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

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College of Health Sciences

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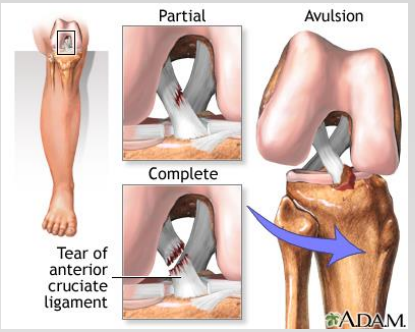
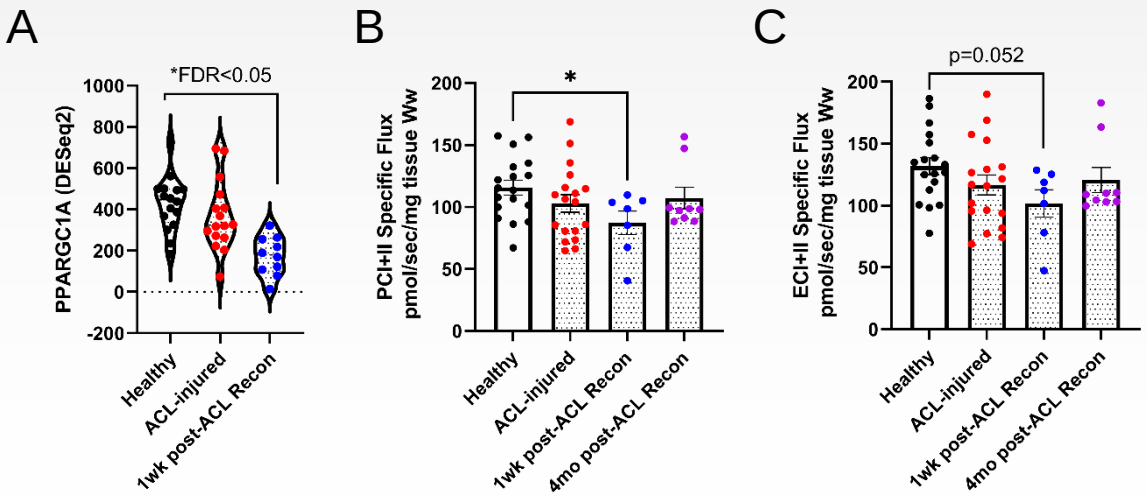
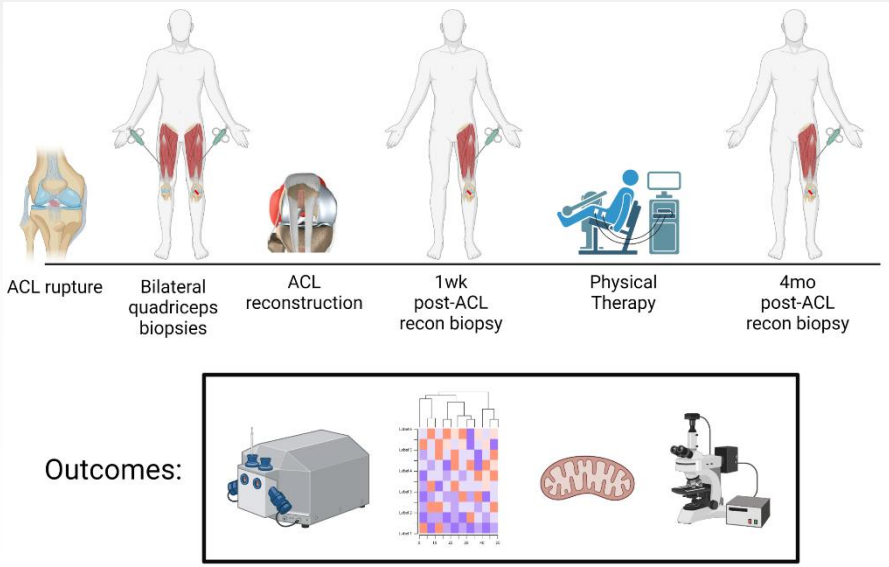
Muscle recovery following knee injury

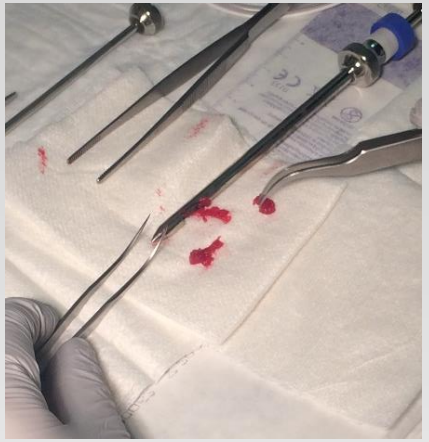


- ACL rupture → protracted quadriceps atrophy and weakness
  - ~50% of ACL-injured knees progress to osteoarthritis within 5–15 years
  - Despite surgical reconstruction and rehabilitation
- Localized quadriceps muscle fatigue after ACL injury → poor knee mechanics and contributes to the development 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





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

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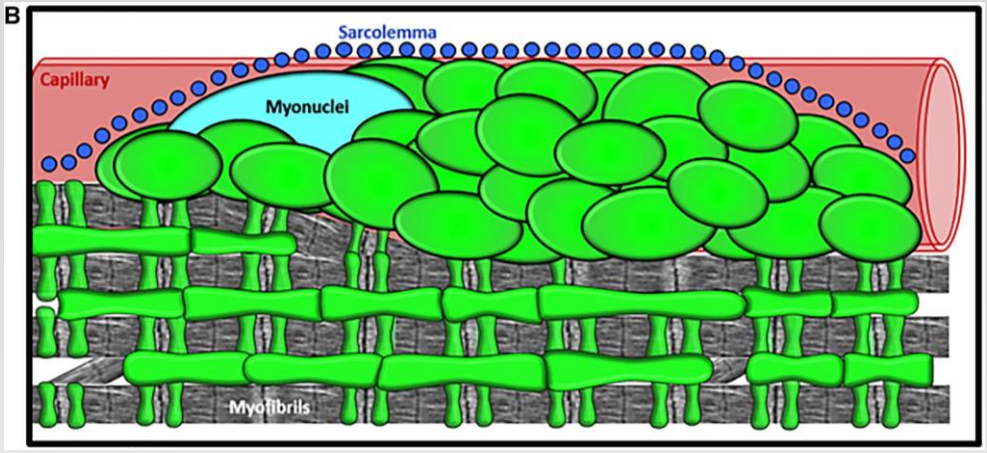
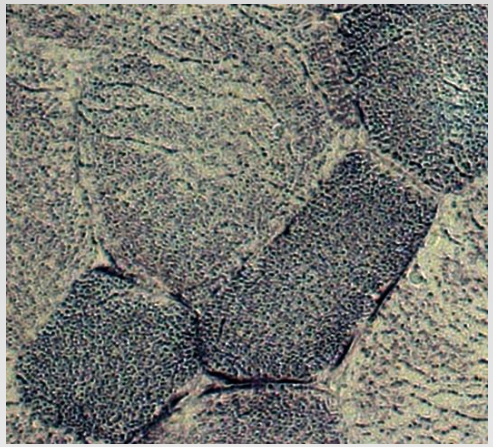
TEM images of quadriceps mitochondria

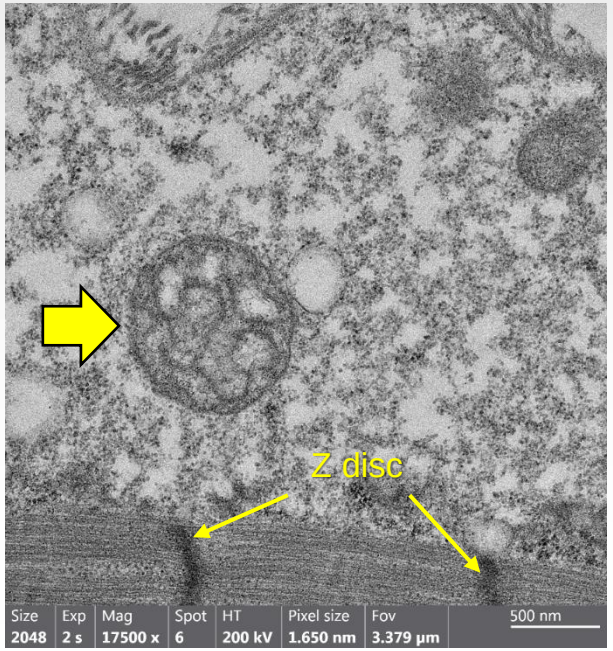


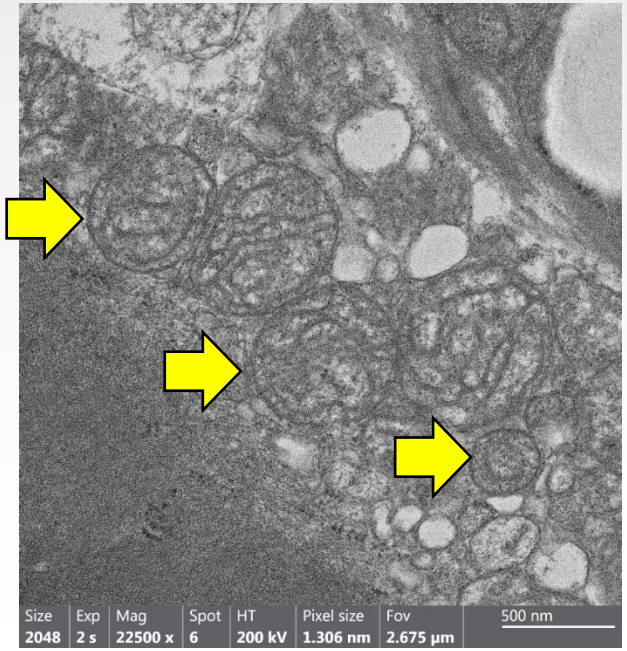
Willingham TB, et al. Front Cell Dev Biol. 2021



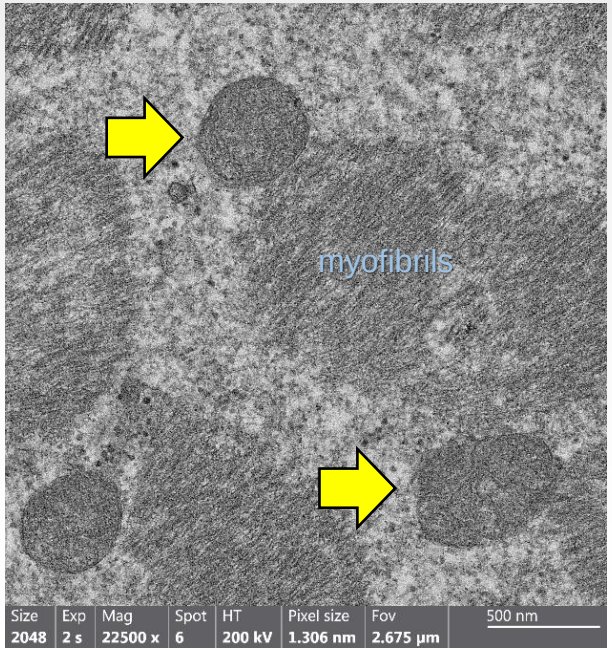
Succinate dehydrogenase Fry lab

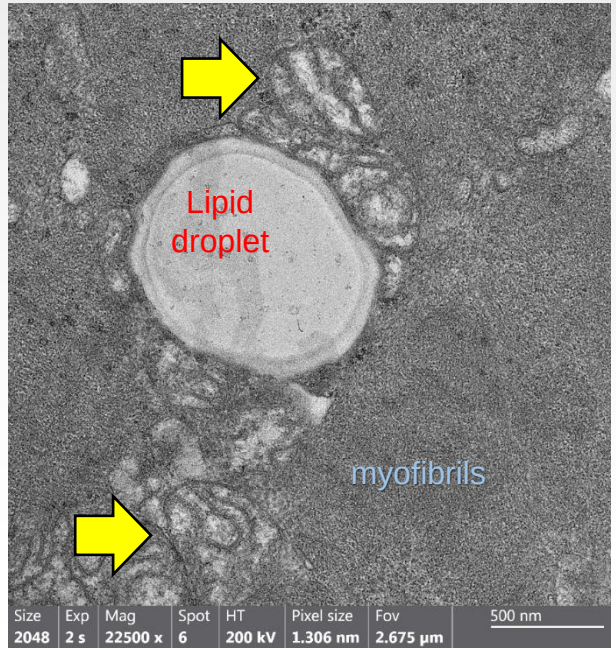
Subsarcolemmal mitochondria





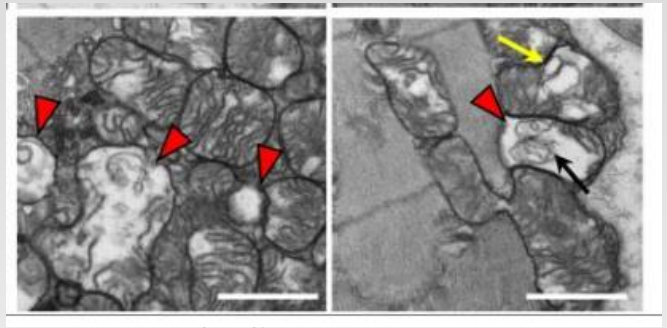
Intermyofibrillar mitochondria



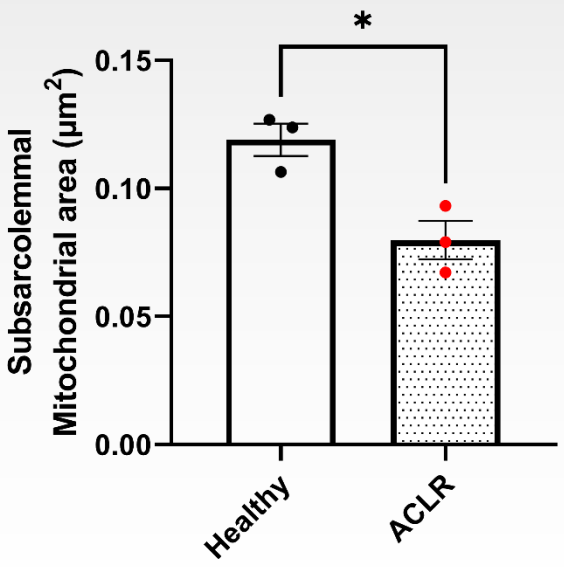


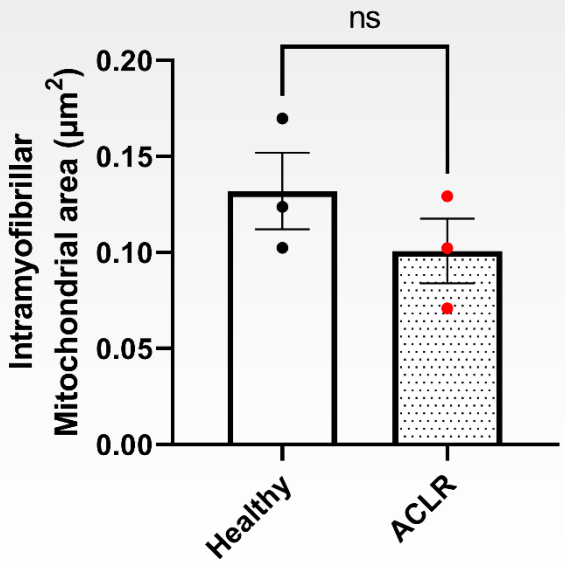
Analysis of TEM images of quadriceps mitochondria

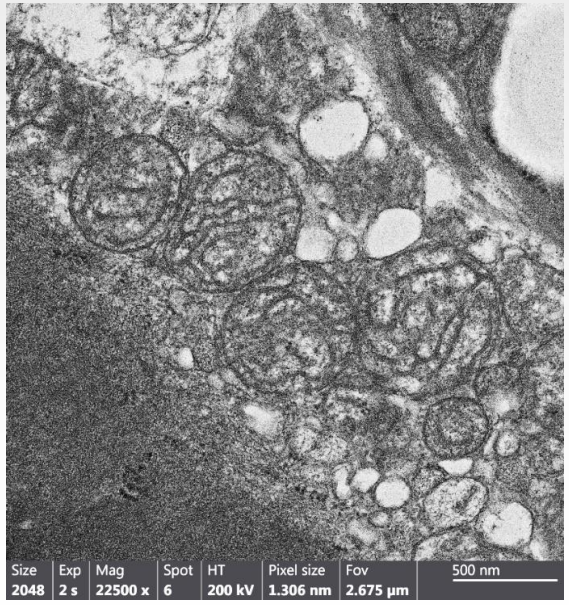
- Assessed area of mitochondria (treated as circular [πr^2] using perimeter)
- Assessed morphology
  - Destruction of cristae with expanded matrix space
  - Concentric 'onion shaped' cristae
  - Compartmentalization into vacuolar structures



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

- Fry lab
  - Christine Latham, PhD
  - Camille Brightwell, PhD
  - Alex Keeble, BS
  - Nick Thomas, MS
  - Haley Noehren
  - Peyton Balawender
  - Madi O'Daniel
- Brian Noehren PT, PhD
- Jim Begley
- Jillian Cramer

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