Muscle and Oxidative Stress

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Disclosures

- Co-founder and Chief Scientific Advisor
- SAB member DMD charities
- Research funding – GW Pharmaceutical
Dystrophin and the Muscle Membrane

Adapted from Allen et al. Physiol Rev. 2016 Jan;96(1):253-305
Dystrophin Functions

• Membrane Stabiliser
  – Inside to outside (across membrane)
  – Through DAPC to Biglycan and $\alpha 7\beta 1$ integrin
  – Integrity of membrane – more resilient to stretch
  – Membrane repair and general membrane vesicle coordinator

• Shock Absorber
  – Spectrin-like repeats unfold with force (springs) – reduces stretch related damage

• Signalling
  – Localising signalling molecules (e.g. nNOS)
  – Localising molecules that localise other signalling molecules
    • $\beta$-dystroglycan/dynamin and vesicle trafficking; phosphorylation targets proteosomal breakdown of DPC
    • $\alpha$-dystrobrevin re phosphorylation sites
    • Syntrophins (multiple isoforms – coordinating ion channels, nNOS localisation)
  – Scaffolding for other signalling molecules (e.g. Spectrin-like repeats Par-1 phosphorylation)
Dystrophinopathy and Oxidative Stress

**Generating ROS**

- **Muscle Membrane**
  - Pro-oxidant (Nox2) generates ROS
  - Membrane Ca$^{2+}$ pores become leaky
  - Mislocalised nNOS amplifies Ca$^{2+}$ levels in the cell

- **Mitochondria compromised by abnormal Ca$^{2+}$ levels**
  - When not making ATP generates ROS
  - Imbalance of mitochondrial fuels (NADH/NAD ratio) generates ROS

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**Oxidative Stress**

<table>
<thead>
<tr>
<th>Oxidants</th>
<th>Anti-oxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide $\cdot O_2^-$</td>
<td>SOD/Catalase</td>
</tr>
<tr>
<td>RNS NO$^+ \cdot O_2^-$</td>
<td>Glutathione</td>
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Impact of ROS and Abnormal Ca2⁺ Levels

• Precipitated by abnormal [Ca2⁺]i

• Impaired Mitochondria Function
  – Reduced energy production
  – ROS amplified when ATP not produced OR abnormal ratio of mitochondrial ‘fuels’ (NADH/NAD)
  – Ca2+ homeostasis further perturbed

• Apoptosis/Necrosis/Autophagy
  – Cell programmed cell death (increased susceptibility to ROS)/cell death precipitated by external cell signals
  – Proteases (slow increases in Ca2⁺ from leaky channels)
  – Autophagy (clearing out the debris) impaired

• Inflammation
  – ROS promotes inflammatory cells escaping from blood vessels
  – Inflammation amplifies iNOS

• Altered muscle environment
  – Fibrosis
  – Fat deposition (Fibro-adipogenic progenitors)
  – Muscle repair

All Impact on Muscle Function
Dystrophinopathy: Impact on Muscle Function

• Damaged muscle - exerts less force
• Higher % muscle fibre splitting/branching after repair (exerts less force)
• Fibre alignment loss (sarcomere register) – fibres not pulling altogether
• Resting \([\text{Ca}^{2+}]_i\) higher impairs force production
  – Hypercontracted ‘clots’
  – Misclocalised nNOS enhance muscle wasting
• Infiltration of non contractile tissue
  – Fatty fibrotic lesions
• Fatigue
  – \(\text{Ca}^{2+}\) impacts ATP (energy output fuel)
  – High NADH/NAD ratios (mitochondrial imput fuels)
• Impaired blood flow
  – Less fuel
  – Oxidative stress
Impact of Loss of Dystrophin

Complicated by different:
- Dystrophins
  - Other Tissues
- nNOS
- Syntrophins
- Dystrobrevins
- DPC associated proteins
Impact of Loss of Dystrophin

- Loss of Dystrophin
- Oxidative Stress/Ca^{2+}
  - Perfusion
  - Inflammation
  - Fatty-Fibrosis
- Muscle Damage/Loss of Function
- Tadalafil/Sildenafil
- Simvastatin
- Raxone
- CoQ10
- Nemo Binding Protein
- Vamorolone
- Edasalonexent
- HT-100
- N-Acetylcysteine
- Poloxamer 188
- Biglycan
- Mitsugamin
- Quercetin
- Resveratrol
- Cannabidiol
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Muscle adaptations due to ‘stress’
Inactivity enhances fatigue

Exercise and Calorie Restriction

- More aerobic, more energy production
  - Mitochondria = Cell’s Internal Battery

- Less Fatigue
- Increases muscle mass
- Increases blood flow
- Increase blood vessel development
- Anti-inflammatory, anti-fibrotic

All key problems of DMD
Need to ‘mimic’ exercise/calorie stress?
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Gene Augmentation: Oxidative Stress

Can we find a master switch to make aerobic muscle?

- Orphan nuclear hormone receptor
- Improves muscle pathology
- Improves vascular support to muscle
- Better delivery of drug?

Matsakas FASEB J. 2013 Oct;27(10):4004-16
**Gene Augmentation: Oxidative Stress**

**Number of Fibers**
- Control: 1000 ± 50
- Treated: 1500 ± 100

**Percentage of Central Nuclei**
- Control: 100 ± 10
- Treated: 8 ± 2

**Percentage of Positive Fibers**
- Control: 48 ± 2
- Treated: 2 ± 0.5

**Succinate Dehydrogenase**
- 5 fold reduction; p=0.039

**TNF-alpha**
- 2 fold reduction; p=0.039

**IL-1β Expression**
- 5 fold reduction; p=0.039

**Embryonic Myosin**
- 2 fold reduction; p=0.039
Too Much of A Good Thing

- Anti-oxidant
  - Increase mitochondrial function
  - Need to ‘fuel’ the system
  - Key fuels are low in DMD
Thank You