INTRODUCTION
Heart disease, as the leading cause of death worldwide, kills a million people every year. Among several kinds of heart disease, heart failure, which means the heart fails to provide adequate blood flow to body, is a major disease. Cardiac muscle weakness (i.e. cardiomyopathy) is usually responsible for heart failure. Decreased workload on heart or increased cardiac force production by cardiac contractile regulation is vital for treatment of cardiomyopathy.

The current therapy of cardiac contractile regulation is mainly to increase intracellular Ca\(^{2+}\) concentration through post-translational modifications such as phosphorylation and dephosphorylation of ion channels or Ca\(^{2+}\) handling proteins.\(^1\) But the treatment has some adverse consequences. Therefore, our lab is interested in an alternative approach and acetylation (another kind of post-translational modifications) of myofilament proteins has recently been discovered as a new pathway capable of regulating cardiac muscle contraction. Increased acetylation can lead to greater force production for a given physiological calcium concentration due to increased calcium sensitivity.\(^2\) There are two possible causes for increased calcium sensitivity: altered dynamics of calcium-induced myofilament activation and altered dynamics of the actin-myosin interaction (cross-bridge cycle).

The cross-bridge cycle is a major process in cardiac force generation and thus plays an important role in cardiac contractile regulation. During the cross-bridge cycle, myosin heads attach to thin filaments forming cross-bridges and pull the thin filaments causing cardiac muscle contraction. The mechanics of cross-bridge dynamics are described by two aspects: recruitment (relationship between the number of cross-bridges formed and length changes) and distortion (relationship between cross-bridge stretch and length changes).\(^3\) The total force generated equals the product of recruitment and distortion. Therefore, a mathematical model can be built to describe the relationship between force generated and length changes.

However, the exact biophysical mechanisms of cardiac contractile regulation by myofilament acetylation is still not understood. It is believed that a better understanding of the mechanisms of cardiac muscle contraction is important in improving heart failure treatments because if we know how the force production could be affected we can determine a better way to create larger force. And that’s the goal of our project.

OBJECTIVE
The objective of the project is to determine whether myofilament acetylation alters cross-bridge dynamics (recruitment and distortion) which would be done by experimental data (force changes versus length changes) collection and model-based analysis (optimization based on Ford model).

HYPOTHESIS/SUCCESS CRITERIA
It was hypothesized that the parameters in the muscle contraction model would change due to the increased myofilament acetylation, which means the cross-bridge dynamics would be altered through changes in recruitment and distortion.

METHODS
In order to accomplish the project, the left ventricular papillary muscle from mice was chosen as the model since the fibers are aligned in the muscle which avoids complex force directions. Each papillary muscle was cut into strips under a microscope. These strips were skinned in detergent to remove all membranes. Skinned muscles were separated into two groups and treated at 4\(^\circ\)C overnight with DMSO (control group: vehicle treated) and trichostatin-A (TSA), a histone deacetylase-inhibitor that increases myofilament acetylation.

Treated muscles were attached to a force transducer and motor arm which were used to apply 0.5%, 1%, 1.5% and 2% stretches and releases. The calcium concentration of the bathing solution was held fixed (pCa=5.65 or 5.72, sub-maximal activation). The data of force changes and length changes were recorded by computer at a sampling rate of 2000 Hz.

The mechanical relationship between force changes versus length changes is described using a mathematical model: the Ford model consists of five parameters describing the cross-bridge cycle processes: recruitment (magnitude: \(\lambda_d\), kinetics: b), distortion (magnitude: \(v_0\), kinetics: c), and recruitment-distortion interaction (\(\gamma\)).\(^4\) A custom program written in MatLab was used to estimate model parameters based on an iterative optimization algorithm. The value of magnitude and kinetics of recruitment and distortion were compared between vehicle treated and TSA treated muscles in order to identify whether the cross-bridge dynamics was altered by myofilament acetylation. Student’s t-tests were done for statistical analysis and significant difference is determined when P value is below 0.05.

RESULTS
Figure 1 shows the raw data of the relationship between force changes and length changes with respect of time at 1% stretch, where we can see the force increased sharply and then decreased to a steady state when a stretch was applied. And the force at the steady state is higher than the force before the stretch.
Figures 2 and 3 show mean values plus or minus standard error for the estimated parameters. The values for magnitude and kinetics of recruitment ($\lambda_d$ and b, respectively) are significantly increased (Student’s t-test, P<0.05) in TSA treated fibers ($\lambda_d$: 0.87±0.01 vs. 0.81±0.01, TSA vs. Veh) (b: 22.9±1.7 vs. 17.8±1.3, TSA vs. Veh). Furthermore, the value for magnitude of distortion ($v_0$) was significantly decreased (Student’s t-test, P<0.05) in TSA treated fibers (0.032±0.001 vs. 0.045±0.004, TSA vs. Veh), while the kinetics of distortion (c) were significantly increased (Student’s t-test, P<0.05) in TSA treated fibers (62.2±1.4 vs. 53.1±3.7, TSA vs. Veh).

DISCUSSION

The results of this project support the hypothesis that myofilament acetylation alters cross-bridge dynamics. Increased acetylation results in higher magnitude of recruitment which means the number of cross-bridges increased and the force generated by single cross-bridge also increased due to the decrease of magnitude of distortion. These two changes of magnitudes result in a larger force generated during cardiac contraction. Increased kinetics of both recruitment and distortion show an increased rate constant representing a faster rate of force generation and relaxation. However, whether these changes are beneficial is still under debate.

However, this research was limited by several factors. The Ford model we used to model the mechanics of cardiac contraction doesn’t fit the data perfectly which leads to larger standard error when optimizing, especially with larger stretches. Also, there could be some physical differences between mice and humans that might affect our results of cross-bridge dynamics.

In the future, further studies need to be done to examine the changing patterns for each stretch and release in order to optimize the model and get a better optimization.

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REFERENCES


