Assessing thin filament lengths in young healthy human skeletal muscle – a role for fiber type?

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INTRODUCTION: Skeletal muscle force production is mediated by interactions and overlap between actin (thin) and myosin (thick) filaments in the sarcomere[1]. As such, myofilament lengths are critical in determining fundamental contractile properties of skeletal muscle[2]. Since whole muscle contractile performance can be accurately predicted from sarcomere-level myofilament properties[3], obtaining high-resolution measurements of myofilament lengths in human muscles, to construct models of contractile performance that are muscle, sex, and age-specific, has profound clinical significance[4,5]. Animal studies have confirmed that, while thick-filament lengths are constant across muscles and species (1.65 µm), thin-filament lengths are highly variable between species, and between muscles of a single species, with type I (slow) muscles typically having longer thin filaments than type II (fast) muscles[6]. However, there have only been limited studies of thin-filament lengths in humans [7,8] especially in young healthy subjects. Recently, it was demonstrated that thin-filament lengths were negatively correlated with the percentage of type IIX myosin heavy chain within older human biopsies, with shorter thin-filaments in type IIX myosin heavy chain-positive fibers, establishing the existence of a relationship between thin-filament lengths and fiber types in human muscle[7]. Interestingly, pure type IIX fibers are uncommon in healthy human skeletal muscle (~0.1-6% of total fibers[9,10]). The objective of the current study is to compare thin-filament lengths between the two major fiber types (pure MHC type I (slow) and pure MHC type IIA (fast oxidative glycolytic)) in young healthy human skeletal muscle biopsies.

METHODS: Human *Semitendinosus* and *gracilis* biopsies are initially being used for all immunofluorescence and image analysis. As of the submission date n=1 semitendinosus biopsy has been fully processed and analyzed. Muscle bundles were thawed, stretched to resolve Tmod and immersed in a fixation solution at 4°C. Samples were cryoprotected, embedded in OCT, frozen in liquid N2 cooled isopentane and sectioned (12µm). For immunostaining, sections were washed for 20 min in PBS + 0.1% Triton X-100 (PBST), permeabilized for 20 min in PBS + 0.3% Triton X-100 and blocked overnight at 4°C in 2% BSA + 5% goat serum in PBST. Sections were labeled with the following primary antibodies diluted in blocking solution overnight at 4°C: BA-D5 (MyHC I) IgG2B, 1:100 (DSHB); SC-71 (MyHC IIA) IgG1, 1:500, (DSHB), supernatant; 6H-1 (MyHC IIX), IgM, supernatant, 1:1, (DSHB); rabbit polyclonal Tmod1 antibody, 1:25. The secondary antibodies included: Alexa Fluor 350, GαMs IgG2B, 1:200 (MyHC I); Alexa Fluor 488, GαMs IgG1, 1:200 (MyHC IIA); Alexa Fluor 594, GαMs IgGM, 1:200 (MyHC IIX); Alexa Fluor 647, GαR IgG (H+L) (Tmod1) 1:200; in blocking buffer for 2 h at room temperature. Tissues were then washed in PBST, preserved in an aqueous mounting medium, and cover slipped. Images of single optical sections were collected on a Nikon AXR confocal microscope mounted on a Nikon eclipse Ti2-E microscope using a 60× NA 1.4 oil objective lens. Plot profiles of the antibody labeled images were fit with Gaussian curves to determine the epitope peak position using Fityk software. Thin-filament length was determined from the Tmod1 epitope positions across the Z-disk. Data were analyzed using unpaired, two tailed t-test. Normality was confirmed using the Shapiro-Wilk test. Data are presented as mean (±SEM) and significance was set to α=0.05. This study was approved by the UC San Diego Human Research Protections Program. All recruited patients were consented prior to enrollment.

RESULTS: A total of 52 fibers (29 type 1; 23 type IIA) were statistically analyzed. The mean (±SD) thin-filament lengths (µm) based on Tmod1 localization were: 1.27 (±0.15); range = 0.87-1.54 for type I fibers and 1.25 (±0.17); range = 0.96-1.57 for type IIA fibres; no significant differences existed between fiber types (p=0.41) (Figure 1).

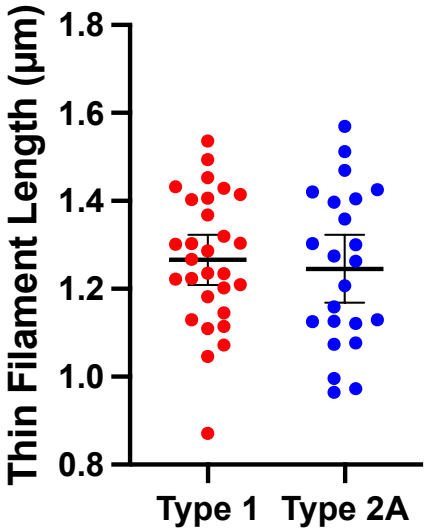

Figure 1. Average (±SD) thin-filament lengths determined by Tmod1 localization in pure MHC type 1 (n=29) and MHC type IIA (n=23) positive fibers from the semitendinosus.

DISCUSSION: Obtaining high-resolution measurements of myofilament lengths in human muscles is critical to our understanding of the fundamental contractile properties of skeletal muscle. To our knowledge, this is the first study to initiate a systematic analysis of thin filament lengths in young healthy human skeletal muscle. Our preliminary findings suggest that the mean and range of thin-filament lengths (based on Tmod1 localization) from young healthy skeletal muscle agrees with a previous study in older healthy skeletal muscle biopsies[7]. In contrast to previous reports suggesting thin-filament lengths are fiber type dependent in animals[6] and humans[7], our preliminary findings suggest that thin-filament lengths may not differ between the two most common fibre types in healthy human skeletal muscle (type I and type IIA), which contrasts with the only other report comparing type I and type IIX fibers in older human muscle biopsies[7]. However, our analysis is currently limited to a single biopsy. More samples, muscles, and age groups will be required to draw definitive conclusions.

SIGNIFICANCE/CLINICAL RELEVANCE: Ongoing data collected from this study will enhance musculoskeletal models that aim to predict 1) stresses imposed on the musculoskeletal system 2) movement in the contexts of sports medicine and 3) rehabilitation and optimizing surgical tendon transfers.

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