Effect of Stretching on Thoracolumbar Fascia Injury and Movement Restriction in a Porcine Model

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Objective: Stretching of fascia is an important component of manual and movement therapies. We previously showed that in pigs, a unilateral thoracolumbar fascia injury combined with movement restriction (hobble) produced contralateral loss of fascia mobility (shear strain during passive trunk flexion measured with ultrasound) similar to findings in human subjects with chronic low back pain. We now tested whether such abnormalities could be reversed by removing the hobble with or without daily stretching for 1 mo.

Design: Thirty pigs were randomized to control, injury, or injury + hobble for 8 wks. The hobble restricted hip extension ipsilateral to the injury. At week 8, the injury + hobble group was subdivided into continued hobble, removed hobble, and removed hobble + stretching (passively extending the hip for 10 min daily).

Results: Removing hobbles restored normal gait speed but did not restore fascia mobility. Daily passive stretching was not superior to removing hobbles, as there was no significant improvement in fascia mobility with either treatment group (removed hobble or stretching).

Conclusions: Reduced fascia mobility in response to injury and movement restriction worsens over time and persists even when movement is restored. Reversing fascia abnormalities may require either longer than 1 mo or a different treatment "dose" or modality.

Key Words: Fascia, Connective Tissue, Stretching, Manual Therapy, Movement Therapy, Rehabilitation, Back Pain

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For the past few decades, there has been a growing interest in the role of fascia in the mechanism of manual and movement-based therapies.[1–6] However, to know whether manual and/or movement therapies impact fascia, it is necessary to first identify that a pathological abnormality of fascia can be measured quantitatively and then test whether this can be improved by the therapy. As a first step in investigating this question, our research group focused on the thoracolumbar fascia, which is an important connective tissue structure in the back that forms a biomechanical link between the shoulders and the pelvis.[1] We first conducted cross-sectional studies in humans to determine whether any abnormality of the thoracolumbar fascia could be detected in human subjects with chronic low back pain. Using ultrasound, we showed that human subjects with chronic low back pain of more than 12-mo duration have increased thickness and decreased shear plane mobility (shear strain) of the thoracolumbar fascia.[7,8] We hypothesized that this pathology was a result of an initial injury of fascia (e.g., "sprain" or "strain") followed by reduced mobility and/or altered movement patterns due to tissue stiffness, pain, or fear of pain.[9] In this model, ongoing pain contributes to the persistence of chronic inflammation, which leads to fibrosis, connective tissue adhesions, and reduction in shear plane mobility. A previous study showed in rodent models that stretching of the back for 10 min once a day has both anti-inflammatory and antifibrotic effects.[10–13] In a murine model of scleroderma, ultrasound was more sensitive than histology in detecting tissue pathology in both the inflammatory and fibrotic phases of the disease.[13] We therefore hypothesized that (1) an animal model combining an initial injury followed by movement restriction of the back would produce abnormalities of the thoracolumbar fascia similar to those observed in humans with low back pain and (2) restoring movement and stretching would reverse these abnormalities. In an initial study, we developed a porcine model in which an initial injury of fascia was combined with movement restriction of the back achieved by connecting a unilateral leg strap to a chest harness, herein referred to as a "hobble."[14] This allowed the pigs to walk, but restricted full hip extension and pelvic side-bend, and thus decreased the stretching of the thoracolumbar fascia that normally occurs during gait. We found that after 8 wks, the injury + hobble group had both increased thoracolumbar fascia thickness and reduced mobility (shear strain) during passive trunk flexion compared with controls.[14] In the current study, we tested whether these abnormalities could be reversed by removing the hobbles for 1 mo or with the addition of daily stretching.

METHODS

Animals and Experimental Design

Experimental protocols were approved by the University of Vermont Institutional Animal Care and Use Committee. Castrated male domestic swine ($n = 20$) (4–6 wks old, 4–7 kg)

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were acquired from E.M. Parson's, Hadley, Massachusetts. Pigs were group housed in 8 × 8-ft pens at the University of Vermont animal facility on a 12-hr light-dark cycle, and food intake was adjusted to gain approximately 1 lb/day. Animals were trained to stand on scales without restraint to monitor weight weekly. All pigs were let out of their pens and encouraged to walk freely for 15 min twice a day. A stabilization period of 7 days was allowed before subsequent randomization (week 0) into one of following three groups: (1) control (C) (n = 6), (2) injury alone (I) (n = 6), and (3) injury plus hobble (I + H) (n = 18). At the end of week 8, the I + H group was further subdivided into the following three groups (n = 6 per group) for an additional 4 wks: continued hobble (I + H), removed hobble (RH), and RH plus stretching (RH + S).

Study Interventions

Fascia Injury

At week 0, pigs in the injury groups underwent a unilateral fascia injury in the dorsal trunk (side randomized) at the L3–4 vertebral level, 2 cm from the midline as previously described.[14] In brief, anesthesia was induced by intramuscular injection of ketamine (20 mg/kg) and atropine (0.05 mg/kg) followed by 4% isoflurane inhalation. Pigs were shaved and surgically prepared with betadine scrub and isopropyl alcohol. A 4-cm longitudinal skin incision was made 2.0 cm lateral to midline. Target depth of incision was the deep subcutaneous tissue layer between the subcutaneous membranous layer and the perimuscular fascia stopping at, but not incising, the perimuscular fascia. Blunt and microsurgical dissection tools were used to detach the perimuscular fascia from the adjacent deep subcutaneous tissue, producing a 4 × 4-cm injury centered 2 cm lateral to midline. The incision was closed with five interrupted nylon skin sutures, and the animals were monitored daily. There were no wound infections among any of the groups.

Movement Restriction (Hobble)

Custom nylon hobbles were created in-house as previously described.[14] At week 0, a conventional dog harness was fitted and a nylon cuff was placed on one hind limb (side randomized), which was connected to the chest harness by interchangeable links that allow for a custom fit. With proper adjustment, the hobble device restricted hind-limb positioning so that the standing distance between forelimb and hind limb was approximately two thirds the distance of an unrestrained animal. The hobble restricted hip extension during the gait cycle. Additional adjustments to the hobbles were made accordingly as the animals increased in size to maintain the restricted hind-limb positioning. Hobbles were kept in place for 8 or 12 wks. Pigs underwent daily inspection for any sign of chafing, and hobbles were adjusted accordingly. When in place, the hobble prevented full hip extension and pelvic lateral flexion in the transverse plane during gait.

Fascia Injury and Movement Restriction

At week 0, pigs randomized to I + H underwent the perimuscular microsurgical technique described previously. On the day after the surgical procedure, a hobble device was fitted on the same side as the injury.

No Intervention

Animals in the C group were fitted with a harness but not an ankle cuff and did not receive any surgical intervention or movement restriction.

Stretching

Pigs randomized to stretching were habituated to be placed in a Panepinto sling during the course of 4 wks preceding the onset of stretching. Once a day, pigs were placed and suspended in the sling. Both hind legs of the pig were then pulled gently into extension such that the hind legs were 25–45 degrees from the horizontal. In this position, the shoulders are held in a fixed position by the armholes of the sling, and thus passively extending the hind-limbs pulls on the thoracolumbar fascia as well as the hip. This position was maintained for up to 10 min as tolerated. Most pigs were able to relax and quiet down in this position, with some animals briefly falling asleep. Stretching sessions were terminated early if a pig struggled or vocalized to express discomfort.

Measurement Methods

Gait Analysis

Before fascia injury, pigs were trained to walk on a rubber mat alongside plastic tubing with space markers. Hobbles were removed (if present) during training and data collection. At week 8 and 12, 7 trials per pig were videotaped. Videos were downloaded and analyzed using VideoPad Video Editor Version 2.41. Gait speed was calculated by dividing the walking distance by the time duration between the pig's first foot entering past the first marker and the last foot passing the final marker.

Ultrasound Data Acquisition

All ultrasound imaging was performed immediately after euthanasia by intravenous lethal injection of Fatal Plus (100 mg/kg) to prevent respiration artifacts during imaging. Pigs were placed prone on a surgical table. Ultrasound imaging was performed as previously described[14] with a Terason 3000 (Terason, Burlington, MA) scanner with a 4.0-mm, 10-MHz linear array transducer. Ultrasound images were acquired bilaterally at the L3–4 level with the ultrasound probe oriented transversely, and the edge of the probe aligned with the lateral border of the vertebral body. To measure tissue displacement within the connective tissue layers of the thoracolumbar fascia, ultrasound cine-recordings were acquired during passive flexion of the trunk. After the ultrasound image acquisition described previously, the pigs were repositioned so that the L4 level was at the edge of the table. The transducer was placed longitudinally on the side of the dorsal trunk contralateral to the intervention (injury and/or hobble, with side randomized in control animals) at the level of the L3/L4 interspace, 2 cm from midline. A cine-loop (25-Hz frame rate) was captured for a 10-sec period, while the hips of the pig were manually flexed 90 degrees and returned to neutral position for 5 cycles at 0.5 Hz.

Ultrasound Data Analysis

Measurement of Thoracolumbar Fascia Shear Strain

Ultrasound radio frequency data were captured and analyzed on the nonintervention side during the third flexion/

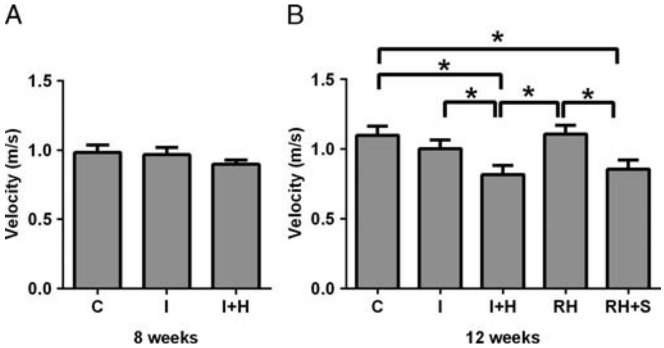

FIGURE 1. *A,* Gait velocity measurements at week 8 in control (C), injury (I) or injury plus hobble (I + H) for 8 weeks. *B,* Gait measurements at week 12 in control (C), injury (I), injury plus hobble (I + H), removed hobble (RH), and removed hobble plus stretching (RH + S) groups. Significant pairwise differences were present at week 12 but not week 8 (*$P < 0.05$).

extension cycle as previously described.[8] Subsequent analysis was conducted using an in-house program written in MATLAB (2010a; The MathWorks, Natick, MA). A 10 × 20-mm region of interest encompassing the dermis, subcutaneous fat, fasciae layers, and muscle was identified. Shear strain was calculated in ten half-millimeter increments using a moving window starting 1 mm deep to the muscle boundary. The average shear strain was calculated among all the window positions, and the average value was used for subsequent statistical analysis.

Tissue Thickness Measurements

Ultrasound images on the injury side were visually inspected to ensure consistency of intervention. All quantitative ultrasound measurements were performed on the side contralateral to the intervention (hobble and/or injury). This allowed examination of connective tissue remodeling away from the injury itself, with and without movement restriction. In control animals, the measured side was randomized. Ultrasound images were measured using a custom software Matlab program. In all images, the thickness of tissue layers was measured 2 cm from the lateral border of the vertebral body by identifying the deep aspect of the dermis and the superficial edge of the muscle as previously described.[14]

Statistical Analyses

One-way analyses of variance were used to compare mean gait speed, shear strain, and tissue thickness among the three treatment conditions at the 8-wk assessment. Similarly, analyses of variance were used to compare outcome measures at 12 wks across the five treatment conditions. Fisher's least significant difference procedure was used to perform pairwise comparisons among treatment means. All statistical analyses were conducted using SAS Version 9.4 (SAS Institute, Cary, NC).

RESULTS

Week 8

The first 8 wks of this experiment was a partial replicate of our previous study, i.e., a comparison of I + H compared with C and I. Although we did not find significant differences between groups for gait speed (Fig. 1A, Table 1), we did reproduce our previous results for thoracolumbar shear strain, which were significantly reduced in the I + H group compared with controls (Fig. 2A, Table 1). We also observed a similar trend for increased thoracolumbar thickness in the I + H group as in our previous study, although differences were not statistically significant (Table 1).

At 8 wks, the I + H group was subdivided into the following three groups: I + H for 4 more wks (I + H), RHs for 4 wks (RH), and RHs plus daily stretching (RH + S). At the end of 12 wks, gait speed was similar to controls in the RH group, but not in the stretching group (Fig. 1B, Table 2). Fascia mobility (shear strain) decreased in all groups from week 8 to week 12, and significant differences remained between the I + H group and controls. The two groups that had their hobbles removed (RH and stretching groups) were not significantly different from the group that remained hobbled for 12 wks (Fig. 2B, Table 2). We also examined change scores for shear strain from 8 to 12 wks. In all groups, shear strain decreased from 8 to 12 wks, consistent with increasing pig age. Although the RH and stretching groups had smaller decreases in shear strain compared

TABLE 1. Week 8 measurements for C, I, and I + H groups

	C	I	I + H	ANOVA
	n = 6	*n* = 6	*n* = 18	*P*
Gait speed, m/sec	0.98 (0.06)	0.97 (0.06)	0.90 (0.06)	0.38
Shear strain, %	59.9 (5.4)	55.1 (5.4)	40.8 (5.4)	0.008
Thickness, cm	0.31 (0.04)	0.32 (0.04)	0.38 (0.04)	0.25

Results are shown as mean (SE).
ANOVA, analysis of variance.

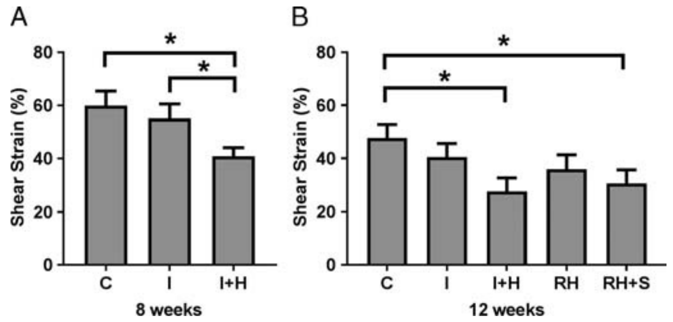

FIGURE 2. *A,* Thoracolumbar fascia shear strain measurements at week 8 in control (C), injury (I) or injury plus hobble (I + H) for 8 weeks. *B,* Shear strain measurements at week 12 in control (C), injury (I), injury plus hobble (I + H), removed hobble (RH), and removed hobble plus stretching (RH + S) groups. Significant pairwise differences were present at week 8 and week 12 (*$P < 0.05$).

with the other groups, there was no evidence that these differences were statistically different across the groups ($P = 0.55$).

Fascia thickness increased overall from week 8 to week 12, but there were no significant differences in thickness between groups at week 12 (Table 2).

DISCUSSION

In this porcine model, although removing hobbles for 1 mo was sufficient to restore normal gait speed, it was not sufficient to restore fascia mobility. This shows that reduced fascia mobility in response to injury and movement restriction is a plastic phenomenon that worsens over time and persists even when movement is restored. Importantly, as in our previous study, all ultrasound measurements were made on the side contralateral to the injury. Thus, observed reductions in shear plane mobility were not due to immediate scarring but rather reflected a more diffuse response of fascia to the injury that was exaggerated by the movement restriction. These results suggest that persistent and extensive involvement of fascia after a minor back injury may cause long-term biomechanical abnormalities in the back.

The passive stretching method used in this study was not superior to simply removing the hobble. The lack of benefit of stretching contrasts with our previous studies in several rodent models in which daily stretching of the back for 10 min reduced both inflammation[11,12] and collagen deposition after an injury.[10] More recently, we showed that daily stretching reduced inflammation and improved subcutaneous tissue mobility in a murine model of scleroderma.[13] Lack of response to stretching in our porcine model may be due to differences in the manner in which stretching was applied to rodents compared with pigs. In rodents, the thoracolumbar fascia was stretched by partially suspending the animals by the tail allowing them to grasp the edge of the table and extend their hind legs, which simultaneously extends the forelimb and hind limb and increases the distance between shoulders and hips, thus directly stretching the thoracolumbar fascia.[10–12] Because this method cannot be applied to pigs, we used a different stretching method that passively extended the hip with the pig suspended in a sling that immobilizes the shoulder through the arm hole of the sling. An important difference between this and our rodent model is that in the pigs, we were not able to simultaneously extend the shoulder because this would have resulted in breathing difficulties because of pressure of the sling on the neck and upper chest. However, because the shoulder was in a fixed position, extending the hip did stretch the thoracolumbar fascia, although not as much as in the rat model where both forelimb and hind limb are extended. It is important to note that although this method focused on stretching the tissues of the hip and lower back whose movement had been previously restricted, it may not have exerted sufficient traction on the thoracolumbar fascia to mimic our rodent model. Thus, it is possible that our stretching method excessively focused on the hip, and a different method that would distribute the stretch throughout the whole back may have been more effective. Another important difference between pigs and rodents is the structure of the connective tissue in the back, where the pig is more similar to humans. In contrast to rodents, both pigs and humans do not have a subcutaneous muscle in the back, and their skin is tethered to subcutaneous and perimuscular fascia layers by retinaculae that renders superficial tissues much less mobile relative to deep muscles.[15,16] It is, therefore, also possible that stretching may engage different tissues layers in these different animal models.

In summary, reduced thoracolumbar fascia mobility measureable with ultrasound and similar to that observed in human

TABLE 2. Week 12 measurements for C, I, I + H, RH, and RH + S groups

	C	I	I + H	RH	RH + S	ANOVA
	n = 6	*n* = 6	*n* = 6	*n* = 6	*n* = 6	*P*
Gait speed, m/sec	1.10 (0.07)	1.00 (0.07)	0.82 (0.07)	1.11 (0.07)	0.86 (0.07)	0.009
Shear strain, %	47.7 (5.1)	40.5 (5.1)	27.7 (5.1)	36.2 (5.1)	28.6 (5.1)	0.06
Thickness, cm	0.44 (0.06)	0.51 (0.06)	0.51 (0.06)	0.47 (0.06)	0.55 (0.06)	0.74

Results are shown as mean (SE).
ANOVA, analysis of variance.

subjects with chronic low back pain was induced in pigs by injury and 8 wks of movement restriction but was not reversed by either restoring movement or the addition of daily stretching for 4 wks. Importantly, we do not conclude that stretching in general is not useful, only that the specific stretching method that we used was not effective. Future studies using this porcine model could examine whether reduced thoracolumbar fascia mobility can be reversed using a different "dose" of stretch (i.e., varying amplitude, duration, frequency) or different stretching methods (active vs. passive). Studies in humans should also examine the relationship between thoracolumbar fascia pathology, pain, and function to determine whether restoring fascia mobility is important in the healing of chronic low back pain.

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