Quadriceps mitochondrial dysfunction following anterior cruciate ligament injury and reconstruction: TEM analysis of mitochondria

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Muscle recovery following knee injury

- ACL rupture → protracted quadriceps atrophy and weakness
  - ~50% of ACL-injured knees progress to osteoarthritis within 5–15 years
  - Despite surgical reconstruction and rehabilitation

- Localized quadriceps muscle fatigue after ACL injury → poor knee mechanics and contributes to the development of osteoarthritis

- Mitochondrial dysfunction contributes to muscle weakness and fatigability
  - Following ACL injury & reconstruction
    - ↓ Mitochondrial biogenesis (A)
    - ↓ Reduced oxidative capacity (B-C)

- Need: Ultrastructural analysis of quadriceps mitochondrial volume density and morphology

A

B

C

Outcomes:
TEM analysis of quadriceps mitochondria

- Biopsies from human participants collected in the OR or CCTS
- 1mm$^3$ portions of biopsies, 4-5 pieces from each biopsy
- Samples immediately fixed & processed
- Workflow:
  1. Fry lab
     1. Fixed: 4% paraformaldehyde + 3.5% glutaraldehyde in 0.1 M Sorenson's phosphate buffer (2hr)
     2. Washed in 0.1 M Sorenson’s phosphate buffer + 5% sucrose
  2. UK Imaging Center (Jim Begley)
     1. Treated with 1% OsO$_4$
     2. Resin embedding
     3. Thick and thin sections (70nm)
     4. Stained with a solution of uranyl acetate and lead citrate
  3. UK Electron Microscopy Center (Jillian Cramer)
     1. Transmission Electron Microscope: FEI Talos F200X
     2. Captured 7 fibers from each biopsy in their entirety
     3. Comprised >100 mitochondria per sample
TEM images of quadriceps mitochondria

Subsarcolemmal mitochondria

Intermyofibrillar mitochondria

Analysis of TEM images of quadriceps mitochondria

- Assessed area of mitochondria (treated as circular $\pi r^2$ using perimeter)
- Assessed morphology
  - Destruction of cristae with expanded matrix space
  - Concentric ‘onion shaped’ cristae
  - Compartmentalization into vacuolar structures

Owen AM, et al. eLife. 2019
Issues encountered / Acknowledgements

• Bulk processing of specimens
  • Collection from the OR difficult to predict / plan
  • Length of time samples are preserved post-fix prior to embedding

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