

# EFFECTS OF THE N-HELIX 2-12 DELETION MUTATION IN CARDIAC TROPONIN C ON MYOFILAMENT FUNCTION

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## Results

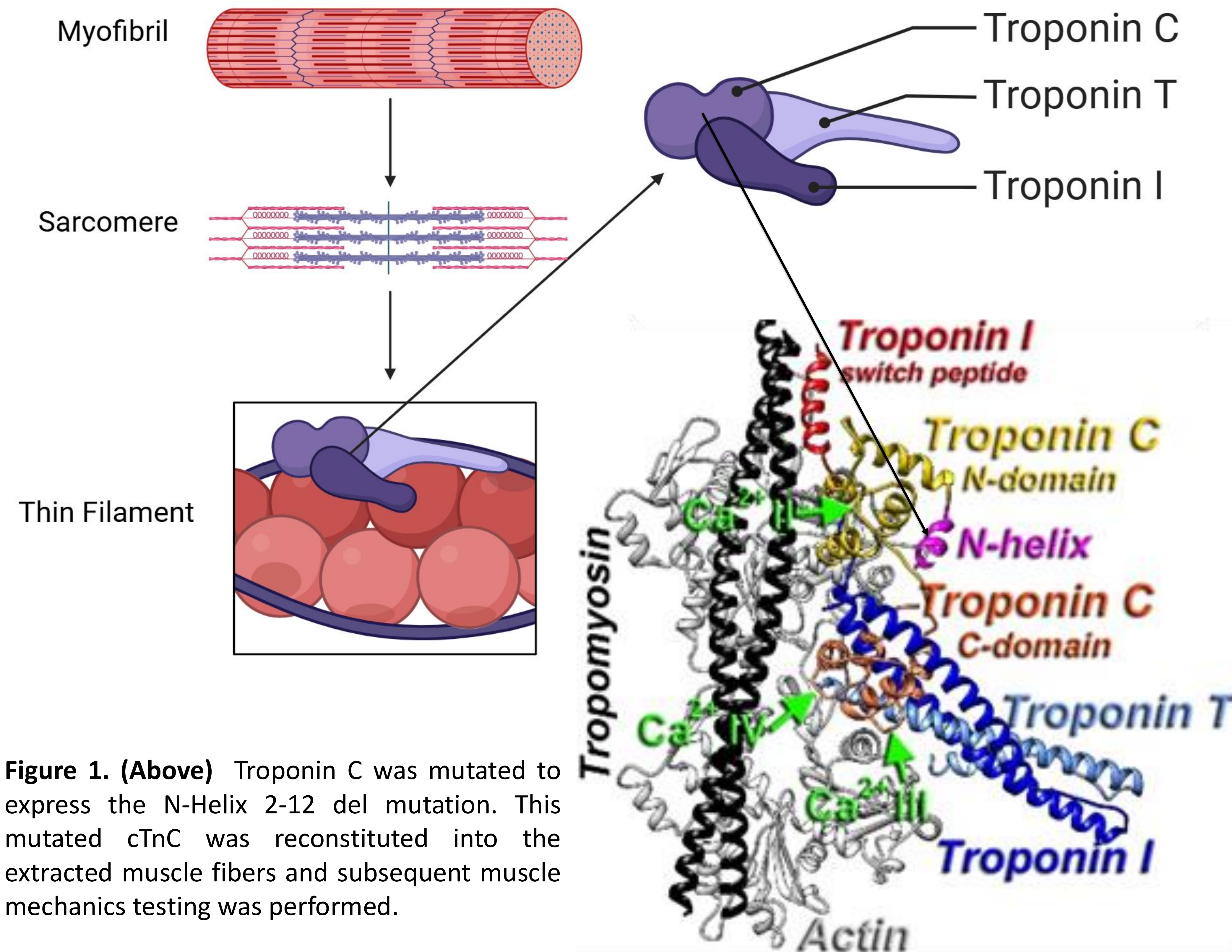
### Abstract

For the past 75 years, since the Framingham Study began, cardiovascular disease (CVD) research has been pivotal in combating one of the deadliest epidemics in American history. Many CVDs involve genetic mutations of proteins within the functional unit of cardiac muscle, the sarcomere. Cardiomyopathy, specifically hypertrophic, is characterized by different mutations that cause enlarged ventricular walls, blocked aortic flow, abnormal ejection fraction, and many other cardiac maladaptation. To explore this, we are looking to evaluate how cardiac troponin C (cTnC), specifically with the "2-12 del" N-helix deletion mutation, affects myofilament function. This is important because cTnC plays a central role in excitation-contraction coupling, and several amino acids within the N-helix can be affected by mutations, leading to cardiomyopathy. To do this, we are extracting endogenous cTnC protein from skinned cardiac papillary muscle and reconstituting their sarcomeres with either exogenous wildtype (control) or N-helix 2-12 del mutated protein. We expect ~80% of the endogenous protein to be extracted by CDTA treatment. Following reconstitution, we measure biomechanical properties including Ca<sup>2+</sup>-activated steady-state isometric force and stiffness, and tension redevelopment kinetics (kTR). In addition to biomechanics, we plan to perform Western blots on the experimental tissue to examine protein composition after extraction-reconstitution. We anticipate seeing differences in the Ca<sup>2+</sup>-dependence of biomechanical properties due to removal of the N-helix.

### Methods

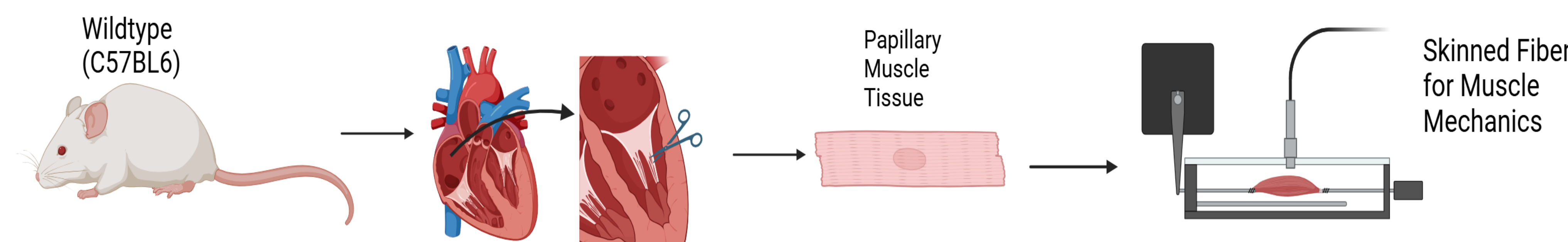
- Muscle mechanics testing was performed on demembrated cardiac muscle bundles.
- ~80% of troponin was removed from the muscle tissue using CDTA and muscle mechanics testing was performed.
- Troponin was reconstituted with Wildtype (WT) or Mutant (MT) protein and muscle mechanics testing was performed.
- Additional tests were implemented on reconstituted single cardiomyocytes using a range of pCa concentrations (pCa 8, 6.5, 6, 5.8, 5.6, 5.4, 5.2, 5, and 4).
- Data was analyzed using DOSBOX program.

### N-Helix 2-12 Deletion on Cardiac TnC



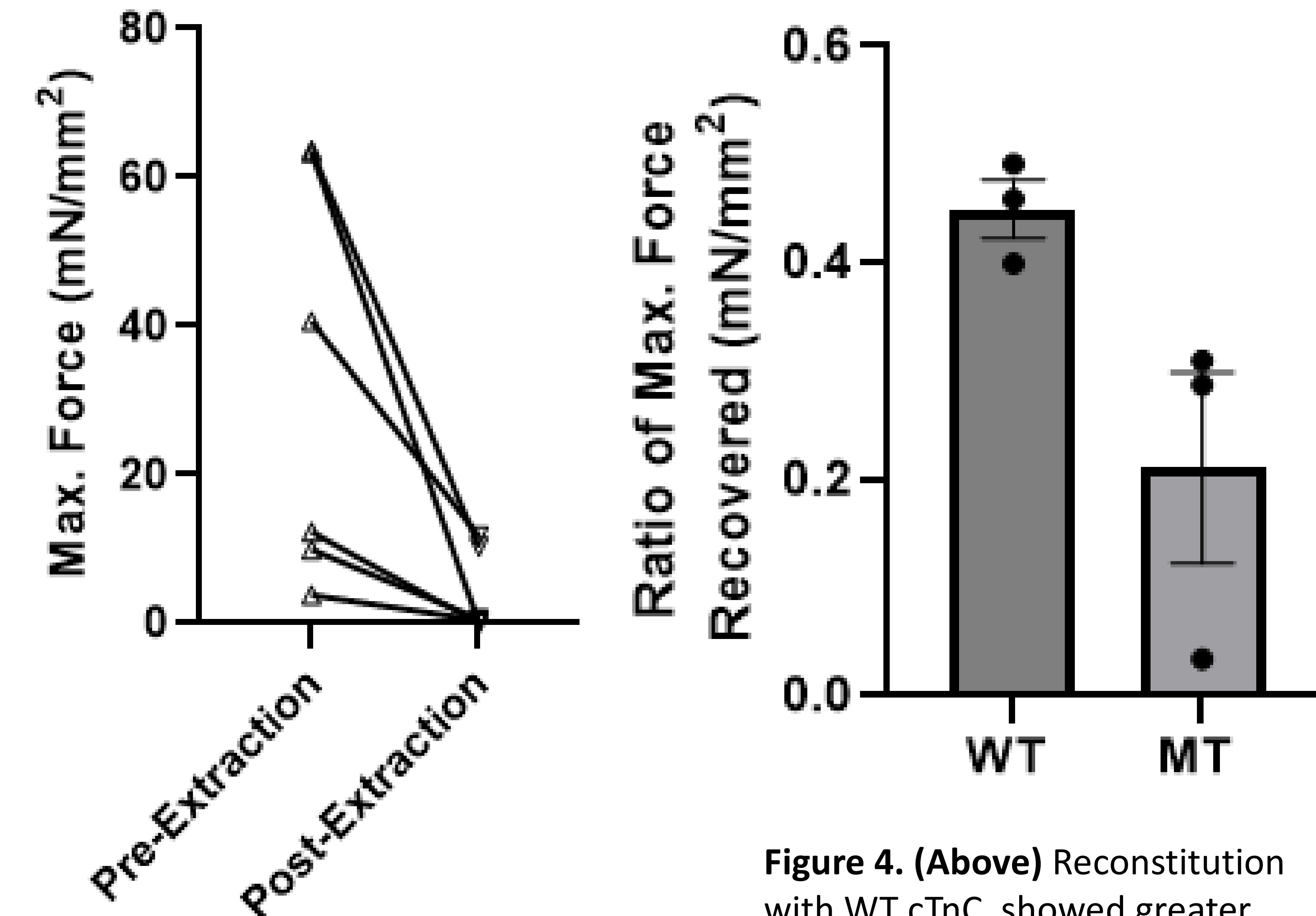
**Figure 1. (Above)** Troponin C was mutated to express the N-Helix 2-12 del mutation. This mutated cTnC was reconstituted into the extracted muscle fibers and subsequent muscle mechanics testing was performed.

### Isolation of Wildtype Papillary Muscle



**Figure 2. (Above)** Papillary muscle extraction protocol. Papillary muscle is isolated from the left ventricle of wildtype C57BL6 mouse and the fibers are trimmed and T-clipped to appropriate size. They are then placed in a "skinning solution" (relaxing solution + 1% TritinX-100) which demembrates the muscle fibers, allowing for precise control of the amount of calcium the fibers are exposed to by removing intracellular calcium, preparing them for muscle mechanics experiments.

### Graphs



**Figure 3. (Above)** Maximum force is greater pre-extraction of cTnC. This indicates that the extraction protocol with CDTA was successful.

**Figure 4. (Above)** Reconstitution with WT cTnC showed greater amounts of maximum force recovery than MT cTnC, a likely result of reduced binding capabilities in MT troponin.

### Conclusion

Altogether, our data suggests that

- 1) The extraction protocol is effective, with maximum force decreasing post-extraction.
- 2) Less force is recovered when reconstituted with MT protein compared to WT.
- 3) We hypothesize that the missing N-Helix in the MT protein causes less binding of troponin, creating a lower maximum force than WT protein and thus requiring higher Ca<sup>2+</sup> concentrations to generate the same amount of force.

### Next Steps

- 1) Continue muscle mechanics experiments to increase sample size.
  - Determine the change in Ca<sup>2+</sup> sensitivity when MT cTnC is incorporated vs. WT cTnC.
  - Determine kTr and sinusoidal stiffness.
- 2) Perform Western Blots to confirm presence of reconstituted protein.