

Non-uniformity in pre-insertional Achilles tendon is not influenced by changing knee angle during isometric contractions

S. Bogaerts^{1,2}  | C. De Brito Carvalho^{3,4,5} | A. De Groef^{1,2,6} | P. Suetens^{3,4} | K. Peers^{1,2}

¹Department of Development & Regeneration, KU Leuven, Leuven, Belgium

²Department of Physical and Rehabilitation Medicine, University Hospitals Leuven, Leuven, Belgium

³ESAT/PSI, UZ Leuven and University Hospitals Leuven, Leuven, Belgium

⁴MIRC, KU Leuven and University Hospitals Leuven, Leuven, Belgium

⁵INESC TEC Porto, Instituto de Engenharia de Sistemas e Computadores-Tecnologia e Ciência, Porto, Portugal

⁶Department of Rehabilitation Sciences, KU Leuven, Leuven, Belgium

Correspondence

Stijn Bogaerts, Department of Development & Regeneration, KU Leuven-University Hospitals Leuven, Leuven, Belgium.
Email: stijn_bogaerts@hotmail.com

Achilles tendinopathy remains a prevalent condition among recreational and high-level athletes. Mechanical loading has become the gold standard in managing these injuries, but exercises are often generic and prescribed in a “one-size-fits-all” principle. The aim of this study was to evaluate the impact of knee angle changes and different levels of force production on the non-uniform behavior in the Achilles tendon during isometric contractions. It was hypothesized that a flexed knee position would lead to a more distinct non-uniform behavior, due to greater differential loading of soleus vs gastrocnemius, and that this effect would be attenuated by higher levels of force production. Contrary to the hypotheses, it was found that the non-uniform deformation, that is, superficial-to-deep variation in displacement with highest displacement in the deep layer, is consistently present, irrespective of the level of force production and knee angle ($n = 19$; mean normalized displacement ratio 6.32%, 4.88%, and 4.09% with extended knee vs 5.47%, 2.56%, and 6.01% with flexed knee, at 25%, 50%, and 75% MVC, respectively; $P > .05$). From tendon perspective, aside from the influence on muscle behavior, this might question the mechanical rationale for a change in knee angle during eccentric heel drops. Additionally, despite reaching high levels of plantar flexion force, the relative contribution of the AT sometimes appears to be decreased, potentially due to compensatory actions by agonist muscle groups. These results are relevant for optimizing AT rehabilitation as the goal is to reach specific local tendon loading.

KEYWORDS

mechanics, rehabilitation, soft tissue, speckle tracking, ultrasound

1 | INTRODUCTION

The Achilles tendon (AT) is the thickest tendon of the human body and is, like every tendon, structured in a hierarchical manner from collagen molecule up to fascicle bundle.¹ The AT is the tendinous continuation of the 3 muscles from the triceps surae: the lateral gastrocnemius, the medial gastrocnemius, and the soleus.² It rotates medially from proximal to distal and inserts on the calcaneus bone at the ankle.³ Despite this design, Achilles tendinopathy

remains a highly prevalent condition among recreational as well as high-level athletes.⁴ Although understanding of the multifactorial etiology of tendinopathy is still incomplete, mechanical loading appears to be of crucial importance in managing this injury, and rehabilitation through active exercise has become the gold standard.^{5,6} However, these exercises are often generic and prescribed in a “one size fits all” principle. The classic exercise used in patients with mid-portion Achilles tendinopathy is the eccentric heel drop.⁷ Interestingly, minor modifications to an exercise can make

an important clinical outcome difference for patients with insertional tendinopathy.⁸

In the currently most often used Alfredson eccentric heel drop program, the calf muscle is eccentrically loaded both with the knee straight and also with the knee bent.⁷ The latter has been included to maximize the activation of the soleus muscle.⁷ Considering the multi-muscle and rotatory anatomy of the tendon, it can be assumed that changing the knee angle at which the heel drop is performed can influence the local AT deformation pattern, due to differential impact on soleus vs gastrocnemius. Recently, it was indeed confirmed that a change in knee angle has an impact on the non-uniform behavior of the different layers in the Achilles tendon.⁹ This non-uniform behavior of the AT,¹⁰ that is, a superficial-to-deep variation in displacement and strain of the layers of the AT, has been shown to be present during different contraction modes, for example, passive,¹¹ eccentric,⁹ and isometric.¹² An eccentric and passive motion in a flexed knee position have been shown to lead to relatively more displacement in the deep and middle layer of the AT than in the superficial layer, when compared to the extended knee position.⁹ This is hypothesized to be linked to proportionally more loading of the soleus muscle in the flexed knee position, with relatively less gastrocnemius contribution, following its origin proximal to the knee joint.⁹ Given the fact that the middle and deep layers of the AT are considered to be originating from the soleus muscle,¹³ relatively higher activation of the soleus muscle is hypothesized to result in relatively higher displacement in the middle and deep layers of the AT.⁹

However, it is unclear to what extent these intratendinous changes occur due to the positional changes or due to the subsequent biomechanical advantages or disadvantages that arise. It has been shown that knee flexion causes a drop in force production of ankle plantar flexion because of the more isolated contribution of the soleus muscle, as the gastrocnemius can contribute less because of its origin proximal to the knee joint.¹⁴ Additionally, Finni et al¹⁵ described the influence of active vs passive muscle contribution to the behavior of muscle shear, which is believed to have an influence on local tendon behavior. During passive conditions, there is a slack and compliant connection between muscle bellies of the triceps surae, leaving margin for a heterogeneous non-uniform behavior, which might also be the case at low levels of force production. During more active conditions, the tensing of muscle connections would lead to more uniform behavior, which will potentially cancel out any heterogeneous loading at the tendon level, associated with a specific knee position.

Therefore, the goal of this study was to evaluate whether there is a mechanical rationale behind the currently used rehabilitation protocol to perform the eccentric heel drop both with knee in extension as well as in flexion. Also, another goal was to evaluate whether higher levels of force production

would attenuate a possible impact of knee angle on local AT deformation. To reach this goal, the local AT deformation pattern was evaluated in an extended as well as flexed knee position, both at 3 levels of force production (25%, 50% and 75%). To facilitate interpretation and maximize standardization, activation was achieved by isometric contractions, performed on an isokinetic device. The local AT deformation was evaluated by means of high-frequency ultrasound based speckle tracking.¹² Firstly, it was hypothesized that a flexed knee position would lead to a more distinct non-uniform deformation, and, secondly, that this non-uniform deformation would be attenuated by higher levels of force production.

2 | MATERIAL AND METHODS

The study was approved by the UZ/KU Leuven ethics committee (s-number 59330).

2.1 | Subjects

A convenience sample of healthy subjects was recruited from a group of co-workers at the department of Physical and Rehabilitation Medicine of the University Hospitals Leuven. Candidates were asked to participate and given the opportunity to read the study protocol and sign the informed consent. Subjects with previous history of Achilles tendinopathy, rupture, surgery, and/or systemic or neuromuscular diseases were excluded. Nineteen (19) subjects agreed to participate.

2.2 | Procedure

Participants were asked to refrain from physical activity, other than normal ambulation required in daily life, the day before and the day of the test. During the testing, the participant lay prone on a table, knees extended, with the foot fixated to an isokinetic testing device (Biodex system 4 PRO, Biodex Medical Systems, Inc., Shirley, New York).

The knee was extended in a comfortable range for the subject, with goniometer —aligned with the midline of the femur at the iliotibial band and the midline of the fibula along the axis between the fibula head and lateral malleolus —confirming not more than 5° flexion was reached. The axis of rotation of the dynamometer was aligned with the lateral malleolus. The ankle was set in a neutral position. A standardized warm-up of 5 repetitions of concentric plantar and dorsiflexion through 20° range of motion was performed. A maximal isometric voluntary contraction (MVC) was performed, firstly to get acquainted to the device and secondly to estimate the 25%, 50%, and 75% MVC values. Subjects received visual feedback on force development during all tests. They were asked to reach the required level of force production during approximately 2 seconds. Two trials were

recorded per level of force production, with exclusion of a trial if there was insufficient image quality (eg, probe slipping). After reaching 2 trials at 25%, 50%, and 75% MVC in the extended knee position, the same was repeated in a flexed knee position. Subjects were kneeling upright and again a maximal MVC was performed to get acquainted with this new position and estimate the 25%, 50% and 75% MVC levels. Knee angle was again measured with the goniometer, confirming $90 \pm 5^\circ$ was reached. Afterwards, procedure was similar to the extended position. Torque data for each motion trial were extracted from the Biodex machine after finishing all testing procedures.

2.3 | Ultrasound

A high-spatial and high-temporal resolution US system (Vevo 2100, FujiFilm VisualSonics Inc., Toronto, Canada) was used to acquire 2D + time US images during each motion. The transducer (MS250, FujiFilm VisualSonics Inc., Toronto, Canada) had a central probe frequency of 21 MHz and images thus had a spatial image resolution of 0.02×0.09 mm. The images were acquired in the pre-insertional tendon area, with the edge of the calcaneus bone always in sight, in the sagittal plane (Figure S1).

2.4 | Tendon deformation evaluation

The entire speckle tracking approach used in this work was described elsewhere.^{12,16} In short, after acquisition, ultrasound images were visually reviewed to ensure that there were no artifacts (eg, probe release, air bubble) and a stable speckle pattern was visualized. A region of interest (ROI) containing only tendon material was selected, to limit the calculation of deformation to the tissue of interest. To reduce computational effort, an interval out of the 500 available frames was selected. The total length of the interval was on average 200 frames, as subjects were asked to reach the level of force in approximately 2 seconds and considering a frame rate of 100 frames/s. This interval was then long enough to capture the complete displacement, always starting registration just before the cue was given to the subject to start the contraction. Point-wise displacement maps were obtained along the major deformation direction representing the tissue displacement in the longitudinal direction (in mm), relative to the starting position in the first frame. The ROI selected at the pre-processing step was then re-used to automatically define 6 sub-regions (3 proximal and 3 distal), consisting of a deep, middle, and superficially located sub-region.

2.5 | Outcome parameters

Based on the speckle tracking output, 2 outcome parameters were calculated. First, *local tendon tissue displacement* (mm) of the different layers was defined separately, computed

directly from the point-wise displacement maps as an average displacement within each sub-region along the major deformation direction. Intraclass correlation coefficient (ICC) for this parameter has been shown to be 0.86 for intraday and 0.72 for interday measurements, leading to a Standard Error of Measurement (SEM) of 0.35 mm and 0.44 mm, respectively.¹² Second, the *normalized displacement ratio* (%) was calculated. The normalized displacement ratio is computed as the difference in local tendon tissue displacement between the deep and the superficial layer and then divided by the average displacement in all 3 layers combined.¹⁷ This is supposed to take probe slipping and displacement during acquisition, and therefore relative displacement errors, into account. Participants were asked to fill in a document asking their body mass, body height, hours of activity per week, and the validated VISA-A questionnaire,¹⁸ questioning tendon health. Subjects with VISA-A score lower than 90 were excluded from this study.

2.6 | Statistics

All statistics were performed using Statistical Package for Social Sciences (SPSS Inc. version 24., Chicago, Illinois, USA), and the alpha level for all tests was set at 0.05.

First, to evaluate the presence of non-uniform behavior, local tendon tissue displacement of the different layers of the AT was compared with a two-way ANOVA with repeated-measures, separately at each level of force production (25%, 50% and 75% MVC) and knee angle (extended and flexed). Second, to evaluate the magnitude of non-uniform behavior, the normalized displacement ratio in extended and flexed knee position at 3 levels of force production was compared, using a repeated-measures ANOVA with post-hoc Bonferroni's adjustment for multiple comparisons.

A paired t-test was used to compare the mean force levels reached at the pre-set levels.

The influence of length, weight, and hours activity per week was evaluated using a linear regression.

3 | RESULTS

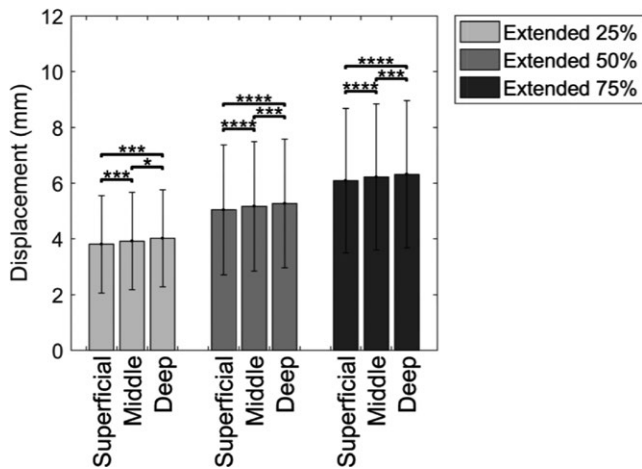
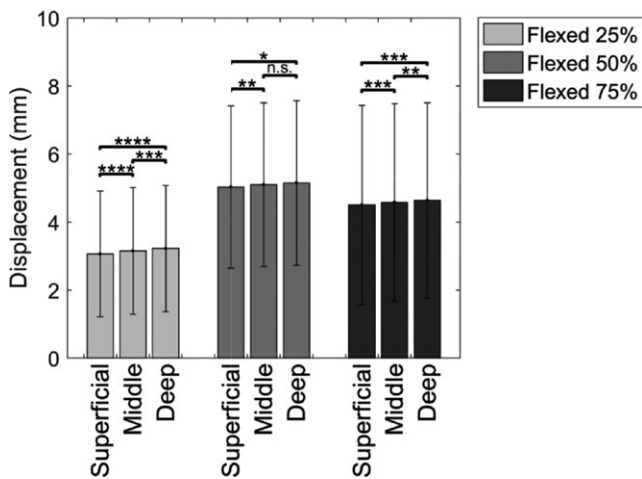
Subject characteristics ($n = 19$) are given in Table 1.

An overview of outcome parameters is given in Table S1.

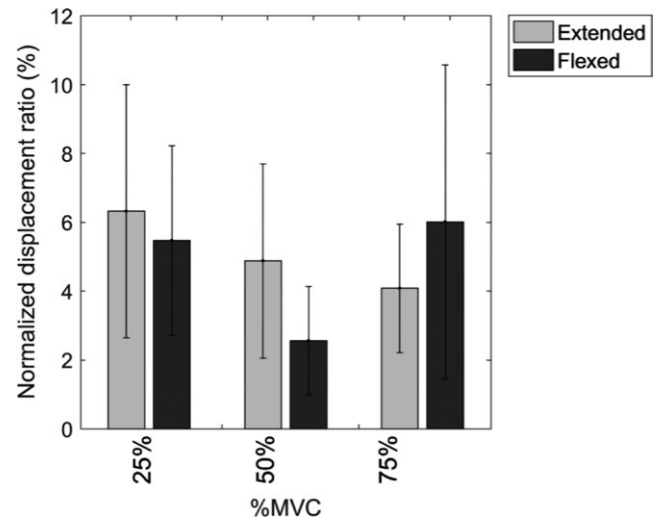
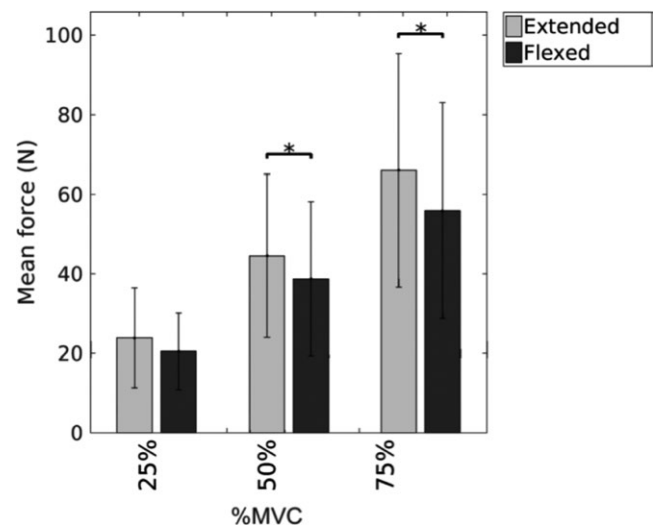
For the different layers separately, mean (\pm SD) local tendon tissue displacement is given in Table S1 and Figures 1 (knee in extended position) and 3 (knee in flexed position). The results show a significantly different displacement between all 3 layers for both knee positions at all 3 levels of force production, except for middle-to-deep at 50% MVC in a flexed knee position (Figure 2). These differences are generally greater than the previously calculated SEM of 0.35 mm for intraday measurements.¹²

TABLE 1 Subject characteristics (mean \pm standard deviation)

Subjects	11 Male:8 Female
Body height (cm)	176 \pm 9
Body mass (kg)	71 \pm 14
Age (y)	28 \pm 3
Activity per week (h)	6 \pm 4
VISA-A (/100)	99 \pm 1

**FIGURE 1** Mean local tendon tissue displacement during trials with knee in extended position \pm standard deviation. (* $P < .05$; *** $P < .005$; **** $P < .001$)**FIGURE 2** Mean local tendon tissue displacement during trials with knee in flexed position \pm standard deviation. (* $P < .05$; ** $P < .01$; *** $P < .005$; **** $P < .001$; n.s., non significant)

The amount of non-uniform behavior, as expressed by the normalized displacement ratio, did not significantly differ between different settings, that is, flexed or extended knee position at 3 levels of force production (Figure 3). A trend can be seen towards a more uniform behavior (ie, decrease

**FIGURE 3** Mean normalized displacement ratio \pm confidence interval**FIGURE 4** Mean levels of force at different %MVC at both knee angles. (* $P < .05$)

in ratio) at higher levels of force production, except for 75% MVC in a flexed knee position. Also, the flexed knee position appears to lead to a more uniform tendon deformation, except at the 75% MVC where the non-uniform behavior rises again.

Linear regression analysis revealed that none of the independent variables had a significant impact on normalized displacement ratio (body mass: $P = .28$; body height: $P = .27$; hours activity per week: $P = .77$).

Mean levels of force subjects reached at the pre-set levels of 25%, 50%, and 75% MVC can be found in Figure 4. The mean level of force reached is always higher in the extended knee position when compared to the flexed knee position, but only significantly different at 50% and 75% MVC ($P < .02$), not at 25% MVC ($P = .08$).

4 | DISCUSSION

This study evaluated the impact of different levels of force production and a change in knee angle on the non-uniform behavior in the Achilles tendon of healthy subjects during an isometric contraction. It was hypothesized that a flexed knee position would lead to higher normalized displacement ratio, and that this effect would be attenuated by higher levels of force production. Contrary to these hypotheses, it was found that the non-uniform deformation in the AT, that is superficial-to-deep variation in displacement with highest displacement in the deep layer, is consistently present, irrespective of the level of force production and knee angle.

The results of this study confirm the presence of non-uniform behavior at all levels of force production (25%, 50% and 75% MVC) and both tested knee angles (extended and flexed). More specific, there is always significantly more local tissue displacement in the middle layer compared with the superficial layer, the deep compared with the middle layer, and the deep compared with the superficial layer. Only deep-to-middle local tissue displacement during trials at 50% MVC in the flexed knee position is not significantly different (Figure 2). This is generally in line with previous research where the same behavior was found during passive,¹¹ eccentric,⁹ and isometric loading.¹² Multiple potential reasons for this non-uniform behavior of the AT have been described. Besides a possible influence from the rotatory anatomy of the tendinous area,² the multi-muscle origin, with differences in neural activation and cross-sectional area of the separate muscles, is hypothesized to contribute.¹⁰ Because of this potentially differential impact of soleus vs gastrocnemius, it could be expected that a change in knee angle, which affects the bi-articular gastrocnemius more than the uni-articular soleus, would impact the intratendinous non-uniform deformation pattern. Indeed, it has previously been shown that with the knee joint in extension, the displacement in proximal gastrocnemius musculotendinous region was higher than in soleus; with the knee joint in flexion this was reversed.¹⁹ In general, total displacement of the proximal musculotendinous area was also higher in extension vs flexion.¹⁹ Previous research by Slane et al⁹ has also shown that when the knee was flexed, eccentric loading significantly altered motion in the mid and deep portions of the tendon, but not in the superficial tendon.

However, results in the current study show no significant impact of changes in knee angle on the amount of non-uniformity in the AT. The amount of non-uniform behavior, as expressed by the normalized displacement ratio, did not significantly differ between different settings, that is, flexed or extended knee position at 3 levels of force production. This is contrary to the hypothesis where it was expected that a flexed knee position would lead to a more distinct non-uniformity due to greater differential loading of soleus vs gastrocnemius.

This could be an indication that the assumption of soleus and gastrocnemius having separate sub-tendons is incorrect. Indeed, there is still uncertainty whether the tendinous portions arising from each muscle are still morphologically distinguishable in the free AT. Some papers indicate discernable sub-tendons in the free AT,¹³ while others do not.²⁰

Importantly, there are a few differences in methods used to evaluate the non-uniform behavior between the current study and previous research. Firstly, the region of the Achilles tendon under investigation is different. Others⁹ evaluated the midportion of the tendon, whereas this study evaluated the pre-insertional region. It has been shown that there are differences in mechanical behavior between these regions,^{21,22} so a different non-uniform behavior could be expected. Furthermore, the rotational component has a high degree of inter-individual variation. Three types of torsion are described,¹³ but they commonly demonstrate the most pronounced rotation at the midportion level.³ This could cause a more distinct non-uniform behavior at this level of the tendon. Towards the insertion, tendon rotation appears to be less present,¹³ corroborating a different morphology and mechanical behavior of this area.^{21,22} Secondly, the study of Slane et al⁹ evaluated local tendon behavior during an eccentric (dynamic) deformation, whereas this study used an isometric (static) contraction in a neutral ankle position. A dynamic motion will cause changes in calcaneus position, an effect that is limited in the fixed position during isometric contractions. The calcaneus position has an important impact on local tendon deformation, as Lersch et al²³ showed that eversion will differentially impact the deformation of the proximal and distal tendon. Therefore, future studies should evaluate the differences in local tendon behavior of the Achilles tendon in vivo during different angles of the ankle joint during isometric contractions. Finally, ultrasound images in this study are obtained at fiber level,¹² whereas others^{9,11} have evaluated this deformation at fascicle level, using lower resolution ultrasound. Thorpe et al²⁴ state, summarizing ex vivo and in vitro research, that "... at low force, there is sliding between fibers/fibril, but no real fiber extension. At higher force, there is interfascicular matrix sliding." Consequently, the higher level of ultrasound resolution used in this study might capture a different level of ultrastructural behavior.

In this study, the local AT deformation pattern was not altered by a change in knee angle and no attenuation of the non-uniform behavior at higher levels of force production could be shown. However, a trend can be seen towards a more uniform behavior (ie, decrease in normalized displacement ratio) at higher levels of force production, except at 75% MVC in a flexed knee position, where this trend is broken. This rise in normalized displacement ratio (Figure 3), and also drop in local tissue displacement (Figure 1), at 75% MVC in the flexed position, is unexpected. A possible explanation for this finding might be compensatory actions

by subjects trying to reach the requested force level. The kneeling upright position used during the knee flexed trials is an adverse position to develop maximal plantar flexion force. Therefore, subjects might be tempted to use agonist muscle groups (eg, tibialis posterior, flexor hallucis longus, gluteus maximus, etc.). Similar compensatory actions have been found after AT rupture where a high plantar flexion torque appears to be achieved by compensatory action of the flexor hallucis longus muscle.^{25,26} Therefore, the AT might be relatively under-used during these trials and the local AT displacement consequently unexpectedly low. During trials with the knee in extension, the position is adequate enough to reach the requested levels of force with sufficient contribution of the AT. It is generally accepted that knee angle has an important influence on force generation capacity in the triceps surae,^{14,27} resulting in a higher force output in extended position. This was also found in this study as subjects reached statistically significant higher levels of force production during trials with knee in extension when compared to the flexed position (Figure 4). This is again supposed to be linked to the biomechanically more beneficial position for the gastrocnemius muscle-tendon unit in the extended position, whereas the muscle partly loses its contribution in the flexed knee position due to a drop in fascicle length.²⁸

It is generally known that tendons react to loading with changes in their metabolism,⁵ although it remains unclear if changes are to be expected in material or mechanical properties.²⁹ Nonetheless, to achieve a positive outcome with therapeutic loading programs, the data strongly suggest that loading magnitude in particular plays a key role for tendon adaptation, with the need for an adequate local stimulus to the tendon.³⁰ However, as discussed above, having a patient perform a high load exercise does not necessarily reflect adequate local AT deformation. Too heavy exercises may induce compensation mechanisms using relatively less local AT contribution. Then again, current evidence shows that heavy slow resistance training³¹ is equally effective as the isolated eccentric loading program.⁷ As discussed in a clinical commentary by Couppé et al³² parameters such as load magnitude, speed of movement, and recovery periods between exercise sessions should be more carefully controlled. Therefore, the tool used in this study might be ideally positioned to monitor local AT loading during rehabilitation exercises. However, the clinical relevance of the non-uniform deformation pattern of the AT remains to be further investigated. It has been hypothesized that less non-uniform behavior at older age,¹⁷ due to crosslinks at interfascicular level,³³ would limit the possibility for stress dissipation through the tendon by interfascicular sliding. Tendon fascicles themselves then become more vulnerable to tensile damage. A more distinct non-uniformity might therefore be a sign of

tendon health, but more research is needed to investigate this hypothesis.

A few limitations should be considered. Firstly, an inherent limitation of studies using speckle tracking to evaluate local tendon mechanics is out-of-plane motion. However, the high frame-rate that is used in these experiments limits this potential influence¹² and all acquired images were visually reviewed to ensure there was a stable speckle pattern without artifacts. Secondly, the isometric contraction in a neutral ankle position is not exactly a functional position and careful interpretation is warranted. Future research should investigate the local AT deformation pattern during an isometric contraction in different ankle joint angles and during more functional movements like walking or during eccentric heel drops. Thirdly, the mechanical behavior of normal ATs differs from that of pathological ATs.³⁴ Therefore, translation of results from this study to the clinical rehabilitation setting should be done with care. A strength of the present study is the use of high-frequency ultrasound-based speckle tracking, leading to evaluation of local tendon mechanics at a smaller hierarchical level than performed so far in the literature.¹²

5 | PERSPECTIVES

Results of this study show that non-uniform behavior is consistently present within the Achilles tendon, but that its magnitude is not significantly influenced by changes in level of force production or knee angle. From clinical perspective, this might question the mechanical rationale for a change in knee angle during the Alfredson eccentric heel drop program.⁷ However, further research is needed to evaluate the local AT deformation patterns during more functional movements. Additionally, it was found that despite reaching high levels of force, the contribution of the AT might sometimes be relatively smaller, potentially due to compensatory actions. These results are relevant for optimizing AT rehabilitation as the goal is to reach specific local tendon loading. Therefore, the tool used in this study might be ideally positioned to monitor local AT loading during rehabilitation and individually tailor tendon exercises.

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ORCID

S. Bogaerts  <http://orcid.org/0000-0001-5708-2439>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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