

METHODS & TECHNIQUES

Micro-biopsies: a less invasive technique for investigating human muscle fiber mechanics

Paige E. Rice^{1,2,3,*}, Sophia Nimphius¹, Chris Abbiss¹, Kevin A. Zwetsloot⁴ and Kiisa Nishikawa²

ABSTRACT

The purpose of this investigation was to demonstrate that muscle fiber mechanics can be assessed on micro-biopsies obtained from human medial gastrocnemii. Three micro-biopsy samples were collected from female dancers ($n=15$). Single fibers and fiber bundles were isolated and passively stretched from 2.4 to 3.0 μm at 0.015 and 0.04 $\mu\text{m s}^{-1}$ ($n=50$ fibers total) and in five increments at 0.12 $\mu\text{m s}^{-1}$ ($n=42$ fibers total). Muscle fibers were then activated isometrically at 2.4 μm ($n=4$ fibers total) and 3.0 μm ($n=3$ fibers total). Peak stress and steady-state stress were significantly greater ($P<0.0001$) after stretching at 0.04 $\mu\text{m s}^{-1}$ than at 0.015 $\mu\text{m s}^{-1}$. Furthermore, peak stresses and steady-state stresses increased non-linearly with fiber length ($P<0.0001$). We conclude that active and passive muscle fiber mechanics can be investigated using tissue from micro-biopsies.

KEY WORDS: Stress, Gastrocnemius, Dancers, Myofibers

INTRODUCTION

Muscle fiber mechanics inform both the scientific community and the general public on clinical (Mathewson et al., 2014), athletic (Trappe et al., 2000) and situational (Trappe et al., 2008) aspects of muscle properties (Hyunseok and Jong-Hee, 2017). Traditionally, muscle samples obtained from Bergström needle percutaneous biopsies (Bergström, 1975) are compulsory for performance of mechanical experiments on single muscle fibers or fiber bundles (Power et al., 2016; Malisoux et al., 2006; Krivickas et al., 2011; Lim et al., 2019; Harber et al., 2004; Widrick et al., 2001; Larsson and Moss, 1993). One important requirement for these analyses is that muscle fibers must be sufficiently long (2–3 mm) to mount onto force transducer and length-controlling levers. Further, muscle fibers must be undamaged to accurately measure force–length and force–velocity properties. Lastly, there must be an adequate number of useable (long and undamaged) muscle fibers in each biopsy sample. In general, a Bergström biopsy necessitates medical supervision, administration of injectable lidocaine, and a 1 cm surgical incision (Shanely et al., 2014). A single pass Bergström biopsy typically yields approximately 60 mg of tissue and can contain anywhere between 200 and 800 muscle fibers (Larsson and Moss, 1993). Although Bergström biopsies are an overall safe, outpatient procedure that does not pose a risk to the large majority of

individuals, research participants are often intimidated by the size of the needle, invasive nature of the procedure and amount of tissue harvested, not to mention participants can experience soreness for up to 1 week following the biopsy (Hessel et al., 2020). In an effort to increase the willingness of subjects to participate in studies involving collection of muscle tissue, we investigated whether micro-biopsy samples might be a viable means of obtaining human gastrocnemius muscle samples for use in mechanical experiments. Much of the data on human muscle fiber mechanics is from the quadriceps muscles of male subjects (Binder-Markey et al., 2021). In contrast, for this study, we performed micro-biopsies on highly trained female dancers for mechanical experiments on the medial gastrocnemius muscle.

Micro-biopsy needles are roughly two to three times smaller (2.1 mm) than standard Bergström biopsy needles (5–6 mm). For highly vascular muscles such as the gastrocnemius, greater caution must be taken during biopsies to avoid rupturing blood vessels. Thus, a smaller needle may reduce the risk and injury to the participant. Given that micro-biopsies are less invasive, topical lidocaine is usually sufficient to numb the area of the biopsy as an alternative to injectable lidocaine. To perform the micro-biopsy, only two components are necessary: a co-axial introducer needle and the semi-automatic instrument (Fig. 1A). In contrast, eight components are necessary to perform the suction-based Bergström biopsy (Shanely et al., 2014). Additionally, the micro-biopsy needle is a pre-sterilized, single-use device, whereas typical percutaneous biopsy instruments require autoclave equipment for sterilization, which is expensive and not always available to researchers. Even with an autoclave, the concerns of thoroughly cleaning and sterilizing the instrument remain. The muscle micro-biopsy technique has been validated against the Bergström technique for a variety of morphological, immunohistological and bioenergetics analyses (Hayot et al., 2005; Townsend et al., 2016; Isner-Horobeti et al., 2014; Hughes et al., 2015). Existing data suggest that properties such as muscle fiber type, RNA sequencing or mitochondrial respiration can be determined from micro-biopsy samples (Hughes et al., 2015; Grankvist et al., 2020). However, a limitation of micro-biopsies is the relatively small muscle sample obtained. This appears to not be a problem for genetic, histology or proteomic research, but may be a primary concern for fiber mechanics research as a requisite for mechanical experiments is that muscle fibers be undamaged.

Limited data exist not only on fiber mechanics from healthy female athletic and clinical populations, but also from the human gastrocnemius in general. To our knowledge, muscle fiber mechanics have only been reported from human latissimus dorsi ‘fine needle’ biopsy samples (Paoli et al., 2010) and not medial gastrocnemius micro-biopsy samples. Peak stress from male gastrocnemius single fibers adjusted to an average sarcomere length of 2.5 μm ranges between 72 and 51 mN mm^{-2} (Harber et al., 2004; Widrick et al., 2001). In these studies, competitive running and spaceflight have been shown to positively and negatively influence gastrocnemius fiber mechanics, respectively

¹School of Medical and Health Sciences, Edith Cowan University, Joondalup, WA 6027, Australia. ²Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA. ³Department of Health and Exercise Science, Wake Forest University, Winston Salem, NC 27109, USA. ⁴Department of Health and Exercise Science, Appalachian State University, Boone, NC 28608, USA.

*Author for correspondence (ricep@wfu.edu)

© P.E.R., 0000-0002-8899-5385; S.N., 0000-0002-3524-0245; C.A., 0000-0003-3940-5542; K.A.Z., 0000-0002-7995-8769; K.N., 0000-0001-8252-0285

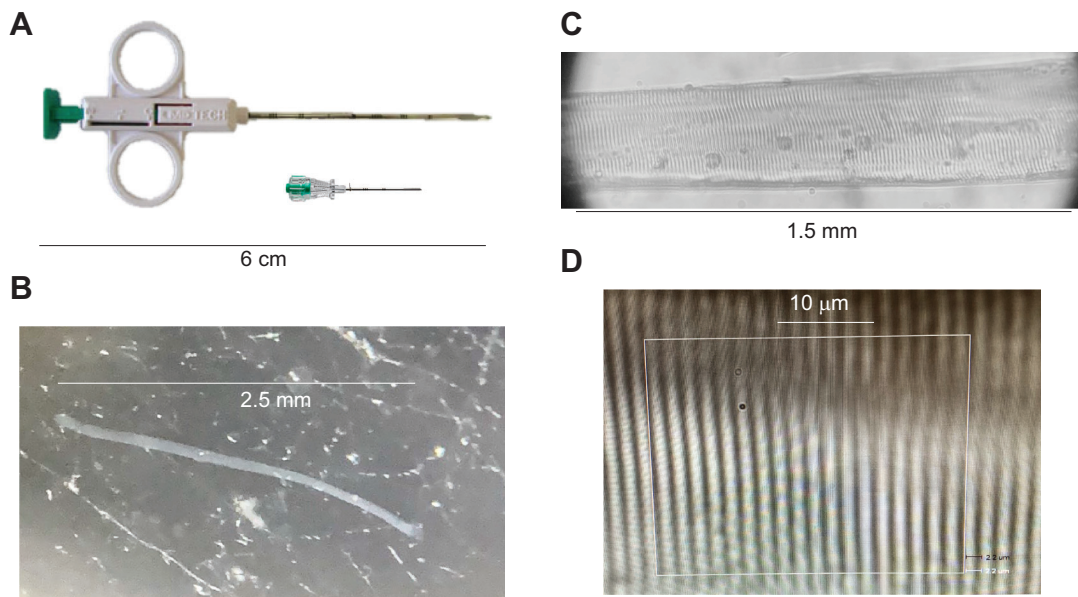


Fig. 1. Biopsy instrument and muscle obtained from a micro-biopsy. (A) Argon SuperCore™ Semi-Automatic Biopsy Instrument 14 G×9 cm, with 13 G×3.9 cm co-axial needle. (B) A single muscle fiber teased from a micro-biopsy sample (~2.5 mm in length) in a Petri dish. (C) A single muscle fiber in relaxing solution glued to length and force levers visually inspected for damage prior to experimentation (50× magnification). (D) Image from the high-speed camera after sarcomere length was adjusted to 2.4 μm.

(Harber et al., 2004; Widrick et al., 2001). Although active fiber mechanics from females exist for the soleus and the vastus lateralis, a paucity of data on female muscle properties persists. Peak stress from younger women ranges from 81 to 142 mN mm⁻² for the soleus (Trappe et al., 2008) and from 116 to 164 mN mm⁻² for the vastus lateralis (Raue et al., 2009). Resting medial gastrocnemius sarcomere lengths measured from cadaver muscle are reported to average 2.59±0.26 μm (mean±s.d.; Ward et al., 2009) and 2.73±0.08 μm (Cutts, 1988), while sarcomere lengths in humans during locomotion are estimated to range from 2.75 to 2.92 μm (Fukunaga et al., 2001). Passive tension of gastrocnemius single fibers appears to be higher in children with cerebral palsy compared with non-disabled children (Mathewson et al., 2014). Furthermore, children with cerebral palsy possess significantly longer sarcomere lengths (3.71±0.44 μm) than previously reported sarcomere lengths of non-disabled adults (Larsson and Moss, 1993). We attempted to perform both passive and active muscle fiber mechanics in these sarcomere length ranges to better understand the mechanical properties of the medial gastrocnemius. Owing to the ambiguity of optimal sarcomere length in the medial gastrocnemius and in dancers' medial gastrocnemius (Ward et al., 2009; Cutts, 1988; Moltubakk et al., 2018; Rice et al., 2021; Frasson et al., 2008), we measured fiber mechanics at average sarcomere lengths of 2.4 and 3.0 μm.

It is vital that muscle fiber mechanics are not only reported from young or non-disabled male vastus lateralis muscles, but also that a greater diversity of populations and muscles are explored. Medial gastrocnemius fiber mechanics have yet to be reported from females or dancers. The purpose of this study was to determine whether passive and active muscle fiber mechanics could be performed on micro-biopsies from the medial gastrocnemii from humans. We secondarily sought to explore the medial gastrocnemius muscle fiber characteristics of female dancers, a population that is generally underrepresented in the literature, despite their unique and extensive training history as a source for understanding the capabilities and adaptability of human muscle. We hypothesized that passive and active mechanical experiments from micro-biopsies would be possible, and thus a new

technique for research laboratories to obtain muscle tissue and assess muscle fiber mechanics. By demonstrating the feasibility of this micro-biopsy technique, we believe that researchers will be more confident and equipped to investigate muscle fiber properties in a larger variety of muscles and populations.

MATERIALS AND METHODS

Female dancers ($n=15$; age=24.2±5.0 yr; height=163.8±4.6 cm; body mass=61.1±8.9 kg; dance training=19.6±4.9 yr; means±s.d.) volunteered to participate in this study. The dancers were required to have a minimum of 10 yr of experience in ballet, jazz, modern, lyrical or contemporary dance. All dancers were training at least three times per week. Dancers were not to have any neuromuscular disease or lower leg injuries within the past 6 mo or exercise for 24 h prior to the muscle micro-biopsy. Ethics approval was granted by the Edith Cowan University Committee (no. 21,229) prior to commencement of data collection. All dancers were informed of the risks and provided signed consent forms prior to the muscle micro-biopsy.

Dancers identified their preferred take-off leg during a split leap (i.e. a preferred right leap means the left leg is the take-off leg/leg to be biopsied) to determine which leg would be used for micro-biopsies. Dancers laid prone on an athletic training table with the legs fully extended and relaxed. Using an ultrasound probe (ProSound F75, Hitachi Healthcare Americas, Twinsburg, OH, USA), the pennation angle of the medial gastrocnemius was identified and marked with a marker to ensure that fiber bundles (2–3 mm in length) would be obtained. The ultrasound was also used to avoid any observable blood vessels during the micro-biopsy. After circling the area of insertion with a marker, a thick layer of topical anesthetic (Emla cream, 5%, AstraZeneca, Södertälje, Sweden) was applied where the micro-biopsy needle would be inserted. After 30 min, the excess anesthetic was removed and the area was cleaned with Povidone-Iodine Swabsticks (Medical Industries Australia, Kemps Creek, NSW, Australia). Sterile equipment was used, and aseptic techniques were then maintained throughout the duration of the procedure. A sterile drape

(Polybacked Towel, 40×60 cm with circular fenestration, Defries Industry, Dandenong South, VIC, Australia) was placed just below the marked area of insertion, and above the sterile field, the ultrasound probe was placed over the area of muscle where the needle would insert to visualize needle placement, which was inserted in parallel with the pennation angle. The co-axial introducer needle was inserted into the marked area in the middle of the muscle belly at an angle of approximately 30 deg. From here, three muscle samples (totaling ~45 mg) were collected from the medial gastrocnemius with micro-biopsy needles (Argon SuperCore™ Semi-Automatic Biopsy Instrument 14 G×9 cm, with 13 G×3.9 cm co-axial needle, Culpan Medical Pty Limited, Moorabbin, VIC, Australia; Fig. 1A). The muscle samples were obtained in a clockwise fashion from the left, right and deep parts of the muscle belly; this was to ensure that a sufficient number of long enough fibers would be obtained. After each pass, the semi-automatic biopsy instrument was removed from the co-axial introducer needle. Refer to Fig. S1 for a visual depiction of the micro-biopsy method. Muscle samples were carefully removed and placed into vials with 2 ml of skinning solution and put on ice immediately. The skinning solution contained 125 mmol l⁻¹ propionic acid, 2.0 mmol l⁻¹ EGTA, 1 mmol l⁻¹ MgCl₂, 4.0 mmol l⁻¹ ATP, 20 mmol l⁻¹ imidazol (pH 7.0) and 50% glycerol (vol/vol) and was stored in a cold room at 4°C. On the day of the muscle micro-biopsy, 50 µl of protease inhibitor cocktail was added fresh to every 2 ml of solution. After 1 h of incubation, the muscle samples were placed in fresh skinning solution and stored at -80°C until further analysis. With a small amount of pressure, gauze, a Tegaderm™ bandage and Coban™ dressing (both Neuss, Germany) was then applied to the dancers' leg.

On the day of fiber mechanics experiments (approximately 1 yr after tissue collection), muscle samples were placed in a Petri

dish with relaxing solution to isolate single muscle fibers and muscle fiber bundles (Fig. 1B). The relaxing solution contained 170 mmol l⁻¹ potassium propionate, 20 mmol l⁻¹ MOPS, 2.5 mmol l⁻¹ magnesium acetate and 5 mmol l⁻¹ K₂EGTA, and was brought to a pH of 7.0 with 8 mol l⁻¹ KOH prior to adding 2.5 mmol l⁻¹ of ATP. One tablet of protease inhibitor (Complete, Roche Diagnostics, Mannheim, Germany) was added per 100 ml of relaxing solution. Muscle fibers were rinsed several times with relaxing solution prior to commencing experiments. Single muscle fibers and muscle fiber bundles were attached with acetone-based glue (Agar Scientific Ltd, Essex, UK) to a length lever controller (Aurora Scientific Inc., model 315C, Ontario, Canada) and force transducer lever (Aurora Scientific, model 400A). The muscle fiber rig sat atop an inverted microscope for experimentation, similar to other systems previously used for fiber mechanics (Rehorn et al., 2014; Hessel et al., 2019). First, muscle fibers were visually inspected for damage under a microscope. For intact, undamaged fibers (Fig. 1C), sarcomere length was determined with a high-speed camera (Aurora Scientific, HVSL 901C) and adjusted to an average starting sarcomere length of 2.4 µm (Fig. 1D). Muscle fiber cross-sectional area was determined with an ocular micrometer (under 50× magnification), assuming the fiber takes on a cylindrical shape when suspended in air.

Three to five preconditioning stretches were performed prior to commencing experimental mechanical tests. For all passive tests, muscle fibers were passively stretched from an average sarcomere length of 2.4 to 3.0 µm at three different velocities in a bath of relaxing solution at room temperature. First, muscle fibers were stretched at 0.015 µm s⁻¹ (per sarcomere) and held for 60 s to reach an isometric steady state before releasing back to a resting length of 2.4 µm (Fig. 2A). Muscle fibers were then stretched at 0.04 µm s⁻¹ (per sarcomere) and held for 6 s (Fig. 2B). Lastly, we performed a

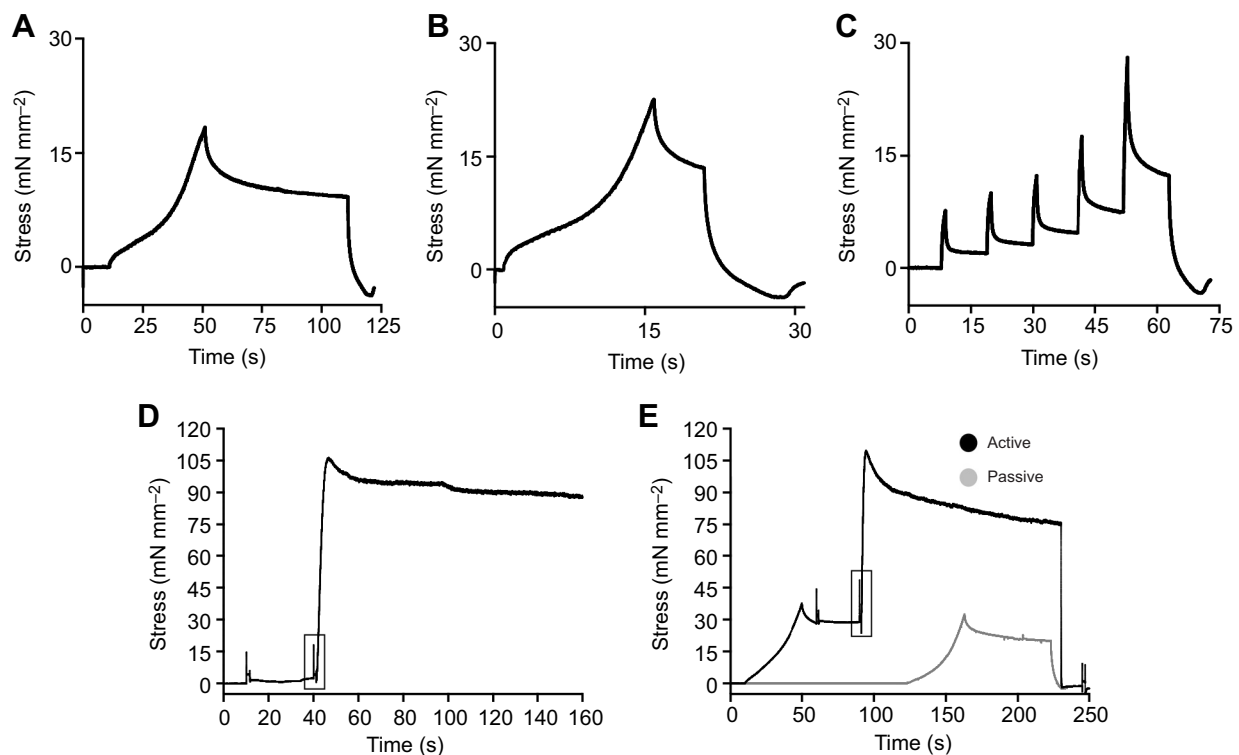


Fig. 2. Example data from passive and active muscle fiber mechanical experiments. Example data from a muscle fiber during passive stretching from an average sarcomere length of 2.4 to 3.0 µm with (A) slow (0.015 µm s⁻¹), (B) moderate (0.04 µm s⁻¹) and (C) ramp stretches (0.12 µm s⁻¹). Example data from an isometric muscle fiber activated at an average sarcomere length of (D) 2.4 µm and (E) 3.0 µm. The rectangles indicate when the fiber was activated.

ramping stretch protocol, wherein muscle fibers were stretched in five increments of $0.12\ \mu\text{m}$ length starting at $2.4\ \mu\text{m}$ and ending at $3.0\ \mu\text{m}$ at $0.12\ \mu\text{m s}^{-1}$ (per sarcomere) and held for 11 s during each ramp (Fig. 2C). Two minutes of rest separated all trials.

Active muscle mechanics were then investigated. The muscle fibers were adjusted to an average sarcomere length of $2.4\ \mu\text{m}$ in relaxing solution, moved to a washing solution for 30 s, and then moved to an activating solution for 120 s before returning to the relaxing solution (Fig. 2D). The relaxing solution was manually changed several times while the muscle fiber rested for 3 min. The muscle fiber was then stretched from 2.4 to $3.0\ \mu\text{m}$ for 40 s and held for 20 s in the relaxing solution. The fiber was moved to a washing solution for 30 s and then transferred to the activating solution for 140 s (Fig. 2E). The washing solution contained $185\ \text{mmol l}^{-1}$ potassium propionate, $20\ \text{mmol l}^{-1}$ MOPS and $2.5\ \text{mmol l}^{-1}$ magnesium acetate. The activating solution contained $170\ \text{mmol l}^{-1}$ potassium propionate, $10\ \text{mmol l}^{-1}$ MOPS, $2.4\ \text{mmol l}^{-1}$ magnesium acetate and $50\ \text{mmol l}^{-1}\ \text{Ca}^{2+}$ EGTA. Both the washing and activating solutions were brought to a pH of 7.0 with $8\ \text{mol l}^{-1}$ KOH prior to adding $2.5\ \text{mmol l}^{-1}$ of ATP to each. We reported activation data only for fibers that activated above $50\ \text{mN mm}^{-2}$ at $2.4\ \mu\text{m}$ (Harber et al., 2004).

Muscle fiber data (passive stretches: $n=50$; passive ramps: $n=42$; active $2.4\ \mu\text{m}$: $n=4$; active $3.0\ \mu\text{m}$: $n=3$) were analyzed with a custom-designed LabVIEW program (version 19.0, National Instruments, Austin, TX, USA). Muscle force was divided by cross-sectional area to determine muscle fiber stress (mN mm^{-2}). For passive muscle fiber mechanics, peak stress was measured from each of the stretches as well as steady-state stress in the last second of each hold. Active peak stress was measured as the highest fiber

force while in activating solution, and steady-state stress was measured from the last second of force in the activating solution.

Statistical analyses were performed in SPSS (version 25.0, SPSS Inc., Chicago, IL, USA). All data were inspected for outliers prior to computing statistics. We ran paired sample *t*-tests between peak stress after stretching at a velocity of 0.015 and $0.04\ \mu\text{m s}^{-1}$ as well as steady-state stress between the different stretching velocities. We then performed repeated-measures ANOVA between the five ramp stretches for peak stress and steady-state stress. An LSD *post hoc* test was used for ANOVA tests to determine significance. Student's *t*-tests were also performed for peak stress and steady-state stress of activated fibers at 2.4 and $3.0\ \mu\text{m}$. Significance was set *a priori* at $P\leq 0.05$.

RESULTS

Our results demonstrate that muscle fiber mechanics can be performed on micro-biopsy samples from human medial gastrocnemius muscles. Peak stress after stretching at $0.04\ \mu\text{m s}^{-1}$ ($18.50\pm 7.30\ \text{mN mm}^{-2}$) was significantly greater than peak stress after stretching at $0.015\ \mu\text{m s}^{-1}$ ($16.18\pm 5.73\ \text{mN mm}^{-2}$) (Fig. 3A). Steady-state stress after slow ($10.70\pm 3.57\ \text{mN mm}^{-2}$) and moderate ($13.42\pm 5.16\ \text{mN mm}^{-2}$) stretching velocities were also significantly different (Fig. 3B).

Peak stresses from ramp stretches were overall significantly different from one another ($P<0.0001$) (Fig. 3E). *Post hoc* comparisons revealed further significant differences between ramp 1 ($3.96\pm 1.70\ \text{mN mm}^{-2}$), ramp 2 ($6.14\pm 2.08\ \text{mN mm}^{-2}$), ramp 3 ($9.20\pm 2.76\ \text{mN mm}^{-2}$), ramp 4 ($14.13\pm 4.43\ \text{mN mm}^{-2}$) and ramp 5 ($22.27\pm 7.84\ \text{mN mm}^{-2}$). Steady-state stresses from ramping stretches were also significantly different from one

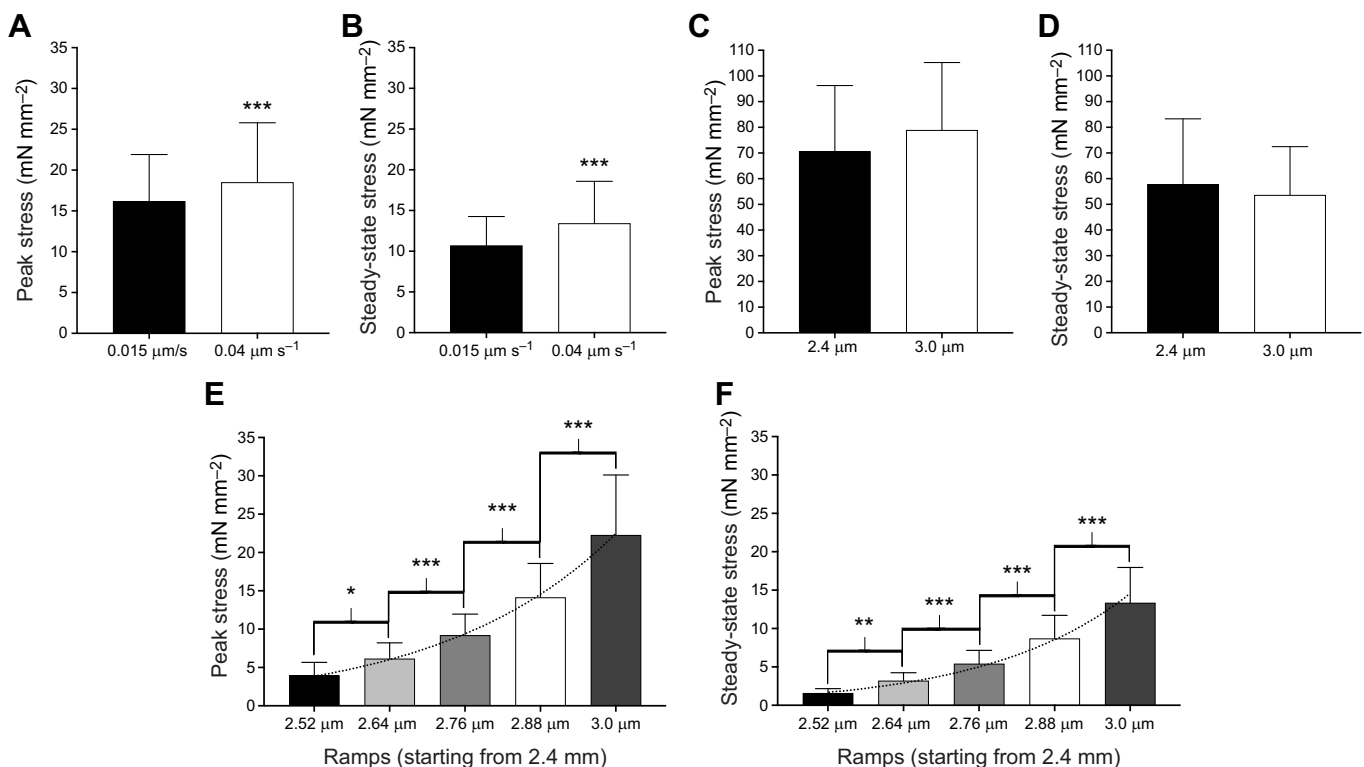


Fig. 3. Results from passive and active muscle fiber mechanical experiments. (A) Passive peak stresses and (B) steady-state stresses at the end of holding at an average sarcomere length of $3.0\ \mu\text{m}$ after stretching at a velocity of 0.015 and $0.04\ \mu\text{m s}^{-1}$. (C) Total active peak stress and (D) steady-state stress at the end of activation at 2.4 and $3.0\ \mu\text{m}$ average sarcomere lengths. (E) Passive peak stresses and (F) steady-state stress during incremental ramps at a velocity of $0.12\ \mu\text{m s}^{-1}$. SS: steady state. Significant differences are indicated by asterisks (* $P=0.024$, ** $P=0.005$, *** $P<0.0001$).

another overall ($P<0.0001$) (Fig. 3F). Similarly, *post hoc* comparisons further revealed significant differences between ramp 1 (1.58 ± 0.58 mN mm $^{-2}$), ramp 2 (3.20 ± 1.05 mN mm $^{-2}$), ramp 3 (5.40 ± 1.76 mN mm $^{-2}$), ramp 4 (8.69 ± 3.02 mN mm $^{-2}$) and ramp 5 (13.34 ± 4.62 mN mm $^{-2}$).

No significant differences between activated fibers at 2.4 and 3.0 μ m were found for peak stress ($P=0.695$) or steady-state stress ($P=0.819$) when passive peak stress is not subtracted from active peak stress. Total isometric peak stress slightly differed between 2.4 μ m (70.66 ± 25.61 mN mm $^{-2}$) and 3.0 μ m (78.88 ± 26.33 mN mm $^{-2}$). Steady-state stress at the end of activation was slightly higher at 2.4 μ m (57.84 ± 25.44 mN mm $^{-2}$) than at 3.0 μ m (53.61 ± 18.86 mN mm $^{-2}$).

DISCUSSION

The primary purpose of this study was to determine whether medial gastrocnemius micro-biopsy samples from humans would yield a sufficient number of long enough (2–3 mm) and undamaged muscle fibers necessary for mechanical experimentation. Here, for the first time, we demonstrated that passive and active muscle fiber mechanics can be performed on micro-biopsy samples collected from human gastrocnemius muscles. Moreover, we present novel mechanical data on medial gastrocnemius muscle fibers from female dancers. Our peak and steady-state stress data are comparable to results from other studies of passive and active muscle fiber mechanics (Harber et al., 2004; Noonan et al., 2020), suggesting that the micro-biopsy technique is a practical, yet less invasive means of obtaining muscle samples from humans suitable for mechanical experiments. Additionally, each muscle sample contained at least 10 undamaged muscle fibers that were viable for mechanical experimentation. The implications for our findings open the possibility for scientists to less invasively investigate muscular adaptation to various interventions, muscular differences among clinical and athletic populations, and force–length and force–velocity properties in different human muscles. Furthermore, our results add to the literature by expanding the available information on fiber mechanics from females, dancers and the medial gastrocnemius muscle. Future research is needed to determine the feasibility of muscle micro-biopsies.

Similar to previous results (Rehorn et al., 2014), we found that passive stress is velocity dependent in human medial gastrocnemius muscle fibers (Fig. 3A,B). Our passive ramping fiber mechanics additionally demonstrate that the stress-relaxation properties of dancers' medial gastrocnemii are non-linear (Fig. 3E,F), as has also been shown in previous studies (Malisoux et al., 2007; Meyer et al., 2011). Performing passive stretches and ramp stretches on muscle fibers provides valuable information about the viscoelastic properties of different muscles from different species (Malisoux et al., 2006; Lim et al., 2019; Rehorn et al., 2014; Prado et al., 2005). Such information supports a role for the giant spring-like protein, titin, which has been repeatedly recognized for its variable stiffness (Li et al., 2016) and energy-storing capabilities (Linke, 2018). Recently, Vera et al. (2020) detected 16 TTN (titin) gene variants from dancers and suggested the altered gene is possibly related to greater muscle elasticity (i.e. range of motion and flexibility) in this population. Dancers, like most aesthetic athletes, must possess highly flexible and strong muscles to achieve athletic success (Koutedakis and Jamurtas, 2004). Comparative muscle fiber research between dancers and other athletic, clinical or untrained populations may inform scientists on the effects of chronic stretching and physical training at a cellular level.

Owing to the extreme ankle-joint range of motion dancers possess, previous researchers have debated whether dancers either: (1) operate at shorter sarcomere lengths and have a greater number of sarcomeres in series (Moltubakk et al., 2018; Rice et al., 2021) or (2) operate at longer sarcomere lengths and have fewer sarcomeres in series (Frasson et al., 2008). Our preliminary findings suggest that total peak fiber stress (Fig. 3C) is higher at an average sarcomere length of 3.0 μ m ($n=3$) than at 2.4 μ m ($n=4$), but not when passive peak stress is subtracted from active peak stress. From these data, we are unable to conclude at what sarcomere length dancers' peak tension is highest, but it may be that the hypothesis of dancers operating at shorter sarcomere lengths is more likely (Moltubakk et al., 2018; Rice et al., 2021). In the future, it would be interesting to further investigate dancers' optimal sarcomere length with dynamometry in combination with single fiber activation experiments in 1.0 μ m increments from dancers' medial gastrocnemii. Optimal sarcomere lengths for humans are reported to be slightly longer than for other species of animals (Rassier et al., 1999), which would align with our notion that optimal sarcomere length is somewhere between 2.4 and 3.0 μ m. Interestingly, our peak stress values were more comparable with those of slow twitch muscle fibers (Power et al., 2016; Harber et al., 2004). A predominance of slow twitch muscle fibers in the medial gastrocnemius aligns with vastus lateralis fiber type data on dancers from Dahlstrom et al. (1987) and medial gastrocnemius fiber type data from Fry et al. (1997). It should be acknowledged that the force shape of activated fibers (Fig. 3) were somewhat atypical from other data (Paoli et al., 2010), which may be a function of the storage time. Regardless, additional research is warranted before conclusions can be reached regarding active fiber mechanics from dancers.

Muscle is a highly plastic tissue with several paradoxes that have yet to be elucidated (Lieber et al., 2017). We believe that the micro-biopsy technique will aid in solving some of the ambiguities surrounding muscle in a more practical fashion than percutaneous Bergström biopsies. For our present work, the amount and quality of tissue yielded from micro-biopsies was satisfactory to perform muscle fiber mechanics. We acknowledge that the storage time may have affected the low yield of successful active fiber mechanical data. As always, further research is needed on human muscle to better understand intrinsic properties, adaptations to different stimuli, and in various athletic populations and clinical conditions. Specifically, we aim to conduct additional studies on the passive and active muscle fiber properties of the medial gastrocnemius of dancers for a better understanding of chronic adaptation to training.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: P.E.R., S.N., K.N.; Methodology: P.E.R., K.A.Z., K.N.; Formal analysis: P.E.R.; Investigation: P.E.R., C.A.; Writing - original draft: P.E.R.; Writing - review & editing: P.E.R., S.N., C.A., K.A.Z., K.N.; Supervision: S.N., K.A.Z., K.N.; Funding acquisition: P.E.R., S.N., K.A.Z.

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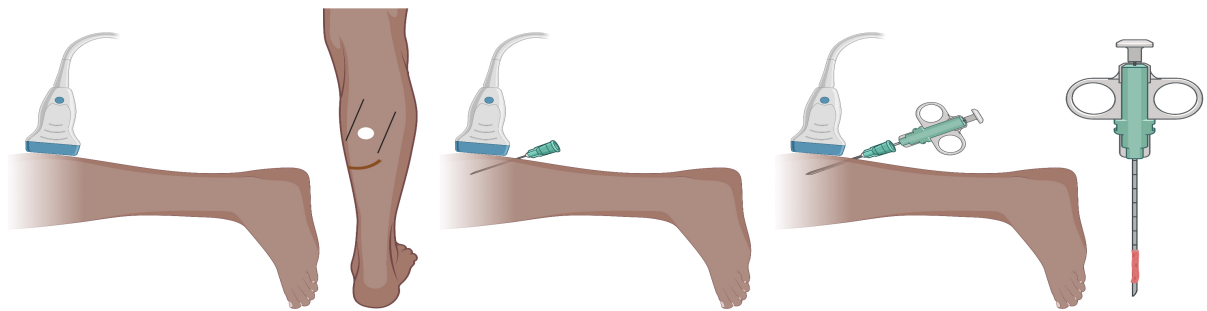


Fig. S1. Sequential steps of preparing for the medial gastrocnemius micro-biopsy are shown from left to right. Ultrasound was used to determine fascicle pennation angle as a guide for parallel needle insertion as well as any larger blood vessels that needed to be avoided. After local anesthetic was applied, the introducer needle was inserted and monitored with ultrasound. Three micro-biopsies were then taken in a clockwise fashion from the medial gastrocnemius muscle and placed in skinning solution.