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Chronic effects of muscle and nerve-directed stretching on tissue mechanics

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Tissue-directed stretching interventions can preferentially load muscular or nonmuscular structures such as peripheral nerves. How these tissues adapt mechanically to long-term stretching is poorly understood. This randomized, single-blind, controlled study used ultrasonography and dynamometry to compare the effects of 12-wk nerve-directed and muscle-directed stretching programs versus control on maximal ankle dorsiflexion range of motion (ROM) and passive torque, shear wave velocity (SWV; an index of stiffness), and architecture of triceps surae and sciatic nerve. Sixty healthy adults were randomized to receive nerve-directed stretching, muscle-directed stretching, or no intervention (control). The muscle-directed protocol was designed to primarily stretch the plantar flexor muscle group, whereas the nerve-directed intervention targeted the sciatic nerve tract. Compared with the control group [mean; 95% confidence interval (CI)], muscle-directed intervention showed increased ROM (+7.3°; 95% CI: 4.1–10.5), decreased SWV of triceps surae (varied from –0.8 to –2.3 m/s across muscles), decreased passive torque (–6.8 N·m; 95% CI: –11.9 to –1.7), and greater gastrocnemius medialis fascicle length (+0.4 cm; 95% CI: 0.1–0.8). Muscle-directed intervention did not affect the SWV and size of sciatic nerve. Participants in the nerve-directed group showed a significant increase in ROM (+9.9°; 95% CI: 6.2–13.6) and a significant decrease in sciatic nerve SWV (> –1.8 m/s across nerve regions) compared with the control group. Nerve-directed intervention had no effect on the main outcomes at muscle and joint levels. These findings provide new insights into the long-term mechanical effects of stretching interventions and have relevance to clinical conditions where change in mechanical properties has occurred.

NEW & NOTEWORTHY This study demonstrates that the mechanical properties of plantar flexor muscles and sciatic nerve can adapt mechanically to long-term stretching programs. Although interventions targeting muscular or nonmuscular structures are both effective at increasing maximal range of motion, the changes in tissue mechanical properties (stiffness) are specific to the structure being preferentially stretched by each program. We provide the first in vivo evidence

that stiffness of peripheral nerves adapts to long-term loading stimuli using appropriate nerve-directed stretching.

ankle range of motion; mechanical properties; muscle stretching; sciatic nerve; triceps surae; ultrasound shear wave elastography

INTRODUCTION

Stretching of human skeletal muscles is commonly used in sports and clinical practice with the aim of increasing muscle-tendon extensibility and maximal joint range of motion (ROM). Although it is widely accepted that chronic stretching (i.e., specific repeated sessions over weeks) induces an increase in maximal ROM, the large body of literature has failed to demonstrate an effect of chronic interventions on the muscle-tendon unit mechanical properties [Freitas et al. (21)]. The absence of mechanical adaptations has led to the contention that increased ROM following chronic stretching interventions is mostly explained by an increase in stretch tolerance (21, 59). However, despite this evidence, three major elements have to be considered when interpreting the lack of changes in tissue mechanical properties (21).

First, studies of chronic stretching have implemented relatively short stretching training periods (i.e., < 8 wk); hence, it is possible that the duration of the stretching intervention has not been sufficient to trigger mechanical adaptations within the muscle-tendon unit (21, 43). Second, the chronic adaptations in mechanical properties of muscle-tendon units (e.g., hamstring and plantar flexors) are typically assessed by measuring the passive torque (21), a measure indicative of global resistance to stretch at the joint level (23, 37). However, stretching may trigger regional effects. Such effects may not be observable using traditional research techniques such as a passive-torque angle assessment utilizing a dynamometer (e.g., Biodex). Regional effects in response to stretch could lead to heterogeneous adaptations within specific muscles of a muscle group (27) or even within specific regions within individual muscles (35). Thus, in some instances, global measurements of passive torque may not be sensitive enough to detect changes in

mechanical properties induced by tensile loading within individual muscle-tendon unit structures. Third, nonmuscular structures such as peripheral nerves and fascia have recently been suggested as potential candidates to explain the limitation of maximal ROM in some multijoint limb positions utilized in stretching (43, 63). For example, a recent study found that the maximal dorsiflexion ROM can be acutely increased with a sciatic nerve stiffness decrement in the absence of any change in tensile properties of plantar flexor muscles and the ankle passive torque (3). Although the mechanical properties of peripheral nerves are thought to influence the stretching amplitude in some multijoint configurations (40, 43), to our knowledge, no previous studies have investigated the adaptations in mechanical properties of peripheral nerves induced by a longer-term stretching intervention in humans.

The present study was designed to investigate whether chronic stretching interventions can induce changes in the passive mechanical properties of either nerve or muscle (depending on which structure was preferentially loaded during the stretch). We compared the outcomes of two different 12-wk interventions, named muscle-directed stretching or nerve-directed stretching, versus a control group. The stretching interventions included exercises similar to those traditionally performed in sports and rehabilitation programs. In addition to the traditional measures, e.g., maximal ankle dorsiflexion ROM and passive torque, a novel feature of the current work was that the passive stiffness of both sciatic nerve and plantar flexor muscles was estimated using ultrasound shear wave elastography. We hypothesized that both chronic stretching protocols would improve the maximal dorsiflexion ROM but trigger different mechanical adaptations in the nerve and muscular tissues.

MATERIALS AND METHODS

Participants, Ethical Approval, and Randomization

Sixty human volunteers of both gender (means \pm SD; age: 20.5 ± 2.0 yr, height: 172.2 ± 9.1 cm, mass: 63.8 ± 9.0 kg) participated in this study. All participants were students recruited from the Faculty of Sports Sciences between September and October 2016 utilizing posted flyers. Inclusion criteria comprised 1) healthy and asymptomatic status and 2) ages between 18 and 30 yr. Exclusion criteria comprised 1) recent (< 3 yr) musculoskeletal injury/disability in spine or lower limbs; 2) severe cardiovascular, neurological, or psychiatric disease; 3) a positive passive straight leg raising test (e.g., pain elicited with passive hip flexion with the knee in full extension), which can be indicative of neuropathic pain and/or sciatic nerve or nerve root pathology; and 4) recent engagement in any flexibility training.

The Institutional Ethics Committee (Tours Ouest I, reference: no. CCP MIP-08) approved the study, and all procedures conformed to the Declaration of Helsinki.

After completion of the baseline assessment, participants were randomly assigned to either sciatic stretching (nerve-directed; $n = 21$; 9 females), muscle stretching (muscle-directed; $n = 21$; 11 females), or control (control, $n = 18$; 9 females) groups. Randomization was performed by an investigator, not involved in recruitment or assessments, using a computer-generated algorithm. All participants reported their typical physical activity level to be greater than 8 h/wk before and during the period of the study, and were informed about methods before providing informed written consent.

Experimental Procedure

Dynamometry. An isokinetic dynamometer (System 3; Biodex Medical Systems, Upton, NY) was used to perform passive ankle rotations and measure the ankle torque during testing sessions performed before and after stretching training period. The lateral malleolus was considered as an estimate of the ankle center of rotation and aligned with the axis of the ergometer. The neutral position of the ankle (0°) was defined as the angle of 90° between the footplate and the shank. The foot was firmly strapped to minimize the potential heel displacement from the dynamometer footplate during the ankle rotations. The chest and the waist were strapped to minimize trunk motion during ankle rotations. All measurements were performed on the right ankle joint.

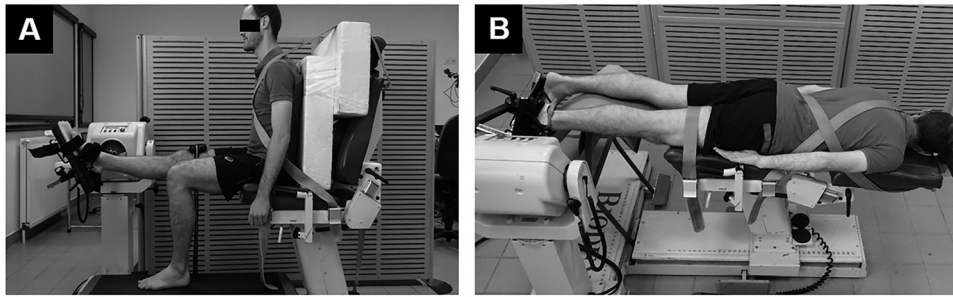
Angle and torque data were collected at 100 Hz with an analog/digital converter (Biopac Systems Inc., Goleta, CA). Maximal dorsiflexion ROM was tested in two hip joint positions: flexed at 90° (HIP-flexed; Fig. 1A) or neutral (HIP-neutral, 180° ; Fig. 1B). Because pilot testing demonstrated low reliability for sciatic elastography measurements during full ankle ROM assessment in the HIP-flexed position (unpublished data), elastograms were only performed in the HIP-neutral position. A standard goniometer (MSD, Londerzeel, Belgium) was used to measure the hip joint angle. For both conditions, the knee joint was placed in full extension (i.e., 180°).

Ultrasound recordings: elastography and B-mode imaging. The shear wave velocity of the sciatic nerve and triceps surae muscles [gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and soleus] was assessed using an Aixplorer ultrasound scanner (version 6.1; Supersonic Imagine, Aix-en-Provence, France) coupled with a linear transducer (a 50-mm, 4–15 MHz transducer for GM and GL muscles; and a 38-mm, 2–10 MHz transducer for the sciatic nerve and soleus muscle; Super Linear, Aix-en-Provence, France). Shear wave velocity was used as an index of tissue stiffness [see details in Hug et al. (29)]. Briefly, the shear wave velocity (V_s) is directly linked to the shear modulus (μ): $\mu = \rho V_s^2$, where ρ is the estimated density of soft tissues ($1,000 \text{ kg/m}^3$). In an isotropic homogenous medium, the Young's modulus (E) can be approximated as three times the shear modulus (μ), as follows: $E = 3 \mu$. Ultrasound shear wave elastography (longitudinal plane measurements) has been shown to provide an accurate estimation of the biomechanical properties of anisotropic musculoskeletal soft tissues, including muscles (19, 25) and, more recently, peripheral nerves (50). During the past decade, shear wave elastography has been used to reliably measure the shear wave velocity of plantar flexors muscles (20, 35) and sciatic nerve (5, 24, 31).

To map the effects of stretching in individual structures (Fig. 2), shear wave elastography was measured in different regions of triceps surae muscles and sciatic nerve tract. These regions were initially identified using B-mode. Specifically, participants laid prone with the knee of the tested lower limb in full extension (180°). The ankle was positioned in a neutral position (0°). Three landmarks were first identified to define the regions for the measurements in the sciatic nerve: 1) the midpoint between the ischial tuberosity and the greater trochanter, 2) the point where sciatic nerve divides into tibial and common peroneal nerves, and 3) the lateral femoral condyle. The sciatic nerve was then divided into two equidistant proximal (sciatic_{proximal}) and distal (sciatic_{distal}) regions between *points 1* and 2. The elastography assessments were performed in the midpoint of each region. The tibial nerve was assessed between *points 2* and 3. The sciatic nerve and its tibial branch were considered as the sciatic nerve tract. Additional B-mode images (ankle position set at 0°) were acquired in both longitudinal and transverse views to assess cross-sectional area and thickness, respectively, within the different nerve regions.

For each gastrocnemius muscle, the shear wave velocity was measured at three equidistant regions of $\sim 33\%$ of each muscle length as reported in Le Sant et al. (35): proximal (GM_{proximal} and

Ankle ROM assessments



Intervention

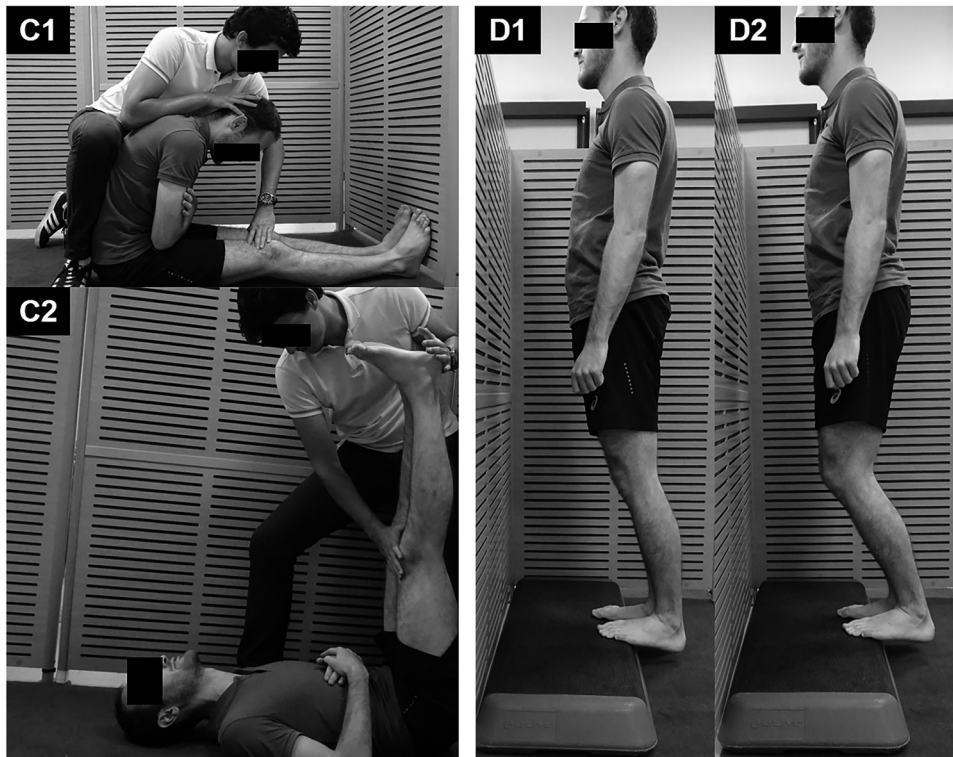


Fig. 1. *A* and *B*: testing positions used to measure the maximal ankle dorsiflexion range of motion (ROM). *A*: hip flexed at 90° (HIP-flexed position). *B*: hip neutral (HIP-neutral position). *C* and *D*: stretching interventions. *C1* and *C2* correspond to the stretching exercises performed by participants in the nerve-directed group to stretch the sciatic nerve tract; *D1* and *D2* show the stretching exercises performed by the muscle-directed group to load the plantar flexors.

GL_{proximal}), intermediate (GM_{intermediate} and GL_{intermediate}), and distal (GM_{distal} and GL_{distal}). Soleus shear wave velocity was assessed in two different regions (i.e., proximal and distal). Two landmarks were utilized to define these regions: 1) mid-distance between lateral and medial malleoli and 2) popliteal fossa. The shear wave velocity of both soleus_{distal} and soleus_{proximal} was assessed at 10% and 50% of the total length between *point 1* and *point 2*, respectively. Soleus_{distal} was assessed in the medial compartment of the lower leg, and soleus_{proximal} was measured underneath the GM muscle. B-mode images of the intermediate regions of GM and GL were acquired (ankle in neutral position) to measure the muscle architecture, including fascicle length and thickness.

To scan each structure and region, the ultrasound transducer was handheld by an experienced examiner. For all shear wave elastography measurements at both nerve and muscle structures, the transducer was aligned parallel to the tissue fibers with minimal tissue compression (Fig. 2). When necessary, the transducer orientation was slightly adjusted in real time to maintain the longitudinal view of each structure throughout ankle rotation.

The following shear wave elastography acquisition parameters were set in the Aixplorer: penetration mode: 100% opacity, no

temporal smoothing (persistence = OFF), and intermediate spatial smoothing of 5/9. The maps of the shear modulus were obtained at 1 sample/s and with a spatial resolution of 1 × 1 mm. An analog trigger signal originating from the ultrasound scanner at each elastography measurement was used to synchronize shear wave velocity data with mechanical and surface electromyography (EMG) data.

Electromyography. Myoelectrical activity was assessed using surface EMG to check that the lower leg muscles remained passive during the measurements. A pair of surface electrodes (Kendall 100 Series Foam Electrodes, Covidien, Mansfield, MA) was placed over the GM and soleus muscles at the location recommended by the Surface EMG for Non-Invasive Assessment of Muscles guidelines (26). EMG data were acquired simultaneously with the mechanical data (MP35, Biopac Systems Inc., Goleta, CA) and sampled at 1 kHz. The EMG signals were amplified (gain = 1,000) and band-pass filtered between 5 and 500 Hz.

Study Assessments

Participants were asked to avoid intense exercise (other than the trial intervention) 48 h before the testing sessions performed before and after

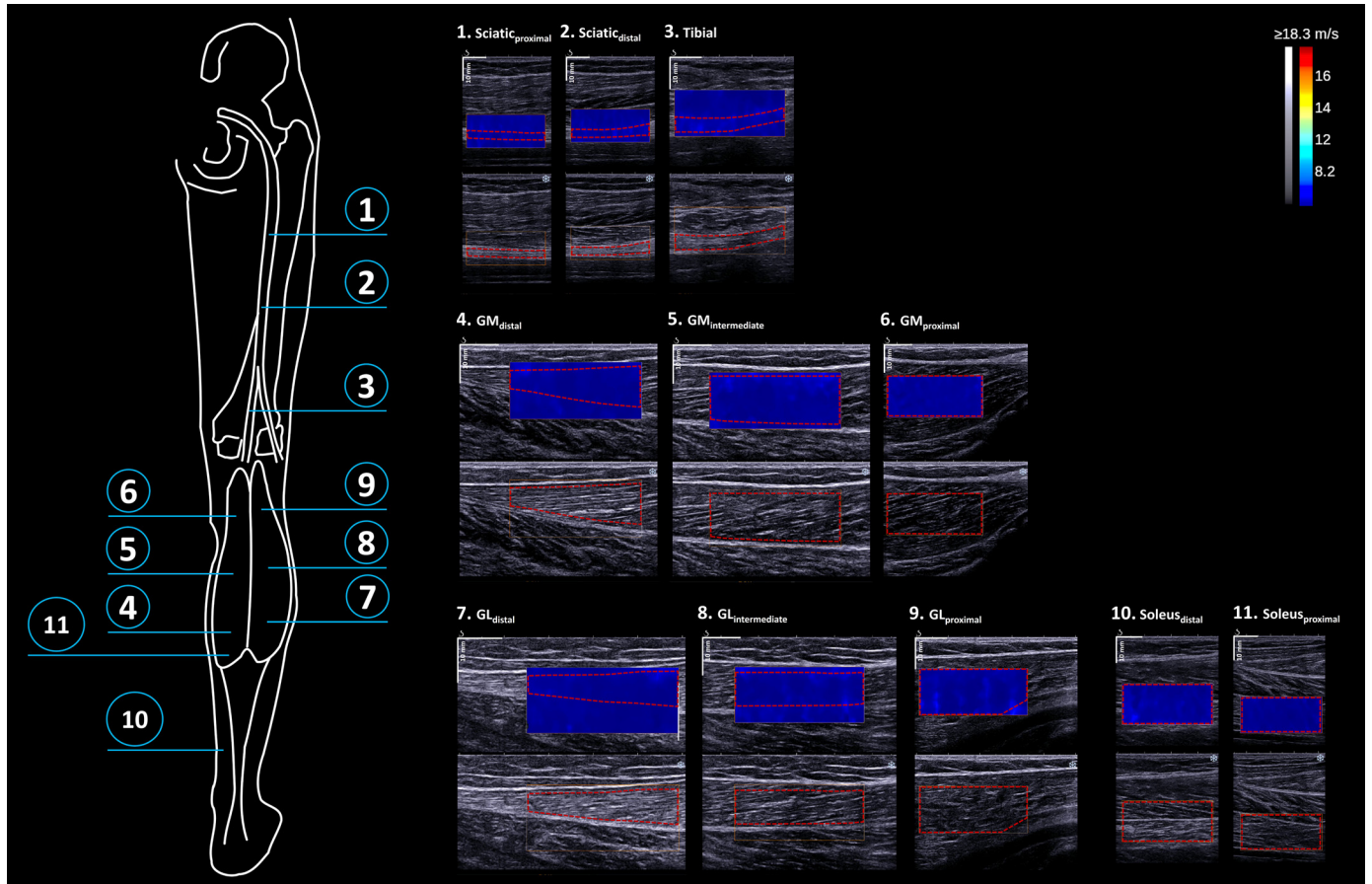


Fig. 2. Typical images of ultrasound shear wave elastography data for each muscle and nerve at a matched plantar flexion angle (participant no. 59). The numbers represent the locations at which ultrasound measurements were taken for each structure. There are 2 panels per ultrasound image. *Top*, shear wave velocity maps (colored maps; scale ranging from 0–18.3 m/s). *Bottom*, ultrasound B-mode. Regions of interest are outlined in dotted red in both B-mode and shear wave elastography panels. GL, gastrocnemius lateralis; GM, gastrocnemius medialis.

the stretching training. Thirty minutes before each testing session, participants were familiarized with the testing setup and maximal ankle dorsiflexion ROM assessments. Static stretching was avoided during familiarization. Pre- and posttraining testing sessions were performed 24 h before the first stretch intervention and 24 h following the last stretch intervention. Both testing sessions were conducted at the same period of the day (± 3 h). Assessors at baseline were blinded to the intervention allocation. For practical purposes, the assessor who performed the ultrasound scans was not blinded to the group after the allocation and throughout the study.

At the start of each testing session, five preconditioning ankle rotations were performed (at $5^\circ/s$) in the HIP-neutral position between 40° of plantarflexion and 15° of dorsiflexion (44). Thereafter, the testing sessions were divided into four steps. First, the passive maximal ankle dorsiflexion ROM was assessed in both HIP-neutral and HIP-flexed positions (randomized order, Fig. 1, *A* and *B*). Participants were asked to completely relax with their eyes closed while the ankle was passively rotated at $2^\circ/s$ from 40° plantar flexion toward maximal dorsiflexion. When the participants reached their maximum tolerable stretch limit (4), they pressed a button to stop the passive movement immediately and release the footplate to avoid static stretching occurring. For each hip position, participants performed two trials of maximal dorsiflexion ROM with 1-min rest in between. The greatest maximal dorsiflexion ROM attained during these trials was utilized for further analyses (8). Second, real-time elastography measurements (i.e., ultrasound video clip) were performed for the different structures and regions with a random order. Measurements were performed while the ankle was passively rotated

($2^\circ/s$) from 40° of plantar flexion to the maximal dorsiflexion ROM in the HIP-neutral position, as assessed previously. A 1-min rest interval was given between passive stretches, which allowed the ultra-sonographer to reposition the transducer for a new region or structure between measurements. Third, two ultrasound B-mode images of each gastrocnemius muscle and sciatic nerve tract were performed to assess the effects of stretching protocols on muscle architecture and nerve size. B-mode measurements were performed in a randomized order. Finally, to normalize the EMG signals, two maximal voluntary isometric plantarflexions were performed with the ankle in a neutral position (0°). Participants were positioned in supine. There was a 1-min rest interval between each maximal effort trial.

Outcome measures. All outcome measures at pretesting were collected before the allocation of participants into intervention and control groups. After the 12-wk intervention ceased, the assessments were repeated for all groups (posttesting). The coprimary outcomes were passive ankle dorsiflexion ROM and sciatic nerve and triceps surae muscles' shear wave elastography. Secondary outcome measures were ankle passive torque and ultrasonographic architectural properties (GM and GL fascicle length and thickness; cross-sectional area and thickness of the sciatic nerve) and GM and soleus root mean square (RMS) EMG.

Intervention

Participants in nerve-directed and muscle-directed experimental groups were involved in a 12-wk stretching protocol, performed five

times per week between 10:00 AM and 3:00 PM. In each session, participants performed two different static stretching exercises (described below) according to their allocated group (i.e., sciatic or muscle stretching). Each stretching exercise was maintained for 45 s and performed five times. Participants were instructed to start the next repetition immediately after release of the previous stretch (< 5-s rest interval between stretches). The duration of stretch exposure was identical between muscle-directed and nerve-directed groups (7.5 min), leading to a total stretch duration of 7.5 h over the 12-wk training period. Stretching exercises were performed at the maximal stretch tolerance, defined as the onset of pain during each exercise. The stretching exercises that were chosen are commonly used in rehabilitation and sports practice. Three independent training supervisors were responsible for: 1) delivering the nerve-directed intervention and supervising the muscle-directed stretching sessions and 2) ensuring that participants complied with the intervention.

Nerve-directed stretching group. Each session of the nerve-directed group was composed of the following stretch exercises, performed in a randomized order. *Sciatic stretching 1*: from a seated position, the supervisor passively flexed the hip toward a long sitting position, followed by lumbar, thoracic, and cervical flexion. The knee joints remained in full extension, and the ankle's joint angle was maintained in a neutral position (0°) to minimize the stretch of the plantar flexor muscles (Fig. 1C1). *Sciatic stretching 2*: from a supine position with the knees fully extended, the participant's legs were passively raised (i.e., hip flexion) by a supervisor with the ankle joints in a relaxed position (Fig. 1C2). These exercises have been shown to be effective at inducing acute changes in sciatic nerve mechanical properties without affecting the mechanical properties of plantar flexor muscles (3).

Muscle-directed stretching group. Participants in the muscle-directed group performed the following plantar flexor stretch exercises in a randomized order. *Triceps surae stretching 1*: the participant was instructed to stand on the step, with the knees in full extension with their heels hanging over the edge and the forefeet supporting body weight. Then, the plantar flexor stretch was performed bilaterally by dropping the heels slowly toward the floor until the maximal stretch tolerance was achieved (Fig. 1D1). *Triceps surae stretching 2*: the same exercise was performed with the knees flexed approximately at 70° (Fig. 1D2). These exercises were designed to preferentially stretch the gastrocnemii (*triceps surae stretching 1*) and soleus muscles (*triceps surae stretching 2*) (35). Participants were allowed to touch a wall (anterior to participant's body) with their hands to minimize active plantar flexor muscle activity and keep the balance. For both stretching exercises, participants were also asked to maintain a relaxed state in their calf muscles throughout the stretching time.

Control group. Participants in the control group were not engaged in any specific flexibility training and were asked to maintain the same level of physical activity for the duration of the stretching training protocol.

Data Analysis

All data were processed using custom-made MATLAB scripts (The MathWorks Inc., Natick, MA). All data remained coded so that the data analyses could be undertaken in a blinded manner. Angle and torque signals were low-pass filtered (10 Hz) with a second-order Butterworth filter. The torque was corrected for gravity. Clip videos were exported from the Aixplorer's scanner in mp4 format and then sequenced in jpeg images (1 Hz). The image processing converted each pixel of the color map into a shear wave velocity value based on the recorded color scale (scale = 0–18.3 m/s on our version of the scanner). A region of interest (ROI) was first defined on the first map as the largest muscle or nerve area (Fig. 2). Then, this ROI was manually tracked image-by-image. Each image was then inspected for artifacts (i.e., saturation) and signal void areas, and the ROI was manually adjusted to be sure that artifacts were not included in the

averaged shear wave velocity. The shear wave velocity data extracted from the ROI were then averaged, and this value was used for statistical analysis. Importantly, in thin and stiff structures it is possible that the shear wave propagation is guided, and hence the thickness is likely to affect the group shear wave velocity (11). For this reason, nerve thickness was assessed to ensure that shear wave velocity would not have been influenced by pre- to posttraining thickness changes (3).

The root mean square of the EMG signal (RMS EMG) was calculated every second over a 500-ms window throughout the testing procedures. Artifacts originating from the ultrasound beams (1 sample/s) were removed from the EMG signal. The RMS EMG values were normalized to the maximal voluntary isometric muscle activation test.

For each participant, the pre- and postsciatic nerve (3 regions), GM (3 regions), GL (3 regions), and soleus (2 regions) shear wave velocity, ankle passive torque, and RMS EMG (2 muscles) data were calculated every 2°, from 40° of plantarflexion to the maximal ankle ROM in dorsiflexion. The maximal dorsiflexion angle attained in the pretesting was used to examine the pre-to-post effects of interventions. Pre- and postshear wave velocity, torque, and RMS EMG outcomes measured at this ankle angle were then used for further statistical analysis. In a few cases, the dorsiflexion angle at posttesting was lower than the pretest angle; hence, the first was used to examine the outcomes. A linear interpolation method was used to calculate the outcome values at the selected angle. The effects of interventions on ankle dorsiflexion ROM were further examined at the same passive torque pre- and postintervention across groups.

Measurements of nerve size and muscle architecture were performed using the static B-mode images. The examiner was blinded to participant's identity and group allocation. The fascicle length and muscle thickness were calculated simultaneously from B-mode images of GM_{intermediate} and GL_{intermediate} muscles. Specifically, two different images were processed for each muscle. Two straight lines were superimposed digitally on each image to highlight superficial and deep aponeuroses planes. The fascicle length was measured as the length of the fascicle (straight line distance) between their intersections with the aponeuroses. When this length exceeded the ultrasound field of view, an extrapolation of the fascicle length was conducted using trigonometry (47). In each image, three distinct fascicles were measured (at proximal, intermediate, and distal regions, respectively), and the average length between the three fascicles was used in the statistical analysis. Muscle thickness was calculated as the average of a straight line between superficial and deep aponeuroses. Nerve thickness was calculated using B-mode ultrasound images of sciatic_{proximal}, sciatic_{distal}, and tibial. It was calculated as the mean of six different straight lines between superficial and deep nerve boundaries (superficial and deep epineurium, respectively) along the longitudinal view of each nerve B-image. Nerve cross-sectional area was measured three times on the same image, and it was calculated as the area of nerve in a transversal B-mode image; the average was used for further analysis. All nerve size and muscle architectural parameters were calculated in two B-mode images. An average of two measurements was used in the results.

Statistics

The IBM SPSS software (version 20.0; IBM Corporation, Armonk, NY) was used for the statistical procedures. All data are reported as means ± SD. The Kolmogorov–Smirnov test was used to confirm whether all variables were normally distributed. For all outcome variables, the effect of the intervention was determined using one-way analysis of covariance (ANCOVA) with group (nerve-directed vs. muscle-directed vs. control) as the between-subject factor (i.e., independent variable), postintervention values as the dependent variables, and preintervention values as covariate factors. The model was checked for assumptions (data distribution, independence of covariate

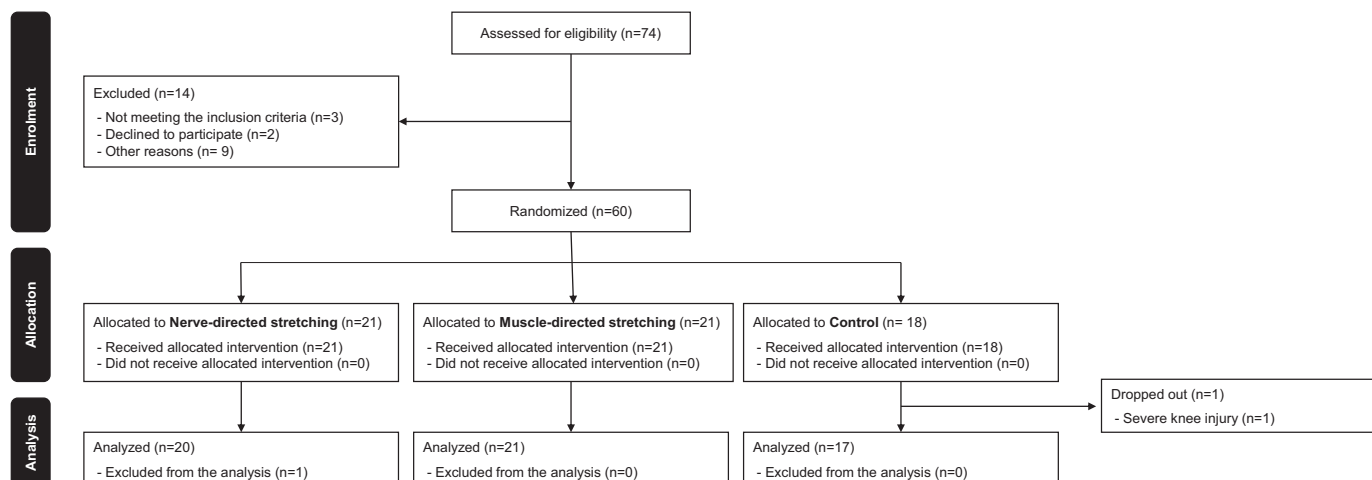


Fig. 3. Flow chart of study enrolment, allocation, and analysis.

and treatment, homogeneity of regression slopes and homoscedasticity). Bonferroni adjustments were performed to account for multiple comparisons between groups. Between-group ANCOVA-derived (adjusted) mean differences, P values, and 95% confidence intervals (CIs) are presented. The partial η -squared ($\rho\eta^2$) values were reported as a measure of the effect size of the ANCOVA's findings. Values of 0.01, 0.06, and 0.14 were considered benchmarks for small, medium, and large effects (48), respectively. Typical error and intraclass correlation coefficient (ICC) (1, 2) were calculated to express the within-subject reliability of maximal dorsiflexion ROM assessments (*trial 1* vs. *trial 2*). Typical error represents the within-subject standard deviation and was calculated as described by Hopkins (28). The ICC indicates the error in measurements as a proportion of the total variance in scores, and as a general rule we considered an ICC over 0.9 as high. The statistical significance was set at $P < 0.05$.

RESULTS

Participants

Figure 3 depicts a flowchart of this study. A total of 60 participants were enrolled and randomly allocated to the nerve-directed ($n = 21$), muscle-directed ($n = 21$), and control ($n = 18$) groups. Characteristics of participants are described in Table 1. One participant from the control group withdrew due to anterior cruciate ligament tear unassociated with the stretching intervention. The number of participants completing the study was 59 (21 in nerve-directed group, 21 in muscle-directed group, and 17 in control group). One participant (nerve-directed group) was excluded from data analysis because of imaging acquisition artifacts. Data from 58 participants were analyzed (nerve-directed: 20; muscle-directed: 21; control: 17).

Effects of the Intervention

Table 2 presents the means \pm SD for all outcome measures at baseline (preintervention) and after the 12 wk (postintervention) per group, together with the adjusted mean changes for nerve-directed and muscle-directed groups versus the control group.

Maximal ankle dorsiflexion ROM. Adjusting for baseline data, significant main effects of group with large effect sizes were observed for the maximum ankle dorsiflexion ROM assessed for both HIP-neutral [$F(2,54) = 17.7$, $P < 0.001$, $\rho\eta^2 = 0.40$] and HIP-flexed [$F(2,54) = 30.5$, $P < 0.001$, $\rho\eta^2 = 0.53$] positions. In the HIP-neutral condition, post hoc analysis showed a significant increase in ankle dorsiflexion ROM in the muscle-directed group compared with the control group with a mean difference of 7.3° (95% CI: 4.1–10.5; $P < 0.001$), whereas the mean difference between the nerve-directed group and the control group was not statistically significant ($P = 0.313$). In the HIP-flexed condition, participants in the nerve-directed group showed a significant increase in ankle dorsiflexion ROM compared with the control group, with a mean difference of 9.9° (95% CI: 6.2–13.6; $P < 0.001$). There was no significant difference between the muscle-directed and the control groups in this testing position ($P = 1$). Between-group differences and individual data are depicted in Fig. 4. In this trial, typical error was 2° and 2.2° for HIP-neutral and HIP-flexed, respectively. ICC was 0.932 (95% CI: 0.903–0.952) and 0.963 (95% CI: 0.947–0.974) for HIP-neutral and HIP-flexed, respectively.

For the HIP-neutral condition, there was a significant main effect of group for the maximal dorsiflexion ROM at the same

Table 1. Participant characteristics at baseline for the nerve-directed, muscle-directed, and control groups

	Total Sample	Nerve-Directed	Muscle-Directed	Control
n	60 (31 men, 29 women)	21 (12 men, 9 women)	21 (10 men, 11 women)	18 (9 men, 9 women)
Age, yr	20.5 \pm 2	19.5 \pm 1.1	21 \pm 2.4	21.1 \pm 2
Height, cm	172.2 \pm 8.7	174.4 \pm 8.7	171.4 \pm 9.3	171.5 \pm 8.5
Weight, kg	63.7 \pm 9.1	65.8 \pm 8.9	62.8 \pm 9.4	62.2 \pm 8.9
BMI, kg/m ²	21.3 \pm 1.6	21.5 \pm 1.6	21.3 \pm 1.9	21 \pm 1.2

Data are presented as means \pm SD. BMI, body mass index.

Table 2. *Effects of intervention*

Outcomes	ANCOVA									
	Baseline			12-Wk			Nerve-Directed Minus Control		Muscle-Directed Minus Control	
	Nerve-Directed	Muscle-Directed	Control	Nerve-Directed	Muscle-Directed	Control	Adj. Mean Diff. (95% CI)	P Value	Adj. Mean Diff. (95% CI)	P Value
<i>Ankle dorsiflexion range of motion</i>										
ROM, HIP-neutral, °	37.9 ± 7	36.9 ± 7.6	40.6 ± 8.1	41.9 ± 6.6	46.3 ± 7.2	41.7 ± 5.8	2.1 (-1.1 to 5.3)	0.313	7.3 (4.1 to 10.5)	<0.001
ROM, HIP-flexed, °	8.8 ± 9.2	14 ± 10.7	11.4 ± 12.5	16.7 ± 9.8	11.9 ± 11.4	9.4 ± 13.3	9.9 (6.2 to 13.6)	<0.001	0 (-3.6 to 3.7)	1
<i>Ankle dorsiflexion range of motion at the same ankle torque</i>										
ROM, HIP-neutral, °	35.7 ± 6.6	34.7 ± 7	39.5 ± 6.6	36.3 ± 6.1	37.7 ± 7.9	38.5 ± 6.2	1 (-2.1 to 4.2)	1	3.3 (0.1 to 6.4)	0.037
ROM, HIP-flexed, °	8.6 ± 8.4	12.2 ± 10.3	8.7 ± 14	5.5 ± 12	9.6 ± 10.6	7.7 ± 14.6	-2 (-7.4 to 3.4)	1	-1.4 (-6.7 to 4)	1
<i>Triceps surae stiffness</i>										
GM _{proximal} SWV, m/s	8.7 ± 1.8	9.3 ± 1.9	9.7 ± 1.6	8.8 ± 2.2	8.4 ± 2.3	10.5 ± 1.9	-0.7 (-1.9 to 0.5)	0.493	-1.6 (-2 to 0.2)	0.004
GM _{intermediate} SWV, m/s	9.8 ± 2.1	10.7 ± 1.9	11.2 ± 1.6	9.8 ± 2.4	9.3 ± 2.2	11.6 ± 1.6	-0.6 (-1.6 to 0.5)	0.553	-1.9 (-2.9 to -0.9)	<0.001
GM _{distal} SWV, m/s	10.3 ± 2	11.2 ± 2.2	12.2 ± 1.9	9.8 ± 2.4	9.7 ± 0.6	12.8 ± 2.6	-1.1 (-2.7 to 0.4)	0.208	-2.2 (-3.6 to -0.7)	0.001
GL _{proximal} SWV, m/s	7.4 ± 2	7.9 ± 1.8	8.7 ± 1.8	7 ± 1.9	6.7 ± 2.1	9.5 ± 2.2	-1.8 (-3.2 to -0.4)	0.009	-2.3 (-3.7 to -1)	<0.001
GL _{intermediate} SWV, m/s	7.8 ± 2.1	8.3 ± 1.9	9.2 ± 2	7.2 ± 2.1	7.5 ± 2.2	9.4 ± 1.8	-1.0 (-2.1 to 0.1)	0.109	-1.2 (-2.2 to -0.1)	0.028
GL _{distal} SWV, m/s	8.6 ± 1.9	9 ± 2	9.8 ± 2.1	8.1 ± 1.9	7.9 ± 2	10 ± 2.2	-1 (-2 to 0)	0.059	-1.5 (-2.5 to -0.4)	0.003
Soleus _{proximal} SWV, m/s	3.6 ± 1.1	3.9 ± 0.9	4.1 ± 1.4	3.0 ± 0.8	3.6 ± 0.8	4.1 ± 1.1	-0.8 (-1.3 to -0.2)	0.004	-0.3 (-0.9 to 0.2)	0.372
Soleus _{distal} SWV, m/s	3.8 ± 0.9	4.4 ± 1	4.5 ± 1.3	3.8 ± 1.1	3.8 ± 0.8	4.7 ± 1.1	-0.6 (-1.5 to 0.2)	0.198	-0.8 (-1.6 to 0)	0.037
<i>Sciatic nerve stiffness</i>										
Sciatic _{proximal} SWV, m/s	6.1 ± 1.5	6.2 ± 0.8	6.7 ± 2	4.9 ± 1.3	6.4 ± 0.8	7.1 ± 1.9	-1.8 (-2.4 to -1.2)	<0.001	-0.4 (-0.9 to 0.2)	0.439
Sciatic _{distal} SWV, m/s	7.2 ± 1.7	7.2 ± 1.2	8.1 ± 1.4	5.3 ± 1.5	7.2 ± 1.1	8.4 ± 1.5	-2.4 (-3.2 to -1.6)	<0.001	-0.6 (-1.3 to 0.2)	0.235
Tibial SWV, m/s	7.3 ± 1.4	7.7 ± 1.6	9 ± 1.5	6.3 ± 1.3	8 ± 1.5	9.6 ± 1.2	-2.2 (-3.1 to -1.3)	<0.001	-0.7 (-1.6 to 0.1)	0.127
<i>Mechanical properties at ankle joint level</i>										
Ankle torque, HIP-neutral, N-m	29 ± 14.4	28 ± 12.6	33.7 ± 13.8	27.6 ± 13.7	23.7 ± 13.3	36 ± 16.9	-3.9 (-9.1 to 1.2)	0.191	-6.8 (-11.9 to -1.7)	0.005
Ankle torque, HIP-flexed, N-m	20.2 ± 9.1	17.2 ± 11	15.3 ± 8.7	20.8 ± 8.9	19.8 ± 10.2	16.5 ± 7.4	0.7 (-3.8 to 5.2)	1	1.9 (-2.5 to 6.2)	0.897
<i>Muscle architecture</i>										
GM fascicle length, cm	6.2 ± 0.9	6.1 ± 1	5.9 ± 0.8	6.2 ± 0.8	6.6 ± 0.9	6 ± 0.8	0 (-0.3 to 0.4)	1	0.4 (0.1 to 0.8)	0.017
GL fascicle length, cm	7.3 ± 1.3	7.5 ± 1.4	7.1 ± 1	7.1 ± 1.4	7.9 ± 1.5	7.1 ± 1.1	-0.1 (-0.7 to 0.4)	1	0.4 (-0.1 to 0.9)	0.220
GM thickness, cm	1.7 ± 0.2	1.6 ± 0.3	1.5 ± 0.2	1.7 ± 0.2	1.7 ± 0.2	1.6 ± 0.1	0 (-0.1 to 0.1)	1	0.1 (0 to 0.2)	0.196
GL thickness, cm	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.1	0 (-0.1 to 0.1)	1	0.1 (0 to 0.2)	0.078
<i>Sciatic nerve size</i>										
Sciatic _{proximal} CSA, cm ²	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0 (0 to 0)	1	0 (0 to 0)	0.932
Sciatic _{distal} CSA, cm ²	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0 (0 to 0)	1	0 (-0.1 to 0)	0.442
Tibial CSA, cm ²	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0 (0 to 0)	1	0 (0 to 0)	1
Sciatic _{proximal} thickness, cm	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0	0 (0 to 0)	0.972	0 (0 to 0)	1
Sciatic _{distal} thickness, cm	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0 (-0.1 to 0)	0.273	0 (-0.1 to 0.1)	1
Tibial thickness, cm	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0 (-0.1 to 0)	0.365	0 (-0.1 to 0)	0.299
<i>Myoelectrical activity (RMS EMG)</i>										
GM, %	0.9 ± 0.9	1 ± 1.3	0.6 ± 0.4	0.8 ± 1.2	0.8 ± 0.9	0.6 ± 0.4	0.2 (-0.6 to 0.9)	1	0.1 (-0.6 to 0.8)	1
Soleus, %	0.9 ± 1	1.1 ± 1.4	0.8 ± 0.8	0.7 ± 0.4	0.8 ± 1	0.9 ± 0.8	-0.2 (-0.7 to 0.2)	0.650	-0.3 (-0.7 to 0.2)	0.502

Table shows mean between-group differences, adjusted for baseline values, with 95% confidence intervals (95% CI). CSA, cross-sectional area; EMG, electromyography; HIP-flexed, hip flexed at 90°; HIP-neutral, hip neutral; GL, gastrocnemius lateralis; GM, gastrocnemius medialis; RMS, root mean squared; ROM, range of motion; SWV, shear wave velocity. Bold values indicate $P < 0.05$.

ankle torque [$F(2,54) = 3.7, P = 0.032, \rho\eta^2 = 0.12$]. Post hoc analysis revealed a significant increase in dorsiflexion ROM of 3.3° (95% CI: 0.1–6.4; $P = 0.037$) in the muscle-directed group compared with the control group. There were no statistical significant group effects on maximal dorsiflexion ROM at the same ankle torque for the HIP-flexion condition [$F(2,54) = 18.9, P = 0.649, \rho\eta^2 = 0.016$].

Muscle shear wave velocity. There was a significant main effect of group in triceps surae shear wave velocity for all assessed muscle locations with moderate to large effects sizes: GM_{proximal} [$F(2,54) = 5.9, P = 0.005, \rho\eta^2 = 0.18$], GM_{intermediate} [$F(2,54) = 12.4, P < 0.001, \rho\eta^2 = 0.32$], GM_{distal} [$F(2,54) = 7, P = 0.002, \rho\eta^2 = 0.21$], GL_{proximal} [$F(2,54) = 9.3, P < 0.001, \rho\eta^2 = 0.26$], GL_{intermediate} [$F(2,54) = 3.9, P = 0.026, \rho\eta^2 = 0.13$], GL_{distal} [$F(2,54) = 6.3, P = 0.003, \rho\eta^2 = 0.19$], soleus_{proximal} [$F(2,54) = 5.9, P = 0.005, \rho\eta^2 = 0.18$], and soleus_{distal} [$F(2,54) = 3.5, P = 0.036, \rho\eta^2 = 0.12$]. Overall, partici-

pants receiving muscle-directed intervention showed significant decreased triceps surae shear wave velocity (at all measured muscle regions with the exception of soleus_{proximal}) compared with those in the control group (all $P < 0.037$). There were no significant mean differences in shear wave velocity of triceps surae in participants receiving nerve-directed intervention compared with those allocated to the control group, with the exception of GL_{proximal} ($P = 0.009$) and soleus_{proximal} ($P = 0.004$). Figure 5A depicts the between-group differences with individual data points per intervention group for GM_{intermediate} and GL_{intermediate}. Supplemental Figs. S1–S3 (all Supplemental material is available at <https://doi.org/10.6084/m9.figshare.7963517>) describe the pre- to postintervention relationships between the ankle angle and the muscles' shear wave velocity for each intervention.

Sciatic nerve shear wave velocity. Significant main effects for group, with large effects sizes, were observed for all sciatic nerve locations: sciatic_{proximal} [$F(2,54) = 31.3, P < 0.001, \rho\eta^2 = 0.54$],

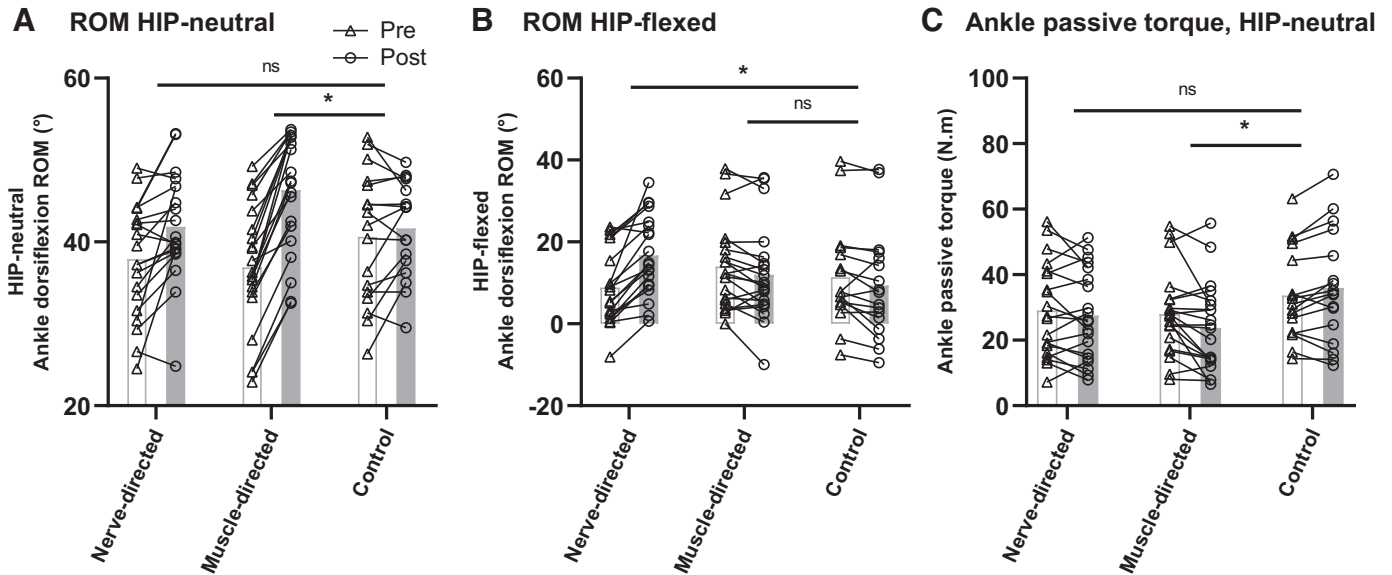


Fig. 4. Effects of interventions on maximal ankle dorsiflexion range of motion (ROM) assessed in both hip neutral (HIP-neutral; A) and hip flexed at 90° (HIP-flexed; B) positions. A: in the HIP-neutral condition, data showed a significant increase in ankle dorsiflexion ROM for the muscle-directed group compared with the control group. B: in the HIP-flexed condition, participants in the nerve-directed group showed a significant increase in ankle dorsiflexion ROM compared with the control group; positive and negative values correspond to ankle dorsiflexion and plantar flexion angles, respectively. C: effects of stretching intervention on ankle passive torque assessed in HIP-neutral. Compared with the control group, the ankle passive torque significantly decreased in the muscle-directed group (* $P = 0.005$). The lines denote individual data. Δ , individual data point at baseline (pretesting); \circ , individual data point after the 12-wk intervention (posttesting). White and gray bars represent averaged ROM for each group before and after the intervention, respectively. * $P < 0.001$; ^{ns} $P > 0.05$.

sciatic_{distal} [$F(2,54) = 35.6$, $P < 0.001$, $\rho\eta^2 = 0.57$], and tibial [$F(2,54) = 21.4$, $P < 0.001$, $\rho\eta^2 = 0.44$]. Post hoc analysis showed that the nerve-directed group exhibited significantly decreased shear wave velocity at all nerve locations (all $P < 0.001$) compared with the control group (Fig. 6), whereas nerve shear wave velocity did not differ between the muscle-directed and control groups (all $P > 0.127$). Supplemental Figs. S5–S7 describe the pre- to postintervention relationships between the ankle angle and the nerve shear wave velocity for each group.

Ankle passive torque. There was a significant main effect of group on the ankle passive torque with a large effect size for the HIP-neutral condition [$F(2,54) = 5.4$, $P = 0.007$, $\rho\eta^2 = 0.17$]. Compared with the control group, post hoc analysis showed that the ankle passive torque significantly decreased in the muscle-directed group with a mean difference of -6.8 N·m (95% CI: -11.9 – -1.7 ; $P = 0.005$; Fig. 4C). There was no evidence of a significant nerve-directed intervention effect over the control group in ankle passive torque ($P = 0.191$).

There were no statistically significant group effects on ankle passive for the HIP-flexed position [$F(2,54) = 0.6$, $P = 0.562$, $\rho\eta^2 = 0.021$].

Supplemental Fig. S4 depicts the pre- to postintervention relationships between the ankle angle and the passive torque for each group and hip condition.

Muscle architecture. There were significant main effects of group in fascicle length observed for the fascicle length of both GM [$F(2,54) = 5.5$, $P = 0.006$, $\rho\eta^2 = 0.17$] and GL [$F(2,54) = 3.6$, $P = 0.034$, $\rho\eta^2 = 0.12$]. Compared with the control group, post hoc analysis showed a significant increase in GM fascicle length of 0.4 cm (95% CI: 0.1–0.8; $P = 0.017$) after the muscle-directed program (Fig. 5B), whereas no sta-

tistically significant mean differences were detected between control and nerve-directed groups ($P = 1$). There was no significant changes in fascicle length of GL following the muscle-directed intervention [0.4 cm (95% CI: -0.1 – 0.9 ; $P = 0.220$)]. When compared with the control group, there were no significant effects of muscle-directed and nerve-directed interventions on gastrocnemii thickness.

Nerve size. There were no statistically significant group effects on nerve cross-sectional and thickness measures for all nerve locations ($P > 0.05$), indicating that sciatic nerve size was not affected by the nerve-directed and muscle-directed long-term interventions.

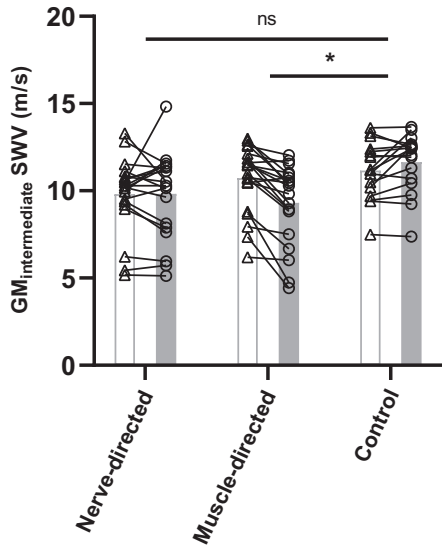
RMS EMG. Soleus and GM RMS EMG were analyzed to ensure that the effects of the intervention could be attributed to changes in passive mechanical properties. There was no significant main effect of group for both muscles. The average RMS EMG was $\sim 1\%$ for both muscles across testing sessions and experimental groups (Table 2). These results suggest that muscle activity levels did not significantly change after the intervention.

DISCUSSION

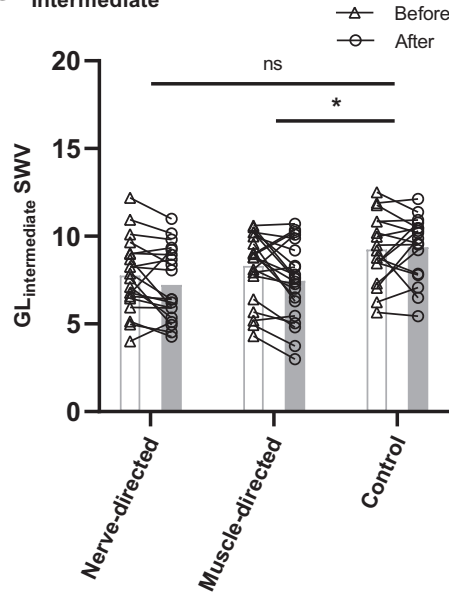
The results of this trial revealed that muscle-directed and nerve-directed 12-wk stretching interventions were an effective way to 1) improve maximal ankle dorsiflexion ROM and 2) induce chronic adaptations in the passive mechanical properties of either muscle or nerve tissues. The trial provides evidence that the nature of the intervention (muscle-directed or nerve-directed) determined whether the mechanical changes occurred at the nerve, muscle, or joint elements. Whereas participants in the nerve-directed group substantially improved maximal dorsiflexion ROM in HIP-flexed and had a significant

A Muscle shear wave velocity

GM_{Intermediate}

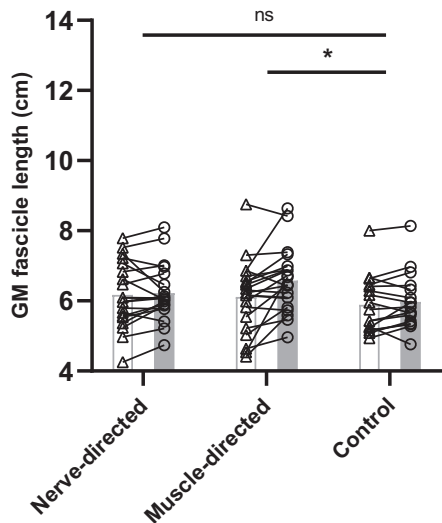


GL_{Intermediate}



B Fascicle length

GM_{Intermediate}



GL_{Intermediate}

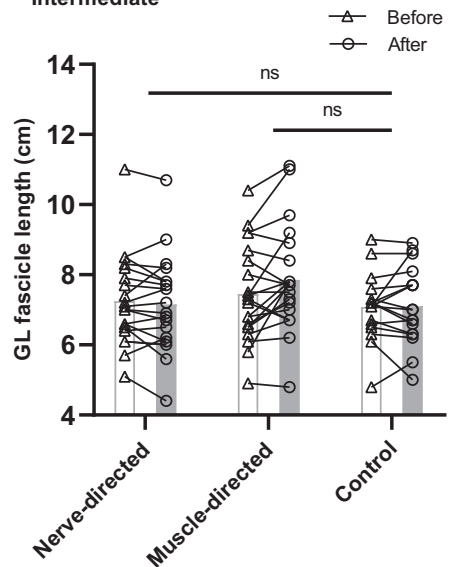


Fig. 5. A: effects of interventions on muscle shear wave velocity (SWV) of the intermediate regions of both gastrocnemius medialis (GM_{intermediate}) and gastrocnemius lateralis (GL_{intermediate}). Compared with the control group, participants receiving the muscle-directed intervention showed a significant decrease in SWV of GM_{intermediate} (* $P < 0.001$) and GL_{intermediate} (** $P = 0.028$). B: effects of stretching interventions on fascicle length of GM_{intermediate} and GL_{intermediate}. Compared with the control group, data show a significant increase of GM_{intermediate} fascicle length following the muscle-directed program (** $P = 0.017$). The lines denote individual data. Δ , individual data point at baseline (pretesting); \circ , individual data point after the 12-wk intervention (posttesting). White and gray bars represent averaged range of motion for each group before and after the intervention, respectively. $^{ns}P > 0.05$.

decrease in sciatic shear wave velocity (an estimate of stiffness), the muscle-directed group increased dorsiflexion ROM in HIP-neutral and exhibited an overall reduction in plantar flexors' shear wave velocity. These findings were in line with the concept that adaptations in mechanical properties are specific to the tissue that is preferentially loaded by a particular intervention.

The maximal dorsiflexion ankle ROM improved in both nerve-directed and muscle-directed groups after the 12-wk training protocols (Table 2). These results are in line with most of the studies examining the long-term effects of stretching interventions on joint mobility in both healthy [e.g., reviewed in (6, 21, 62)] and clinical cohorts (45, 46), but not in reviews of clinical cohorts (33). An interesting and novel finding was

that the muscle-directed group significantly improved the maximal ROM assessed in HIP-neutral by 7.3° (95% CI: 4.1–10.5), but not in HIP-flexed. Conversely, the sciatic nerve-directed intervention elicited a significant increase of 9.9° (95% CI: 6.2–13.6) in maximal ROM assessed in HIP-flexed in the absence of a significant effect in the HIP-neutral position. Together, these results indicate that improvements in joint motion are specific to the limb configuration (i.e., positioning of joints) used during a given stretching exercise. This suggests that stretching effects are dependent on the main structures being loaded during the exercise, which may be predominately of muscular or nonmuscular soft tissue types. The present findings support this rationale, which has been discussed in previous literature (4, 40, 43).

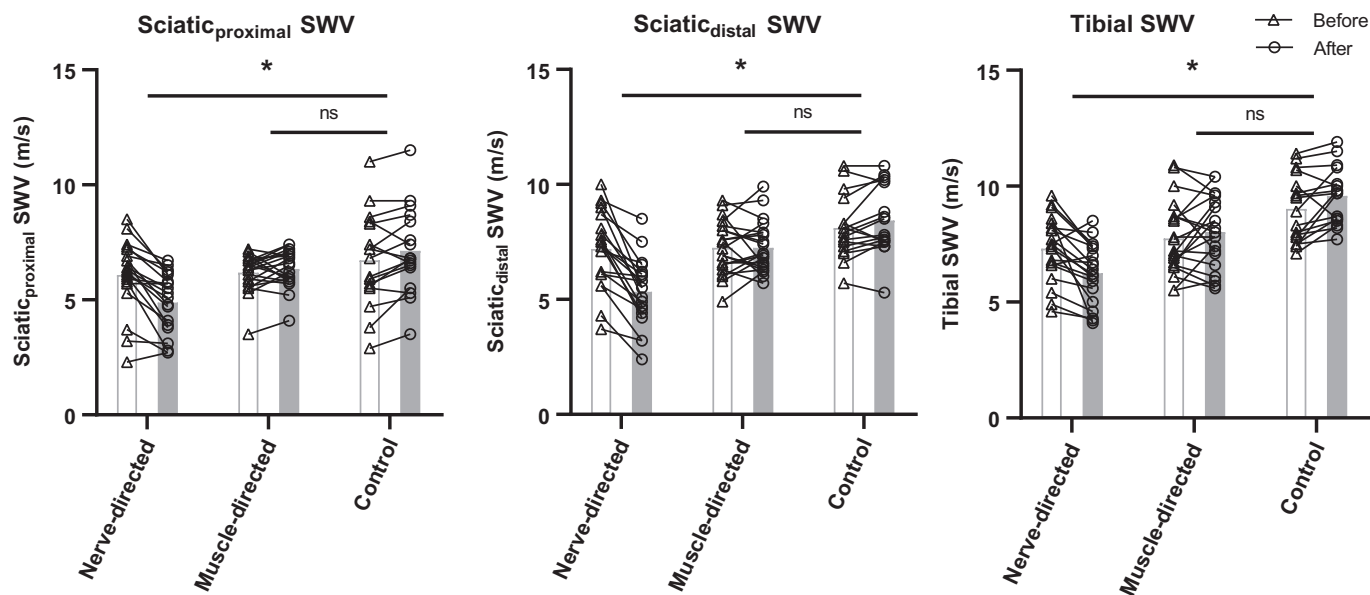


Fig. 6. Effects of the interventions on shear wave velocity (SWV) of the sciatic nerve tract. Compared with the control group, data show that the nerve-directed group exhibited significantly decreased SWV following the intervention at sciatic_{proximal} (A), sciatic_{distal} (B), and tibial (C) nerve regions. The lines denote individual data. Δ , individual data point at baseline (pretesting); \circ , individual data point after the 12-wk intervention (posttesting). White and gray bars represent averaged range of motion for each group before and after the intervention, respectively. * $P < 0.001$; ^{ns} $P > 0.05$.

It should be noted that a modification in stretch tolerance has also been suggested as a potential mechanism for increasing maximal ROM following chronic stretching and cannot be totally excluded from our findings (6, 21, 54, 59). Because several studies have reported improvements in variables such as range of motion in the absence of mechanical adaptations at the joint level, chronic changes associated with long-term stretching have most often been explained by an alteration of sensation, which is referred to as the “sensory” theory in the literature (21, 59). It is thought that stretching of either skeletal muscles or peripheral nerves can modify their mechanosensitivity threshold (10, 38), which could in turn provoke changes in the stretch amplitude by increasing the tolerance for a sensation (e.g., onset of pain).

Our results related to the different testing positions (i.e., hip flexed and hip neutral) align with previous studies (3, 4, 40), which have shown that maximal ankle dorsiflexion ROM assessed in HIP-flexed position is decreased (mean differences ~ 18 – 22°) when compared with that measured at HIP-neutral. Such findings have suggested that whereas the maximal dorsiflexion ROM may be primarily influenced by the passive stretch of plantar flexor muscle-tendon units in HIP-neutral (23, 43, 59), nonmuscular structures spanning multiple joints can potentially influence the dorsiflexion stretch amplitude in HIP-flexed position (3, 43). Indeed, some stretching exercises or postures have been shown to load the sciatic nerve tract independently of surrounding mechanical interfaces such as muscle-tendon units (3, 13, 14, 24).

Studies evaluating the effects of chronic stretching on mechanical properties of muscle-tendon units have traditionally implemented stretching protocols shorter than 8 wk (59), and often they did not observe mechanical adaptations (21). The present trial examined the effects of stretching over a longer intervention period (12 wk). Interestingly, the muscle-directed group showed changes in the mechanical properties of the

plantar flexor muscles, which can potentially explain the mechanical adaptations also observed at a joint level. The decrease in ankle passive torque may reflect the stiffness decrease that occurred within the main plantar flexor muscles units acting across this joint (23, 37). In contrast, we observed a nerve stiffness decrease accompanied with a lack of changes in ankle passive torque in the nerve-directed group. This finding provides additional evidence that nonmuscular structures may contribute only marginally to global torque measurements (43).

Participants in the muscle-directed group showed a decrease in triceps surae stiffness following intervention compared with the control group. These results are in line with previous findings reporting reductions in the shear modulus of hamstring (30) and plantar flexor (2) muscle groups. Interestingly, our results revealed that triceps surae (with the exception of soleus_{proximal}) exhibited significant stiffness adaptations across all tested muscles and regions within individual muscles. The magnitude of these changes differed substantially between triceps surae muscles and across different regions of the same muscle. The decrease in shear wave velocities ranged from 0.8–2.2 m/s. These findings (and variability) have not been possible to observe in vivo using the traditional passive torque-angle procedures. They highlight the importance of using localized approaches, such as shear wave elastography, to better quantify the effects of stretching on mechanical properties of musculoskeletal structures.

The muscle stiffness adaptations to stretching may be at least partly explained by a greater fascicle length (59). We observed a significant increase in fascicle length of GM following the muscle-directed intervention, in the absence of significant changes in muscle thickness. A tendency for an increase in fascicle length (albeit not significant) was also observed on the GL. This finding is consistent with previous studies that utilized ultrasound imaging to assess the muscle architectural adaptations to long-term exercise programs, such as passive

stretching (22, 46), and eccentric muscle actions (7) in both healthy and clinical populations. The increase in fascicle length is also in line with studies involving animals subjected to prolonged immobilization in a lengthened position [e.g., (56, 60, 61)]. Evidence from such studies suggests that increased fascicle length is related to a greater number of sarcomeres in series, which occurs at the ends of the muscle fibers (56). The changes in sarcomere number are likely dependent on the muscle length used during the immobilization and the time of immobilization (56) rather than the active muscle tension (16).

Another important finding was that the sciatic nerve adapted mechanically to the nerve-directed intervention (decrease in shear wave velocity >1.8 m/s for all assessed locations). Our results also demonstrate that nerve size parameters, including cross-sectional area and thickness, were unaffected by the intervention. From a technical perspective, as changes in these parameters could influence the shear wave velocity independently of any change in mechanical properties (11), the absence of adaptations in nerve thickness and cross-sectional area provides evidence that the change in shear wave velocity was most likely related to actual nerve stiffness changes. Where those stiffness changes might be occurring can only be speculated. Most of the mechanical resistance of peripheral nerves to loading is provided by three well-organized layers of connective tissue (endoneurium, perineurium, and the epineurium) (57). Among these, the perineurium is considered to be the primary load-bearing structure of the nerve to stretching in the longitudinal axis (49, 57), and hence it is most likely to be affected by our intervention. It has been reported that peripheral nerves can adapt in length when subjected to loads following limb lengthening procedures (12). Indeed, recent studies involving animals have shown that moderate tensile loads (under physiological limits) can accelerate both nerve growth capacity and rate of elongation (1, 36), without impaired function (52). Consistent with such findings and in line with our results, in a nerve repair experiment Bora et al. (9) showed that although there was an initial increase in nerve tension immediately after excising a segment, the nerve returned to its uncut state of tension after seven weeks, with increased compliance within neural connective tissue observed. Additionally, the stiffness adaptations may also be related to changes in neural collagen fibril type and density (39) and in the meso-epi-perineurium connections (55). However, such evidence is either indirect or based on studies with animals, whose growth and adaptation patterns differ from humans.

From a clinical standpoint, the concept of structural differentiation is an essential part of neurodynamics testing, which has been largely used by health professionals (e.g., physical therapists) to make a distinction between the involvement of neural and nonneural structures in nerve disorders (57). Evidence for neurodynamics is supported by studies involving animals and human cadavers, which have demonstrated that nerves can be tensioned mechanically while they are elongated in a longitudinal direction. This is possible by combining movements of one or several joints that induce displacement of the nerve endings in opposite directions (51, 57). Our results are of importance to human movement and health fields (e.g., orthopedic and physical therapy) because they clearly show, for the first time, that it is possible to reduce nerve stiffness and improve joint ROM. Such interventions could be particularly beneficial in neuromusculoskeletal disorders that induce local-

ized mechanical dysfunction, including increased local nerve stiffness in conjunction with impaired maximal joint ROM (e.g., carpal tunnel syndrome in upper limb or disk pathology within the lumbar spine) (17, 32, 42). In many instances, however, it is difficult to determine whether nerves or fascia may be responsible for limiting stretch amplitude (43). Indeed, the fascial system and the main peripheral nerves in the lower limb have similar biomechanical behavior when under load. They act mechanically as a continuum, spanning joints of axial and appendicular human body segments (53, 63). For instance, the displacement of the GM deep fascia has been shown to be highly correlated with pelvic motion (i.e., forward tilting) confirming, *in vivo*, a myofascial connectivity between the trunk and lower leg (15), as reported in some anatomical studies (53). Therefore, it is reasonable to assume that the nerve-directed exercises could also influence the fascia of the leg (i.e., crural fascia), contributing to the mechanical changes observed in muscle and nerves (63). Further research is needed to clarify the role of fascial tissue in joint function and its adaptations to mechanical stretching stimuli.

We acknowledge the following study limitations. First, the sample comprised healthy young adults, so the findings cannot be generalized to older aged groups where architecture and tissue mechanical properties are likely to be different. Second, a small eccentric active lengthening component during the stretching intervention (i.e., heel-drop phase) cannot be totally excluded for the muscle-directed group. We did not measure the muscle activity during the stretching sessions. However, the time duration for this muscle activity is small compared with the time spent in the fully loaded position of the stretch. Participants were also asked to maintain a totally relaxed state in their calf muscles during the stretch. Additionally, we ensured that minimal muscle activation was present during the ankle rotations performed on the pre- and posttesting sessions (18, 34). Specifically, EMG RMS of GM and soleus was lower than 2%, and we did not observe an intervention effect on this variable. Thus, involuntary muscle activity is unlikely to have biased the primary outcome measures and ankle passive torque. Finally, fascicle length was derived from a linear extrapolation method. This process is prone to error, as fascicle and aponeurosis curvature may exist and muscle architecture is not homogeneous throughout the muscle (58). However, gastrocnemii fascicles present a relatively linear arrangement, and the measurement error associated with the fascicles' curvature has been demonstrated to be negligible in resting GM muscle (41). Furthermore, typical gastrocnemii fascicle length is approximately the size of the (linear array) transducer used for the assessments; i.e., 5 cm. A further analysis of our data indicated that the amount of extrapolation was $13.2\% \pm 10.9\%$ and $26.4\% \pm 13.6\%$ for the GM and GL, respectively. Additionally, we ensured that the B-mode pre- and postintervention images were performed in the same muscle region, with minimal pressure and the same positioning of subjects.

Conclusions

Our findings showed that in healthy adults it is possible to target and induce chronic changes in the mechanical properties of nerve or muscular structures through specific stretching exercises. These tissue adaptations were associated with improvements in maximal joint ROM. Additionally, this study

demonstrates for the first time that human peripheral nerves can adapt chronically to mechanical loading. These findings provide new perspectives for understanding chronic effects of stretching interventions and have relevance to clinical conditions where a shortening of soft tissue structures has occurred.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.J.A., S.R.F., F.H., G.L.S., L.L., R.G., P.M., and A.N. conceived and designed research; R.J.A., G.L.S., and J.-B.Q. performed experiments; R.J.A., S.R.F., J.-B.Q., and A.N. analyzed data; R.J.A., S.R.F., F.H., G.L.S., L.L., R.G., J.-B.Q., P.M., and A.N. interpreted results of experiments; R.J.A. prepared figures; R.J.A. drafted manuscript; R.J.A., S.R.F., F.H., G.L.S., L.L., R.G., J.-B.Q., P.M., and A.N. edited and revised manuscript; R.J.A., S.R.F., F.H., G.L.S., L.L., R.G., J.-B.Q., P.M., and A.N. approved final version of manuscript.

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