Complex Intramuscular Connective Tissue Organization is Required to Explain Active Force and Sarcomere Shortening in the Human Gracilis Muscle

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INTRODUCTION: Computational musculoskeletal models are used to predict muscle performance after surgical procedures (*e.g.,* tendon lengthening, tendon transfer, surgical release). However, most computational muscle models are not explicitly validated on human muscle but are based on assumptions from small animal models. This is because direct measurements of human muscle properties are extremely rare and logistically complex. We recently measured human gracilis isometric contractile properties, resting sarcomere length, and muscle dimensions during a free functioning gracilis surgical transfer [1,2] and created a high-fidelity data set that enables explicit testing of several musculoskeletal modeling approaches. The current study uses our anatomical and physiological data previously acquired to compare three computation approaches used to predict muscle passive and active contractile properties.

METHODS: After IRB approval and patient consent (n=12, 10M:2F), intraoperative data were collected as the lower limb was placed in four joint configurations (JC1 to JC4) that gradually lengthening the gracilis through its anatomical range. At each JC, we measured *in-situ* muscle-tendon unit (MTU) length, passive sarcomere length, passive tension, and active tetanic tension [1,2]. After muscle removal from the thigh, we measured MTU slack length, external tendon length, and muscle volume. The active sarcomere length-tension curve was then calculated for each subject using their patient-specific active tension relationship and the human muscle force-length relationship which assumes maximum tension occurs at a sarcomere length of 2.7 µm [3].

A subject-specific Hill type-muscle model was created for each subject [4]. Experimentally determined optimal fiber length was input and tendon slack length adjusted so optimal fiber length occurred at the MTU length where maximal force was produced, (Model 1) (Fig. 1A). Next, a subject-specific modified Hill type-muscle model (Model 2) was created for each subject that added a compliant internal connective tissue (ICT) structure (Fig. 1A). Finally, a subject-specific model was created for each subject that included two parallel elastic elements (Model 3) that allowed different mechanical properties of ICT and MTU. (Fig. 1A). The root mean squared error (RMSE) between experimental and model predictions for passive and active forces were calculated across subjects. Data are presented as mean±standard error of the mean. Significance level was set to $p < 0.05$.

RESULTS: MTU length and passive force increased as the limb was moved from JC1 to JC4 (Fig. 1B, blue symbols). Measured MTU length increased 24%, from an average of 41.8 cm to 51.7 cm (Fig. 1B). In contrast, sarcomere length only increased 13%, from an average of 3.2 µm to 3.6 µm (Figs. 1B and 1C) (Table 1), implying that some other structure(s) "absorbed" the MTU length change. Predicted active sarcomere operating range covered ascending and descending limbs of the curve (Fig. 1C). To account for these forces, sarcomere shortening with muscle activation was predicted to be greatest for the shortest sarcomere length (48±3%) while at the longest sarcomere length, no shortening was predicted (-2±3%) (Fig. 1C, dashed black lines).
Model 1: A compliant muscle in series with a stiff tendon predicted the passive and active length-tension curves well (RMSE =0.13±0.01 and 0.15±0.02 respectively, Table 2), but passive sarcomere lengthening was not observed between JC 1 and 2 (Fig 1D) as seen experimentally (Fig. 1B).
Model 2: Compliant ICT in series with muscle fibers somewhat corrected changes in sarcomere length (Fig. 1E), but passive and active force predictions were quite different from the experimental values (RMSE =0.19±0.03 and 0.29±0.03, Table 2).
Model 3: An added independent parallel elastic element replicated sarcomere length change and the passive and active length-tension relationships (Fig 1F) and had the lowest RMSE across all models (RMSE =0.09±0.01 and 0.14±0.02, Table 2).

DISCUSSION: The purpose of this study was to compare three computational approaches to musculoskeletal modeling by comparing predictions directly to the *in-situ* muscle data. Measured *in-situ* human gracilis force and sarcomere lengths indicate that significant intermuscular compliance was required to explain the data since the change in MTU length measured was 24% (Fig. 1B), while sarcomere length changes was only 13% (Fig. 1B, Table 1). However, simply adding compliance in series with muscle fibers to account for this discrepancy (Model 2) did not result in better predictions of force or sarcomere length changes (Fig. 1E). Only a more complex biomechanical model, in which a compliant parallel structure (likely representing connective tissue) was added to the muscle (Model 3) actually replicated muscle force and sarcomere length changes. Future work is required that measures sarcomere shortening during muscle activation to validate this modeling approach. Structural studies are also required to define the ICT geometry and biomechanical properties.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrated that, in order to reproduce experimentally measured data in human gracilis muscles, a model with complex passive mechanical and structural properties is required. This has significant implication for models used to predict surgical outcomes where lengths are often referenced to passive sarcomere length, and both are assumed to be tightly coupled to active mechanics. In addition, this calls into question functional predictions made from simple muscle anatomical measurements [5].

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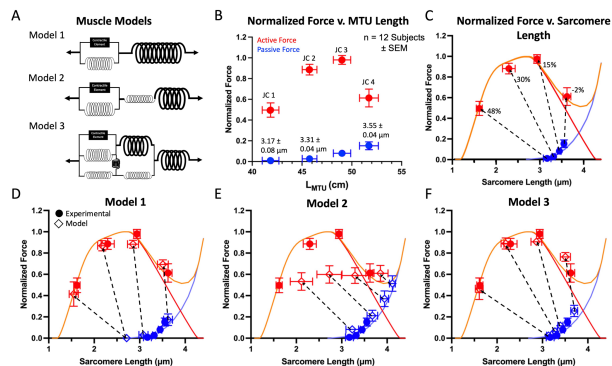
Figure 1: A) Illustrations of model designs B) Intraoperative data collected at each of four joint configurations (JC) in the passive state (**blue**) and stimulated active state (**red**) as a function of muscle tendon unit (MTU length) C) Measured passive force and sarcomere length with estimated sarcomere shortening percentage overlaid on the normalized muscle force *vs*. sarcomere length curve. Modeled normalized force, sarcomere length, and sarcomere shortening using Models 1(D), 2(E), or 3(F). (n=12 mean±SEM).

Table 1: Change in Passive sarcomere length ± SEM

Experimental	13% ± 4%
Standard Hill	34% ± 3%
Modified Hill	29% ± 4%
Parallel ECM	18% ± 3%

Table 2: Average RMSE ± SEM

	Passive	Active
Standard Hill (1)	0.13 ± 0.01	0.15 ± 0.02
Modified Hill (2)	0.19 ± 0.03	0.29 ± 0.03
Parallel ECM (3)	0.09 ± 0.01	0.14 ± 0.02

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