Effects of stroke injury on the shear modulus of the lower leg muscle during passive dorsiflexion

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Running head: lower leg shear modulus after stroke

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ABSTRACT

Contractures are common complications of a stroke. The spatial location of the increased stiffness among plantar flexors and its variability among survivors remain unknown. This study assessed the mechanical properties of the lower leg muscles in stroke survivors during passive dorsiflexions. Stiffness was estimated through the measurement of the shear modulus. Two experiments were independently conducted where participants laid supine: with the knee extended (experiment 1, n=13 stroke survivors and n=13 controls), or with the knee flexed at 90° (experiment 2, n=14 stroke survivors and n=14 controls). The shear modulus of plantar flexors (*gastrocnemius medialis* [3 locations], *gastrocnemius lateralis* [3], *soleus* [2], *flexor digitorum longus*, *flexor hallucis longus*), *peroneus longus*) and dorsi flexors (*tibialis anterior*, and *extensor digitorum longus*) was measured using ultrasound shear wave elastography during passive dorsiflexions (2°/sec). At the same ankle angle, stroke survivors displayed higher shear modulus than controls for *gastrocnemius medialis* and *gastrocnemius lateralis* (knee extended); and *soleus* (knee flexed). Very low shear modulus were found for the other muscles. The adjustment for muscle slack angle suggested that the increased shear modulus was arising from consequences of contractures. The stiffness distribution between muscles was consistent across participants with the highest shear modulus reported for the most distal regions of *gastrocnemius medialis* (knee extended) and *soleus* (knee flexed). These results provide a better appreciation of stiffness locations among plantar flexors of stroke survivors, and can provide evidence for the implementation of clinical trials to evaluate targeted interventions applied on these specific muscle regions.

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NEW & NOTEWORTHY

The shear modulus of 13 muscle regions was assessed in stroke patients using elastography. When compared to controls, shear modulus was increased in the *gastrocnemii* when the knee was extended and in the *soleus* when the knee was flexed. The distal regions of GM and SOL were the most affected. These changes were consistent in all the stroke patients, suggesting that the regions are a potential source of the increase in joint stiffness.

KEY WORDS: shear modulus – stroke – elastography – muscle – contracture

INTRODUCTION

Stroke injury is a world-leading cause of premature mortality and long-term disabilities (42). For most survivors of a stroke, adaptations in soft tissues arising from non-neurological and neurological pathological processes (20, 21, 38) lead to muscle contracture defined as an increased passive muscle stiffness and a reduction in joint mobility (22, 31). Consequently, contractures potentially influence motor function. For instance, when affecting plantar flexors at the ankle joint they prevent an appropriate foot position at initial contact during gait. This abnormal posture from hindfoot to forefoot contact with the walking results in a limited rollover and a restricted ability of the plantar flexor muscles to generate forces at longer muscle lengths during the stance phase of the gait cycle (4).

Clinical and instrumented assessments including passive joint torque measurements (30, 59) report increase levels of stiffness among stroke survivors. However, such findings cannot provide individual quantification of the stiffness of the numerous muscles that contribute to the passive torque (61). Consequently, the muscle locations affected by increase stiffness remain unknown. Therefore, the clinician still does not know if some muscles require individualized treatment (28). For instance, it is possible to enhance the effect of stretching on

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the *gastrocnemius medialis* muscle by specific positioning of the ankle and subtalar joints (18). Another scenario might be when patients do not respond to conservative interventions, surgical release is an option, but ideally it would be performed only on a selected part of an aponeurosis that was most affected. Currently, procedures are more "global" (e.g. Vulpius, Strayer, Baumann, Baker, Green techniques), and may not be targeting the most affected structures/locations. Being more precise during such surgical procedures would potentially limit weakness and reduce the likelihood of complications such as wound infection (1).

Ultrasound shear wave elastography (SWE) provides a unique opportunity to estimate the mechanical properties of a muscle region (5). Through the estimation of shear wave velocity propagating in a muscle (or through the calculation of a shear modulus, see in Methods), SWE has been reported to provide a reliable local quantification of an individual muscle stiffness (16, 46) and the estimation of its slack angle (the angle of the onset of passive tension) (36, 37). Consequently, SWE is a promising tool for the characterization of muscle mechanical properties among stroke survivors.

This study aimed to measure the shear modulus among lower leg muscles in stroke survivors compared with matched controls. For that purpose, passive ankle rotations were performed with the knee extended (experiment 1) and with the knee flexed (experiment 2). We hypothesized that stroke survivors would exhibit higher muscle stiffness than controls.

MATERIALS AND METHODS

Participants

Twenty-seven patients who had suffered a stroke and 27 age and sex matched control subjects volunteered to participate in experiment 1 (knee extended, n=13) and experiment 2 (knee flexed, n=14). Patients were recruited through the neurological rehabilitation department of the university hospital (in- and out- patients) and controls were recruited through email

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advertising via a university network. Volunteers were included if they: 1) were over 18 years old; 2) had suffered a cortical or subcortical stroke which affected their ankle mobility. Their demographic information is presented in Table 1. Potential participants were excluded if they had cognitive or speech impediments that affected their ability to follow instructions associated with the protocol; or moderate motor disability quantified as a score <4 on Modified Rankin Handicap Scale (58). Clinically, the spasticity of the *triceps surae* was assessed by the modified Ashworth Scale (7). The isometric strength in plantar- and dorsiflexion of stroke participants was approximately 50% of that recorded in the control group (see Table 1), which is similar to the levels reported in the literature (14). The protocol was approved by the local Institutional Ethics Committee. Participants were informed of the nature of the study before providing a written informed consent. The procedures conformed to the Declaration of Helsinki.

Instrumentation

Dynamometer

An isokinetic dynamometer (Biodex 3 Medical, Shirley, New York, USA) was used to passively rotate the ankle of participants, with the knee fully extended (hip at 0°, experiment 1) and the knee flexed at 90° (hip at 90°, experiment 2) (see Figure 1 of Le Sant et al. (43)) . The ergometer axis was aligned with the estimated ankle center of rotation (8). The neutral position (i.e., the sole of the foot at right angle to the tibia) defined was defined as 0°. Ankle angle and joint torque signals were transmitted from the *dynamometer* to an external 16-bit analog/digital converter (1 kHz, PowerLab ADInstruments Inc., Colorado Springs, U.S.A.) and visualized and stored on a computer for later analyses.

Surface electromyography (sEMG).

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sEMG signals of *gastrocnemius medialis* and *gastrocnemius lateralis* (GM and GL, respectively), *soleus* (SOL) and *tibialis anterior* (TA) were simultaneously recorded (1000 Hz, ME 6000, MEGA Electronics Ltd, Kuopio, Finland) using hydrogel adhesive surface electrodes (KendallTM 100 foam-series, Covidien, Mansfield, USA). Electrodes were placed according to the SENIAM guidelines (35). Ultrasound imaging ensured that electrodes were placed over the considered muscle. Both sEMG activities and the ankle torque were visualized in real-time to ensure that there were no increases in muscle activity during the stretching procedures. If so, the trial was not accepted and repeated. Signals were stored for later off-line analyses.

Elastography

The technique used to measure the shear modulus has been previously described in detail (5, 26). Briefly, SWE relies on the measurement of shear waves velocity that result from mechanical perturbations applied to the tissue. The shear modulus (μ) is directly related to the shear wave velocity (Vs) (Equation 1): $\mu = \rho Vs^2$

where μ is the shear modulus of the tissue, and ρ the density of the tissue (1000 kg.m-3 for muscle).

Shear modulus has been shown to display a strong linear relationship with Young's modulus (R^2 between 0.916-0.988), as shown with conventional material testing procedures (16, 40). Thus, the shear wave velocity is directly related to the shear modulus, that is, the stiffer the tissue, the faster the shear wave propagation.

An Aixplorer ultrasound scanner (Supersonic Imagine, v. 6.1, Aix-en-Provence, France) was coupled with one linear transducer (2–10 MHz, SL10-2 or 4–15 MHz, SL15-4, Supersonic Imagine, Aix-en-Provence, France). A transistor-transistor logic pulse was sent by the ultrasound scanner at each shear modulus measurement (i.e. each second) to synchronize

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shear modulus measurements with the ankle angle, passive torque and sEMG signals. The transducer was aligned along the longitudinal axis of the leg (ie, corresponding to physiological plane of lower leg muscle shortening/lengthening direction) and perpendicular to the skin so that the image plane intersects perpendicularly the muscle aponeurosis. Thus, the stiffness measurement was always performed in the estimated muscle shortening/lengthening direction, as done with conventional material testing (16, 26). The main plantar flexors [GM, GL, SOL, *flexor digitorum longus* (FDL), *flexor hallucis longus* (FHL), *peroneus longus* (PL)] and dorsi flexors [TA, and *extensor digitorum longus* (EDL)] were scanned. Three proximo-distal regions were determined for the *gastrocnemii* (distal, mid, and proximal regions on the muscle), and two for the *soleus* (distal and proximal). These regions were chosen based on anatomical guidelines used for botulinum toxin injections to treat muscle over activity in neurological conditions (55, 63). SWE has very good reliability for measuring shear modulus of superficial and deep muscles, especially during stretching (15, 43, 51). A previous study showed good inter-day reliability for shear wave measurements at all the locations used in this study (43).

Protocol

Firstly, for both experiments, the maximal angle in dorsiflexion was measured as the dynamometer moved the ankle joint during a slow passive stretch (2°/sec). When participants felt "maximal tolerable stretch" in the calf (i.e. onset of pain), they pushed a button that stopped the motion. During this motion, subjects were blindfolded. Participants undertook three trials and the maximum angle recorded was utilized in the subsequent calculation of 80 % of range of dorsiflexion. Second, five ankle rotations from 40° of plantarflexion to 80% of the predetermined maximal ankle dorsiflexion angle were performed for muscle conditioning purposes (54). Third, one shear modulus measurement was performed for each location in a randomized order during ankle dorsiflexion at a velocity of 2°/sec, from 40° of plantarflexion

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178 to 80% of the maximal dorsiflexion. This range was used as passive motion beyond this point
179 often invokes unwanted muscle activation (48). Between each measurement, a 1-min of rest
180 was observed. At the end of the procedure, participants were asked to perform three voluntary
181 maximal isometric contractions (MVC) in plantarflexion and dorsiflexion and the root mean
182 square (sEMG-RMS) of the associated sEMG signals were utilized to normalize activity
183 recorded during passive motion to 80% dorsiflexion ROM.

184 **Data analysis and statistics**

185 Data were processed using Matlab® scripts (The MathWorks Inc., Natick, USA). Ultrasound
186 videos exported from Aixplorer's software were sequenced in 'jpeg' images. Then, each pixel
187 of the color map was converted into a shear modulus value established from an image
188 processing algorithm (26). Shear modulus values were averaged over the largest region of
189 interest (ROI) that avoided aponeurosis and artifacts. The mean area of the ROIs ranged
190 between 60 mm^2 (FDL, stroke survivors) and 180 mm^2 (TA, controls).

191 The following analyses were performed for each muscle region, and are depicted in Appendix
192 A. First, shear modulus values were compared at two points: 1) at the maximal common angle
193 in dorsiflexion that was attained by patients and controls, and 2) at 80% of maximal
194 dorsiflexion. Second, to provide information about muscle contracture, the "slack angle" was
195 visually determined as the onset of increase in shear modulus during the passive dorsiflexion.
196 This was performed for each muscle region and each participant by an experienced examiner
197 blinded to the muscle region and the participant. The visual approach for determining the
198 slack angle has been shown to be reliable in previous studies including those of our research
199 group (36, 37, 43). Third, in order to account for the potential change in slack angle, the shear
200 modulus values corresponding to the maximal common dorsiflexed position from the slack
201 angle were compared between patients and controls. This latter analysis is known to give
202 insights into the mechanical behavior of muscle tissue in vivo (34). Since the maximal ankle

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dorsiflexion was highly variable among stroke survivors (Table 1), these analyses were performed for each patient and his/her matched control subject in order to determine the maximal common angle for each pair of patient/control.

The sEMG-RMS were calculated over a 300 ms window centered on each shear modulus measurement (1 Hz) and normalized to the maximal values reached during the MVCs. The sEMG over the stretch ROM and the value reached at 80% of the maximal ROM were assessed in the statistical analyses.

Since distributions failed to pass the Shapiro-Wilk test for normality, Mann-Whitney U tests were conducted to analyze between-group differences in shear modulus, slack angle and muscle activity. The statistical significance was set at $p<0.05$. The 0.05 value was adjusted for multiple tests using a Bonferroni correction (shear modulus: 0.05/13, slack length: 0.05/11, EMG: 0.05/4). When significant, between-groups differences (stroke – controls) and bootstrapped confidence intervals (95% CI) (n=1000 samples) (19) were computed on shear modulus, slack angle, and muscle activity. Effect sizes were estimated using the General Mann-Whitney measure (θ) ($\theta=U/nm$, where U is the Mann-Whitney statistic, n and m the sample sizes of both groups, respectively). A value of $\theta=0.5$ indicates a perfect concordance (i.e. equal distribution of the population data), while $\theta=0$ or $\theta=1$ no overlap between the group distributions (52). In addition, data were displayed pictorially by the decreasing order of shear modulus values recorded for all muscle regions among individuals, to qualitatively appreciate the location of the stiffest muscle regions among leg muscles. The muscle region displaying the highest shear modulus was presented in black while a pale pink color corresponded to the muscle region where the lowest shear modulus was recorded. Data are presented descriptively for these analyses.

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RESULTS

All subjects completed the protocol of experiment 1 or experiment 2. However, a low recording quality was observed for 3% of the elastography measurements (artifacts or void areas within the ROI; 10/338 videos of experiment 1 and 10/364 videos of experiment 2, respectively; details provided in Figure 1). These data were excluded from the analyses to reduce a potential risk of bias.

Experiment 1 (Knee extended)

Shear modulus-ankle angle relationships are provided for each transducer location of experiment 1 in Figure 2. Between-groups differences only appeared significant at the maximal common angle in dorsiflexion (Figure 1A), where the shear modulus was higher for stroke survivors than for controls for *gastrocnemii* at the distal and mid muscle regions (all p-values<0.002; θ between 0.11 and 0.14) and was indicative of higher muscle-tendon unit stiffness. The between-group difference (stroke-controls) of shear modulus was: 31.2 kPa (CI 95% 29.1;96.2 kPa) for GMdistal, 26.7 kPa (CI 95% 18.1;86.0 kPa) for GMmid, 20.42 kPa (CI 95% 17.0;25.2 kPa) for GLdistal, and 24.6 kPa (CI 95% 18.9;30.4 kPa) for GLmid, respectively. The slack angle (Table 2 and Figure 2) occurred at a more plantar flexed angle in stroke survivors within GMmid ($p=0.045$, $\theta=0.17$) and GL (all p-values <0.019, θ ranges from 0.12 to 0.14). The between-group difference of slack angle was: -5.6° (CI 95% -8.8;-1.5°) for GMmid, -6.16° (CI 95% -7.7;-3.1°) for GLdistal, -5.7° (CI 95% -7.5;-1.9°) for GLmid, and -5.6° (CI 95% -8.6;-1.2°) for GLproximal, respectively.

The highest shear modulus was measured on GM for each group (Figure 3A), and consistently found at the most distal site (85% of stroke survivors).

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Experiment 2 (Knee flexed)

Shear modulus-ankle angle relationships are provided for each transducer location of experiment 2 in Figure 4. At the maximal common angle in dorsiflexion (Figure 1C) shear modulus was higher for stroke survivors for SOLdistal ($p=0.001$, $\theta=0.13$) with a between-group difference of 18.2 kPa (CI 95% 9.1;24.6 kPa). At 80% of maximal ROM (Figure 1E) a lower shear modulus was found among stroke survivors on *dorsiflexors* (TA: $p=0.002$, $\theta=0.14$, between-group difference -3.8 kPa [CI 95% -6.6;-1.4 kPa]; EDL: $p=0.001$; $\theta=0.11$, between-group difference -6.0 kPa [CI 95% -9.7;-4.2 kPa]).

The slack angle (Table 2 and Figure 4) was measured at a more plantar flexed angle in stroke survivors in SOL (distal, $p<0.001$, $\theta=0.10$, between-group difference -6.4° [CI 95% -10.1;-2.7°]; proximal $p<0.001$, $\theta=0.02$, between-group difference -9.1°[CI 95% -11.7;-6.2°]).

The highest shear modulus values were measured for SOL for each group (Figure 3B) and were consistently found at the most distal site (86% of stroke survivors).

Muscle activity

During both experiments, activity of GM, GL, SOL and TA remained below 5% of maximal activation (Table 3). Despite the subjects being asked to stay relaxed, stroke survivors displayed higher averaged sEMG amplitudes than controls (all p-values<0.05, θ between 0.08-0.19 [exp.1] and 0.10-0.21 [exp.2]). At 80% of maximal ROM in dorsiflexion, between-group sEMG differed for plantar flexor muscles (GM, GL and SOL: all p-values<0.05, θ between 0.07-0.14 [exp.1] and GL: $p=0.009$, $\theta=0.21$ [exp.2]). Between-groups differences and CI95% are reported in Table 3.

DISCUSSION

The present study compared the shear modulus between stroke survivors and healthy controls in several locations of the lower leg muscles. In several muscle regions, the shear modulus

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values reached at a given ankle angle were higher for the stroke group compared to the control group, but not at 80% of maximal dorsiflexion. Accounting for the slack angle, there were no between-groups differences in the shear modulus.

These results conform with studies conducted on healthy participants, also reporting a higher shear modulus for GM (knee extended) (10, 36, 43) and SOL (knee flexed) (43) during passive dorsiflexions. Since the muscle force will be influenced by both elasticity and size, the between muscle differences in shear modulus should not be interpreted directly as difference in muscle force. For instance, due to differences in CSA, if two muscles exhibit the same change in shear modulus during stretching, the bigger muscle will exhibit the larger change in passive force. The ~2 times larger CSA of GM compared to GL (23) combined with higher shear modulus values for the GM clearly demonstrate an imbalance of passive force in favor of GM when the ankle is dorsiflexed. In the same way, considering their small CSA (23) and low shear modulus values, the passive muscle force of the small plantar flexors (FDL, FHL, PL) could be considered as negligible. It is more challenging to interpret the balance of passive force between *gastrocnemii* and SOL because of the volume of SOL is much larger than GM (x2.4) and GL (x5.2) (3), while shear modulus values are higher for both *gastrocnemii* with the knee extended. Further studies combining measurements of muscle size and muscle mechanical properties should be performed to better understand how contracture could influence the individual muscle contributions to the passive torque.

The velocity used (2°/sec) and the ROM (80% of the maximal ROM) were set to limit the reflex responses to stretching. However, between-group differences in the amount of sEMG activity were found (Table 3). The values reported remained below 5% of sEMG during MVC. As such, it seems unlikely that muscle activation might influence our measurements, but the actual effect of this factor on our measurements remains unknown. If it did play a notable role, it would lead to an increase in muscle force developed in resistance to stretching

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297 at a short muscle length (61). Thus, it would induce a change in muscle slack angle (Table 3)
298 and an increase in shear modulus for stroke survivors compared to controls (Figures 2 and 4).
299 However, some points need to be considered. Firstly, it should be noted that different
300 thresholds are often used to consider "passive conditions". Regarding the literature, the reader
301 finds notable variability across studies. For instance 1% (48), 2% (50), or 5% (24) or 10%
302 (29). In the absence of a consensus on the threshold that should be used, we firmly
303 encouraged participants to relax, as recommended during clinical examination (28) and in
304 most research studies undertaken in this area. We also carefully checked that there were no
305 increases in sEMG signals during each trial. If an increase in muscle activation was
306 noticeable, the trial was not utilized for analyses and repeated. Secondly, the reflex-mediated
307 increase found among the muscle activity of survivors [through an increased excitability of
308 the alpha motor neuron at the spinal cord level (53)] has been shown to reach its maximum
309 between 1 and 3 months after stroke (6). Studies also reported that the contributions of neural
310 contributors to stiffness during stretching may decrease over time in stroke populations (11,
311 59). At more chronic stages, such as for the sample recruited in the present study, the response
312 observed during slow passive stretching may primarily be due to the passive intrinsic
313 mechanical properties for slow stretching (49, 59). Thus we believe that the results of the
314 present study are marginally influenced by the neural contributions. This belief remains to be
315 validated in further experiments. One possibility would be to test the effects of a transient
316 blocking of motor nerve to eliminate muscle activity during stretching (9). A better
317 understanding of the influence of slight EMG activation on passive mechanical responses (i.e.
318 passive torque, shear modulus and fascicle length) might be then elucidated.
319 Furthermore, we also provide a pictorial of individual responses through a pictorial mapping
320 of the stiffness levels in multiple muscle regions (Figure 4). This is novel, since the few
321 studies using SWE in stroke subjects were focused on one muscle thought to be representative

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of the studied muscle group (17, 39, 44, 47, 62). Our pictorial analyses highlight that stroke survivors' response is commonly observed at the same muscle regions within plantar flexors: the highest values for GM followed by GL (Figure 3A, experiment 1) and for SOL (Figure 3B, experiment 2). Of particular relevance, the studies of Mathevon et al. (47) and Jakubowski et al. (39) focused on GM and reported higher shear modulus on the affected side of stroke survivors. While we measured the shear modulus during standardized dynamic stretches, these studies positioned the ankle in the targeted angle first, before scanning the GM with the transducer. These static measurements could induce a stress relaxation effect (45) which may explain their lower values compared to those of the present study.

Very low shear modulus values were recorded for the other plantar flexors (Figure 1), supporting the concept that *triceps surae* muscles might be preferentially affected during passive dorsiflexion after a stroke. This is close to what is also observed among controls (Figure 3), and elsewhere on healthy participants (43).

Finally, higher shear modulus values were found for TA and EDL in controls (experiment 2, see Figure 4 plots L and M). A number of researchers (2, 27, 56) have commented that the resting position of the foot in a sitting and lying position in stroke survivors is often that of greater plantarflexion than those individuals without stroke. Consequently, a decrease in shear modulus of the dorsiflexors in the more plantar flexed angles could be observed.

The results of the present study are important because they provide evidence of the spatial locations that are the most affected after a stroke among the plantar flexor muscle group. This opens perspectives for future studies to evaluate the efficacy of interventions aiming to restore mobility of the ankle joint. While classical stretching exercises do not have significant effects on muscle contracture (31) it is possible to design more intensive stretching programs targeting GM (and/or SOL). For instance, as mentioned previously, a dorsiflexion position at the ankle coupled with an inverted subtalar position (knee extended) can enhance the effect of

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the maneuver on GM compared to other plantar flexors (18). In addition, when severe contractures are noticeable, release surgeries are offered to survivors to restore ankle joint dorsiflexion (13). However, secondary mobility issues related to such surgery have been observed, including over-lengthening of the muscles (1). Relatedly, there is literature in support of the use of isolated gastrocnemius recession techniques, but the precise location of the most stiff regions prevents the surgeon from being sure of the location to incise (12). In lieu of our findings that the shear modulus values were the highest at the distal muscle regions of GM and SOL it would interesting to investigate the efficacy of mini-invasive isolated recessions close to the distal myotendinous junction on the aponeuroses of GM and/or SOL muscle (60), and compare results to those from more generalized incisions.

Limitations

The present study was designed to measure shear modulus in several locations of the plantar flexors during passive ankle dorsiflexions. Fascicle length measurements might have been an alternative method to investigate muscle mechanical properties (32, 33). However, with the data collected in the present study, it was only possible to measure the fascicle length of the GM and hence this was not pursued. Future studies might compare fascicle length and shear modulus measurements. The shorter slack angle reported in the present study for stroke survivors is in accordance with Gao et al. (25) who reported shorter fascicle length of GM for stroke survivors at 40° of PF. However, the findings are different to those of Kwah et al. (41). who did not report between-group differences in fascicle slack length. Differences in study methodologies (dorsiflexions performed in various knee angle configurations) or participants (sample size, clinical characteristics of participants) might have contributed to contrasting findings. For instance, the between-groups ranges in ankle range of motion were higher in the present study (see Table 1) compared to Kwah et al. (41). A more in-depth comparison of the

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methods used in the present study and those of Kwah et al. is required to better understand the differences. Because it involves the detection of subtle changes, the measurement of the slack length remains challenging, and the most appropriate method still remains to be established. Finally, while our results show that SWE is relevant to detect the effects of the stroke injury in multiple muscle locations, they cannot be used to infer the cause of the increased shear modulus. It is fundamental to better understand these mechanisms to improve therapeutic decisions (57). Potential mechanisms include changes in connective tissue or extracellular matrix. Active force generation may also be involved through impairment of calcium signalling of the muscle cell. Such a potential change in active force during the stretching cannot be detected with EMG.

Conclusion

Our findings suggest that the *gastrocnemii* and *soleus* muscles are most responsible for the increase in stiffness observed in plantar flexors muscles of stroke survivors. Within these muscles, the distal regions of GM and SOL were the most affected. No between-group differences were found when accounting for slack angle, suggesting that the increased level of muscle stiffness can be explained by a decrease in muscle length. In addition, our inter-individual analysis revealed that the most affected locations were similar among stroke survivors. These new results provide a better understanding soft tissue responses after a stroke that affects dorsiflexion of the ankle, a movement critical to the performance of efficient and safe walking.

APPENDIX

Appendix A – Averaged shear modulus–ankle angle (standard deviation bars omitted for clarity) and shear modulus– ankle range of motion in dorsiflexion adjusted from slack angle relationships for both populations (stroke survivors and controls) during the passive

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dorsiflexion of the ankle, in experiment 1 (knee fully extended) for *gastrocnemius medialis* distal muscle region.

The averaged slack angle value is provided and depicted on each relationship for each group with bigger symbols (black circle for stroke participants, and white square for healthy controls).

As described in Materials and Methods section, three comparisons of shear modulus values were performed (1/at the same ankle angle, 2/ at 80% of the maximal ROM and 3/ at the same angle accounting from slack angle), for each muscle region, in order to investigate the between-groups differences of muscle shear modulus.

ACKNOWLEDGMENTS: The authors are grateful to all participants involved in the study, and to Aurélie Sarcher (CHU Nantes / INSERM UMR 131 LaTIM Brest) for assistance with statistical analysis.

GRANTS

The studies were supported by a grant from the University of Nantes (interdisciplinary program), and the Région des Pays de la Loire (QUETE project). The funders had no role in design, data collection and analysis, manuscript redaction and publication. The authors have declared no completing interests.

AUTHOR CONTRIBUTIONS

Conceived and design the study: GLS, FH, RA, AN, and RG. Performed experiments: GLS, RA, TL, and RG. Analyzed and interpreted data: GLS, AN, FH, PMN and RG. Edited manuscript: GLS, AN, FH, RA, TL, PMN, and RG.

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TABLES

Table 1. Characteristics of stroke survivors and control participants

	Experiment 1 (knee fully extended)		Experiment 2 (knee flexed)	
Characteristics	**Stroke survivors (n=13)**	**Controls (n=13)**	**Stroke survivors (n=14)**	**Controls (n=14)**
Sex (male:female)	8:5	8:5	10:4	10:4
Age (y)*	54.3 (12.6)	54.7 (11.9)	58.4 (14.7)	57.1 (14.2)
Height (cm)*	170.0 (6.5)	170.7 (8.8)	171.0 (6.1)	173.6 (8.9)
Weight (kg)*	69.8 (10.4)	68.8 (10.3)	74.6 (12.5)	73.3 (11.6)
Years poststroke*	1.1 (1.3)	NA	3.4 (3.4)	NA
Ankle dorsiflexion ROM (maximal angle) †	16.6 (8.2)	27.8 (7.2)‡	23.2 (5.4)	37.5 (8.3)‡
Affected side (L:R)	7:6	NA	8:6	NA
Spasticity (yes:no)	13:0	NA	13:1	NA
MAS§ (dorsiflexion, knee extended)	1:4 1+:1 2:2 3:6	NA	1:1 1+:2 2:7 3:3	NA
MAS§ (dorsiflexion, knee flexed)	1:5 1+:2 2:3 3:3	NA	1:5 1+:2 2:4 3:2	NA
Isometric strength in plantarflexion (N.m)*	33.4 (21.9)	63.4 (12.0)	26.8 (14.0)	66.0 (32.2)
Isometric strength in dorsiflexion (N.m)*	11.2 (11.5)	27.9 (7.7)	13.0 (8.4)	36.8 (12.6)

Abbreviations: ROM: range of motion; MAS: Modified Ashworth Scale; §1, 1+, 2 and 3 scores refer to the grades of MAS; NA: not applicable. * Mean (SD); † Median (interquartile range); ‡ between-group differences ($p<0.05$).

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Table 2. Slack angle of the plantar flexors measured during dorsiflexion, with the knee fully extended (experiment 1) and with the knee flexed (experiment 2) for each group (stroke survivors and controls).

		Experiment 1 (knee fully extended)		Experiment 2 (knee flexed)	
		Stroke survivors	**Controls**	**Stroke survivors**	**Controls**
Transducer location	**GM distal**	-27.1 (-29.0;-23.8)	-19.8 (-23.0;-19.1)	-11.6 (-13.3;-8.1)	-11.8 (-14.6;-8.9)
	GM mid	-25.2 (-27.2;-21.9)	-19.6 (-20.2;-17.3)*	-10.7 (-12.6;-6.3)	-8.7 (-12.2;-5.9)
	GM proximal	-25.4 (-27.1;-21.0)	-19.5 (-21.7;-18.0)	-9.0 (-13.2;-5.8)	-8.5 (-10.9;-6.6)
	GL distal	-25.3 (-27.0;-23.0)	-19.1 (-21.2;-17.9)*	-10.5 (-14.2;-7.2)	-10.6 (-12.9;-7.5)
	GL mid	-24.3 (-25.0;-23.3)	-18.6 (-19.7;-17.7)	-10.9 (-12.6;-8.5)	-9.6 (-11.7;-7.4)
	GL proximal	-23.2 (-24.8;-22.2)	-17.6 (-19.2;-16.2)	-10.6 (-12.9;-8.0)	-7.9 (-11.0;-6.6)
	SOL distal	-10.0 (-11.3;-6.5)	-5.4 (-9.3;-1.6)	-12.7 (-14.1;-11.4)	-6.4 (-9.7;-3.4)*
	SOL proximal	-6.6 (-7.9;-3.8)	-0.5 (-5.2;-1.3)	-11.4 (-14.5;-7.8)	-2.3 (-4.1;0.4)*
	FDL	-7.5 (-11.2;-2.9)	-4.9 (-7.7;-0.1)	-8.8 (-9.5;-7.7)	-8.1 (-8.6;-5.9)
	FHL	-8.9 (-11.1;-8.1)	-5.6 (-7.8;-2.0)	-11.0 (-12.5;-7.9)	-5.5 (-8.2;-3.3)
	PL	-8.5 (-12.9;-5.9)	-9.1 (-10.1;-5.5)	-10.7 (-13.7;-7.4)	-7.9 (-10.0;-6.9)

Data are shown as median (quartile 1; quartile 3). * $p<0.004$ (value adjusted by Bonferroni correction, 0.05/11).

Abbreviations: GM: *gastrocnemius medialis*; GL: *gastrocnemius lateralis*; SOL: *soleus*; FDL: *flexor digitorum longus*; FHL: *flexor hallucis longus*; PL: *peroneus longus*. distal: distal-leg transducer location; mid: mid-leg transducer location; proximal: proximal-leg transducer location.

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Table 3. sEMG amplitude measured during passive dorsiflexion and normalized to that measured during MVC.

		Experiment 1 (knee fully extended)			Experiment 2 (knee flexed)		
	Muscle	**Stroke survivors**	**Controls**	**Between-group differences [CI 95%]**	**Stroke survivors**	**Controls**	**Between-group differences (CI 95%)**
Averaged sEMG amplitude (%)	**GM**	3.5 (1.2;4.4)	0.5 (0.2;0.8)*	2.9 [0.7;3.9]	4.1 (1.4; 5.4)	1.0 (0.4; 1.6)*	3.1 [0.7;3.9]
	GL	1.8 (0.9;3.2)	0.5 (0.3;1.2)*	1.2 [0.2;2.0]	3.1 (1.5; 6.1)	0.8 (0.4; 1.3)*	1.7 [0.2;2.0]
	SOL	2.2 (1.2;3.4)	0.7 (0.4;1.1)*	1.5 [1.0;1.9]	3.1 (1.7; 4.4)	1.0 (0.5; 1.1)*	2.0 [1.0;1.9]
	TA	0.7 (0.5;1.2)	0.1 (0.1;0.4)*	0.5 [0.1;1.0]	1.1 (0.6; 1.8)	0.3 (0.1; 0.7)*	0.6 [0.1;1.0]
sEMG amplitude at 80% of max dorsiflexion (%)	**GM**	3.7 (1.5;5.9)	0.3 (0.1;0.7)*	3.5 [1.4;5.6]	4.2 (2.1; 5.8)	1.5 (0.4; 2.6)	ns
	GL	2.4 (1.0;4.4)	0.5 (0.3;1.0)*	1.9 [0.5;4.1]	4.4 (1.7; 6.1)	1.0 (0.4; 2.6)*	ns
	SOL	2.8 (1.9;4.9)	1.0 (0.4;1.3)*	1.8 [1.3;2.9]	4.5 (2.8; 6.7)	1.3 (1.0; 4.3)	3.1 [1.3;4.1]
	TA	0.8 (0.7;1.1)	0.4 (0.1;0.7)	ns	1.0 (0.6; 1.4)	1.9 (0.2; 2.2)	ns

Data are shown as median (quartile 1; quartile 3). * $p<0.013$ (value adjusted by Bonferroni correction, 0.05/4). CI 95% : Confidence Interval (95%) about between-group differences in medians from bootstrapping

Abbreviations: GM: *gastrocnemius medialis*; GL: *gastrocnemius lateralis*; ns: not significant; sEMG: surface electromyography; SOL: *soleus*; TA: tibialis anterior.

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FIGURE CAPTIONS

Figure 1 – Box plot of shear modulus values for each transducer location among stroke survivors vs controls, in both experiments (A to C: knee fully extended; D to F: knee flexed at 90°, respectively). Three situations were examined: at the maximal common angle in dorsiflexion displayed among each pair (A and D); at 80% of maximal range of motion (ROM) in dorsiflexion (B and E); and at the maximal common angle in dorsiflexion displayed among each pair accounting from the slack angle (C and F). * $p<0.05$ (adjusted by Bonferroni correction, 0.05/13 [A, B, D and E], and 0.05/11 [C and F]). θ General Mann-Whitney measure of effect size.
Boxplot legend: median (midline), box (25^{th} and 75^{th} percentiles).
Abbreviations: GM: *gastrocnemius medialis*; GL: *gastrocnemius lateralis*; SOL: *soleus*; FDL: *flexor digitorum longus*; FHL: *flexor hallucis longus*; PL: *peroneus longus;* TA: *tibialis anterior;* EDL: *extensor digitorum longus*. distal: distal-leg transducer location; mid: mid-leg transducer location; proximal: proximal-leg transducer location.

Figure 2 – Averaged (standard deviation) shear modulus–ankle angle relationships during the passive dorsiflexion of the ankle, in experiment 1 (knee fully extended) for each muscle region.
Standard deviation bars were omitted for clarity. The averaged slack angle is provided and depicted on each relationship for each group with bigger symbols (black triangle for stroke participants, and white diamond for healthy controls) for each muscle region (except for dorsi flexors).
Abbreviations: GM: *gastrocnemius medialis*; GL: *gastrocnemius lateralis*; SOL: *soleus*; FDL: *flexor digitorum longus*; FHL: *flexor hallucis longus*; PL: *peroneus longus;* TA: *tibialis anterior;* EDL: *extensor digitorum longus*. distal: distal-leg transducer location; mid: mid-leg transducer location; proximal: proximal-leg transducer location.

Figure 3 – Individual stiffness distribution at the same ankle angle (maximal common angle) during both experiments (knee fully extended and knee flexed at 90°) for each transducer location among plantar flexor muscles, in stroke survivors and matched controls (S01 corresponding to stroke survivor participant n°1, and C01 to control participant n°1, respectively). White cells represent missing values.
Abbreviations: GM: *gastrocnemius medialis*; GL: *gastrocnemius lateralis*; SOL: *soleus*; FDL: *flexor digitorum longus*; FHL: *flexor hallucis longus*; PL: *peroneus longus*. distal: distal-leg transducer location; mid: mid-leg transducer location; proximal: proximal-leg transducer location.

Figure 4 – Averaged (standard deviation) shear modulus–ankle angle relationships during the passive dorsiflexion of the ankle, in experiment 2 (knee flexed at 90°) for each muscle region.
The averaged slack angle is provided and depicted on each relationship for each group with bigger symbols (black triangle for stroke participants, and white diamond for healthy controls) for each muscle region (except for dorsi flexors).
Abbreviations: GM: *gastrocnemius medialis*; GL: *gastrocnemius lateralis*; SOL: *soleus*; FDL: *flexor digitorum longus*; FHL: *flexor hallucis longus*; PL: *peroneus longus;* TA: *tibialis anterior;* EDL: *extensor digitorum longus*. distal: distal-leg transducer location; mid: mid-leg transducer location; proximal: proximal-leg transducer location.

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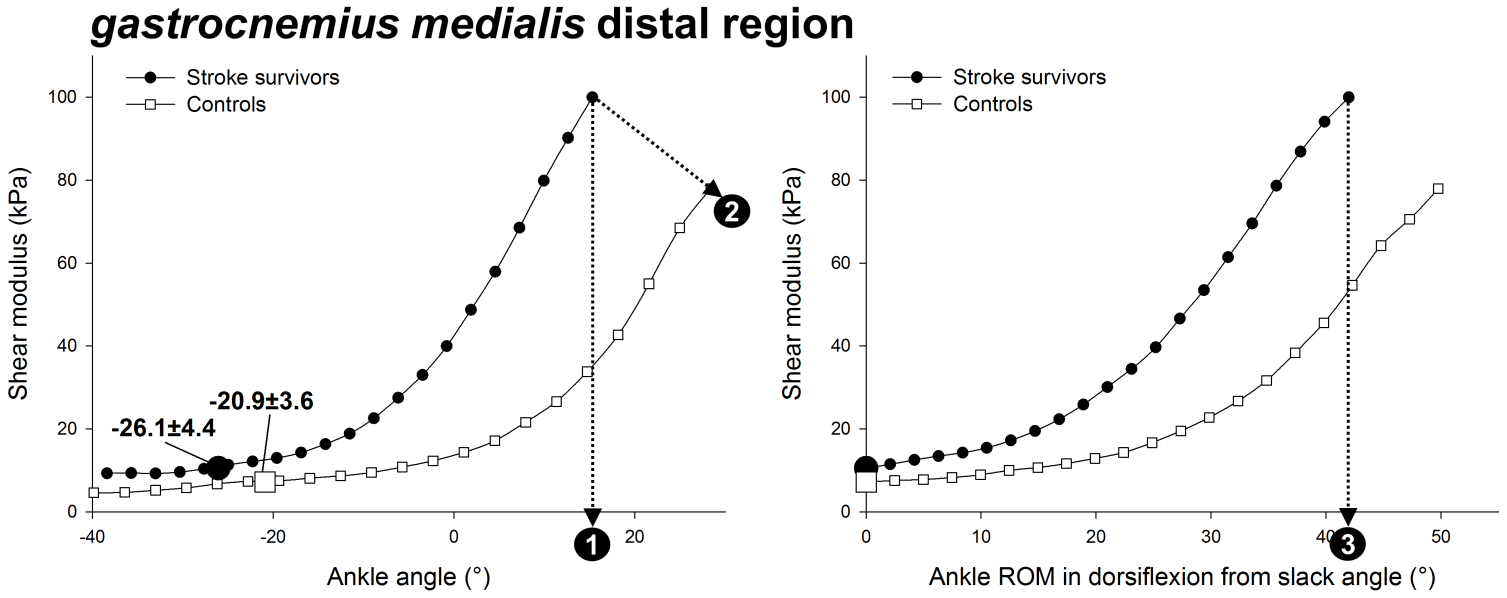

Analyses

1. at the same ankle angle
2. at 80% of maximal ROM in dorsiflexion
3. at the same angle, accounting from slack angle

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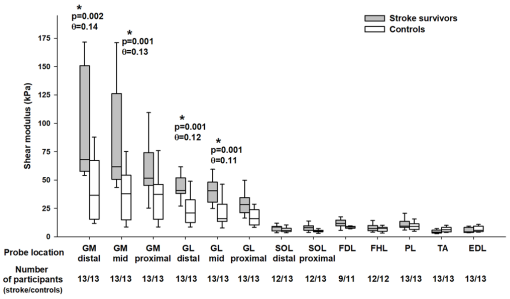
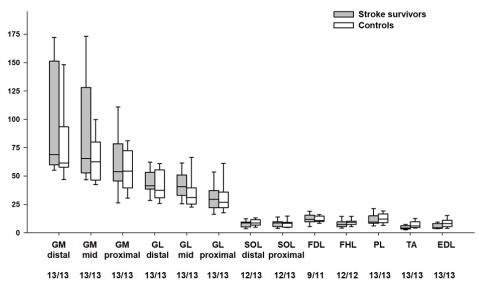
Experiment 1 (*knee fully extended*)

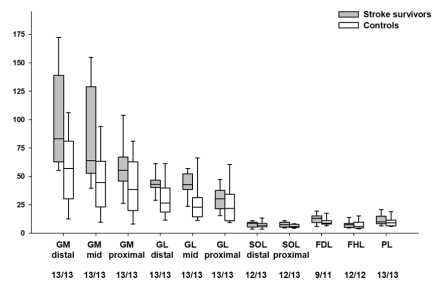
A- Maximal common angle in dorsiflexion



B- 80% of maximal ROM in dorsiflexion

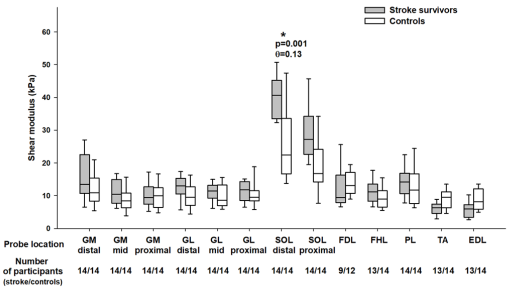


C- Maximal common angle in dorsiflexion, from slack angle

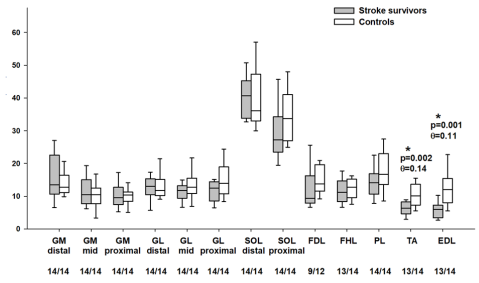


Experiment 2 (*knee flexed at 90°*)

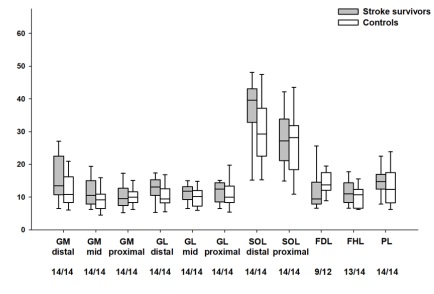
D- Maximal common angle in dorsiflexion



E- 80% of maximal ROM in dorsiflexion



F- Maximal common angle in dorsiflexion, from slack angle



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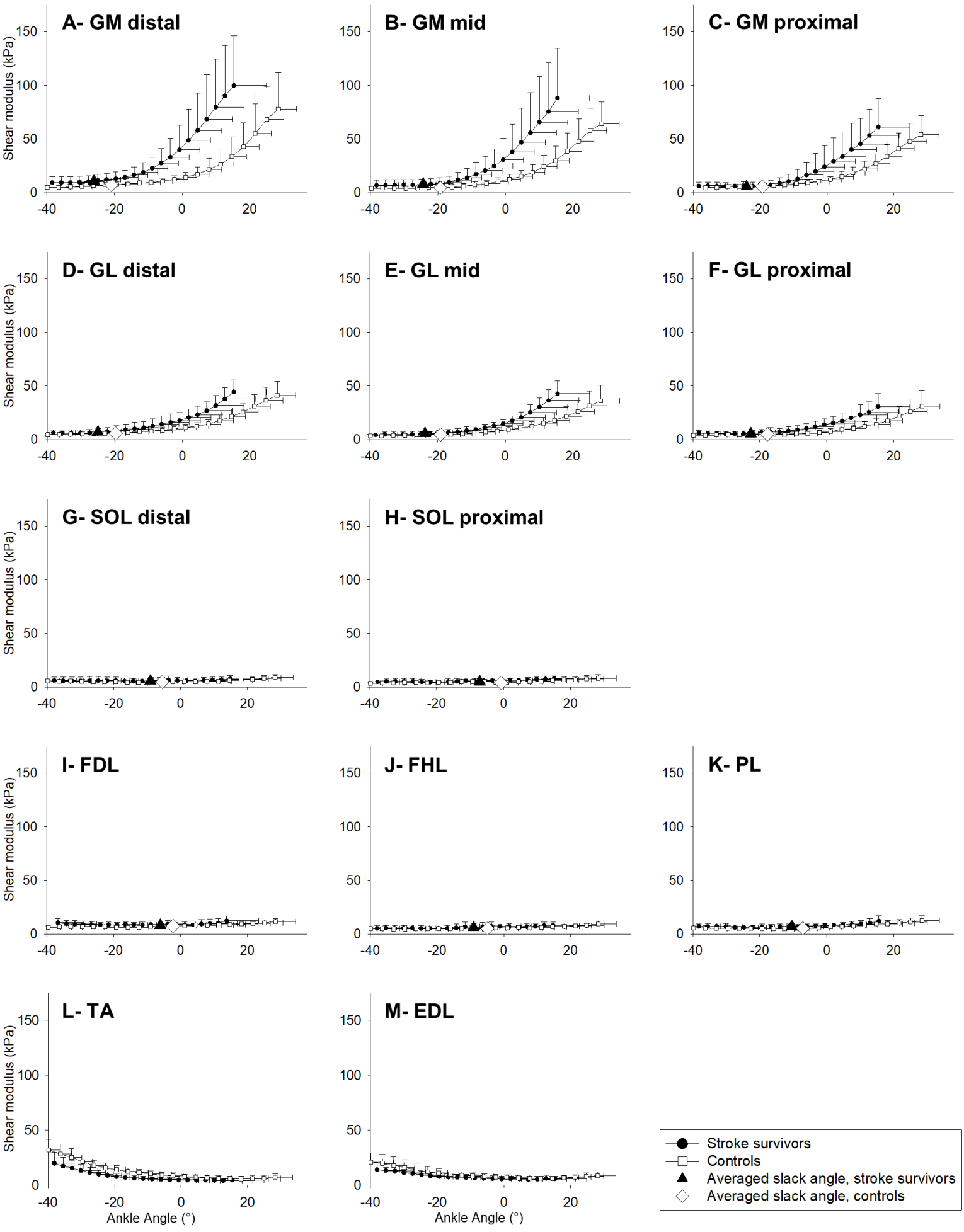

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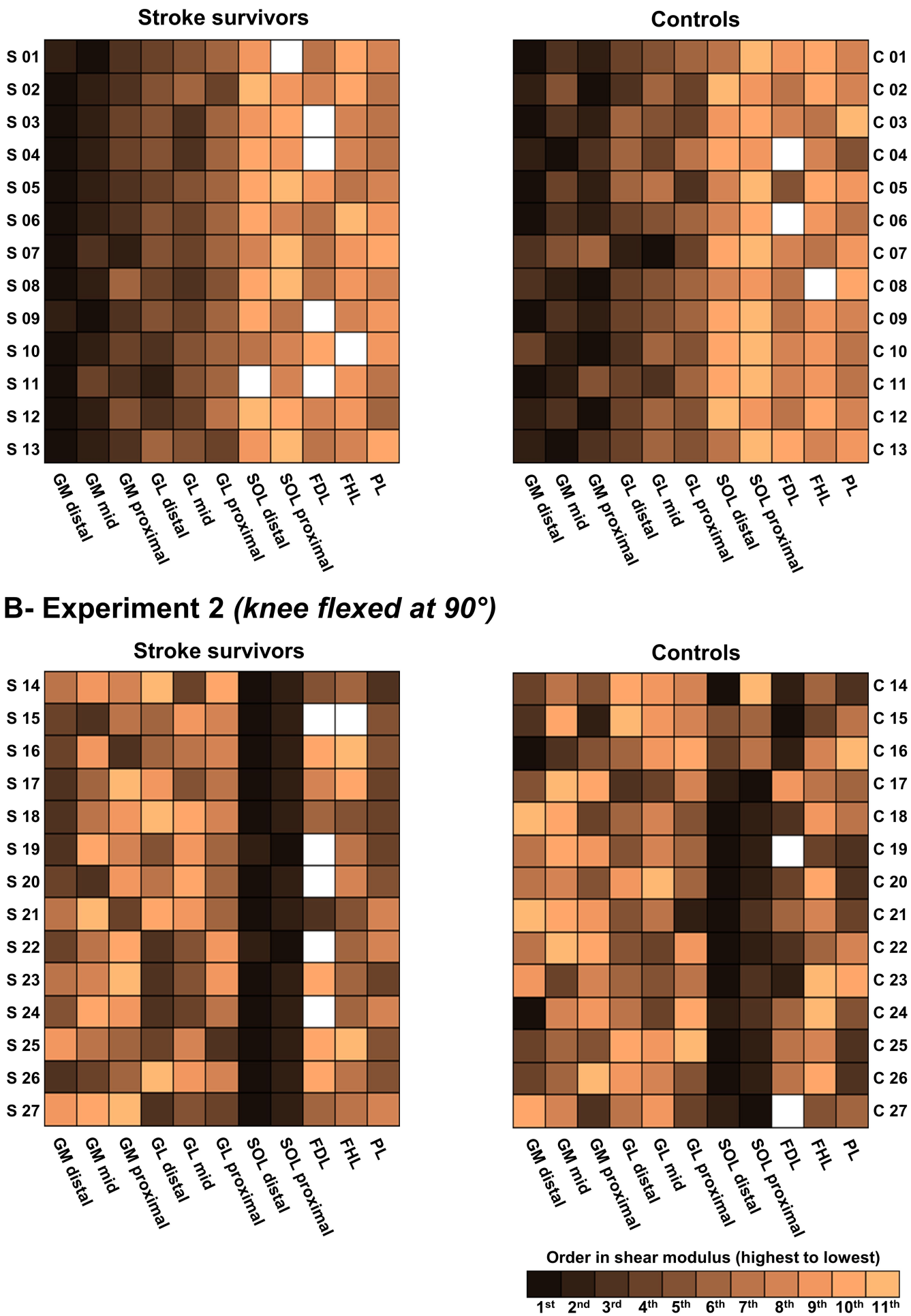

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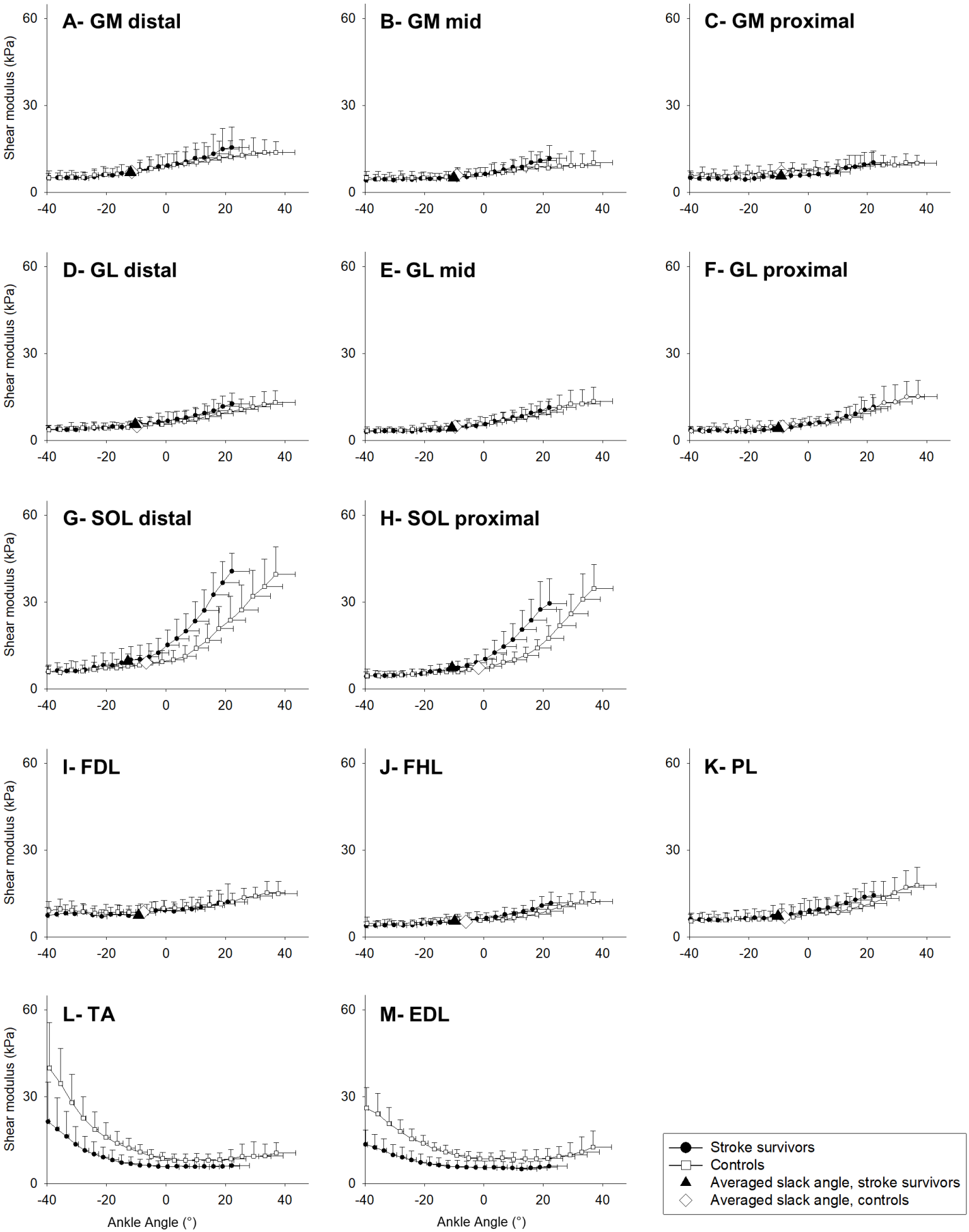

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