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Short communication

Human soleus sarcomere lengths measured using *in vivo* microendoscopy at two ankle flexion angles

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ABSTRACT

The forces generated by the soleus muscle play an important role in standing and locomotion. The lengths of the sarcomeres of the soleus affect its force-generating capacity, yet it is unknown how sarcomere lengths in the soleus change as a function of ankle flexion angle. In this study, we used microendoscopy to measure resting sarcomere lengths at 10° plantarflexion and 20° dorsiflexion in 7 healthy individuals. Mean sarcomere lengths at 10° plantarflexion were 2.84 ± 0.09 μm (mean ± S.E.M.), near the optimal length for sarcomere force generation. Sarcomere lengths were 3.43 ± 0.09 μm at 20° dorsiflexion, indicating that they were longer than optimal length when the ankle was in dorsiflexion and the muscle was inactive. Our results indicate a smaller sarcomere length difference between two ankle flexion angles compared to estimates from musculoskeletal models and suggest why these models frequently underestimate the force-generating capacity of the soleus.

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1. Introduction

The soleus, an ankle plantarflexor, plays an important role in standing (Joseph and Nightingale, 1952), walking (Liu et al., 2006; Neptune et al., 2001), and running (Hamner et al., 2010), and may influence the speed at which the walk-to-run transition occurs (Arnold et al., 2013; Lai et al., 2015). The soleus is the strongest ankle muscle due to its large physiological cross-sectional area (Handsfield et al., 2014; Ward et al., 2009). Despite the biomechanical importance of the soleus, the lengths of its sarcomeres at different ankle flexion angles in healthy individuals are unknown. This gap in knowledge is important to resolve because the lengths of a muscle's sarcomeres have a substantial influence on its force-generating capacity (Gordon et al., 1966). As ankle angle changes, the fibers of the soleus and their constituent sarcomeres change length, which shifts the sarcomeres' positions on the sarcomere force–length curve and affects the force-generating capacity of the muscle.

Musculoskeletal models have been used to estimate sarcomere lengths of muscles at specified joint angles (Arnold et al., 2010; Cutts, 1988) based on sarcomere lengths measured in cadavers at a single joint angle. However, sarcomere lengths in cadavers may be altered by the effects of rigor mortis or fixation (Huang et al., 2011), and the most comprehensive measurements of lower limb sarcomere lengths in cadavers (Ward et al., 2009) included measurements from specimens that were fixed in extreme plantarflexion. To use these measurements in a computer model of the lower limb (Arnold et al., 2010), the sarcomere length–joint angle relationship of the ankle muscles had to be adjusted to match experimentally measured joint moments. Measurements of soleus sarcomere lengths *in vivo* at different ankle angles are needed to provide greater understanding of how ankle flexion influences the force-generating capacity of the soleus in living humans.

Second-harmonic generation (SHG) microendoscopy is a technique for imaging sarcomeres *in vivo* (Cromie et al., 2013; Llewellyn et al., 2008). Sanchez et al. (2015) recently introduced a wearable microendoscopy system and reported soleus sarcomere lengths at one ankle angle. Sarcomere lengths in the soleus have also been measured via laser diffraction in children with cerebral palsy undergoing surgery to treat contracture of the soleus (Mathewson et al., 2015); this study provided unique and valuable insights into muscles with contracture, but the invasive nature of laser diffraction prevented measurement of sarcomere lengths in subjects who were not undergoing surgery. Changes in soleus sarcomere lengths with alterations of ankle angle remain unknown. Minimally invasive SHG microendoscopy allows for recruitment of healthy individuals and repeated measurements in an individual, providing measurements of sarcomere lengths necessary to understand normal muscle function and how pathology changes sarcomere length.

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The goal of this study was to measure *in vivo* sarcomere lengths in the soleus muscles of healthy adults at two ankle flexion angles to better understand how changes in ankle angle affect the force-generating capacity of this important muscle. Using SHG microendoscopy, we made the first *in vivo* measurement of sarcomere lengths in the soleus at two ankle angles.

2. Methods

Seven unimpaired adults (3 men and 4 women; age 27.8 ± 4.6 years; height 171 ± 11 cm; mass 65.4 ± 11.2 kg (mean ± standard deviation)) with no history of ankle injury or neuromuscular disease participated in this study. The Stanford University Institutional Review Board approved the experimental protocol.

After giving informed consent, participants lay on their side with the medial side of the lower leg accessible. This position was comfortable for the subjects to remain still with the microendoscope in the muscle for the duration of the imaging, which took 15–60 minutes. We used a manual goniometer to secure the subject with a custom-made brace at ankle angles of 10° plantarflexion or 20° dorsiflexion. The ankle angle was measured as the sagittal plane angle between the long axis of the tibia and bottom of the participant's foot. We acknowledge that there may be inaccuracy in ankle angle measurement with the manual goniometer. The knee was comfortably flexed to approximately 45°. We used ultrasound to determine the muscle location and fiber orientation. We selected an insertion location in the thickest part of the muscle such that a 1.5 cm long microendoscopic probe could be inserted vertically into the soleus approximately 2 cm distal from the edge of the gastrocnemius (Fig. 1). We instructed the participant to relax and then inserted a probe such that the side-viewing probe imaged a plane parallel to the soleus muscle fiber axis.

We acquired sarcomere images with a wearable SHG microendoscope (Sanchez et al., 2015). The system delivered 200 fs, pulsed laser light at 1030 nm through the wearable microendoscope into the muscle via a microendoscope probe. The microendoscope probe consisted of a 500 μm diameter gradient refractive index (GRIN) objective lens and relay lens in series with a prism to direct the laser beam out the imaging window on the side of the probe. The probe was secured in a 1.5 cm long, 20-gauge stainless steel tube with a sharpened tip. Signals reflected from the muscle were collected through the probe and delivered to a photomultiplier tube sensor via an optical fiber. The microendoscope has a resolution of 1.47 μm within the plane of the 78 × 78 μm field of view, 512 × 512 pixel image (Sanchez et al., 2015). We collected images at varying depths using a custom translation stage fitted to the microendoscope. At some depths there were no visible sarcomeres, likely from blood absorption of the laser light. We observed no visible motion in the images due to involuntary or voluntary activation. After images were collected at one ankle angle, the probe was removed, the ankle angle was changed, the probe was inserted within 1 cm of the initial probe insertion, and images were collected at the new ankle angle.

To determine sarcomere length, we found the fundamental spatial frequency of the sarcomere pattern. The precision of our measurement of mean sarcomere length was in the tens of nanometers and below the resolution limit because we can count multiple sarcomeres present within a field of view (Sanchez et al., 2015). Four successive frames were averaged to reduce noise. We manually selected one region of interest per fiber that included at least 10 successive visible myosin bands and was at least 50 pixels wide. We band-pass filtered the region of interest to consider only physiological sarcomere lengths between 2 and 5 μm, and used a Fourier transform to determine the sarcomere length in the regions of interest. To evaluate repeatability, we analyzed 3 imaging trials of 5 μm spaced rulings and measured mean lengths of 5.00 ± 0.03 μm. The standard deviation of 4 successive frames was less than 30 nm, similar to previous reports (Sanchez et al., 2015). Based on these and previous results, measurement errors were approximately 1% of mean sarcomere length. To account for muscle fiber stretching around the probe insertion site, we multiplied our measurements by 0.91, a scaling factor that was determined by measuring sarcomere lengths near and far from the probe insertion in rat muscles (Chen et al., 2016). In these samples, we measured longer sarcomere lengths only within 2 mm of the probe insertion site, and assume this local effect to be similar in humans. We used a Wilcoxon signed-rank test to test for differences in mean sarcomere lengths between the two ankle angles among the individuals.

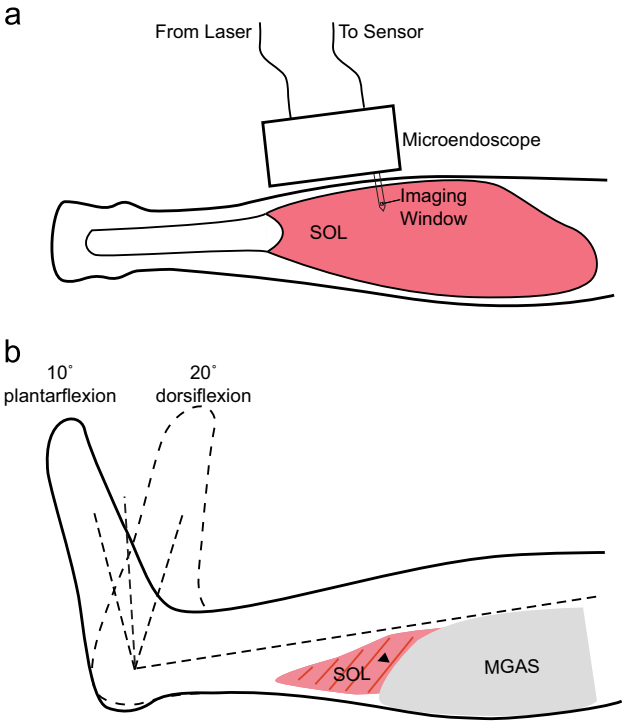


Fig. 1. A wearable SHG microendoscope was used to image sarcomeres in the soleus. Schematic of the right leg in the coronal (a) and sagittal (b) planes showing the imaging location. The microendoscope probe was inserted into the medial side of the soleus, delivering pulsed laser light to the muscle through the imaging window, collecting SHG signal, and delivering it to the light sensor. The imaging probe insertion location is represented as a triangle in panel (b). The imaging probe scans parallel to the muscle fibers of the medial anterior portion of the soleus, approximately 2 cm distal from the gastrocnemius border as identified by ultrasound. Images were acquired with the ankle in 10° of plantarflexion and 20° of dorsiflexion.

3. Results

Resting sarcomere lengths in healthy human subjects (Fig. 2) increased from 2.84 ± 0.09 μm at 10° of plantarflexion to 3.43 ± 0.09 μm at 20° of dorsiflexion (mean ± standard error of the mean, p = 0.016). We measured longer sarcomere lengths at 20° of dorsiflexion in each subject (Fig. 3a). Based on the theoretical sarcomere force–length curve (Gollapudi and Lin, 2009), the force-generating capacity of the soleus at 10° of plantarflexion is 98% of

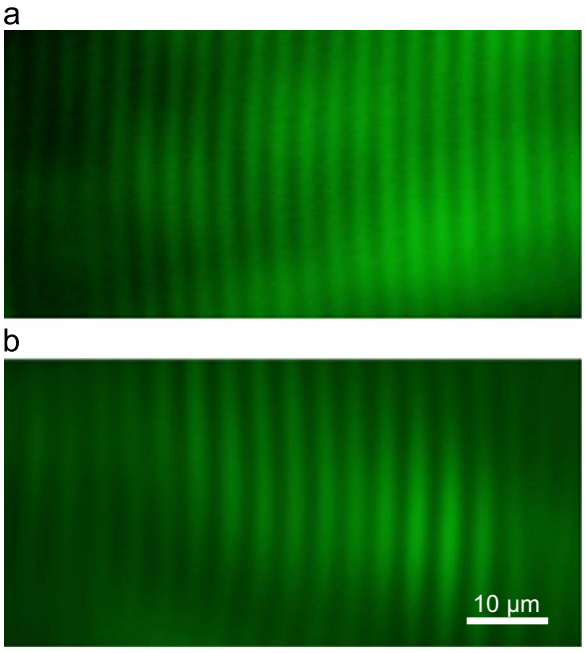


Fig. 2. Second-harmonic generation (SHG) images of sarcomeres in human soleus. (a) SHG image at 10° of plantarflexion with sarcomere length of 2.98 μm. (b) SHG image at 20° of dorsiflexion with sarcomere length of 3.48 μm. Bright regions are myosin-containing bands. Images are cropped, low-pass filtered, and contrast adjusted for presentation. Notice that the dark bands are further separated in panel (b).

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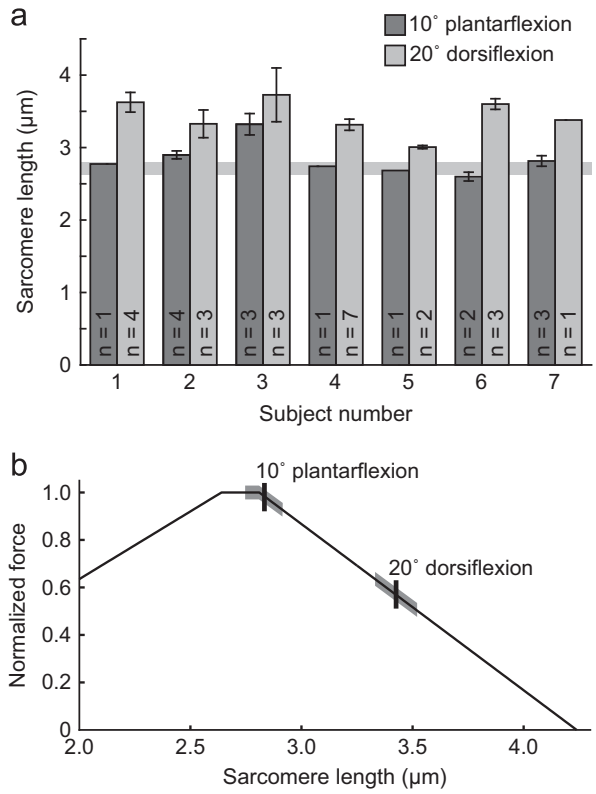


Fig. 3. Resting sarcomere lengths in the soleus increase when the ankle is flexed from 10° of plantarflexion to 20° of dorsiflexion. (a) Sarcomere lengths of the soleus in 7 subjects at 10° of plantarflexion and 20° of dorsiflexion. Mean ± standard error of the mean (S.E.M.) of the n fibers measured. Dark horizontal band represents the plateau region of the force–length relationship. (b) Mean (dashed vertical line) and S.E.M. (shaded highlighting) of mean sarcomere lengths measured in 7 subjects in the soleus at 10° of plantarflexion and 20° of dorsiflexion, plotted with theoretical force–length relationship (Gollapudi and Lin, 2009).

optimal. At 20° of dorsiflexion the force-generating capacity is 57% of optimal (Fig. 3b), and the sarcomeres are on the descending limb of the force–length curve.

4. Discussion

We report resting sarcomere lengths measured in healthy human soleus muscles at two ankle flexion angles and characterize how soleus sarcomere lengths increase with dorsiflexion. The joint angles we investigated span most of the range of ankle angles during walking (Arnold and Delp, 2011; Lai et al., 2015). We show how these sarcomere lengths differ significantly and thus affect muscle force-generating capacity at functionally important joint angles. Our measurements are consistent with Sanchez et al. (2015), who reported a mean passive sarcomere length of 3.15 ± 0.14 μm at 0° of ankle plantarflexion in five subjects.

The change in resting sarcomere lengths with joint angle change we measured is comparable to passive fiber length change measured via ultrasound. Kawakami et al. (1998) reported a 26% increase in passive fascicle length from 15° of plantarflexion to 15° of dorsiflexion. Maganaris (2001) found a 25% increase in fiber lengths from 15° of plantarflexion to 15° of dorsiflexion. These results are comparable to the average 21% increase in resting sarcomere length change we measured over a 30° ankle angle change.

Our measurements of resting sarcomeres suggest that the soleus acts on the plateau and descending limbs of the force–length curve from 10° of plantarflexion to 20° of dorsiflexion, whereas ultrasound and dynamometry studies suggest that the soleus acts on the plateau and ascending limb during active contractions (Maganaris, 2001; Rubenson et al., 2012). We believe that sarcomere shortening due to tendon stretch during muscle activation accounts for this difference. Kawakami et al. (1998) reported a decrease of approximately 30% in soleus fascicle lengths between passive muscle and maximum voluntary activation due to tendon stretch. If we assumed similar shortening in sarcomeres as in fiber lengths from Kawakami et al. (1998), we would expect sarcomere lengths of 2.47 μm at 20° of dorsiflexion and 2.00 at 10° of plantarflexion with maximum voluntary activation. These values represent the shortest functional sarcomere lengths expected at these joint angles, as walking and slow running does not require maximum activation (Lai et al., 2015). Thus, fiber shortening with activation is sufficient to shift the sarcomere lengths measured in this study onto the plateau and ascending limb of the force–length curve, indicating that the difference in sarcomere lengths between passive and active conditions must be considered when interpreting our results. Furthermore, sarcomeres in passive muscle may be slack whereby their lengths may not relate reliably with joint angle. However, slack sarcomere lengths in skinned fibers of the soleus of 1.98 μm (Trombitás et al., 1998) are shorter than our measurements, suggesting that our sarcomeres were under passive tension. Future measurements of sarcomere lengths in passive and active muscle are needed to better understand the effects of muscle activation on sarcomere lengths.

Modeling approaches have estimated soleus sarcomere lengths at various ankle angles (Fig. 4). One musculoskeletal model (Arnold et al., 2010) based on sarcomere lengths measured in cadavers (Ward et al., 2009) estimates that passive soleus sarcomere lengths range from 2.42 to 3.41 μm from 10° of plantarflexion to 20° of dorsiflexion. The model thus predicts that the soleus operates over a wide region of the force–length curve, including the ascending limb, plateau, and descending limb. Another model, also based on cadaver measurements (Cutts, 1988), estimated sarcomere lengths to be 2.03 μm in the anatomical position and 3.36 μm at 18° of dorsiflexion, predicting that the soleus acts on an even broader range of the force-length curve. These models assume that whole muscle can be modeled as a

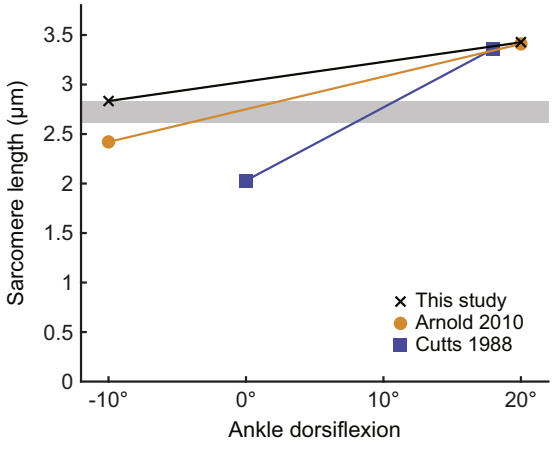


Fig. 4. Sarcomere lengths in the soleus estimated by previous models at different dorsiflexion angles, compared to the mean sarcomere lengths from individuals in this study. Dark horizontal band represents the plateau region of the force–length relationship. Note that we measured smaller sarcomere length change with ankle flexion angle than reported in previous studies.

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scaled up sarcomere such that all the fibers within a muscle are the same length, and all fibers change length by the same amount with joint motion. These assumptions lead to an overestimation of the change in sarcomere length with joint angle compared to our measurements of sarcomere length, measurements of muscle fiber length changes (Kawakami et al., 1998; Maganaris, 2001), and three-dimensional models of muscle architecture (Blemker and Delp, 2006).

Our results provide insight into why musculoskeletal models underestimate the force-generating capacity of the soleus. The force generated by the soleus in musculoskeletal simulations of walking can be compromised because the muscle fibers become shorter than optimal and shorten rapidly (Arnold et al., 2013). If the muscle operates over a narrower range of lengths than predicted by musculoskeletal models, as suggested by our measurements (Fig. 4) and ultrasound data (Kawakami et al., 1998; Lai et al., 2015; Maganaris, 2001), there would be a greater conservation of muscle force-generating capacity during ankle plantarflexion due to reduced force–length and force–velocity effects. Better conservation of force-generating capacity may alleviate the need to artificially inflate the peak isometric force of the muscle (Arnold et al., 2013) to achieve forces that match experimentally measured joint moments and powers.

By measuring sarcomere lengths in the soleus, we have provided insight into the force-generating capacity of the soleus at ankle angles nearly spanning the range of human walking. Our results suggest that simple muscle models used in musculoskeletal simulations overestimate the change of sarcomere lengths with changes in ankle angle resulting in diminished predictions of muscle force-generating capacity. Future studies investigating sarcomere lengths in different regions of the muscle at different joint angles can enable the development of more accurate models of muscle force generation. Studying healthy subjects also provides data needed for comparison with individuals with movement disorders. For example, Mathewson et al. (2015) reported 4 μm long sarcomeres in the soleus of children with cerebral palsy at their maximally dorsiflexed angle, which are longer than sarcomeres we measured in our healthy subjects. Microendoscopy provides a means to compare sarcomere lengths between pathological and healthy muscle and study whether sarcomere lengths relate to the success of interventions designed to improve movement.

Conflict of interest

To disseminate the microendoscopy technology here, S.L.D. is a co-founder of Zebra Medical Technologies Inc. and has a financial interest in the company.

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