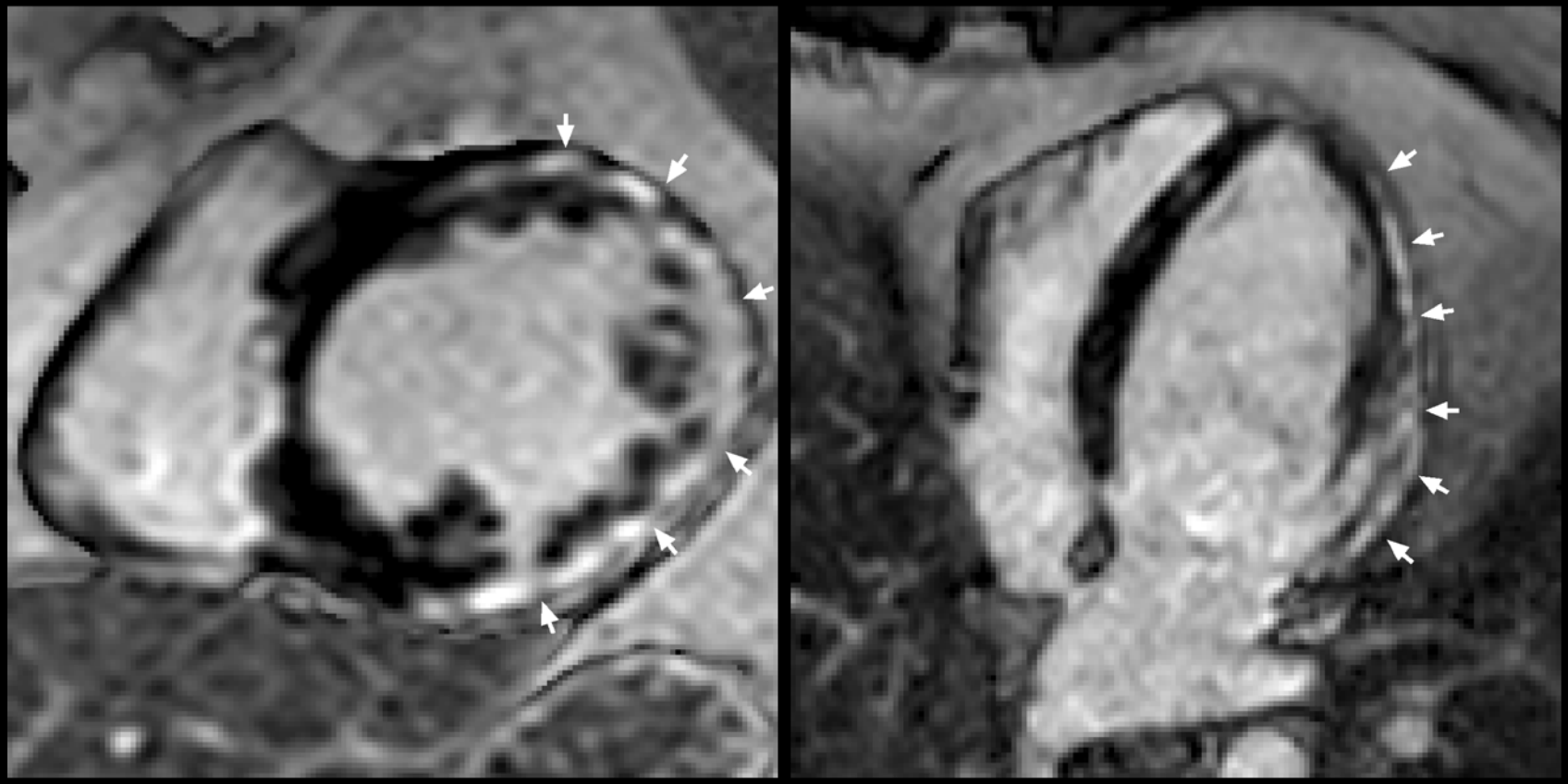


B- Baseline
T- Two Years

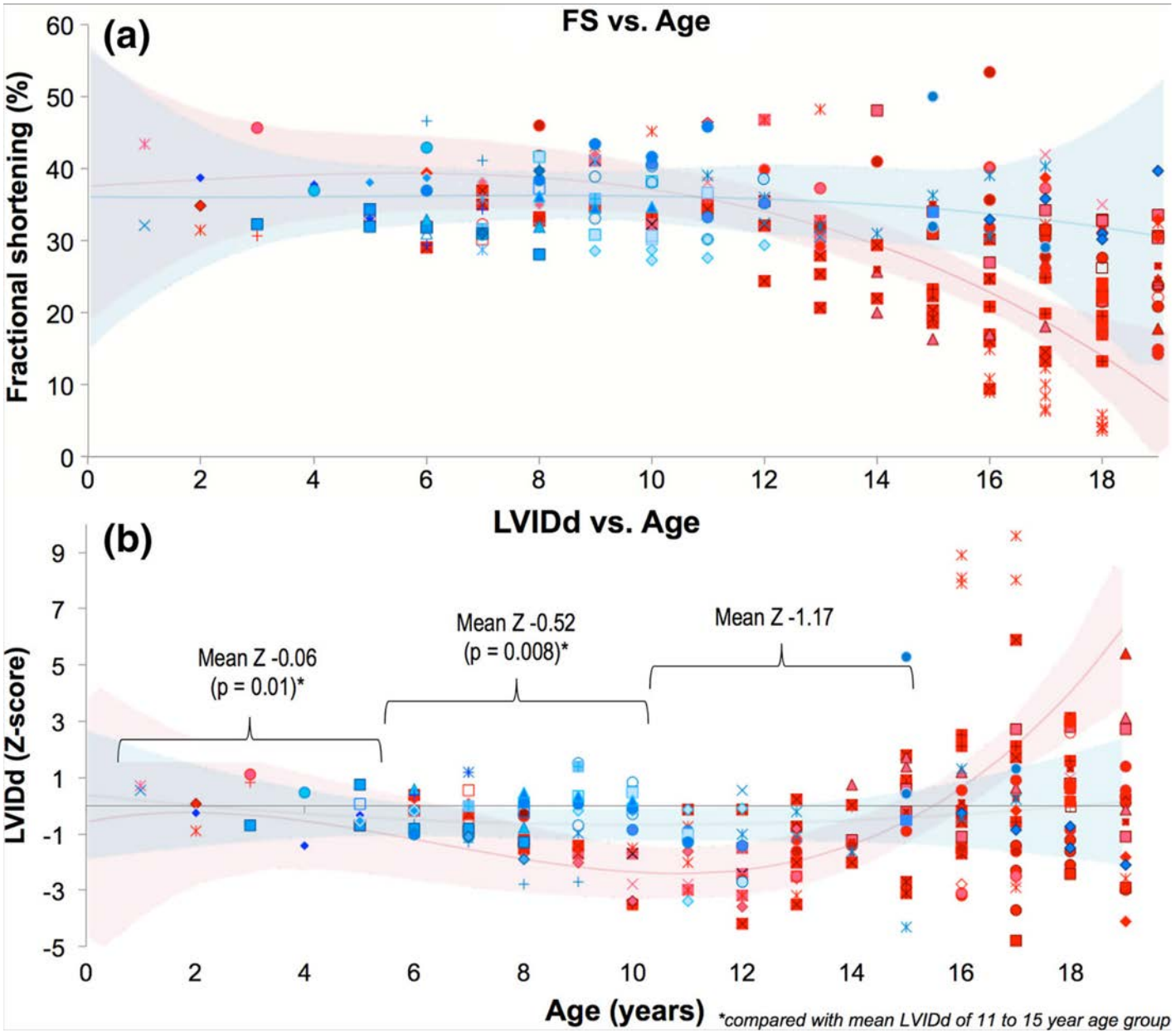




Cardiac MRI showing late gadolinium enhancement in dystrophinopathy heart



“Tonic Contraction” Su *et al.*, *Pediatric Cardiology*, 2015

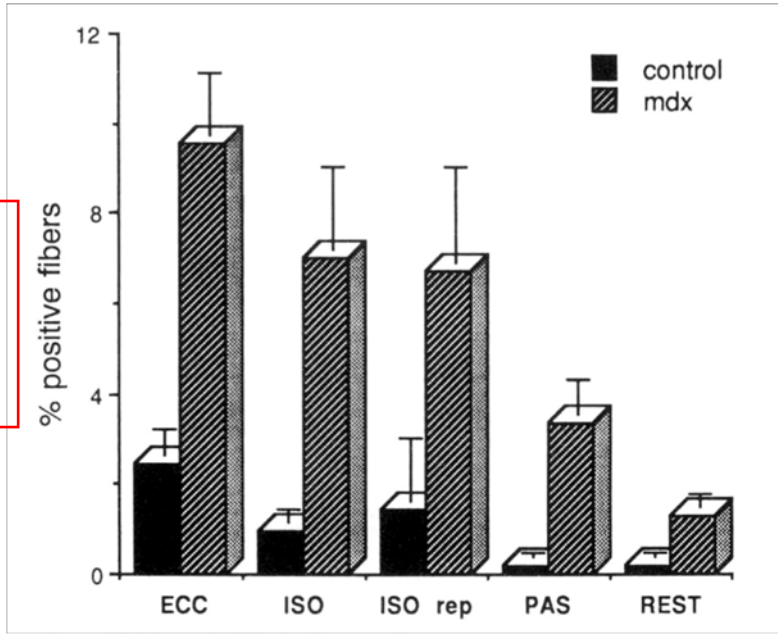


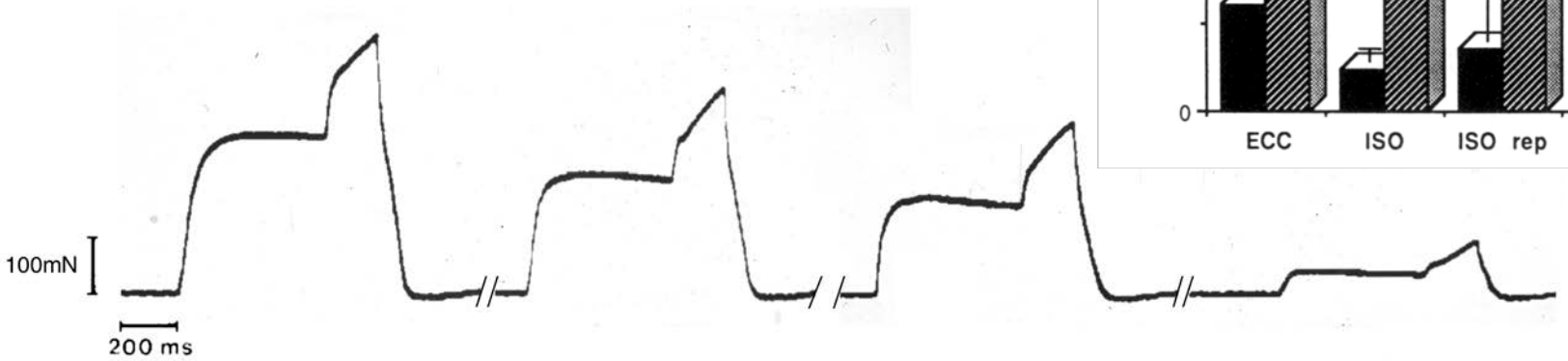
Blue = DMD boys who received ACEi prior to decreased EF Red = DMD boys who received ACEi after EF decreased

The Dystrophin Complex transmits force across the muscle membrane, lowering the threshold for contraction-induced damage to muscle

Contraction and even passive stretch of dystrophic (mdx) muscles causes membrane tears.

(observed by dye entry)





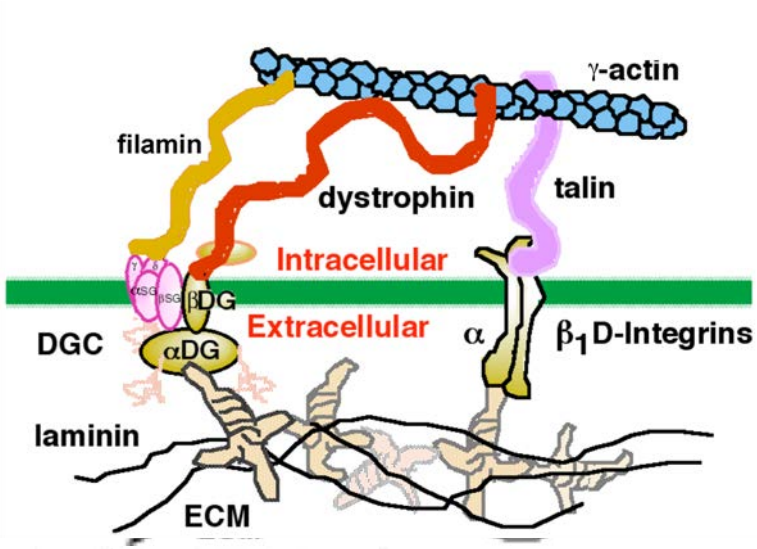
A series of eccentric contractions results in a decrement in force generation in dystrophic muscle, indicating increased susceptibility to damage

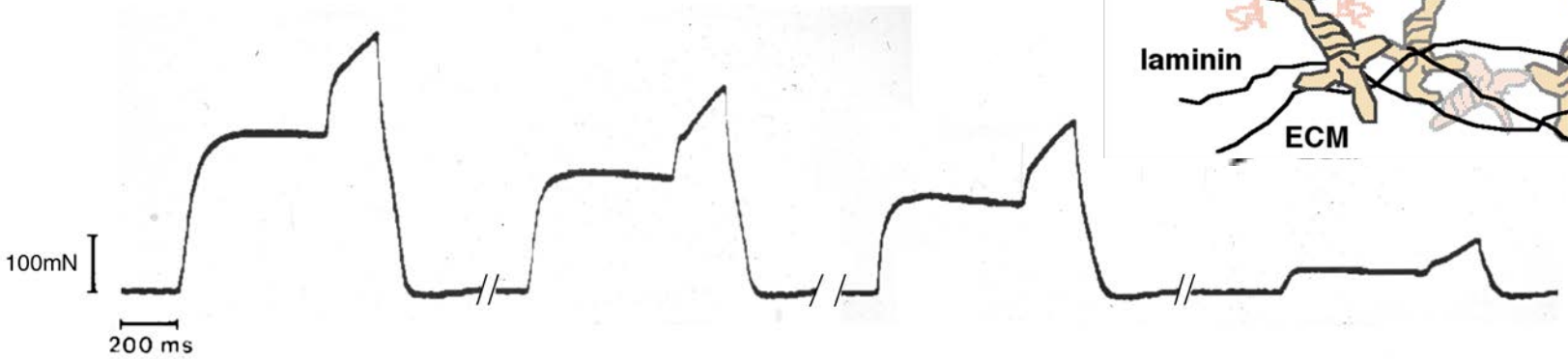
Petrof *et al.* (1993) Dystrophin protects against membrane stress associated with muscle contraction. *Proc. Natl. Acad. Sci. USA* 90: 3710-3714.

The Dystrophin Complex transmits force across the muscle membrane, lowering the threshold for contraction-induced damage to muscle

Contraction and even passive stretch of dystrophic (mdx) muscles causes membrane tears.

(observed by dye entry)





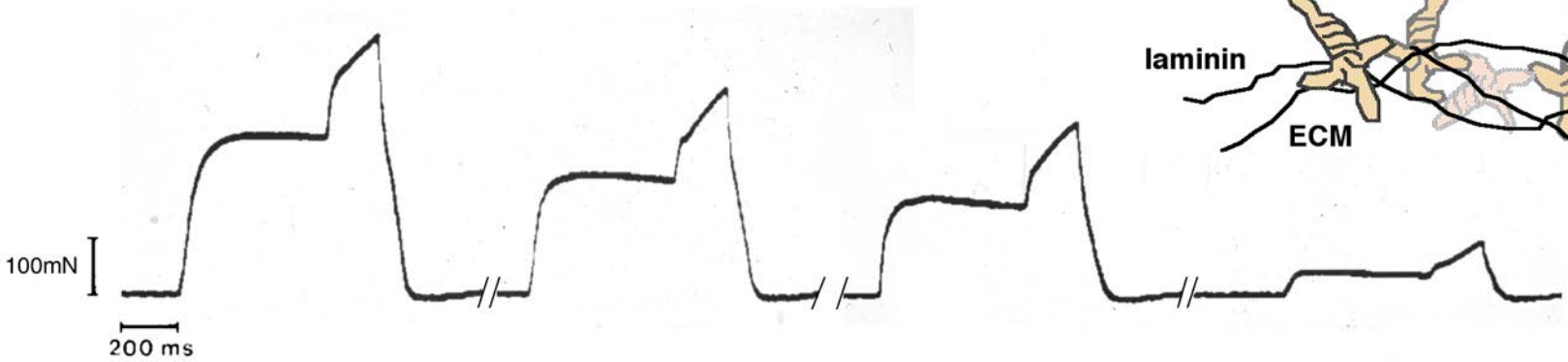
A series of eccentric contractions results in a decrement in force generation in dystrophic muscle, indicating increased susceptibility to damage

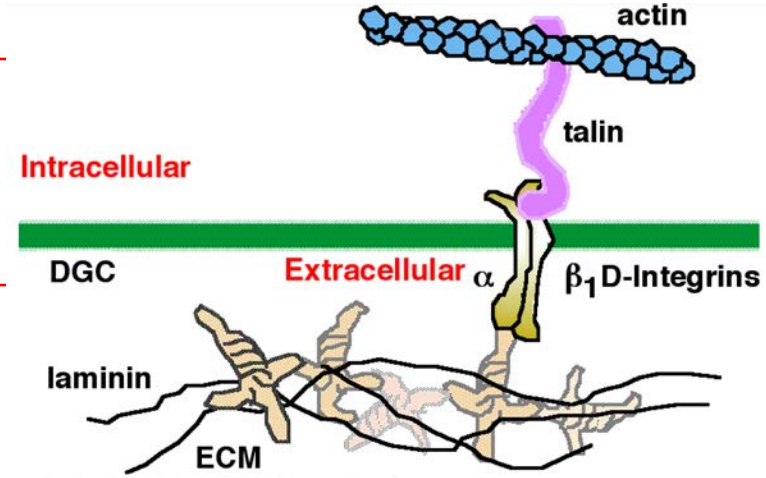
Petrof *et al.* (1993) Dystrophin protects against membrane stress associated with muscle contraction. *Proc. Natl. Acad. Sci. USA* 90: 3710-3714.

The Dystrophin Complex transmits force across the muscle membrane, lowering the threshold for contraction-induced damage to muscle

Contraction and even passive stretch of dystrophic (mdx) muscles causes membrane tears.

(observed by dye entry)

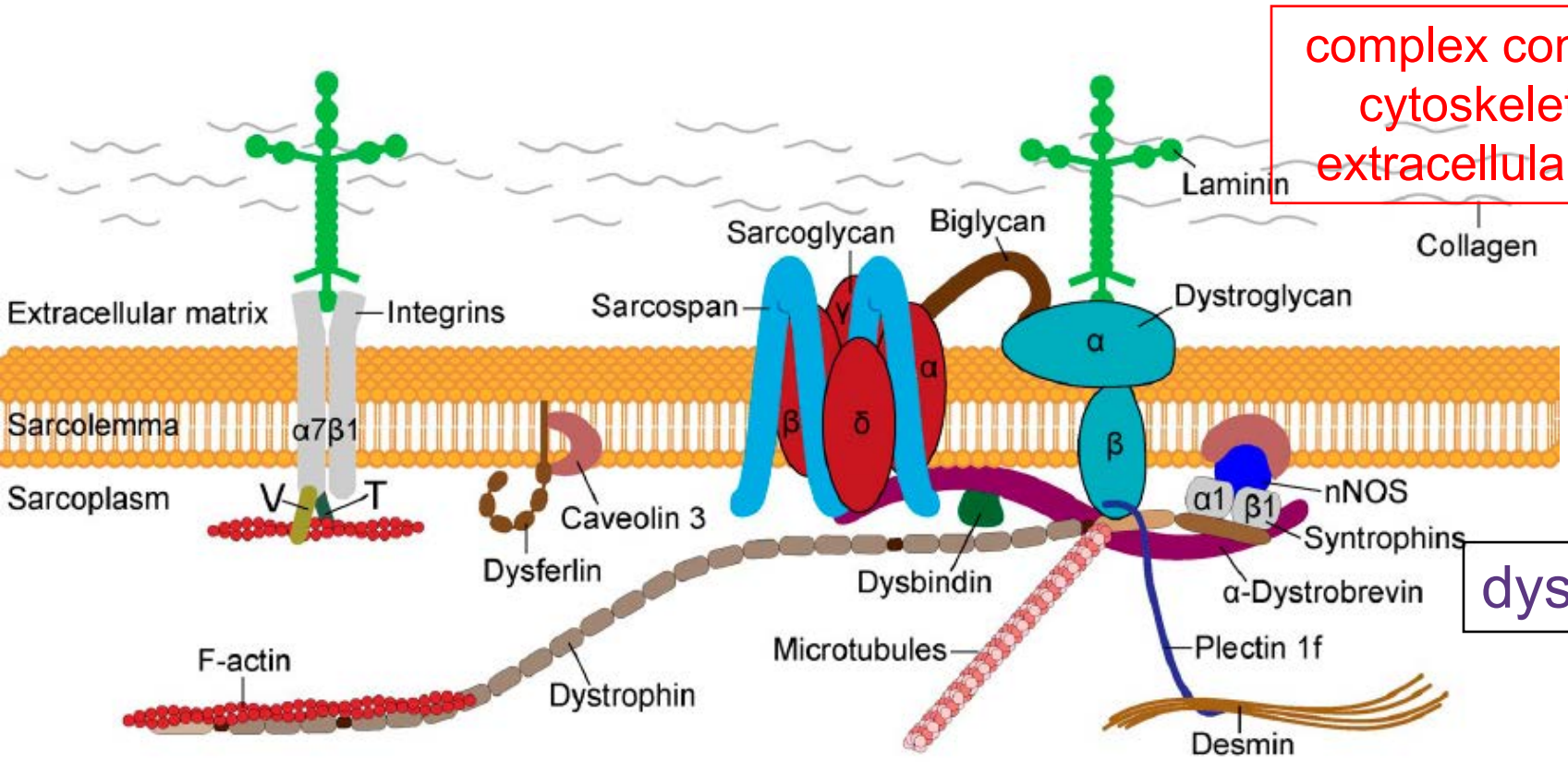




A series of eccentric contractions results in a decrement in force generation in dystrophic muscle, indicating increased susceptibility to damage

Petrof *et al.* (1993) Dystrophin protects against membrane stress associated with muscle contraction. *Proc. Natl. Acad. Sci. USA* 90: 3710-3714.

Dystrophin Complex

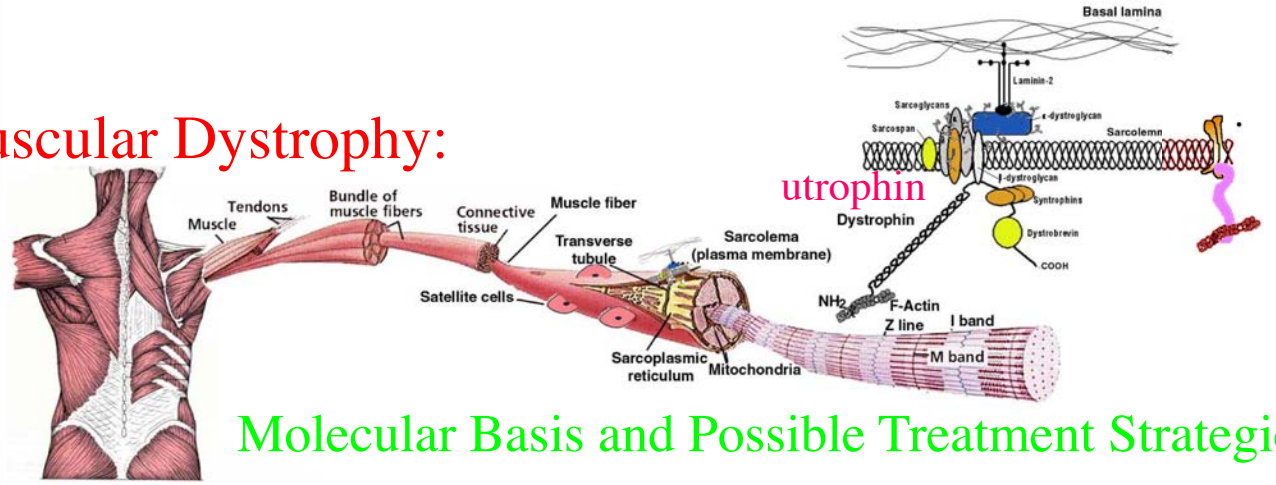


complex connecting cytoskeleton to extracellular matrix

dystrophin

contractile elements

Duchenne Muscular Dystrophy:



Molecular Basis and Possible Treatment Strategies

Functional Roles of Dystrophin:

- Mechanical - transmits force from the contractile apparatus to connective tissue/tendon
- Organizer - positions a number of proteins at the muscle membrane (NOS, ion channels,etc.)
- Signaling - likely plays a number of signaling roles, including a key role in calcium homeostasis

Contraction causes rupture of the muscle membrane, which allows calcium inflow. There also there may be increased flux through ion (TRPC) channels and leakiness of the internal calcium storage compartment (SR) via the ryanadine receptor (SR-calcium release channel).

Excessive calcium activates breakdown of muscle (via calpain and other proteases) and may trigger cell death program.

Cell death triggers an inflammatory response. Activation of fibroblasts can lead to fibrosis, which prevents muscle regeneration (modulated by IGF-I and myostatin).

Considerations for Gene Therapy for the Dilated Cardiomyopathy associated with DMD

Does dystrophin carry a cardiac-specific domain?

Full-length

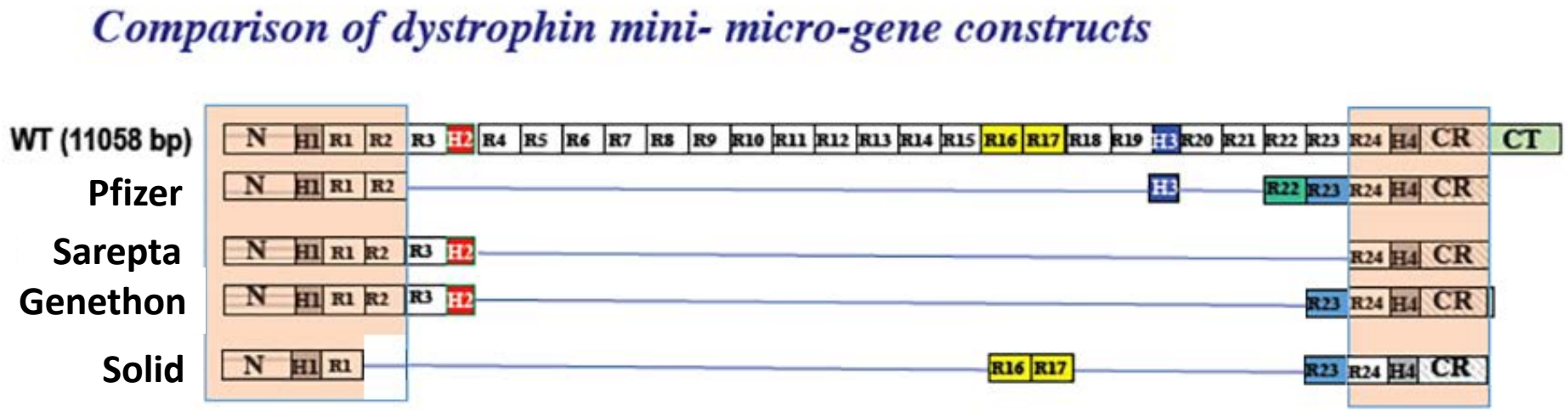
ΔH2-R19

ΔH2-R15

	ΔH2-R19	ΔH2-R15
PR interval	normalized	normalized
QRS duration	not normalized	normalized
QT interval	normalized	normalized
Q amplitude	not normalized	normalized
End-systolic volume	normalized	normalized
dP/dt maximum	normalized	normalized
End-diastolic volume	not normalized	normalized
Ejection fraction	normalized	normalized

Wasala et al 2018 Hum Gene Ther.

Unlikely that AAV.μ-dystrophin will totally rescue the heart

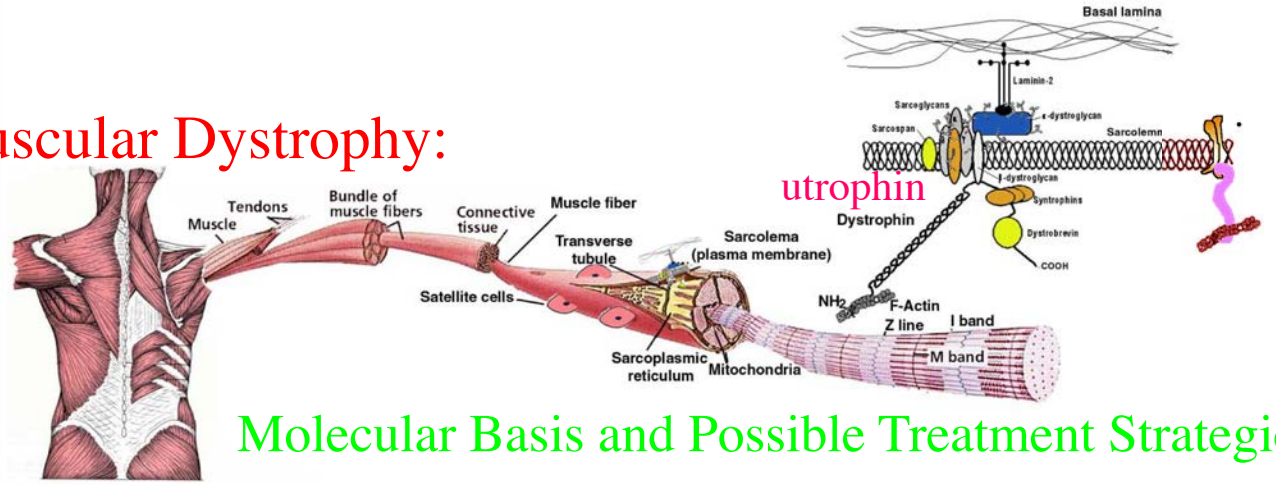


Therapeutic Targets: For DMD skeletal muscle, there are at least six categories of therapies under development.

Current Duchenne muscular dystrophy therapeutic targets can be grouped into six categories. Only the first addresses the primary genetic defect (resulting in the loss of dystrophin protein). The rest address downstream aspects of the pathogenesis.

1) Replacement of dystrophin/utrophin (μdys gene therapy)
2) Increasing muscle mass and regeneration
3) Decreasing inflammation and fibrosis
4) Correcting blood flow regulation
5) Correcting perturbations in calcium handing (more utrophin?)
6) Mitochondria dysfunction and ROS generation

Duchenne Muscular Dystrophy:



Molecular Basis and Possible Treatment Strategies

Functional Roles of Dystrophin:

- **Mechanical** - transmits force from the contractile apparatus to connective tissue/tendon
- **Organizer** - positions a number of proteins at the muscle membrane (NOS, ion channels,etc.)
- **Signaling** - likely plays a number of signaling roles, including a key role in calcium homeostasis

Contraction causes rupture of the muscle membrane, which allows calcium inflow. There also there may be increased flux through ion (TRPC) channels and leakiness of the internal calcium storage compartment (SR) via the ryanadine receptor (SR-calcium release channel).

Excessive calcium is taken up by mitochondria, ultimately contributing to mitochondrial uncoupling, free radical generation, and dysfunction. This can trigger apoptosis of the cardiomyocytes.

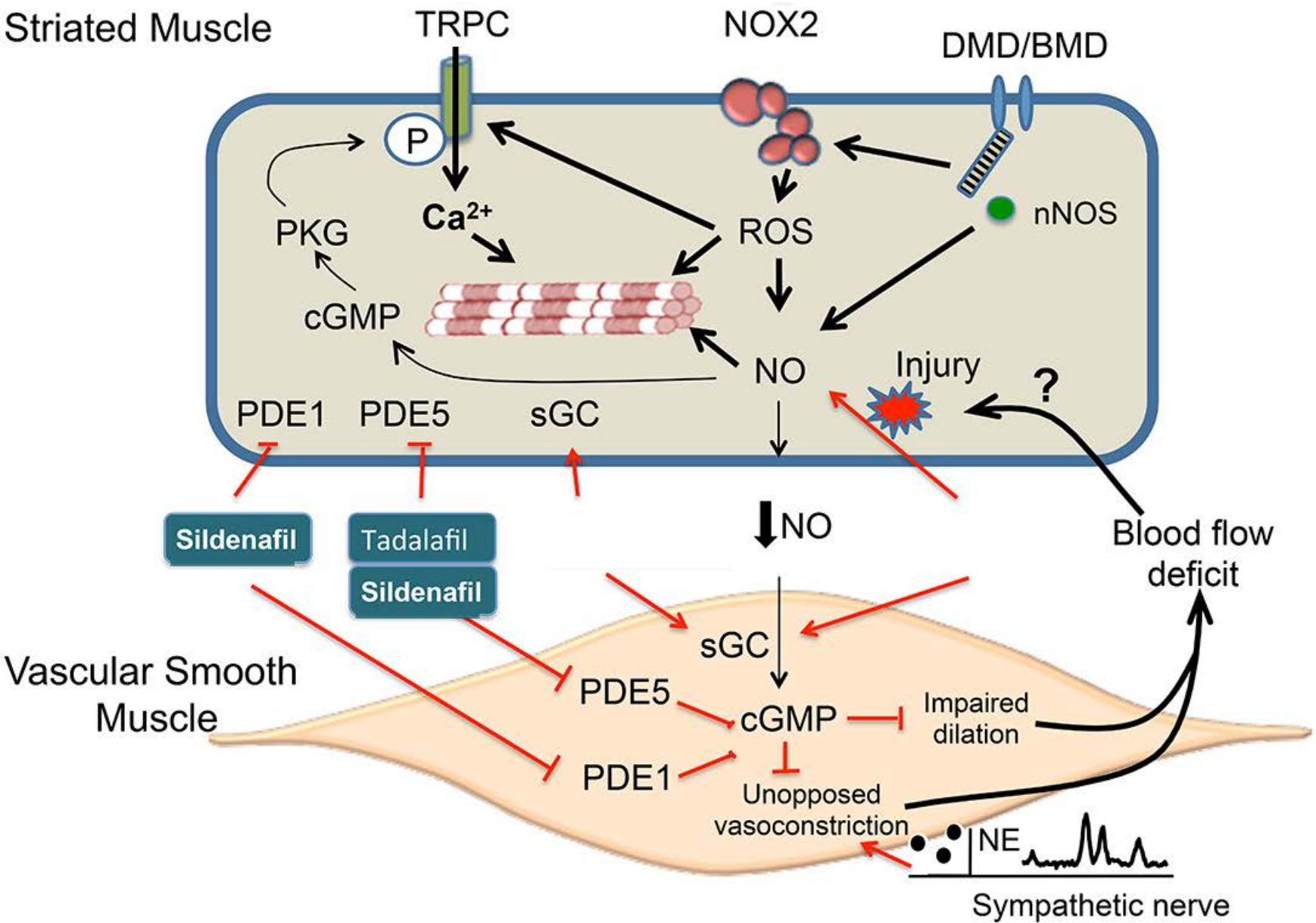
Therapeutic Targets: For the DMD heart, are #5 and #6 the best targets if you cannot deliver full-length dystrophin?

Current Duchenne muscular dystrophy therapeutic targets can be grouped into six categories. Only the first addresses the primary genetic defect (resulting in the loss of dystrophin protein). The rest address downstream aspects of the pathogenesis.

1) Replacement of dystrophin/utrophin
2) ~~Increasing muscle mass and regeneration~~
3) Decreasing inflammation and fibrosis
4) ~~Correcting blood flow regulation~~
5) Correcting perturbations in calcium handing (more utrophin?)
6) Mitochondria dysfunction and ROS generation

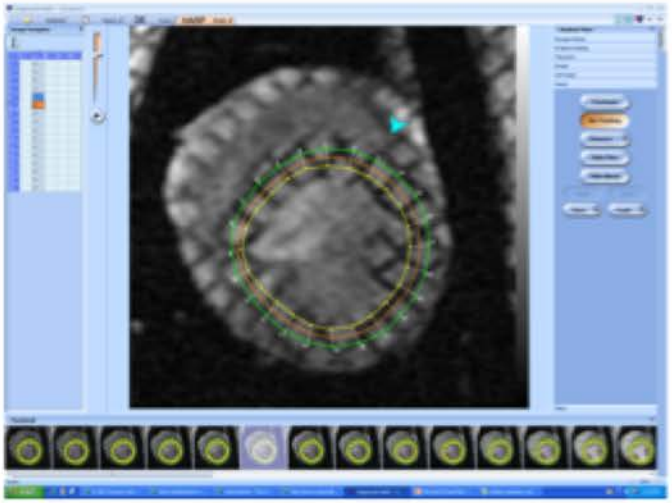
Addressing the calcium leak with tadalafil

Potential Benefits of PDE5 Inhibition

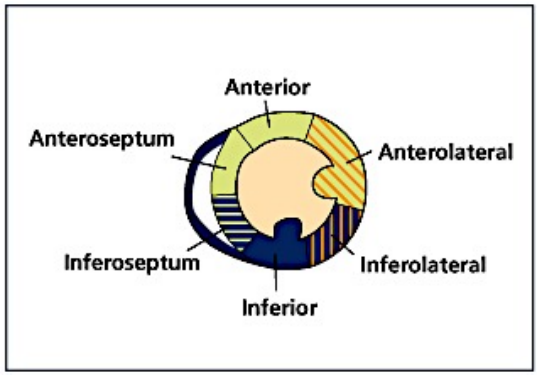


Regional Circumferential Strain Analysis

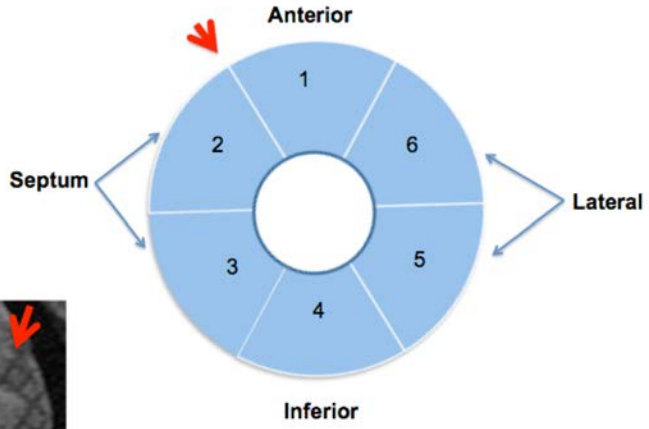
- Performed regional circumferential strain analysis (ECC) using Harmonic Phase method (HARP, Diagnostics Inc)
- Single slices were analyzed from mid-papillary region of left ventricle
- ECC measured at mid-wall of myocardium



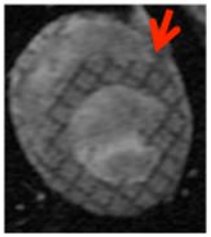
- 16 frames acquired per cardiac cycle; In all cases, tags could be automatically followed beyond end-systole (~frame 4-7), but not always to the end of diastole
- Regional analysis were divided into 6 segments

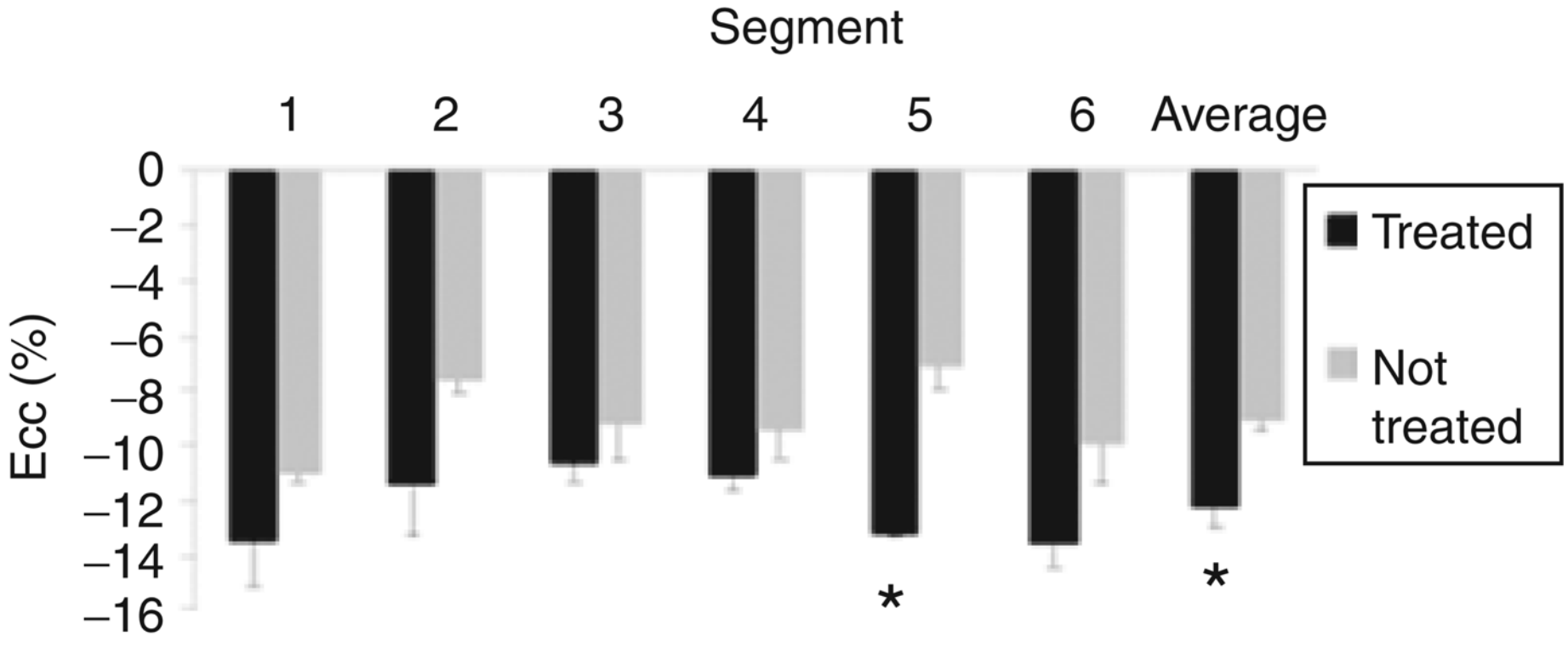


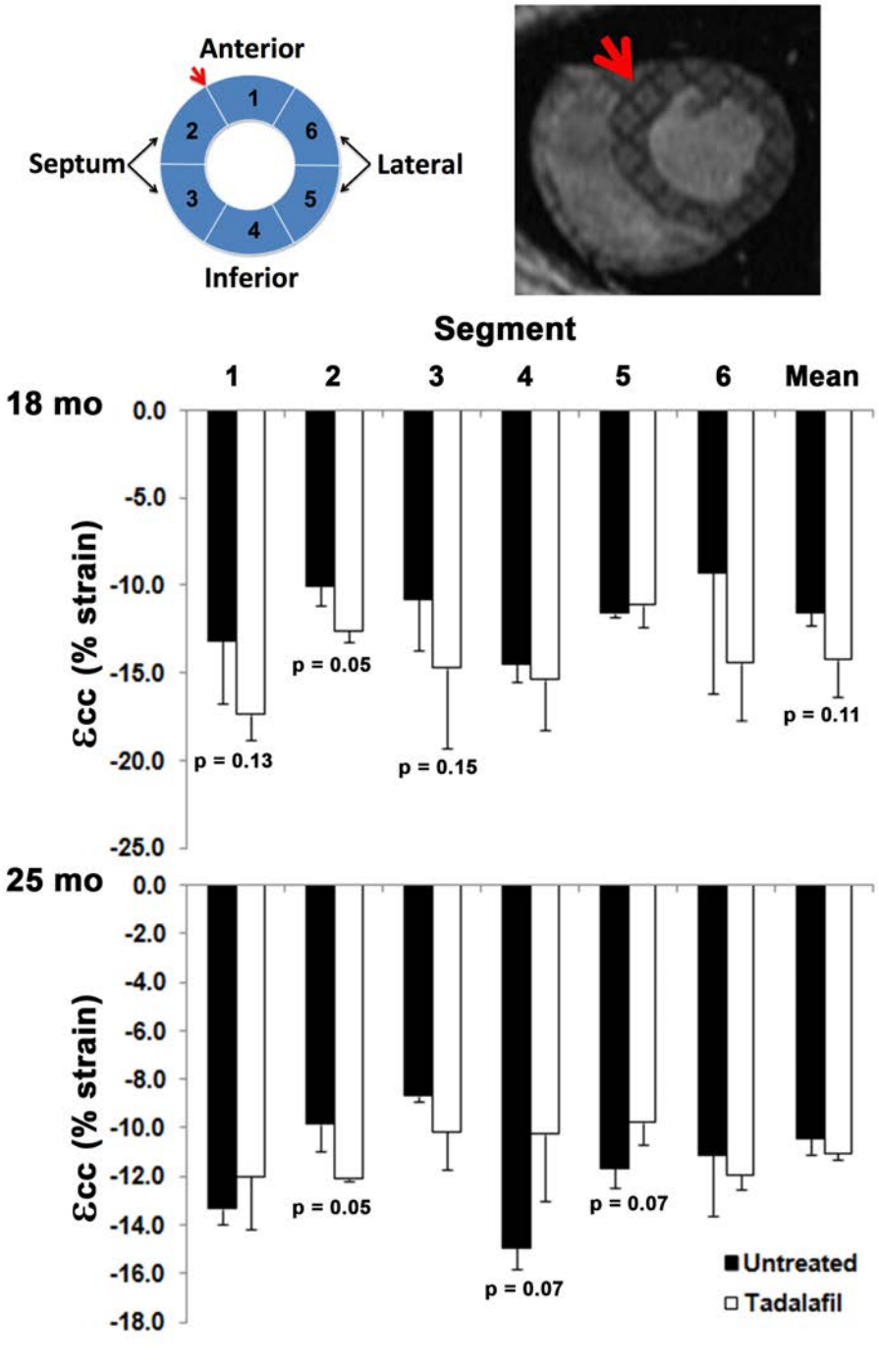
Segments

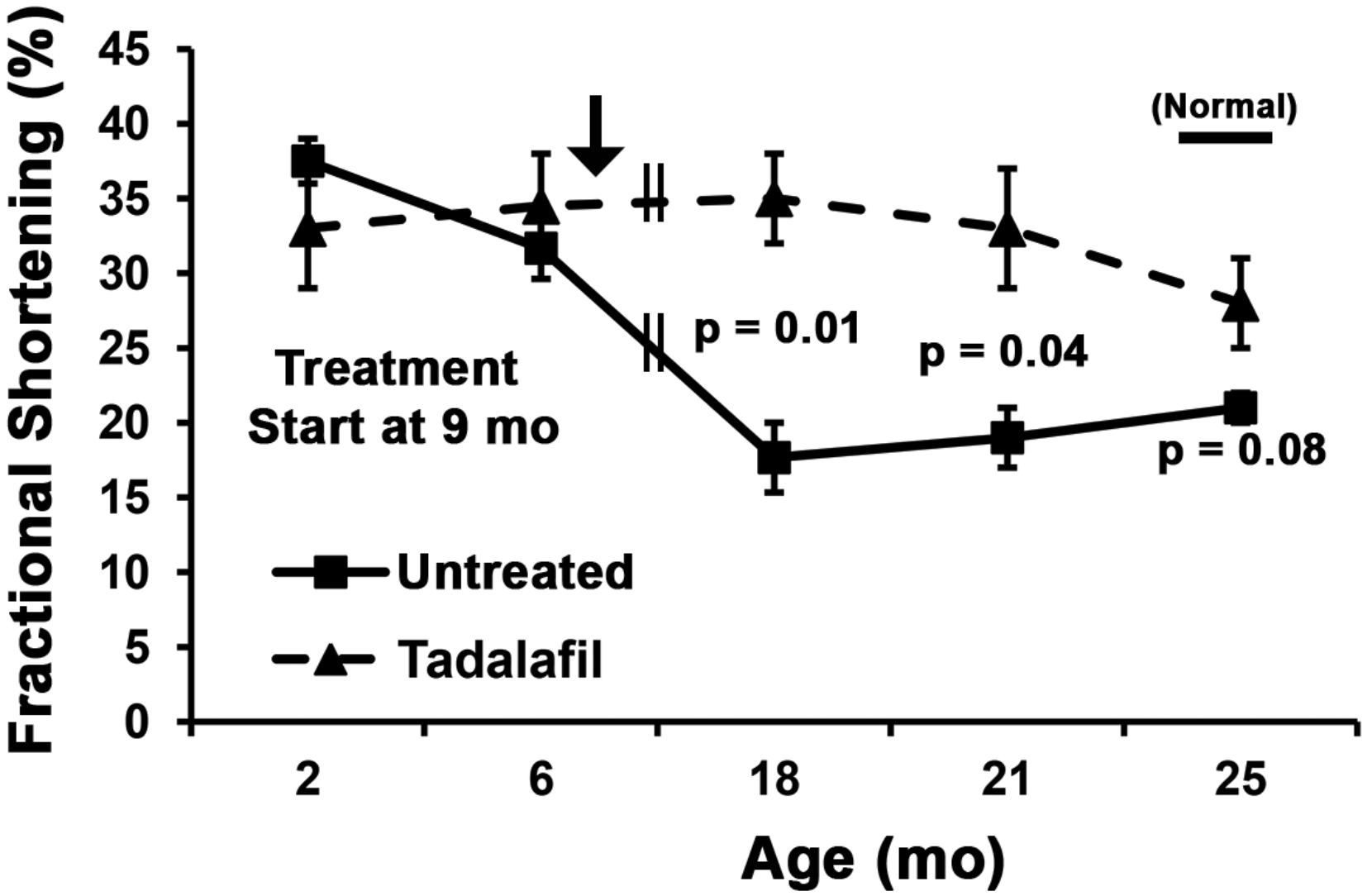


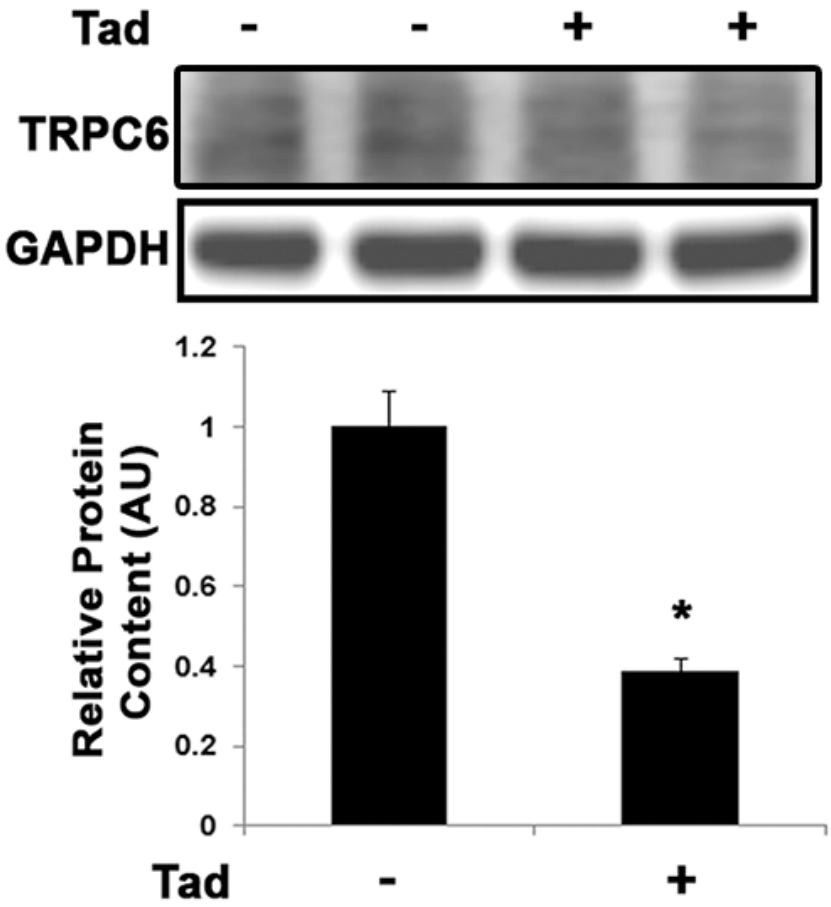
Regional Circumferential Strain Analysis

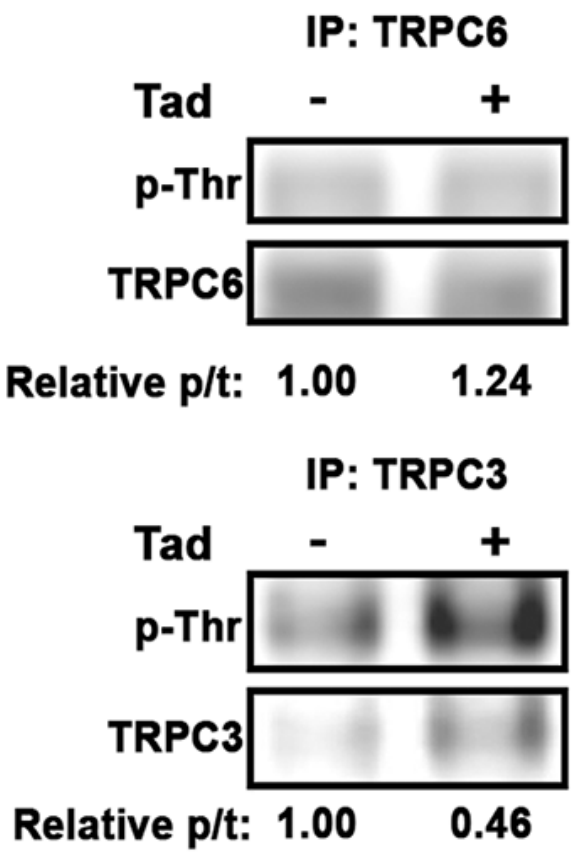


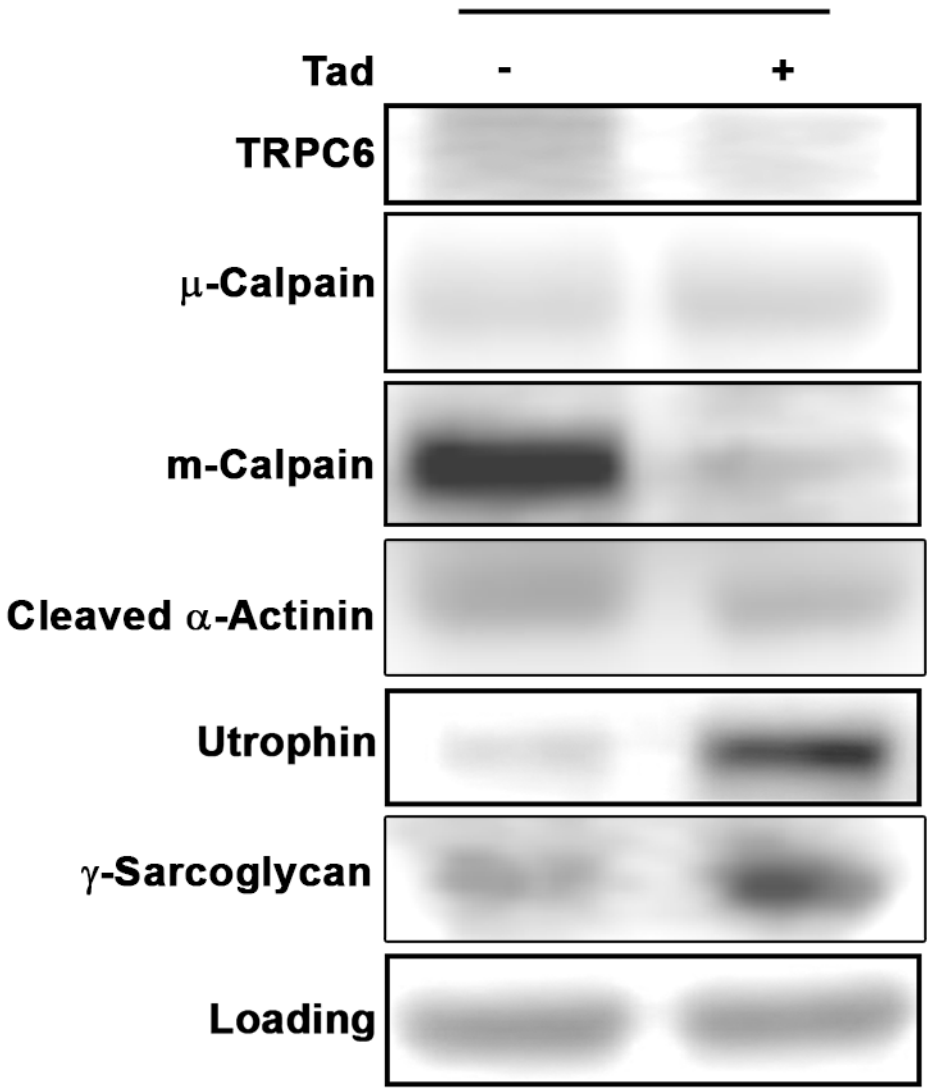


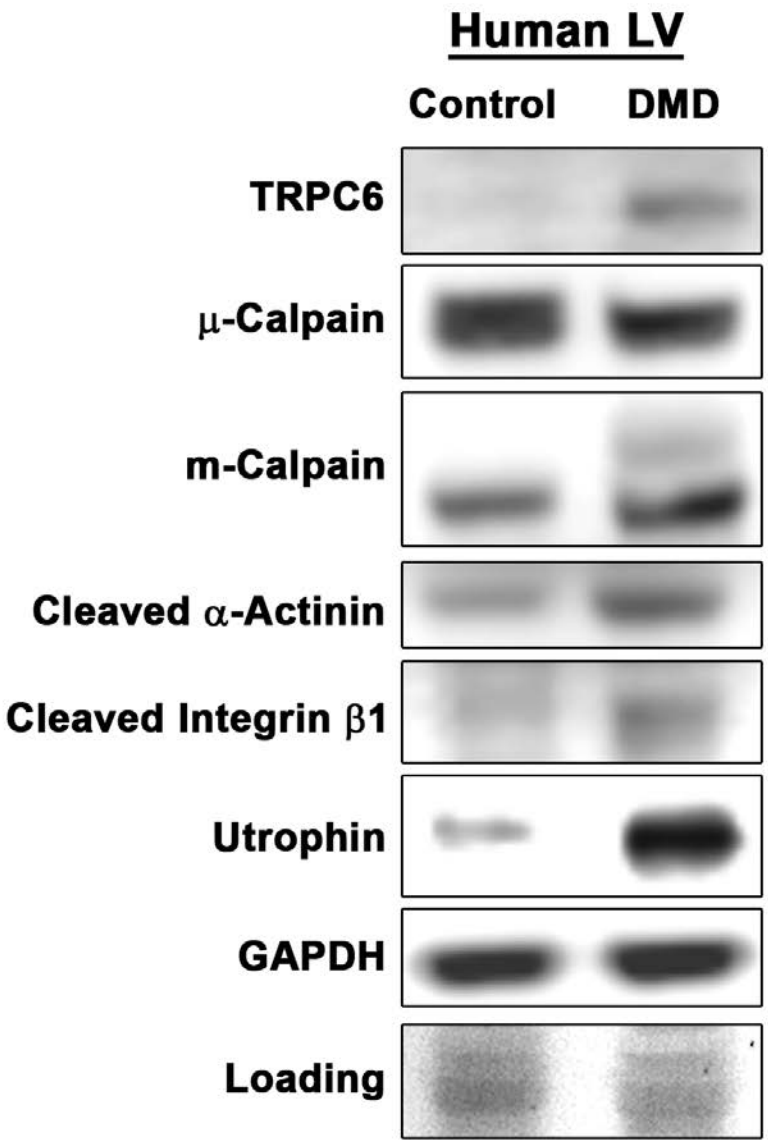

Anterior
Septum
Lateral
Inferior
1
2
3
4
5
6
Segment
1
2
3
4
5
6
Mean
18 mo
25 mo
εcc (% strain)
0.0
-5.0
-10.0
-15.0
-20.0
-25.0
-2.0
-4.0
-6.0
-8.0
-12.0
-14.0
-16.0
-18.0
p = 0.13
p = 0.05
p = 0.15
p = 0.11
p = 0.07
Untreated
Tadalafil


Fractional Shortening (%)
45
40
35
30
25
20
15
10
5
0
2
6
18
21
25
Age (mo)
(Normal)
Treatment
Start at 9 mo
p = 0.01
p = 0.04
p = 0.08
Untreated
Tadalafil


Tad - - + +
TRPC6
GAPDH
Relative Protein Content (AU)
1.2
1
0.8
0.6
0.4
0.2
0
*
Tad - +


IP: TRPC6
Tad - +
p-Thr
TRPC6
Relative p/t: 1.00 1.24
IP: TRPC3
Tad - +
p-Thr
TRPC3
Relative p/t: 1.00 0.46


Tad
-
+
TRPC6
μ-Calpain
m-Calpain
Cleaved α-Actinin
Utrophin
γ-Sarcoglycan
Loading


Human LV
Control
DMD
TRPC6
μ-Calpain
m-Calpain
Cleaved α-Actinin
Cleaved Integrin β1
Utrophin
GAPDH
Loading

PDE5 Inhibition (Tadalafil) Delays Progression of Cardiomyopathy

TRPC6 phosphorylation levels were increased >2-fold

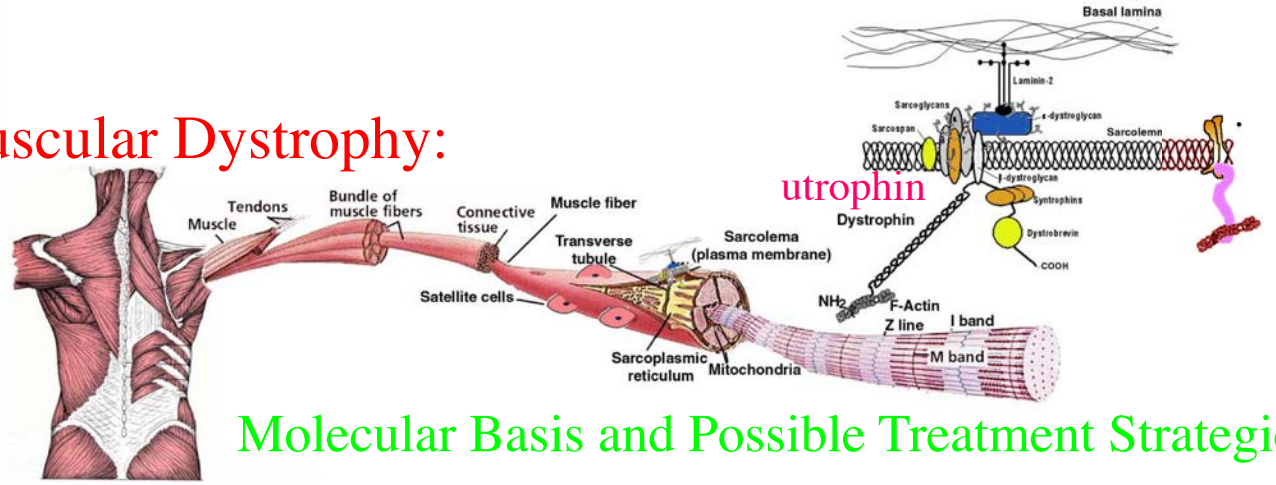
Utrophin levels increased >1.5-fold

Progression of disease delayed by 15 months

Conclusions from PDE5 Study

- PDE5 inhibition with tadalafil can partially silence TRPC6 channels (calcium leak) and decrease breakdown of utrophin (prevents calpain activation) thus slowing progression in dystrophin-deficient hearts.
- Tadalafil may slow cardiac disease progression in DMD/BMD.

Duchenne Muscular Dystrophy:



Molecular Basis and Possible Treatment Strategies

Functional Roles of Dystrophin:

- **Mechanical** - transmits force from the contractile apparatus to connective tissue/tendon
- **Organizer** - positions a number of proteins at the muscle membrane (NOS, ion channels,etc.)
- **Signaling** - likely plays a number of signaling roles, including a key role in calcium homeostasis

Contraction causes rupture of the muscle membrane, which allows calcium inflow. There also there may be increased flux through ion (TRPC) channels and leakiness of the internal calcium storage compartment (SR) via the ryanadine receptor (SR-calcium release channel).

Excessive calcium is taken up by mitochondria, ultimately contributing to mitochondrial uncoupling, free radical generation, and dysfunction. This can trigger apoptosis of the cardiomyocytes.

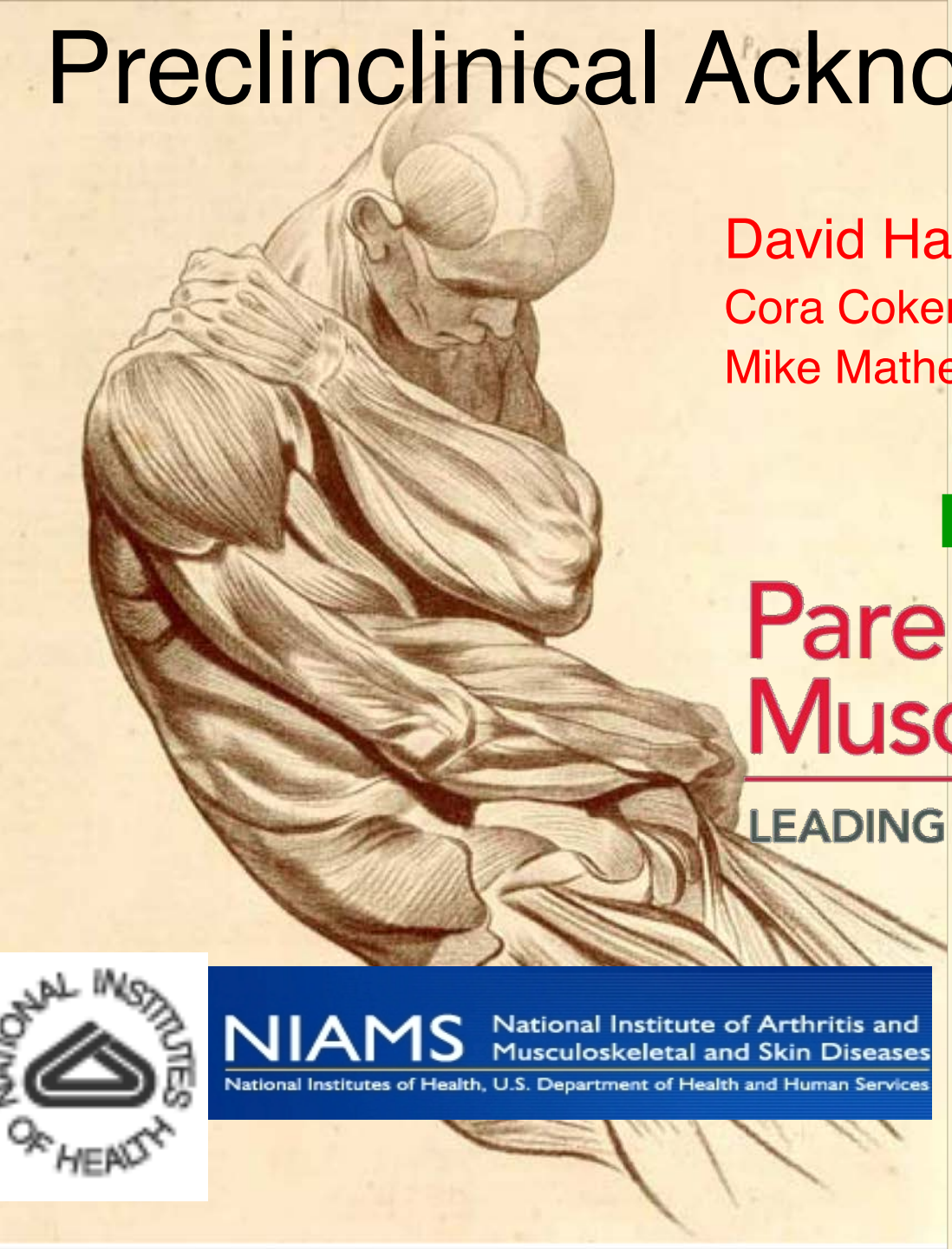
Questions for Gene Therapy for DMD

- Can a micro-dystrophin totally rescue the heart?
- Would addressing calcium handling and/or mitochondrial function with gene therapy, either alone or in combination with micro-dystrophin, provide more benefit than micro-dystrophin delivery alone?
- It may be possible to deliver a cardiac-specific AAV to the heart at the same time a micro-dystrophin is delivered body-wide to the skeletal muscle. Unlike the case of skeletal muscle, the heart would likely never need to be re-dosed.

Parent Project Muscular Dystrophy

LEADING THE FIGHT TO END DUCHENNE

Preclinclinical Acknowledgments:



David Hammers
Cora Coker
Mike Matheny

Meg Sleeper
Beth Barton
Glenn Walter
Sean Forbes

FUNDING:

Parent Project Muscular Dystrophy

LEADING THE FIGHT TO END DUCHENNE

NIAMS National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health, U.S. Department of Health and Human Services

National Institutes of Health