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Non-uniformity in the healthy patellar tendon is greater in males and similar in different age groups

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ABSTRACT

There is increasing evidence that tendons are heterogeneous and take advantage of structural mechanisms to enhance performance and reduce injury. Fascicle-sliding, for example, is used by energy-storing tendons to enable them to undergo large extensions while protecting the fascicles from damage. Reductions in fascicle-sliding capacity may thus predispose certain populations to tendinopathy. Evidence from the Achilles tendon of significant superficial-to-deep non-uniformity that is reduced with age supports this theory. Similar patellar tendon non-uniformity has been observed, but the effects of age and sex have yet to be assessed. Healthy adults (n = 50, 25M/25F) from a broad range of ages (23–80) were recruited and non-uniformity was quantified using ultrasound speckle-tracking during passive knee extension. Significant superficial-to-deep non-uniformity and proximal/distal variations were observed. No effect of age was found, but males exhibited significantly greater non-uniformity than females ($p < 0.05$). The results contrast with previous findings in the Achilles tendon; in this study, tendons and tendon regions at high risk for tendinopathy (i.e. males and proximal regions, respectively) exhibited greater non-uniformity, whereas high-risk Achilles tendons (i.e. older adults) previously showed reduced non-uniformity. This suggests that non-uniformity may be dominated by factors other than fascicle-sliding. Anatomically, the varied proximal attachment of the patellar tendon may influence non-uniformity, with quadriceps passive resistance limiting superficial tendon movement, thus linking flexibility, non-uniformity and injury risk. This study also provides evidence of a differential effect of aging on the patellar tendon compared with evidence from prior studies on other tendons necessitating further study to elucidate links between non-uniformity and injury.

1. Introduction

There is increasing evidence that tendons are complex and highly specialized tissues that take advantage of a variety of mechanisms to enhance performance and reduce injury likelihood. For example, evidence from an equine model has shown that tendons that act primarily as energy-stores take advantage of sliding between fascicles to enable the tissues to undergo the large extensions required in physiological movement, while protecting the fascicles themselves from damage (Thorpe et al., 2015, 2012). Age-based reductions in the capacity for sliding, which have also been observed in the equine model (Thorpe et al., 2013), may thus be a mechanism for predisposing older tendons to chronic injury.

In recent years, ultrasound-based approaches have enabled researchers to study such complexities in tendon mechanics *in vivo* and noninvasively. For example, studies on the human Achilles tendon have shown it to undergo significantly non-uniform displacements during various physiological movements (e.g. passive dorsiflexion, eccentric contraction, etc.; Arndt et al., 2012; Bogaerts et al., 2017; Franz et al., 2015; Slane and Thelen, 2014b), which intriguingly, are reduced with age (Franz and Thelen, 2015; Slane and Thelen, 2015). In the patellar tendon, a number of studies have recently reported patterns of non-uniformity in healthy, young adults that are similar to those found in the Achilles tendon (Lee et al., 2016; Pearson et al., 2017, 2014; Slane et al., 2017a), but it is unclear whether such non-uniformity is affected by age. If non-uniformity is an important factor in protecting tendons from injury, then sex-based differences in

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non-uniformity may also be relevant to consider, as males have been well-established to be a higher risk of patellar tendinopathy than females (de Vries et al., 2015; Lian et al., 2005; Zwerver et al., 2011).

Thus, the goal of this study was to evaluate non-uniformity in the patellar tendons of both males and females over a wide range of ages. We hypothesized that age-based differences in non-uniformity would be similar to previous findings in the Achilles tendon, such that we expected non-uniformity to be reduced with age. We also hypothesized that a reduction in non-uniformity would be observed in tendons at higher risk of injury; in other words, that male tendons would show significantly less non-uniformity than females. To eliminate any potential influence of age- or sex-based differences in muscle contraction patterns or force-generating capacity, we investigated patellar tendon non-uniformity during passive knee flexion in this study.

2. Methods

33 healthy adults over the age of 40 were recruited and gave written consent to participate, as per the guidelines of the Commission for Medical Ethics UZ/KU Leuven (#s58207). Research participants reported no history of knee injury, surgery, or musculoskeletal disorder, and completed the VISA-P survey as a measure of patellar tendon health (Visentini et al., 1998). To enable a broader analysis of age- and sex-based differences, data collected previously from healthy participants under 40 years using the same methodology (Slane et al., 2017a), were also included in the analysis (Table 1).

Passive knee motion was induced via an isokinetic testing device (Biodex Medical Systems, Shirley, NY, USA). Participants were seated with the hip at approximately 90 deg, and the ankle strapped into the system. Preconditioning began by moving the knee through the comfortable range of motion (ROM) for each participant for six minutes (Hawkins et al., 2009). This step also served to acquaint participants with the testing setup and imaging protocol. Following warm-up, the knee ROM was set to 50–90 deg flexion. This small range of motion was necessary to avoid any buckling of the tendon, which occurs when the knee is relaxed and in a more extended posture (Slane et al., 2016), and can create shadowing within ultrasound images making images difficult to track. The ultrasound transducer was then manually held in place over the patellar tendon to enable collection of cine radiofrequency (RF) data. Because the patellar tendon length exceeded that of the 38 mm transducer (L4-15/38, Ultrasonix Corporation, Richmond, BC, Canada), data were collected in a random order from either the proximal or distal patellar tendon, with either the tibial tuberosity or distal edge of the patella in view (Fig. 1). Five repeat trials from each transducer position were collected for each participant, with all data collected by the same researcher.

Following data collection, images were loaded into MATLAB (R2015B, Mathworks, Natick, MA) and each trial was cropped to include one cycle, starting in the extended position, through flexion and extension. Next, anatomical landmarks and tendon borders were manually identified, with the distance between these borders taken as the tendon thickness. Based on these identifications, a regular grid of nodes (3 × 6 nodes) and kernels (1.35 × 1 mm centered on nodes) was generated. Nodes were regularly spaced at 25%, 50% and 75% through the tendon thickness, 2 mm apart, and positioned 9 mm from the anatomical landmark (Fig. 1). This approach produced consistently defined regions of interest that enabled superficial-to-deep comparisons, and ensured that comparisons between participants from this and our prior study (Slane et al., 2017a) were assessing similar regions of the tendon.

Table 1
Subject information presented as mean ± SD (range). Although 53 subjects were initially recruited, three were removed during quality control of the data, so only information from the 50 analyzed subjects is included. Subject information is also separated by age group.

	All subjects	Young adults (21–40 yrs)	Middle-aged (41–60 yrs)	Older (61–80 yrs)
Age (yrs.)	47 ± 8 (23–80)	27 ± 4	50 ± 5	69 ± 7
Sex	25F, 25M	10F, 10M	9F, 6M	6F, 9M
Height (m)	1.73 ± 0.09 (1.55–1.98)	1.74 ± 0.07	1.76 ± 0.12	1.70 ± 0.09
Weight (kg)	73 ± 13 (48–102)	70 ± 11	76 ± 14	74 ± 14
BMI	24.1 ± 2.8 (18.1–30.7)	23 ± 3	24 ± 3	26 ± 4
VISA-P Score	96 ± 9 (56–100)	98 ± 3	99 ± 2	87 ± 13

Note: Young adult data were previously reported (Slane et al., 2017a) and were included in this study to enable the assessment of any differences between age groups.

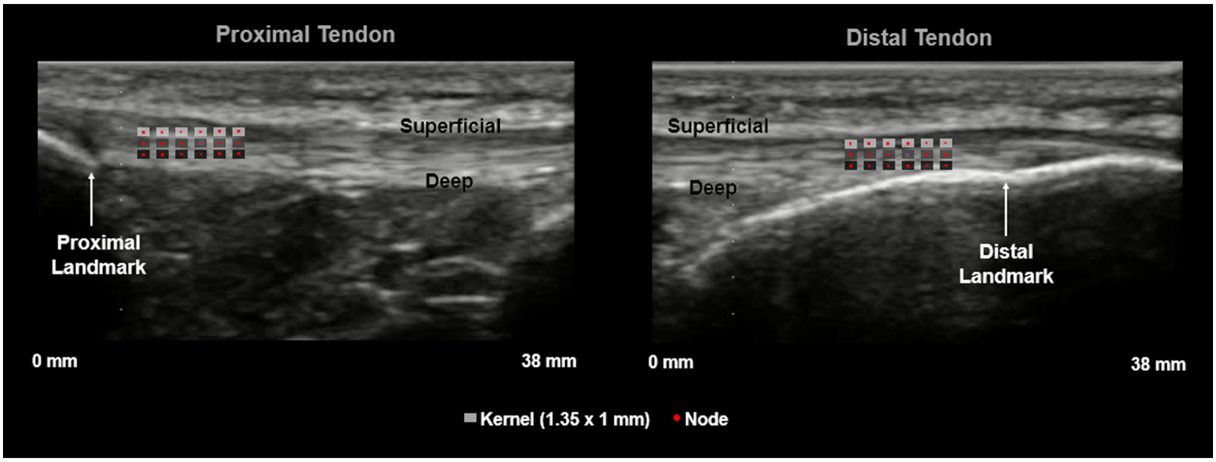

Fig. 1. Representative image of patellar tendon in the most extended posture, with overlaid kernels and nodes. On the right side of the proximal image the very edge of the tibia can be seen demonstrating that there is some overlap between image windows.

Next, regional displacements and strains were computed using a speckle-tracking approach that has been described in detail in prior publications (Chernak and Thelen, 2012; Slane and Thelen, 2014a). Because the maximum knee extension in this study was 50 deg of flexion, it was not possible to compute strains relative to a truly unloaded tendon length. For this reason, the computed values are not true strains, and will henceforth be referred to as percent elongation. As with all other 2D ultrasound approaches, out-of-plane motion remains a challenge and limits the ability to accurately track tissue movement, so any trials with visible out-of-plane motion, observed as the vanishing of underlying structures, or evidence of non-continuous displacements, were removed from analysis. This step resulted in three participants being entirely removed from further analysis. Regional (superficial, mid, deep) displacements and percent elongation were taken at peak flexion from the proximal and distal tendon. To ensure that any inadvertent transducer translation did not influence results, relative regional displacements were computed in which the average displacement of the three regions was subtracted from the regional displacement. Displacement non-uniformity was defined for the proximal and distal tendon as the difference between the maximum and minimum of these relative regional displacements (i.e. range of displacements). Average percent elongation was also computed in the proximal and distal tendon by averaging values from the superficial, mid and deep regions of the tendon.

To assess general patellar tendon behavior, data were first compiled for all participants (n = 50). Repeated measures ANOVAs separately assessed superficial-to-deep differences in relative displacement and percent elongation in the proximal and distal tendon, with significant interactions followed-up with pairwise comparisons using a Sidak correction, and a paired *t*-test compared average proximal and distal percent elongation.

Next, data were separated by sex and age group, with age groups defined as young (aged 21–40), middle-aged (41–60) and older (61–80) adults, to mimic groupings from prior studies on the Achilles tendon (e.g. Slane et al., 2017a). Two-factor ANOVAs tested for sex- and age-based differences, as well as interactions, in terms of displacement non-uniformity and average percent elongation for the distal and proximal tendon. Regression analyses were then used to further test possible effects of aging and tendon thickness on displacement non-uniformity and average percent elongation. Differences in thickness between males and females were assessed with a *t*-test. All statistics were performed in SPSS (IBM Corp., Armonk, NY, USA), with $p < 0.05$ taken as significant.

3. Results

The compiled data (n = 50) revealed significant superficial-to-deep variations in relative displacement in both the proximal and distal tendon during knee flexion. In the proximal tendon, all regions were significantly different ($p < 0.001$), whereas in the distal tendon the deep region was significantly different than the superficial ($p = 0.006$) and mid ($p = 0.01$) regions. In terms of percent elongation, there was a significant difference between the average values in the proximal and distal tendon ($p < 0.001$), with negative percent elongation proximally, and positive percent elongation distally. Significant superficial-to-deep variations in percent elongation were also observed in the proximal tendon ($p < 0.001$), but not in the distal tendon (Fig. 2).

Group-wise comparisons found a significant effect of sex on distal ($p = 0.02$) and proximal ($p = 0.03$) displacement non-uniformity, with males having greater non-uniformity. No effects of age, and no interactions were found (Fig. 3). No effects of sex or age were found on percent elongation in either tendon region. Likewise, regression analyses found no significant correlations between age and displacement non-uniformity, or percent elongation in either the proximal or distal tendon. Tendon thickness, however, did correlate significantly, albeit weakly, with distal displacement

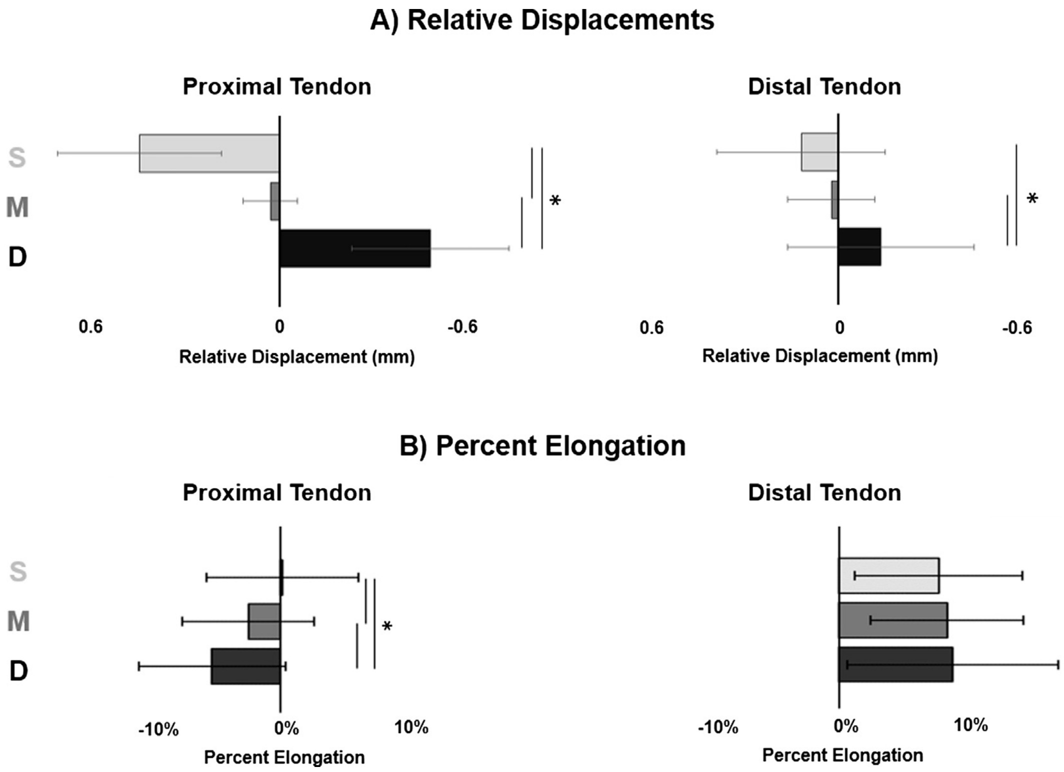

Fig. 2. Average (±SD) for all subjects. (A) Relative displacements were significantly different in the proximal and distal tendon. (B) Significant regional differences in percent elongation were observed in the proximal tendon. $^*p < 0.05$.

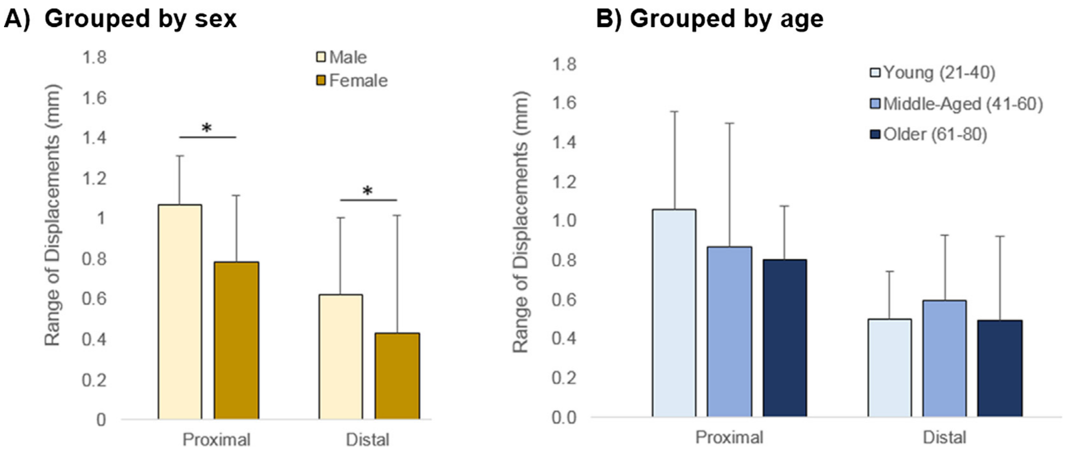

Fig. 3. Non-uniformity (i.e. the range of displacements) average (+SD) grouped by (A) sex and (B) age group. Significant differences ($p < 0.05$) between males and females were observed in both the proximal and distal regions, with no significant differences between age groups, or interactions between sex and age.

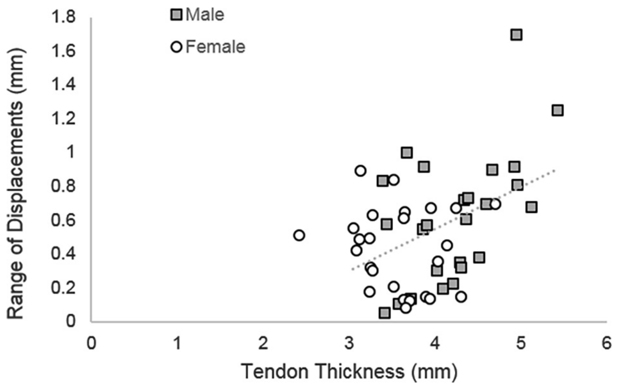

Fig. 4. A significant correlation ($p = 0.004$, $r = 0.43$) was observed in the distal tendon between thickness and non-uniformity (i.e. the range of displacements). Males were also observed to have significantly thicker tendons than females ($p < 0.001$).

non-uniformity (Fig. 4; $p = 0.004$, $r = 0.43$), with no significant correlations with proximal displacement non-uniformity or percent elongation. Males had significantly thicker tendons ($p < 0.001$) than females.

4. Discussion

Consistent with prior observations, in this study we observed significant regional non-uniformity in the patellar tendon. Regional variations included superficial-to-deep non-uniformity in relative displacements and percent elongation, and proximal/distal differences in average percent elongation. In contrast to our hypothesis, we did not observe any effects of age on non-uniformity. We did find a sex-based difference, but this also contrasted with our hypothesis in that we found non-uniformity to be greater in males. Greater regional non-uniformity was observed in the proximal tendon, in terms of both relative displacements and percent elongation, which interestingly is where tendinopathies typically arise (Ferretti, 1986; Johnson et al., 1996). These results contrast with those found previously in the Achilles tendon; in the Achilles tendon, high-risk tendons (i.e. older adults) were found to exhibit reduced non-uniformity, whereas in this study, we found high-risk tendons (i.e. males) and tendon regions (i.e. proximal) to exhibit greater non-uniformity. Such findings suggest that the link between non-uniformity and injury risk may be indirect and more complex than previously thought.

For example, the results suggest that aging differentially affects non-uniformity in the Achilles and patellar tendons, despite their similar functional roles as energy-stores. We previously hypothesized that Achilles tendon non-uniformity was related to fascicle-sliding (Franz et al., 2015; Slane and Thelen, 2015, 2014b), which is key to the functionality of energy-storing tendons (Thorpe et al., 2012), and that the age-based reduction in non-uniformity was linked with a reduced capacity for fascicle-sliding (Thorpe et al., 2013), likely arising from increased collagen cross-linking (Couppé et al., 2009). However, the fact that we did not find the same effect in the patellar tendon suggests that either (a) aging differentially affects fascicle-sliding in the Achilles and patellar tendons, or (b) ultrasound-based observations of non-uniformity are dominated by factors other than fascicle-sliding. Such possibilities will be discussed further in the following paragraphs, though future work with additional evidence will be necessary to support either supposition.

One interpretation of the results of this study is that aging affects non-uniformity in the Achilles and patellar tendons differently. This explanation is somewhat surprising given the prior results from the equine model that suggest that energy-storing tendons depend on fascicle-sliding to enable them to undergo large extensions (Thorpe et al., 2012), and that this functionality is critically impaired with age (Thorpe et al., 2013). Because the Achilles and patellar tendons both serve as energy-stores one would have expected them to be affected similarly by age. Such an interpretation would suggest that it may be that tendons are more varied that previously thought and a two-category approach is insufficient for capturing the range of differences between tendons. This could also help to explain the large inconsistencies in the literature as to the effects of age on tendon mechanical properties; whereas some researchers have reported significant effects of age on strain (Kubo et al., 2007), stiffness (Karamanidis and Arampatzis, 2006; Onambele et al., 2006) and elastic modulus (Couppé et al., 2009; Stenroth et al., 2012), others have found no age-based changes (Carroll et al., 2008; Couppé et al., 2013; Karamanidis and Arampatzis, 2006; Onambele et al., 2006; Stenroth et al., 2015, 2012). Likewise, when considering our results in the context of only patellar tendon studies, our findings of no age-related differences in percent elongation are consistent with the literature (Carroll et al., 2008; Couppé et al., 2009). There is even evidence of tendon-specific differences in terms of injury incidence; though both the Achilles tendon and patellar tendon are susceptible to tendinopathy, the link between Achilles tendinopathy and middle-age is well-established (de Jonge et al., 2011), whereas studies report a lack of evidence for age-based patellar tendinopathy incidence (Ferretti, 1986; Gaida, 2004; Lian et al., 2005; Witvrouw et al., 2011). Thus, we suggest that as we continue to learn about tendon heterogeneity, researchers must be careful in generalizing results from one tendon to understanding another.

On the other hand, an alternate hypothesis is that non-uniformity may arise due to factors other than fascicle-sliding, and thus may or may not be expected to change in a similar way across different tendons. Anatomical differences between the Achilles and patellar tendons may be key here. In the Achilles tendon, it has been hypothesized that the independent actions of sub-tendons arising from the muscles of the triceps surae may contribute to non-uniformity (Finni et al., 2018, Handsfield et al., 2016). In the patellar tendon, the proximal attachment of the patella may affect non-uniformity, with the superficial patellar tendon extending to the quadriceps tendon (Toumi et al., 2006), and the deep tendon inserting directly onto the patella (Yoo et al., 2007). During passive knee flexion, when the tendon is translating distally, the movement of the superficial tendon may be restricted by the passive resistance of the quadriceps. Thus, an alternate hypothesis is that the degree of non-uniformity in the patellar tendon is modulated by the passive resistance of the quadriceps muscles. Likewise, individuals with reduced flexibility and greater passive resistance would exhibit greater non-uniformity within the tendon. Consistent with this hypothesis, there is evidence that males have greater passive stiffness in the knee extensors (Wang et al., 2015; Wu et al., 2016), which may explain the greater non-uniformity we observed in males. Although aging generally contributes to a reduction in flexibility (Chung and Wang, 2009), studies specifically measuring knee extensor passive stiffness have reported no age-based change (Ikezoe et al., 2012; Wu et al., 2016), which is consistent with our observation of no effects of aging on tendon non-uniformity. Reduced flexibility has previously been linked with risk of patellar tendinopathy (Witvrouw et al., 2011), further supporting a potential relationship between reduced flexibility, increased non-uniformity, and risk of tendinopathy. Clinically, this hypothesis lends support to preventative training aimed at improving flexibility, and suggests that noninvasive ultrasound screening may be capable of identifying high-risk individuals. Further study is needed to test this hypothesis. For example, cross-sectional studies could use a dynamometer to measure passive muscle stiffness and non-uniformity to establish whether there is a direct link, and longitudinal studies could identify whether non-uniformity is a predictor of patellar tendinopathy development.

There are other factors that could relate to the greater non-uniformity in males. For example, in this study we observed a link between tendon thickness and increased non-uniformity, with males having significantly thicker tendons. Finding that males have thicker tendons is not surprising, as this occurs naturally (Yoo et al., 2007), but these results suggest that the larger thickness could be a predisposing factor for tendon injuries, contributing to the predisposition of males to tendon injury. On the other hand, many other factors could play a role in the increased incidence of patellar tendinopathy in males including their greater force-generating capacity (Lian et al., 2005) or reduced flexibility (Witvrouw et al., 2011).

It remains challenging to identify the origins and interpret the meaning of the observed non-uniformity. In this study, we found larger non-uniformity in participants who are from populations that are clinically more likely to suffer from a tendinopathy, but it should be reiterated that all tested participants were healthy and asymptomatic (mean VISA-P scores: 96 ± 9). It is possible that this is a meaningful observation, and that non-uniformity could be relevant in the context of injury risk or early-stage degeneration, but as these findings contrast with those found previously in the Achilles tendon, the results are difficult to interpret. One possibility is that non-uniformity is similar to tendon thickness, which is considered both a risk factor for tendinopathy (Jhingan et al., 2011; Visnes et al., 2015), and an early symptom (Cook and Purdam, 2009). However, without further supporting data, such hypotheses remain speculative and more work is clearly needed to better elucidate tendon non-uniformity and its clinical relevance.

There are some limitations to this work. One limitation is that non-uniformity was assessed during passive knee flexion over a small ROM, such that the results may not be representative of non-uniformity during other movements. Also, as many prior studies have focused on evaluating tendon mechanical properties during maximum isometric contractions, it can be challenging to compare our results (e.g. percent elongation) with prior studies. However, choosing this simple loading scenario did enable the assessment of non-uniformity without possible confounders of differing muscle activation patterns or force-generating capacity. Future work could include the assessment of non-uniformity during muscle activation and/or include force data to evaluate passive stiffness of knee extensors. A second limitation of this study is that muscle activity was not monitored during the data collection from middle-aged and older adults, such that the results could have been affected by muscle activation. In our prior work on young adults (Slane et al., 2017a), from which the young adult data for this study were taken, we did assess muscle activity with a single electrode positioned over the vastus medialis. During those collections we found no evidence of muscle activation during trials, so we decided not to continue with monitoring muscle activity. However, it is possible that there was muscle activation in middle-aged and/or older adults during collections. Given that no significant differences were found between age-groups we find this unlikely to be a factor, though unexpected muscle activation of the middle-aged and older adult groups could have masked true age-group based differences. Such age-based differences could also have been missed due to the small sample sizes in this study (n = 15–20 per group). Another limitation, inherent to all 2D ultrasound-based studies, is that collecting dynamic trials without significant out-of-plane motion remains a challenge, and quality analysis is a critical step in our processing approach, which in this study did lead to the removal of three participants from analysis. Although the ability to collect dynamic ultrasound data remains limited, 3D imaging approaches continue to improve (Carvalho et al., 2017, Obst et al., 2014) and may provide an intriguing alternative in the future to limit the influence of out-of-plane motion. As quantitative ultrasound becomes more widespread, we feel strongly about transparently presenting challenges (Slane et al., 2017b), and we recognize that collecting high quality data remains a challenge. Although we strive to be unbiased in the removal of data from analysis, it is certainly possible that we are inadvertently biasing results. One of the key observations in this study was the difference in results in the patellar tendon compared with a previously published study on the Achilles tendon. It should be noted that although the patellar tendon data was collected from a relatively large sample size with a wide age range (n = 50, aged 23–80), the Achilles tendon data comes from a much smaller subject group with a more narrow age range (n = 18, aged 22–54).

This study revealed that the patellar tendon undergoes significant non-uniformity during passive knee flexion that is greater in males, but is similar between individuals in different age groups. Such results contrast with prior observations in the Achilles tendon, where non-uniformity was reduced in older tendons, suggesting that non-uniformity is dominated by factors other than fascicle-sliding, or that aging differentially affects fascicle-sliding in the Achilles and patellar tendons. The observation is particularly relevant because current research has focused on the similarities between the Achilles tendon and patellar tendon, most notably in their roles as energy-stores, but these finding suggest that the similarities between the tendons may be limited. Factors such as known anatomical differences between the tendons could contribute to tendon non-uniformity. The increased passive resistance of the quadriceps in males may contribute to their greater non-uniformity, which would suggest a mechanism as to why reduced flexibility is linked with patellar tendinopathy risk, and support clinical training aimed at improving flexibility. Alternatively, if non-uniformity is related to fascicle-sliding, then this study suggests that aging has a differential effect on fascicle-sliding in two tendons with similar functions as energy-stores. Thus, further work is needed to better elucidate the factors contributing to non-uniformity, and how such non-uniformity may be relevant in the context of injury, aging and function.

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Conflicts of interest

The authors have no conflicts of interest to report.

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