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Patellar Tendon Morphology in Trans-Tibial Amputees Utilizing a Prosthesis with a Patellar-Tendon-Bearing Feature

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PATELLAR TENDON MORPHOLOGY IN TRANS-TIBIAL AMPUTEES UTILIZING A
PROSTHESIS WITH A PATELLAR-TENDON-BEARING FEATURE

By

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A doctoral project submitted in partial fulfillment

of the requirements for the

Doctor of Physical Therapy

Department of Physical Therapy

School of Allied Health Sciences

Division of Health Sciences

The Graduate College

University of Nevada, Las Vegas

May 2019

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Doctoral Project Approval
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The University of Nevada, Las Vegas

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entitled

Patellar Tendon Morphology in Trans-Tibial Amputees Utilizing a Prosthesis with a Patellar-Tendon-Bearing Feature

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Doctor of Physical Therapy

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ABSTRACT

Purpose/Hypothesis: A patellar-tendon-bearing (PTB) bar is a common prosthetic design feature used in individuals with trans-tibial amputations. As the patellar tendon in the knee of residual limb is subjected to the perpendicular compressive force not commonly seen in normal tendon loading, it is possible for tendon remodeling and degeneration to occur over time; however, there is limited data to support this idea. The primary purpose of this study was to use ultrasound imaging to compare the morphological differences between tendons of the residual and intact limb in unilateral trans-tibial amputees who utilize a prosthesis with a PTB feature. We hypothesized that the patellar tendon of the residual limb would have increased thickness, increased cross-sectional area (CSA), a greater proportion of neovascularity, and decreased collagen fiber organization (i.e., reduced peak spatial frequency radius [PSFR]), when compared to the intact limb.

Subjects: Twelve unilateral trans-tibial amputees (age = 53.6 ± 16.9 years; 3 females and 9 males; years after amputation = 16.7 ± 18.2 years) who utilized a prosthesis with a PTB feature, having had it at least 1 year, and weight-bearing at least 1 hour per day.

Materials/Methods: Ultrasound images of each subject's patellar tendons were collected in a single session. Longitudinal and transverse images were taken at the proximal, distal, and mid-portion of each patellar tendon to examine thickness, CSA, neovascularity, and collagen fiber organization. Paired t-tests were used to compare the thickness, CSA, and PSFR between the residual and intact limbs. A chi-square analysis was used to compare the proportion of neovascularity between limbs.

Results: There was a statistically significant difference in mid-portion CSA (residual = 113.71 ± 18.62 mm², intact = 101.79 ± 17.15 mm²; $p = 0.008$), and proximal thickness (residual =

4.76±0.91mm, intact = 4.15±0.96 mm, p = 0.014). There was an increased proportion of neovascularity in the residual limb (residual=66.7%, intact=25%; p=0.018). No difference was found in proximal or distal CSA, mid-portion or distal thickness, and PSFR between residual and intact limbs.

Conclusions : Patellar tendons in residual limbs of individuals using a PTB prosthesis had greater proximal thickness and mid-portion CSA, as well as a greater proportion of neovascularity than intact limbs.

Clinical Relevance: Use of a prosthesis with a PTB feature may cause morphological changes to the patellar tendon. When treating patients with trans-tibial amputations, the health of the patellar tendon may need to be considered.

KEYWORDS: trans-tibial amputee, ultrasound imaging, patellar tendon

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BACKGROUND

There are an estimated 185,000 new amputations each year in the United States and 65% of persons living with limb loss had an amputation of the lower extremity, of which trans-tibial amputation accounts for more than 50%.¹ After a trans-tibial amputation procedure, qualified amputees would be fit with a prosthesis allowing them to perform daily weight-bearing activities.² The patellar-tendon-bearing (PTB) bar is a common and functional prosthetic design used in individuals with trans-tibial amputations.³ This design takes advantage of the patellar tendon as a weight-bearing structure due to its pressure tolerance and reduces loading to more pressure sensitive areas of the residual limb.³ To bear weight, the prosthetic socket is designed with a specific contour that directs weight bearing, compressive forces to the patellar tendon region which are different from the tensile forces a tendon typically experiences during weight-bearing.⁴ In this circumstance the patellar tendon becomes a main load bearing structure for the residual limb during weight bearing activities, and must withstand constant or intermittent perpendicular compression from the prosthetic socket.⁴

As the patellar tendon of the residual limb is subjected to a different loading condition, it is possible that tendon remodeling and/or degeneration can occur over time in amputees using prosthesis with a PTB design.⁴ In animal models, abnormal perpendicular forces applied to tendon tissues have been found to cause morphological changes similar to those observed in a degenerative tendon.⁵ These changes include collagen fiber disorganization, increased water content, increased glycosaminoglycan (GAG) content, thinner collagen fibers, reduced overall collagen content, increased type II collagen, and reduced tendon stiffness.⁵ Using ultrasound imaging, several previous studies on non-amputees have demonstrated that chronic overloading of the patellar tendon results in morphological changes to degenerative tendons, including gross

morphological changes (increased proximal tendon thickness^{6,7}, increased cross-sectional area (CSA)⁸, neovascularity^{9,10}), and intra-tendinous morphological changes (i.e. altered collagen fiber organization¹¹). Although it is likely that these patellar tendon morphological abnormalities would develop in amputees using a prosthesis with a PTB feature, there is limited evidence to support this assumption; To date, only one preliminary study has examined the thickness of the patellar tendon, comparing the residual and intact limbs, and they found increased tendon thickness in the residual limb.⁶ Additionally, no study has examined the intra-tendinous morphological changes in a trans-tibial amputee population. Therefore, the purpose of this study was to examine the gross and intra-tendinous morphological changes that occur in the patellar tendon of unilateral trans-tibial amputees who wear a prosthesis with a PTB feature. We hypothesized that the patellar tendon of the residual limb would have increased thickness, increased CSA, decreased collagen fiber organization, and a higher proportion of neovascularity when compared to the intact limb.

METHODS

Subjects

Participants were all at least 18 years old. They must have had the prosthesis for a minimum of 1 year prior to the study and must have utilized it for at least 1 hour of combined weight bearing (e.g. standing, walking, running, lower extremity weight-bearing resistance training) per day. Participants were excluded from the study if they were non-ambulatory. The data from an existing study was used to estimate the sample size for detecting changes in tendon cross sectional area between normal and degenerative tendons.¹² With 95% power, and an α level of 0.05, using GPower software (GPower, Düsseldorf, Germany) we calculated that four subjects would be needed to detect a difference in tendon CSA between healthy and injured tendons. However, due to the exploratory nature of this study and the fact that more morphological

outcome measures were examined in this work, we recruited a total of 12 subjects with unilateral trans-tibial amputations who use a prosthesis with a PTB feature.

Instrumentation

High-resolution ultrasound images were acquired using a General Electric NextGen LOGIQe scanner (GE Healthcare, Milwaukee, WI, USA) with its musculoskeletal knee preset. Brightness-mode images and power Doppler images were captured using a linear transducer at a central frequency of 10 MHz and depth of 2 cm.

Questionnaires

All participants were asked to complete the following questionnaires to assess their mobility, function, and tendon health: 1) Victorian Institute of Sport Assessment – Patellar Tendon (VISA-P); 2) Prosthesis Evaluation Questionnaire (PEQ); 3) Prosthetic Limb Users Survey of Mobility (PLUS-M) (Table1). The VISA-P questionnaire is a brief survey that utilizes a series of 8 scaled questions to assess symptoms, simple tests of function, and ability to undertake physical activity.¹³ The VISA-P has been found to have excellent test-retest and inter-tester reliability (both, $r = 0.95$).¹³ The PEQ is a self-administered questionnaire with a linear analog scale response 0-100 mm long, where higher numbers indicate better function and quality of life.¹⁴ The PEQ measures prosthesis-related changes in function and quality of life specific to lower limb prosthesis use and has ten sub-scales (ambulation, appearance, frustration, perceived response, residual limb health, social burden, sounds, utility, well-being, usefulness) of which nine have high internal consistency.¹⁴ The PEQ has good content validity and temporal stability (test-retest reliability), validity ranges from .73 to .89 depending on the sub-scale.¹⁴ For our study we used the ambulation sub-scale and scored the responses addressing pain in the residual limb (Table 1). The PLUS-M is a self-report instrument for measuring mobility in adults using lower

limb prosthesis.¹⁵ The PLUS-M has an ordinal-based response option (1 through 5) ranging in level of difficulty to perform (5 being easiest and 1 being unable) on activities that focus on two primary forms of movement, locomotion (i.e., movement in a continuous, repeatable pattern) and/or postural transitions (i.e., movement from one position to another or one type of activity to another).¹⁵ The PLUS-M has good construct validity (strong positive relationship with Prosthesis Evaluation Questionnaire-Mobility Section (PEQ-MS) scores ($p=.78$, $P<.001$), Activities-specific Balance Confidence (ABC) scores ($p=.81$, $P<.001$), and Patient Reported Outcome Measure Information System for Physical Function (PROMIS-PF) scores ($p=.81$, $P<.001$)) for people with lower limb amputation.¹⁵

Procedures

Each subject participated in a one-time data collection session. Upon arrival each subject was given four questionnaires; 1) VISA-P, 2) PEQ, 3) PLUS-M, 4) a Medical History and Activity Questionnaire, prior to the ultrasound imaging protocol. Subjects' weight and height were also measured.

Once complete, ultrasound imaging (depth=2cm, Doppler gain=6dB) was used to obtain images of each subject's patellar tendons on both the residual and intact limbs. Ultrasound imaging was performed by the same trained investigator. The patient was seated with knees and hips flexed to 90 degrees on the edge of a treatment table. A total of twelve images were taken per limb during the imaging session. The proximal attachment of the patellar tendon on the inferior portion of the patella was palpated and marked on the skin at midline. The distal attachment was palpated at the tibial tuberosity and marked on the skin at midline. A tape measure was used to determine the middle of the patellar tendon and was there marked on the skin. To obtain tendon thickness and collagen fiber organization, brightness-mode imaging was

performed longitudinally at the proximal end (patella-patellar tendon junction), mid-portion, and distal end (tibial tuberosity-patellar tendon junction) of the patellar tendon (Figure 1). To measure tendon CSA, brightness-mode imaging was acquired transversely at the same three locations of the patellar tendon (Figure 1). Next, tendons were assessed at all 3 longitudinal and transverse locations for color Doppler signal, and images were captured if neovascularization was observed. Image capture was carefully performed to ensure optimal image quality and minimize movement artifacts.



Figure 1. The left image shows the longitudinal image acquisition. The right image shows the transverse image acquisition.

Data Analysis

All images were exported in JPEG format to a personal computer for analysis of the tendon gross morphology, thickness, and CSA using ImageJ (National Institutes of Health, Bethesda, MD, USA). The tendon thickness was measured on each longitudinal image (i.e.,

proximal, mid-, and distal tendon), which was defined as the perpendicular distance between the borders that outline the patellar tendon (Figure 2). The CSA was determined on each transverse image (i.e., proximal, mid-, and distal tendon). The border of the tendon was outlined manually with a freehand polygon selection tool and the area of the region of interest (ROI) was then calculated (Figure 3).

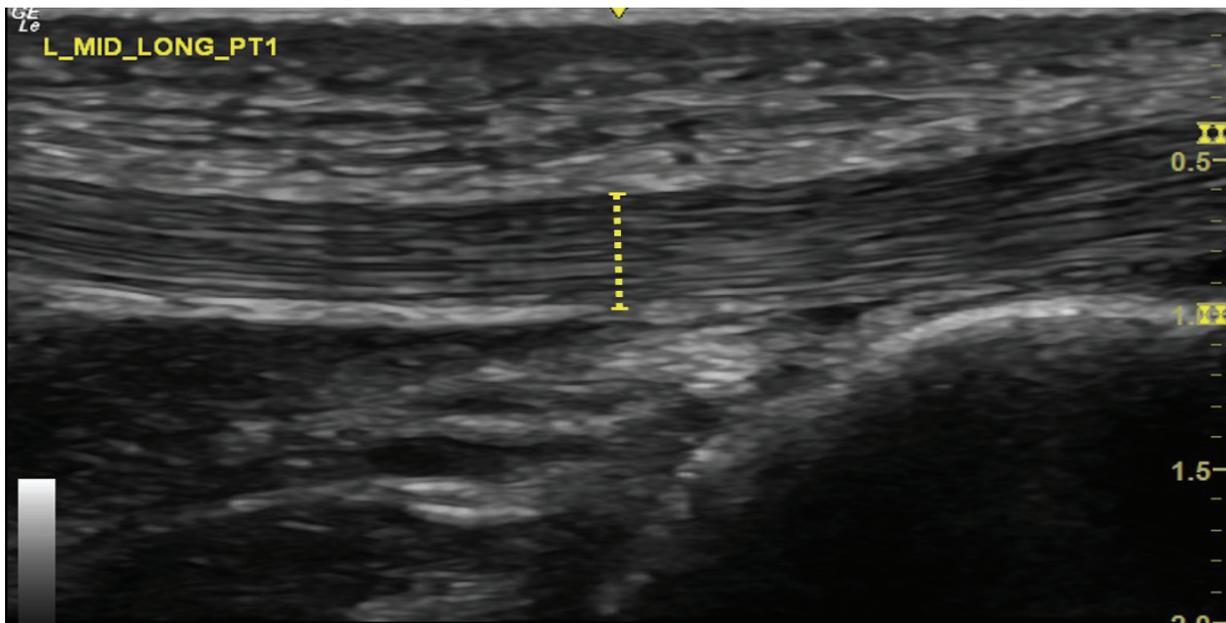


Figure 2. Measurement of the tendon thickness. The thickness is defined as the distance between the borders of the tendon at the center of the image (dashed line).

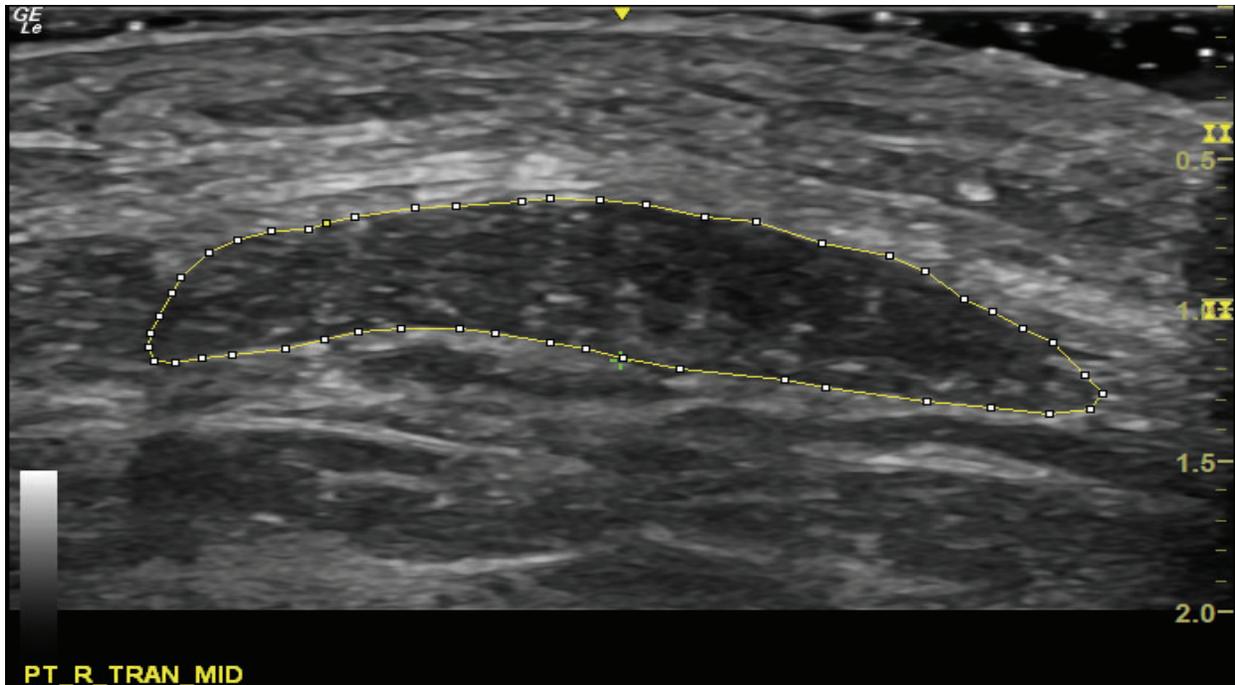


Figure 3. Measurement of the cross-sectional area (CSA) of the tendon. The CSA is defined as the area within the borders of the tendon (dashed line).

For analysis of the intra-tendinous morphology, a custom Matlab program (Mathworks, Natick, MA, USA) was used to quantify collagen fiber organization on the longitudinal image of the mid-portion tendon. In this study, a ROI in the middle 50% of the tendon was manually outlined for the calculation of intra-tendinous morphology (Figure 4). Within the ROI, 32 x 32 pixel kernels were extracted and all possible kernels were processed. A second MATLAB script was used to perform a two-dimensional (2D) Fast Fourier Transform (FFT) of each ROI, from which the peak spatial frequency radius (PSFR) was extracted. The intra-tendinous morphology analysis process has previously been described in full.^{16,17} Typically, a lower PSFR reflects greater collagenous disarray, which is one underlying structural phenomenon of degeneration.¹⁸ A higher PSFR reflects a greater collagenous density, which is associated with greater stiffness and elastic modulus.^{17,19}

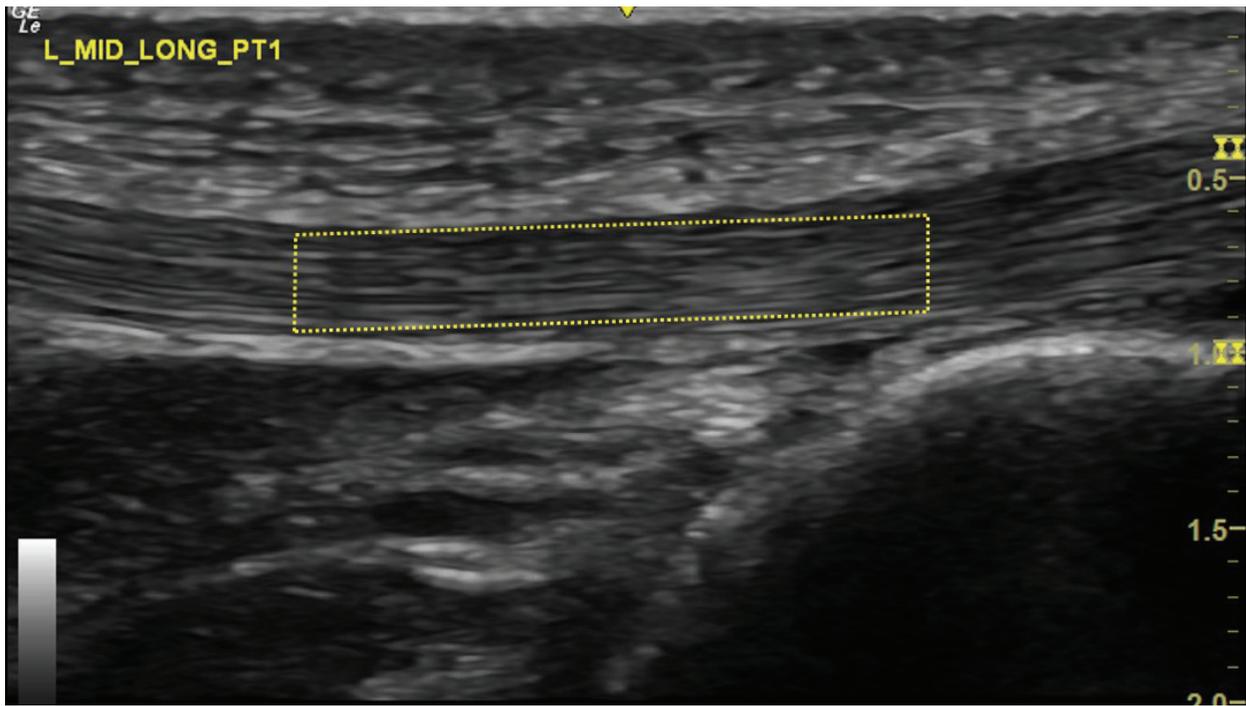


Figure 4. Measurement of intra-tendinous morphology (peak spatial frequency radius [PSFR]). The box outlines the region of interest which is used to analyze the collagen fiber disorganization at each location.

Neovascularity was determined on both longitudinal and transverse images (i.e., proximal, mid-, and distal tendon) using Doppler imaging. Any incidence of neovascularity was noted and determined by the researchers based on the visual evidence of red, pulsed coloration indicating blood flow within the boundaries of the tendon (Figure 5). A scoring of yes or no was used to note incidence of neovascularity.²⁰

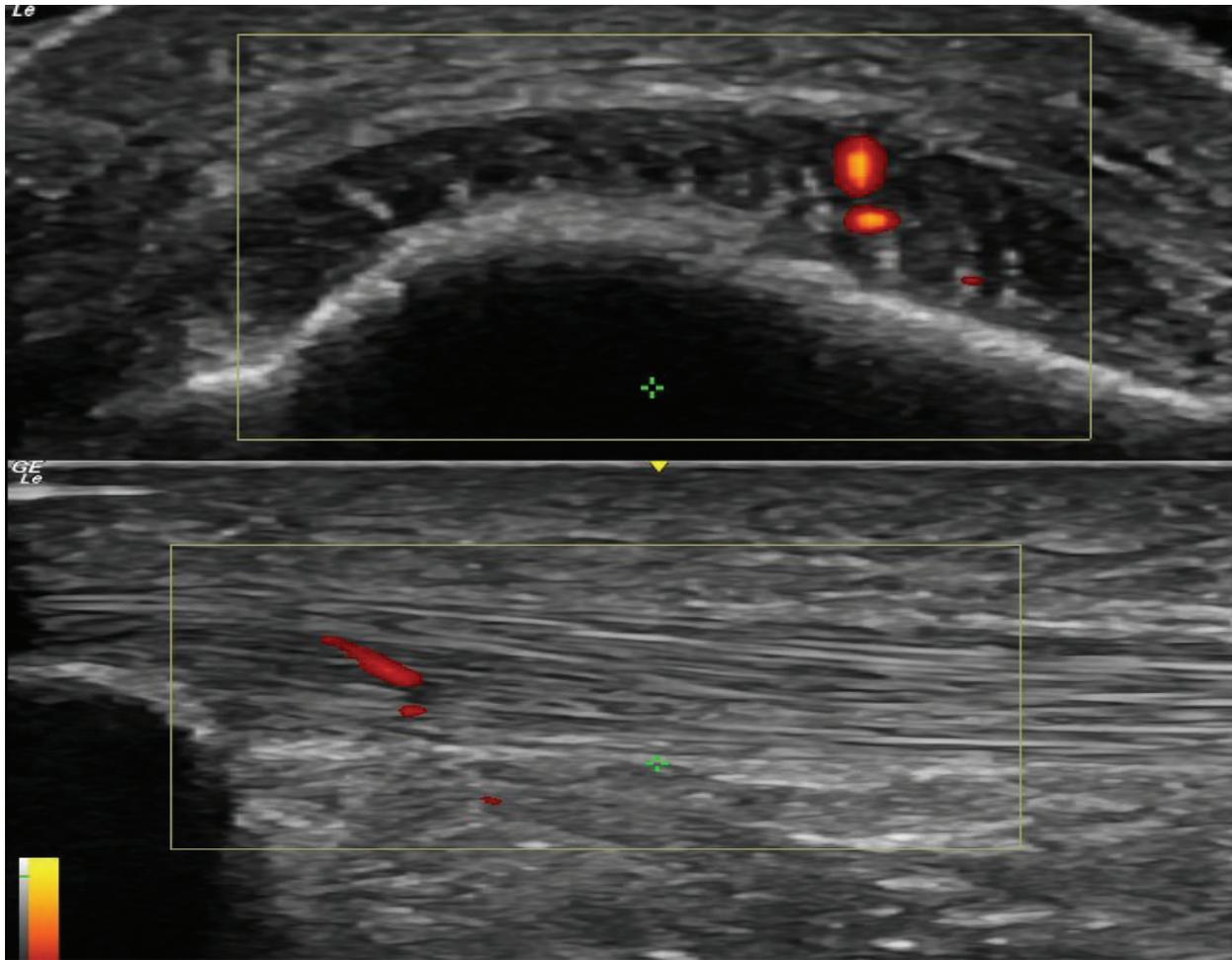


Figure 5. Neovascularity found in the transverse image (top) and longitudinal image (bottom). The red areas indicate blood flow within the tendon.

Measurement Reliability

To establish intra-rater measurement reliability of tendon morphological measures (i.e., thickness, CSA, and PSFR), we performed repeated measurement of the same data from 5 subjects on 2 separate days, 7 days apart. Intraclass correlation coefficients (ICCs) and standard errors of measurements (SEMs) were calculated revealing excellent measurement reliability and low SEMs for PSFR (ICC = 0.998; SEM = 0.00646m⁻¹), CSA (ICC = 0.978; SEM = 1.61mm²) and thickness (ICC = 0.984; SEM = 0.037mm).

Statistical Analysis

Paired t-tests were used to compare the tendons of the residual and intact limbs on each morphological variable. Chi-square was used to compare the proportion of neovascularity between limbs. All statistical analyses were performed with SPSS Statistics 24 for Windows (International Business Machines Corp, Armonk, New York). *A priori* alpha was set at 0.05.

RESULTS

Subject Characteristics

Our participants averaged 53.6 years old, 179.9 cm in height, 93.9 kg in weight, 16.7 years wearing the prosthesis, and were 75% male, see Table 1.

Table 1. Subject Characteristics

| Variable | Subjects (n=12) |
|-----------------------------|--|
| Gender | 3 Females, 9 Males |
| Age, y | 53.6±16.9 |
| Height, cm | 179.9±9.0 |
| Weight, kg | 93.9±14.2 |
| Side of Amputation | 5 Right, 7 Left |
| Years wearing prosthesis | 16.7±18.2 |
| Reason for amputation | 2 Infection 3 Peripheral Artery Disease 7 Trauma |
| VISA-P | 70.8±20.7 |
| PEQ (Pain in residual limb) | 68.5±38.9 |
| PEQ (Ambulation) | 80.3±30.6 |
| PLUS-M | 80.1±20.0 |

Thickness

Tendon thickness was significantly greater when comparing the residual limb (4.76±0.91mm) to the intact limb (intact limb=4.15±0.96) at the proximal portion; (p=0.014;

Figure 6). No differences were found between limbs at mid-portion (residual limb=4.08±0.62mm; intact limb=4.19±0.78mm; p =0.452; Figure 6) and distal portion (residual limb=5.24±1.17mm; intact limb=5.09±1.1mm; p =0.53; Figure 6).

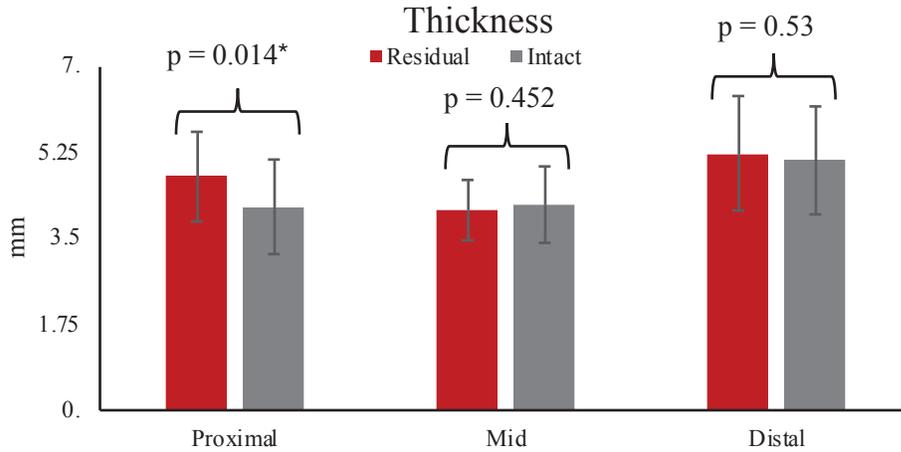


Figure 6. Comparison of tendon thickness between the intact and residual limb. The * indicates a significant difference.

Cross-sectional Area (CSA)

CSA was significantly greater when comparing the residual limb (113.71±22.33mm²) to the intact limb (101.79±17.15mm²) at the mid-portion; (p =0.008; Figure 7). No differences were found between limbs at the proximal portion (residual limb=108.58±18.62mm²; intact limb=114.89±20.79mm²; p =0.164; Figure 7) and distal portion (residual limb=102.11±24.38mm²; intact limb=112.92±26.98mm²; p =0.083; Figure 7).

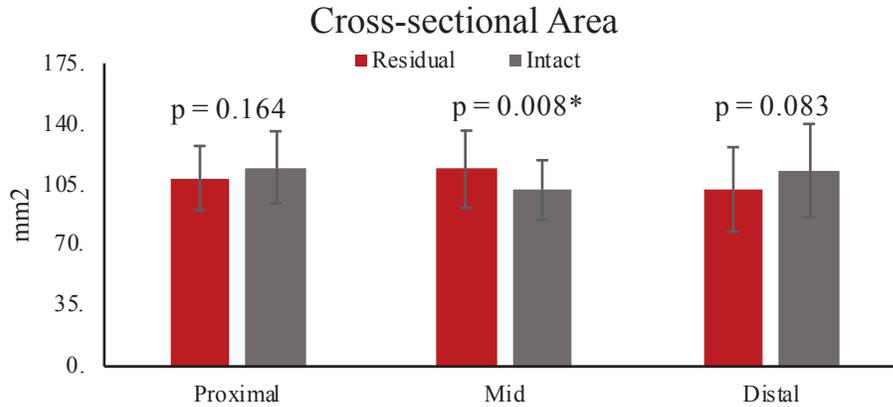


Figure 7. Comparison of CSA between the intact and residual limb. The * indicates a significant difference.

Neovascularization

Our results demonstrated that more residual limbs (66.6%) had neovascularization than intact limbs (25%) using a Chi-square analysis ($X^2= 4.278$ DF =1; $p=0.039$).

Collagen Fiber Organization

The paired t-test revealed that PSFR was not different between residual (1.99 ± 0.15) and intact (1.91 ± 0.14) limbs at the mid-portion of the patellar tendon ($p = 0.215$) (Figure 8).

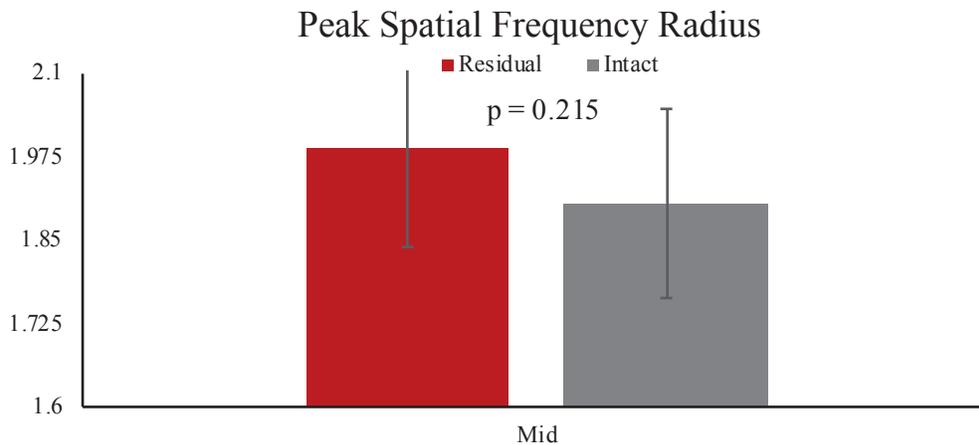


Figure 8. Comparison of PSFR between residual and intact limb.

DISCUSSION

To the best of our knowledge, this is the first study to report comprehensive morphological measures of the patellar tendon in the unilateral trans-tibial amputee population. Our findings support the hypothesis that morphological changes and neovascularity exist in the residual tendons of trans-tibial amputees, likely due to the abnormal loading applied to the patellar tendon. Specifically, we found increases in proximal tendon thickness, mid-portion tendon CSA, and an increased proportion of neovascularity in the residual limb. However, our study revealed no differences in intra-tendinous morphology (i.e., PSFR) between the tendons of the residual and intact limbs.

With respect to the comparisons of morphological measures in trans-tibial amputees between our work and existing literature, our findings agree with results reported by Ozcakar et al that there was a significant increase in tendon thickness on the residual limb, although Ozcakar et al did not specify the location within the tendon.⁶ We also found a significant increase in mid-portion CSA on the tendon of the residual limb compared to the intact limb. While no study has reported on patellar tendon CSA for transtibial amputees, increased mid-portion CSA found in our study may be due to the fact that the location of the weight-bearing feature is close to the mid-portion of the tendon. Animal models have shown that the site that bears abnormal perpendicular loading is prone to more morphological changes.⁵

When comparing our work to the existing literature on tendon degenerative disorders (e.g., tendinopathy), our study agreed with Weinberg et al. who reported an increase in proximal tendon thickness in the tendinopathic tendon.²¹ Our findings of increased mid-portion tendon CSA also agreed with Arya et al (2010) who reported increased CSA in tendinopathic tendons when compared to controls.²² Additionally, our data was similar to previous work showing that

injured tendons have a higher proportion of neovascularity.^{9,20,21,23,24} Previous studies show the proportion of neovascularity ranging from 29% of jumping athletes with patellar tendon pain²⁴, to 42% of volleyball athletes with patellar tendon pain.²³ Our subjects had a neovascularity proportion of 66.7% in the residual limb and 25% in the intact limb. Although the incidence of neovascularity in our study was greater, this may be related to the abnormal perpendicular loading from the patellar tendon bearing prosthesis. It is also possible that the tendon in the residual limb may develop degenerative changes that are commonly observed in tendon overuse injuries, such as tendinopathy.

Our subjects had similar intra-tendinous morphology (i.e., PSFR value) between limbs, which did not agree with Kulig et al. who found that degenerated tendons are characterized by comparably smaller values of PSFR.⁷ The PSFR of the tendons in the residual and intact limbs were between 1.91-1.99 mm⁻¹ in our study, which was lower than the PSFR of healthy patellar tendons (2.20-2.30 mm⁻¹) reported by Pearson et al.²⁵ This may indicate that altered intra-tendinous morphology (increased collagen fiber disorganization) of the patellar tendon was present in both intact and residual limbs in our cohort. The intact limb of transtibial amputees exhibit a higher knee extensor moment during walking compared to their residual limb, which is thought to result in higher tensile loading of the patellar tendon in the intact limb.²⁶ Thus, even though the tendon of the residual limb experiences an abnormal perpendicular force, it is possible that the higher tensile loading experienced in the tendon of the intact limb may result in similar collagen fiber disorganization as seen in the residual limb. Future studies will be needed to examine the difference in collagen fiber organization of the patellar tendon among the limbs of amputees and healthy non-amputees.

With respect to study limitations, first, our study included only individuals from the Las Vegas area which could limit the generalization of our results to other populations. Second, the cause of amputation for a majority of our subjects was trauma; however, trauma is the reason for amputation in about only about 10% of cases in the general population.

CONCLUSION

In conclusion, we observed an increase in proximal thickness and mid-portion CSA of the patellar tendon on the residual limb when compared to the intact limb. As well as an increased proportion of neovascularity on the residual limb. However, we found that there was no difference in PSFR, mid-portion or distal thickness, and proximal or distal CSA. Our findings suggested that use of a prosthesis with a PTB feature may contribute to morphological changes of the patellar tendon.

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CURRICULUM VITAE

Michelle Evers, SPT
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EDUCATION

- University of Nevada, Las Vegas – Las Vegas, NV
Doctorate of Physical Therapy – Expected graduation date May 2019
- California State University, Sacramento – Sacramento, CA
Bachelor of Science in Kinesiology – 2015

CLINICAL EXPERIENCE

- Comprehensive Therapy Centers, Outpatient Clinic – Henderson, NV (1/2019-3/2019)
- Flagstaff Medical Center, Acute – Flagstaff, AZ (10/2018-12/2018)
- HealthSouth Rehabilitation Hospital of Las Vegas, Rehabilitation – Las Vegas, NV (7/2018-9/2018)
- San Luis Sports Therapy, Outpatient Clinic – San Luis Obispo, CA (7/2017-8/2017)

CONTINUING EDUCATION/ SUPPLEMENTAL EDUCATION

- Combined Sections Meeting of the American Physical Therapy Association – Washington, DC 2019
- Combined Sections Meeting of the American Physical Therapy Association – New Orleans, LA 2018
- Combined Sections Meeting of the American Physical Therapy Association – San Antonio, TX 2017
- Advancing Neurological Therapeutics – Las Vegas, NV 2016

PROFESSIONAL MEMBERSHIP

- American Physical Therapy Association 2016 – Present

DOCTORAL DISSERTATION

- Ho KY., Evers M., Kellogg J., Teter K. *Patellar Tendon Morphology in Trans-Tibial Amputees Utilizing a Prosthesis with a Patellar-Tendon-Bearing Feature*. April 2017-May 2019.

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EDUCATION

- University of Nevada, Las Vegas – Las Vegas, NV
Doctorate of Physical Therapy – Expected graduation date May 2019
- University of Nevada, Las Vegas – Las Vegas, NV
Bachelor of Science in Kinesiology – 2016

CLINICAL EXPERIENCE

- Summerlin Hospital, Pediatric Inpatient, Pediatric Outpatient, NICU – Las Vegas, NV
(1/2019-3/2019)
- St. Rose Dominican Hospital, Siena Campus, Acute – Henderson, NV (10/2018-12/2018)
- Boulder City Hospital, Rehabilitation – Boulder City, NV (7/2018- 9/2018)
- Optimal Physical Therapy, Outpatient Clinic – Henderson, NV (7/2017-8/2017)

CONTINUING/SUPPLEMENTAL EDUCATION

- Combined Sections Meeting of the American Physical Therapy Association –
Washington, DC 2019
- “Therapeutic Neuroscience Education” Dr. Adrian Lowe 2017, 2018
- UNLV Distinguished Lecture Series- Dr. Sharon Dunn 2017, Dr. Carolee Weinstein 2016

PROFESSIONAL MEMBERSHIP

- American Physical Therapy Association 2017 - Present
- Member Nevada Physical Therapy Association 2017 - present
- Member Academy of Pediatric Physical Therapy 2019 - present

DOCTORAL DISSERTATION

- Ho KY., Evers M., Kellogg J., Teter K. *Patellar Tendon Morphology in Trans-tibial Amputees Utilizing a Prosthesis with a Patellar-Tendon-Bearing Feature*. April 2017-May 2019.

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EDUCATION

- University of Nevada, Las Vegas – Las Vegas, NV
Doctorate of Physical Therapy – Expected graduation date May 2019
- University of Nevada, Reno – Reno, NV
Bachelor of Science in Neuroscience – 2015

CLINICAL EXPERIENCE

- Therapeutic Associates Medford, Outpatient Clinic – Medford, OR (1/2019-3/2019)
- VA Southern Nevada Hospital, Acute – North Las Vegas, NV (10/2018-12/2018)
- HealthSouth Rehabilitation Hospital of Henderson, Rehabilitation – Henderson, NV (7/2016-9/2016)
- Body Wise Physical Therapy, Outpatient Clinic – Minden, NV (6/2017-8/2017)

CONTINUING/SUPPLEMENTAL EDUCATION

- Combined Sections Meeting of the American Physical Therapy Association – Washington, DC 2019
- Combined Sections Meeting of the American Physical Therapy Association – New Orleans, LA 2018
- Combined Sections Meeting of the American Physical Therapy Association – San Antonio, TX 2017
- “Pain Neuroscience Education” Dr. Adrian Lowe 2017, 2018
- UNLV Distinguished Lecture Series- Dr. Carolee Weinstein 2016, Dr. Sharon Dunn 2017

PROFESSIONAL MEMBERSHIP

- American Physical Therapy Association 2016 – Present

DOCTORAL DISSERTATION

- Ho KY., Evers M., Kellogg J., Teter K. *Patellar Tendon Morphology in Trans-Tibial Amputees Utilizing a Prosthesis with a Patellar-Tendon-Bearing Feature*. April 2017 – May 2019.