

# **The Test-Retest Reliability of Musculoskeletal Ultrasound on the Flexor Hallucis Longus Tendon**

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The test-retest reliability of musculoskeletal ultrasound on the cross-sectional area and thickness of the flexor hallucis longus tendon when measuring healthy adults performed by one health professional.

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Bachelor of Physical Therapy Thesis

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# Preface

This study took place at the Fontys University of Applied Sciences and has played an essential role in obtaining my Bachelor of Science degree in Physiotherapy. It is performed in collaboration with a multidisciplinary group of students from the departments of physiotherapy, podiatry and Medical Imaging and Radiological Techniques. The data was obtained by a final year student from the department of Medical Imaging and Radiological Techniques. This article is unpublished and written by the author Broos Spijkers.

The start of this study was in February 2016 and it was completed in June 2016. During this period I have had the opportunity to learn about musculoskeletal ultrasound and its potential from a physiotherapy perspective. In the future, I would like to learn more about musculoskeletal ultrasound and master its applications on other regions. This study has been an addition for focusing on new ambitions in regards to my development as a physiotherapist after my Bachelor's degree.

Looking back on the process of this study I want to thank everybody who supported me. Firstly I would like to thank my supervisor Marc Schmitz for his inspiration in regards to musculoskeletal ultrasound and giving me the opportunity to learn more about this subject. Secondly I want to thank the colleague student who performed the measurements for this study Younes El-Hamdaoui. I also would like to thank the other researchers and fellow students Vincent Kluitmans, Remon Vijfvinkel, Gard Patursson, Ayra de Waard and Yannick Warnier for being so dedicated to our collaboration. Finally I would like to thank my friends and colleague physiotherapists Jason Power, Daniel Shih, Kunashe Parwada, Jennifer Ko and Shannon Bailey for their remarks and advice during this study.

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# Abstract

**Background:** The flexor hallucis longus (FHL) is one of three muscles that can cause tarsal tunnel syndrome (TTS). TTS is a less common pathology whereby a space-occupying process compresses the tibial nerve. Before it can be determined if musculoskeletal ultrasound (MSU) can be used to diagnose TTS further research needs to be performed. It has to be determined whether MSU is a reliable diagnostic tool in regards to measuring the FHL tendon. Therefore the goal of this study was to determine the test-retest reliability of the FHL tendon for the cross sectional area (CSA) and thickness using MSU.

**Method:** Experimental quantitative cross-sectional research was utilized. Both ankles were measured in 59 participants (30 males, 29 females) for both the CSA as well as for the thickness of the FHL tendon using MSU. For the CSA measurements a transversal image was taken and for the thickness measurements a longitudinal image. To analyze the images the measuring software ImageJ was used. The intraclass correlation coefficient (ICC) with the two-way random model and absolute agreement was used to calculate the test-retest reliability.

**Results:** The median and interquartile range (IQR) of age ( $22 \pm 3$ ) and BMI ( $22.9 \pm 4.2$ ) of the sample population is shown, since this data was abnormally distributed. The data of the weight ( $70.6 \pm 13.3$ ) and height ( $1.74 \pm 0.10$ ) were normally distributed, therefore the mean value and standard deviation (SD) is shown. The test-retest reliability for the CSA was excellent (ICC values were 0.81 and 0.85). For the measurements of the thickness the test-retest reliability was fair (ICC= 0.57) to excellent (ICC= 0.95).

**Conclusion:** The use of MSU on the FHL tendon has excellent test-retest reliability for the CSA and fair to excellent test-retest reliability for the thickness.

**Key words:** test-retest reliability, musculoskeletal ultrasound, flexor hallucis longus, tarsal tunnel, physiotherapy

# Table of contents

<b>Preface</b>	<b>1</b>
<b>Abstract</b>	<b>2</b>
<b>1. Introduction</b>	<b>4</b>
<b>2. Method</b>	<b>6</b>
2.1 Research design	6
2.2 Participants	6
2.3 Data collection and procedures	7
2.4 Measurement instruments	9
2.5 Statistical analysis	9
2.6 Ethical paragraph	10
<b>3. Results</b>	<b>11</b>
<b>4. Discussion</b>	<b>14</b>
<b>5. Conclusion</b>	<b>19</b>
<b>References</b>	<b>20</b>
<b>Appendices</b>	<b>I</b>
Appendix I Information letter (Dutch version)	I
Appendix II Information letter (English version)	III
Appendix III Informed consent (Dutch version)	V
Appendix IV Informed consent (English version)	VI
Appendix V Measurement protocol	VII

# 1. Introduction

In the period from 2006-2010 there has been a total of 108.167 ankle or foot injuries registered in the Netherlands (1). Based on the average cost for diagnosing and treatment of these patients, the costs in total for ankle or foot injuries over this period were €161.817.832 (1). These injuries included fractures, dislocations, ligamentous sprains or strains and muscle-/tendon injuries. This does not even include less common pathologies such as tarsal tunnel syndrome (TTS) which is often missed in the differential diagnosis (2). When pathologies such as TTS are missed in the diagnostic process it is likely that the treatment that is being given to that patient is not specifically targeting the cause of the symptoms. This will increase the number of treatment sessions followed by a logical increase of treatment costs for ankle or foot injuries .

TTS is thus far known as a rare pathology where the posterior tibial nerve or one of its branches becomes entrapped. The tarsal tunnel (TT) is located at the medial side of the ankle. The origin of the TT is located at the posterior side of the medial malleolus and attaches to the anteromedial side of the calcaneus. Through this fibro-osseous tunnel the following structures run: the posterior tibial (PT) tendon, flexor digitorum longus (FDL) tendon, flexor hallucis longus (FHL) and its tendon. Also, in between the FDL tendon and FHL tendon runs a neurovascular bundle containing the tibial nerve, posterior tibial artery and vein (3). The TTS has a broad etiology since several case studies have reported different causes (4-6).

For a typical TTS-patient the symptoms are poorly localized burning sensation or pain and paresthesia at the medial plantar surface of the foot (3). This burning sensation or pain and paresthesia usually worsens after activity (6). Within the physical examination TTS is characterized by one or more of the following findings: positive Tinel sign, pain with passive dorsiflexion or eversion, varus or valgus deformity of the heel, weakness of the foot intrinsic musculature with sustained plantar flexion of the toes (3). However with rather specific symptoms and the physical examination, diagnostic accuracy is still not 100% for these tests (7) . Therefore TTS could still be misdiagnosed as plantar fasciitis or ankle sprain (8).

Currently magnetic resonance imaging (MRI) is the golden standard for diagnostic imaging on soft tissues. The main reason for MRI being the golden standard is because of its multi-planar imaging and high resolution. However because of the high costs and limited availability, the use of MRI at times is not practical from a physiotherapy perspective. Over the last few years a new method for soft tissue imaging has become available within physiotherapy which is called musculoskeletal ultrasound (MSU). The ability to use MSU from a physiotherapy perspective has transformed the role of physiotherapists in the assessment and management of musculoskeletal- and sports injuries. MSU can play an essential role in the diagnosis and monitoring of injuries within the anatomical level of the assessed tissues. (9). Research has shown that MSU can substitute MRI in 100% of the cases for the diagnosis of tendonitis/tenosynovitis

in general (10). MSU has proven itself to be useful, cost-efficient and time-efficient for imaging similar structures in comparison with magnetic resonance imaging (MRI) or computer tomography (CT)(10-11). There can be a difference in thickness, cross sectional area and anatomical anomalies in various tendons. Since this difference is demonstrated, it is important to determine the diagnostic value of MSU on specific tendons before using MSU as a diagnostic tool. This will be helpful to determine if MSU is a valid imaging technique for the diagnosis of tendonitis/ tenosynovitis in specific tendons such as the ones involved in TTS. Various case studies show that the PT tendon, the FDL tendon and the FHL tendon can have an influence on the space-occupying process in the TT (4,5,12). There has been research done for the PT tendon that suggest that MSU can replace MRI in 100% of the cases of dysfunction or tenosynovitis of the PT tendon (10). However the research that has been done for the diagnostic value of MSU on the FHL tendon is minimal.

The purpose of this study is to assess the reliability of MSU on the FHL tendon. MSU can provide a transversal view and longitudinal view which can give an indication for the CSA and the thickness of the FHL tendon. This has led to the following research question: "What is the test-retest reliability for CSA and thickness of the FHL tendon using musculoskeletal ultrasound on healthy adult subjects measured by one health professional?" Ultimately, this could assist the diagnostic process of patients with TTS. Even more specifically, potentially decreasing the amount of misdiagnosed TTS patients that involve the FHL tendon and costs associated with treatment.

## 2. Method

### 2.1: Research design

This research had an experimental quantitative cross-sectional design. It has been executed by a multidisciplinary group of final year students of which four study physiotherapy, two study podiatry and one studies Medical Imaging and Radiographic Techniques (MIRT) at the Fontys University of Applied Sciences in Eindhoven, Netherlands. This research has been set up so that every student obtained the data they needed to answer their own research question. With this in mind, some parts of the data-collection were not applicable nor did they have an influence on this particular study. For this reason only the parts of the research that were relevant and/or influence the data collected for this study are shown.

### 2.2: Participants

Based on a study done by Walter et al., the suitable amount of participants for this reliability study was a minimum sample of 46 participants (13). The participants were included if they were not younger than 18 years old and not older than 30 years old and also have a good understanding of either the Dutch and/or English language. The reason for the minimum age limit of 18 years old was chosen so that the participants can decide for themselves if they participate. Another reason for this minimum age limit was that if participants are younger than 18 years old, their bodies are not fully grown yet. When the bodies of participants are not fully grown and those of other participants are, the anatomical difference would be greater between these participants. Also because this research did not allow participants over 30 years old, the risk of degeneration of the tissues has been minimized. Participants were excluded from this research if they have had any previous injuries, surgeries or neuromuscular disorders concerning their big toes, feet, ankles or lower legs for the past six months. Also participants were excluded from research if they had diabetes mellitus or any kind of rheumatism in the lower extremity. These inclusion- and exclusion criteria have been included into the information letters as can be read in appendices I & II. The recruiting of participants took place at the Fontys University for Applied Sciences. Participants were informed using the information letter, which has been made in an English version (appendix II) for the students in the English stream and in Dutch for the main stream of students (appendix I). The researchers have approached students in the rooms, cafeteria and hallways at the Fontys University of Applied Sciences. Informing the students was done by the use of the information letters (appendices I & II). The students that were potential participants are shown in table 1.

Table 1. Students at Fontys University of Applied Sciences.

Study	Language of the study	Amount of students
<b>MIRT</b>	Dutch	480
<b>Physiotherapy Dutch stream</b>	Dutch	483
<b>Physiotherapy English stream</b>	English	154
<b>Podiatry</b>	Dutch	183
<b>Speech therapy</b>	Dutch	222
<b>Orthopedic technology</b>	Dutch	151
<b>Total</b>	-	1673

*\*\* Abbreviations: MIRT = Medical Imaging and Radiological Techniques*

### 2.3: Data collection and procedures

The researcher at MSU station one was a last year MIRT student and has performed the MSU imaging and measurements for the test-retest reliability (Figure 1). This researcher did not have any experience with MSU prior to the course for this research. All the researchers have spent 60-120 minutes undertaking a theoretical e-learning course. After the theoretical course, an experienced musculoskeletal ultrasonographer supervised all the researchers during two sessions of 90 minutes. This included imaging with the Prosound Alpha 7 from the brand Hitachi Aloka. In the first session with the supervisor, the focus was on transverse imaging. The second session focused on the longitudinal imaging. After the supervised sessions, the whole group jointly practiced without supervision for twenty hours.



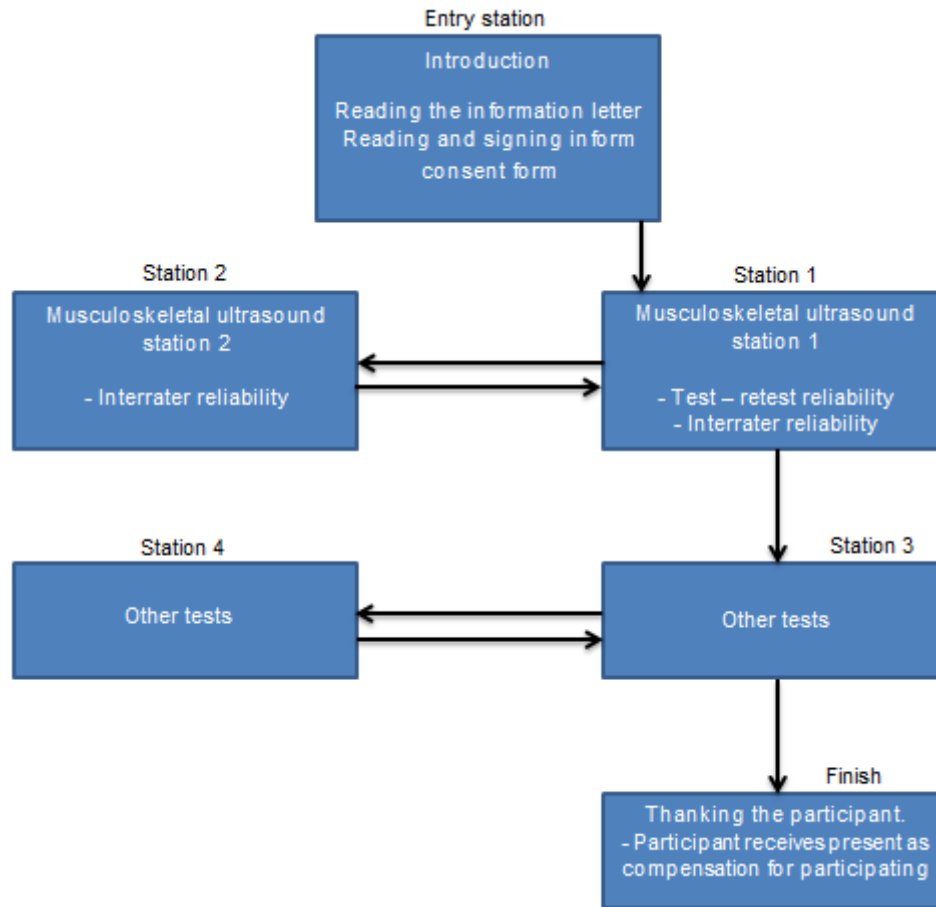


Figure 1. Flowchart

The flow of the research process was constructed so that the stations were not of influence to each other. When the participant came into the room, he or she was welcomed by one of the researchers and assigned with an anonymous code for blinding of the researchers. The participant was instructed to read the information letter if they had not done so already. It was also necessary for the participant to read and sign the informed consent form at this station when the participant chose to participate.

After signing the informed consent form, the participant was sent to station one where the researcher took the images as stated in the measurement protocol (appendix V). The researcher took two images of each ankle noting which one was the left ankle and right ankle. At station one the researcher was testing both for the test-retest reliability as the inter-reliability of the MSU. Afterwards, the participant moved on to the second station where a last year physiotherapy student took two images of each ankle again as stated in the measurement protocol. However the researcher at station two only took the images to study the interrater-reliability. The participant had to go back to the first station again. At the second visit of station one the same researcher as at the first visit to station one took two images of each ankle again (Figure 1).

To the knowledge of the author, there is no evidence that MSU imaging will have a direct effect on the structures. For this reason it was unlikely that the MSU measurements at the second station would have been of influence on the findings from the MSU measurements.

The measurements were performed according to the guideline of the European Society of Musculoskeletal Ultrasound (ESSR) (14). This guideline did not contain information about the measurements of the thickness and CSA of the FHL. Therefore, the research group has carefully composed a measuring protocol based on the ESSR. The thickness was measured in millimeters using a longitudinal view on the FHL. The CSA was measured in square millimeters using the transverse view on the FHL. The landmarks of the transducer, position of the participant and the settings of the MSU can be read in the measurement protocol (appendix V). Because of the lack of time it was not possible to also do the thickness and CSA measurements on the images during the research. In this research, it was decided to only take the images during the research and save them on an USB-stick every hour. This way the researcher had enough time to do the measurements of the thickness and CSA of the FHL. The images were transferred from the USB-stick to a laptop or computer to do the measurements. The time at each MSU station was not more than ten minutes per participant. With this in mind, this research could process one participant every 30 minutes.

## 2.4 Measurement instruments

The MSU imaging was done using Hitachi Aloka's Prosound Alpha 7 (manufactured in Germany in 2012) in combination with the linear transducer from Hitachi Aloka, type UST-5412 36mm high frequency. The location of the transducer needed to be in between the most prominent part of the medial malleolus and the ventral portion of Achilles tendon (appendix V). The linear transducer of Hitachi Aloka's Prosound Alpha 7 fitted in between the most prominent part of the medial malleolus and the Achilles tendon. Because of this, the information received from imaging was more reliable than with a transducer that did not fit in between these landmarks.

## 2.5 Statistical analysis

As can be read in paragraph 2.3, the images that were taken by the researchers were transferred from the MSU device onto a USB-stick. After the research, the images were used to do the measurements of the thickness and CSA of the FHL. The USB-stick was used to transfer the images onto a laptop or computer. When the images were on the laptop the same researcher that took the images did the measurements (appendix V). The data from the thickness and CSA of FHL from the participants were noted in Microsoft Excel by the researcher.

For statistical analysis of the data, the 23<sup>rd</sup> version of the predictive analytics software 'Statistical Package for the Social Sciences' (SPSS) was used. To determine the test-retest reliability of the MSU on the FHL, the researchers used the average of the two measurements at each visit for thickness and CSA per ankle of the participant. The first visit of the participant at station one was compared to the second time this participant visited this station. The test that was used in this reliability analysis was the 'two way random' model. The outcome has been presented in terms of the intraclass correlation coefficient (ICC) for test-retest reliability. This model was combined with the 'absolute agreement' type and 'single measures'. This particular type was chosen because the goal of this research was to determine if the agreement between measurement one and measurement two was absolute. The data was checked for normal distribution with the help of a histogram and a Gaussian curve. If the population was normally distributed it could be described using the mean value and the standard deviation (SD) of this data. The population had to be described using the median and interquartile range (IQR) if it was not normally distributed. The ICC was calculated for both the CSA as the thickness of the FHL tendon. Higher ICC values indicate a better test-retest reliability. An ICC of 1 indicates perfect agreement and an ICC of 0 indicates random agreement. To interpret the results from SPSS, an ICC classification was used. An ICC lower than 0.40 was viewed as poor, in between 0.40-0.59 as fair, in between 0.60-0.74 as good and higher than 0.75 as excellent (15).

## 2.6 Ethical paragraph

In relation to ethical aspects, the participants had to be informed about the research. For this study the researchers recruited verbally in the rooms, cafeteria and hallways of the Fontys University of Applied Sciences. That means that they explained to the participant why this research is important followed by handing the information letter (appendices I & II) to the potential participant. This approach had the advantage that the participant could ask questions for additional information. The information letter was also handed out at the entry station of the research to make sure the participants were well informed prior to the start of the research. This letter contained information regarding the inclusion criteria and exclusion criteria for participation of the research. There were no possible risks for the participant involved in this research. However, the maximal duration of time that the participant could be used for research was 60 minutes according to ethical guidelines. The participant also had to provide informed consent by signing the informed consent form. If the participants signed the informed consent form it meant that they agreed that the researchers could collect, analyse, document and/or present the data obtained from the research for scientific purposes. By signing the informed consent the participant also agreed that personal information will be destroyed after the current research and anonymous data are stored safely for 15 years. After 15 years the anonymous data that was collected will be destroyed as well.

### 3. Results

A total of 68 subjects were recruited for the experiment. Five subjects were excluded because they surpassed the age limit in the inclusion criteria. Another three subjects were excluded because they wanted to leave due to the lack of time. Finally, one more subject was excluded due to the loss of data. In total 59 subjects were included for participation (N=59). Based on gender, this group contained 51% males (N=30) and 49% females (N=29). A total overview of the demographic data of this population is shown in table 2.

Table 2. Demographic data of sample population (N=59) presented as: Mean( $\pm$  SD) + Minimum – Maximum.

	Age (Years)	Weight(kg)	Height (m)	BMI (kg/m <sup>2</sup> )
<b>Male</b>	23 ( $\pm$ 2)	76,0 ( $\pm$ 10,4)	1,80 ( $\pm$ 0,07)	23,3 ( $\pm$ 2,3)
	19 – 30	58,5 – 93,5	1,68 – 1,95	16,3 – 27,6
<b>Female</b>	22 ( $\pm$ 2)	62,5 ( $\pm$ 22,0)*	1,68( $\pm$ 0,07)	22,8 ( $\pm$ 4,8)*
	18 – 27	48,0 – 109,0	1,46 – 1,83	18,0 - 37,7
<b>Total sample population</b>	22 ( $\pm$ 3)*	70,6 ( $\pm$ 13,3)	1,74( $\pm$ 0,10)	22,9 ( $\pm$ 4,2)*
	18-30	48,0 – 109,0	1,46 – 1,95	18,0- 37,7

\* These values are not normally distributed, they are noted as : Median ( $\pm$  IQR) + Minimum – Maximum

\*\* Abbreviations: kg = kilogram, m = meter, kg/m<sup>2</sup> = kilograms per square meter

The MSU data was tested for distribution with the help of a histogram and a Gaussian curve. The results from the histograms and the Gaussian curves show that not all the data is normally distributed. Therefore the median and the interquartile range (IQR) was calculated.

In figure 2 and figure 3 the median and IQR are shown as they have been interpreted into boxplots. However the data from the retest of the CSA measurement at the left side was the only one that was abnormal distributed. Therefore the mean value and standard deviation (SD) of the different CSA data are shown in table 3.

Table 3. Summary of Mean values and SD's of total CSA measurements in mm<sup>2</sup>.

	L-CSA-1*	L-CSA-2	R-CSA-1*	R-CSA-2*
<b>Mean (<math>\pm</math>SD)</b>	13,88( $\pm$ 2,16)	14,08( $\pm$ 1,99)	14,04( $\pm$ 1,47)	13,86( $\pm$ 1,58)
<b>Minimum - Maximum</b>	6,50 – 17,50	10,00 – 17,00	10,50 – 17,00	10,00 – 17,00

\* This data was normally distributed

\*\* Abbreviations: L-CSA-1 = first test of the CSA measurement at the left side, L-CSA-2 = retest of the CSA measurement at the left side, R-CSA-1 = first test of the CSA measurement at the right side, R-CSA-2 = retest of the CSA measurement at the right side, SD = Standard Deviation

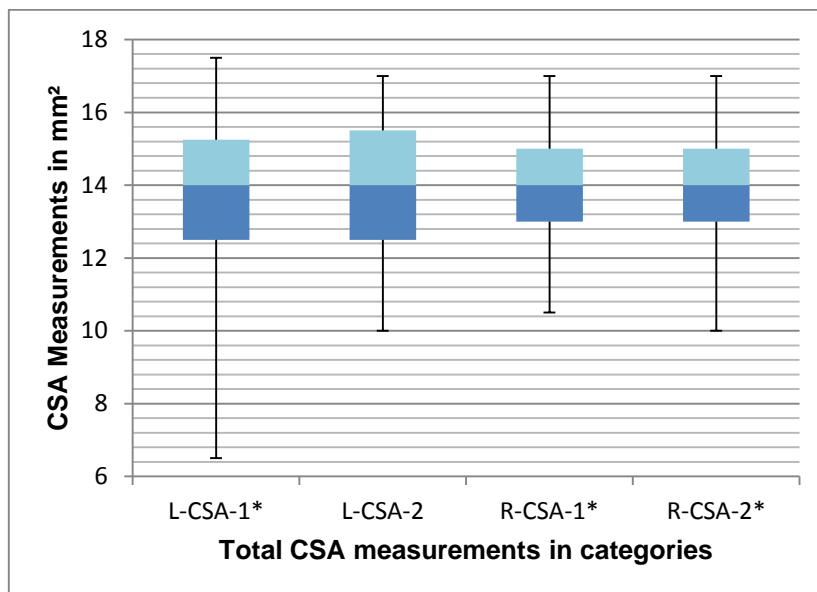


Figure 2. Cross sectional area measurements of the FHL-tendon

\* This data was normally distributed, mean value and SD are shown in table 3.

\*\* Abbreviations: L-CSA-1 = first test of the CSA measurement at the left side, L-CSA-2 = retest of the CSA measurement at the left side, R-CSA-1 = first test of the CSA measurement at the right side, R-CSA-2 = retest of the CSA measurement at the right side, mm² = square millimeter

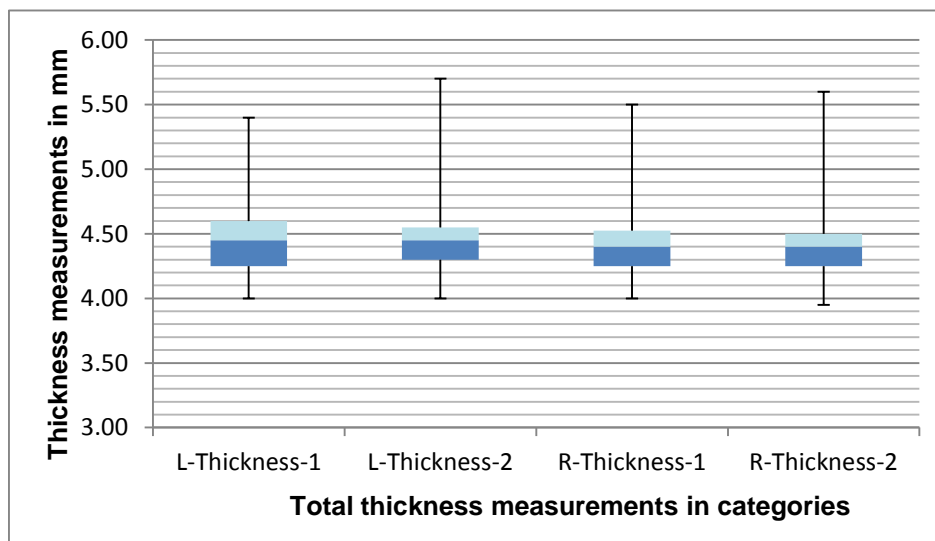


Figure 3. Thickness measurements of the FHL-tendon

\* The data of all four categories were abnormally distributed so the mean value and SD will not be shown.

\* Abbreviations: L-Thickness-1 = first test of the thickness measurements on the left side, L-Thickness-2 = retest of the thickness measurements on the left side, R-Thickness-1 = first test of the thickness measurement at the right side, R-Thickness-2 = retest of the thickness measurements on the right side, mm = millimeter

To be able to determine the test-retest reliability, the intraclass correlation coefficient (ICC) was calculated. As shown in table 4 the ICC value (0,85) determines that the test-retest reliability of the CSA on the left side is excellent. The correlation of the data from the CSA at the left side is significant since the P-value was less than 0,001. The lower bound shows an ICC value of 0,76. This means that even for the subject that had the lowest correlation for test-retest reliability, it still is excellent. At the right side the test-retest reliability of the CSA also is excellent as it showed to have an ICC value of 0,81. The correlation of this data is also significant since it has a P-value of less than 0,05 (16). The lower bound showed an ICC value of 0,71. So even with the subject that showed the lowest ICC-value, there was still a good test-retest reliability.

The data from the thickness measurements on the left side showed that the test-retest reliability is fair since it has an ICC-value of 0,57 ( $P=<0,001$ ). However the lower bound shows an ICC-value of 0,37. This means that the test-retest reliability of the subject with the worst ICC-value for this category has to be viewed as poor. upper bound however has an ICC value of 0,72. The data from the thickness measurements at the right side showed an ICC value of 0.95 ( $P=<0.001$ ).

*Table 4. Test-retest results for MSU measurements on the FHL-tendon defined as ICC's (N=59)*

	ICC (95%)	P-value
<b>CSA left</b>	0,85 (0,76 – 0,91)	< 0,001
<b>CSA right</b>	0,81 (0,71 – 0,89)	< 0,001
<b>Thickness left</b>	0,57 (0,37 – 0,72)	< 0,001
<b>Thickness right</b>	0,95 (0,92 – 0,97)	< 0,001

\* The ICC values shown in this table are the single measures.

\*\* Abbreviations: CSA = cross sectional area, ICC = intraclass correlation coefficient, P-value = probability-value

## 4. Discussion

The purpose of this study was to determine the test-retest reliability of musculoskeletal ultrasound (MSU) on the flexor hallucis longus (FHL) tendon. This includes assessing both the cross sectional area (CSA) and thickness on healthy subjects measured by one health professional. The hypothesis that arises from this purpose is the following : “The test-retest reliability for MSU on the CSA and thickness of the FHL tendon in healthy subjects measured by one health professional is good ( $ICC=0.60-0.74$ ) or excellent ( $ICC=0.75<$ ).”

The results of the CSA measurements confirm the hypothesis, however the results of the thickness only partially confirm the hypothesis. The ICC values for test-retest reliability for the CSA ranged from 0.85 ( $P=<0.001$ ) to 0.81 ( $P=<0.001$ ). For the CSA the ICC-values can be interpreted as excellent test-retest reliability. The ICC for the thickness however ranged from 0.57 ( $P=<0.001$ ) to 0.95 ( $P=<0.001$ ). This means that according to the ICC-values the test-retest reliability of the thickness has to be interpreted as fair to excellent.

During the measurements this researcher was holding the transducer on the left ankle using his right hand and on the right ankle using left hand. The researcher that performed the measurements was ambidextrous. Therefore this study is unable to determine whether the dominant hand had an influence on the test-retest reliability. However the positioning of the researcher was not the same for the right ankle as for the left ankle since the MSU device was set up at the left side of the participant. Therefore the researcher had to extend his left arm to reach to the right ankle of the participant. When measuring the left ankle the researcher had his right arm in a more relaxed position. Prior to the start of the research, when setting up the measurement protocol, the position of the researcher was not considered for standardization. The probe positioning has a great influence on the MSU imaging which was confirmed by the researcher in this study (17). Considering the researcher was in a different position when measuring the left ankle compared to the right ankle, this might have influenced the probe positioning as well. This would indicate that the measurement protocol was not set up optimally since the positioning of the researcher was not standardized. However this hypothesis is contradicted in the results of the measurements on the CSA since the test-retest reliability of the left- and right ankle were both excellent. In addition, the researchers chose to include into the protocol that the participant had to use the towel to keep the ankle in maximal dorsiflexion. This was done to lengthen the FHL tendon during the measurements. However if the FHL tendon needed to be maximally lengthened then the hallux should have possibly been in maximal extension as well (18). With this in mind the measurements might have been more accurate if the towel was kept underneath the hallux, keeping the hallux in an extended position as well as the ankle in dorsiflexion. Even if all of these factors could have been ruled out, the test-retest reliability probably still would not have been perfect.

Although there have been no similar studies done for CSA and thickness measurements on the FHL tendon using MSU. There has been a study done recently for the test-retest reliability of the FHL muscle and the flexor digitorum longus (FDL) muscle on the CSA (19). Prior to the actual research this study did extensive pilot testing to determine the location of the measurements. Eventually the measurements were done on two locations, which were at 40% and 50% of the total length of the tibia starting from the inferior margin of the medial malleolus. This resulted in excellent test-retest reliability ( $ICC=0.98$ ) for the measurements performed at 50% of the total tibia length. However for the measurements that were done at 40% of the total tibia length the test-retest reliability was only fair ( $ICC=0.57$ ). The results for the measurements that were done on the FDL muscle have excellent test-retest reliability for the measurements performed at both 40% ( $ICC=0.92$ ) as 50% ( $ICC=0.98$ ) of the total length of the tibia. The participants in the study done by Mickle et al. had a mean age of 32.1 years with a standard deviation of 10.1 years (19). The software that was used for the measurements was ImageJ as well. These factors have made it more reliable comparing this study with the study performed in this article. For determining the test-retest reliability the intraclass correlation coefficient was used, however the exact model was not described. Therefore the study done by Mickle et al. might have used a different model which would make it less reliable for comparison (19). There were five women and five men recruited for this research. The total amount of participants that were recruited ( $N=10$ ) in the study done by Mickle et al. is considerably less when comparing that to the total amount of participants that were recruited ( $N=59$ ) in the research done in this article.

To the knowledge of the author there was no other research done for the test-retest reliability of MSU on the FHL other than the study done by Mickle et al. However, comparison can still be made with studies that have used similar methodology using MSU on different anatomical structures. A study was performed involving the test-retest reliability of the posterior lower leg and anterior lower leg using MSU. This study focused on regional muscle mass that they measured for a test and retest in standing position, followed by one hour of rest. After the one hour of rest, the measurements were performed in a recumbent position for the test and retest. The test-retest reliability varied from excellent ( $ICC=0.88$ ) for the anterior lower leg to poor ( $ICC=0.34$ ) for the posterior lower leg in recumbent position (20). The participants were lying recumbent in supine position for the measurements on the anterior lower leg. This position is similar to the positioning of participants used in the study performed in this article. The participants in this study had a mean age of 33.8 years with a standard deviation of 9.7. In regards to the BMI of the participants, similar values were measured in comparison to the sample-population that was measured in the study done in this article. The mean age of the participants varies slightly from the age of the participants that was measured in the study done in this article. Also it is unclear which muscles were measured precisely in the study of Thoires et al.(20).



## Strength and Limitations

Within the sample population of the study in this article, the age ranged from 18 to 30 years old. This was chosen to rule out possible outliers since this range of age within students at a university is 17-30 years old (21). However, the sample population was set up to represent the whole population of people that are currently living in the Netherlands and are 18 years old or over. Because of this, the results are not representable for the population in the Netherlands that are over 30 years old. The mean weight of the males (76 kg) and females (65 kg) in the sample study do not represent the mean weight in the total population of the Netherlands in males (84 kg) and females (70 kg) (22). Most likely, this is a result of the relatively young sample population. It is possible that there would have been a difference in the structure of the FHL tendon within the part of the population older than 30 years old. A recent study found that the CSA of the Achilles tendon within people that are over 50 years is significantly greater than that of people that are younger (23). Another study for the Achilles tendon found increased tendon stiffness in elderly subjects (Age  $\geq 65$ ) compared to young subjects (Age 18-40 years). Although this is not the same tendon as that which was studied in this article, it is still a factor to take in consideration for the FHL tendon. Another factor to take into consideration is the influence of BMI on the MSU measurements. When the BMI is 30 kg/m<sup>2</sup> or higher it indicates obesity, which limits MSU in two different ways (24). Firstly, obese patients have increased thickness in body parts, which causes poor penetration of the ultrasound beam beyond the focal depth. Secondly, as the ultrasound beam passes through subcutaneous and intraperitoneal fat, the penetration of the ultrasound beam becomes even more weakened (25). In the sample population 11 participants were moderately overweight and two participants were obese (24). With these participants the imaging of the FHL tendon was more difficult than with the participants who were not overweight.

Difference in the dominant ankle can also cause a difference in the CSA of the tissues that are measured using MSU (23). Therefore it would have been better to categorize the ankles of the participant in terms of dominant- and non-dominant ankle instead of the left- and right ankle. Testing which ankle is dominant would have taken more time and since the time limit was sixty minutes per participant due to ethical reasons this was not possible.

The data from the retest of the CSA measurement on the left ankle and the data from all the thickness measurements were not normally distributed. A possible reason for this could have been that outliers were not ruled out before analyzing the data. However according to Walter et al. the sample size of the population (N=59) in this study was good (13). During the measurements, the researcher also found that there was a considerable difference in the location of where the FHL tendon descends from the FHL. The researcher indicated that in some cases the CSA of the FHL tendon was harder to measure because the FHL tendon would not have descended entirely from the FHL. Therefore the standardization for the location of the transducer in the measurement protocol might have been too strict.

There were no anatomical anomalies found in the participants that were measured.

However there was an anatomical difference found in the bone structure of the medial malleolus. The measurement protocol was standardized so that the positioning of the transducer needed to be with the indicator on the most prominent part of the medial malleolus. In some participants the most prominent part of the medial malleolus was found at the dorsal edge. This anatomical variation created a greater space than usual in between the landmarks for the measurement. The researcher had to fill this space up with ultrasound gel so that it would not cause a problem.

During the study, the researcher that performed the measurements was blinded since the participants were given an anonymous code. The obtained data from the MSU was notated under this anonymous code separately from the demographic data. Therefore the researcher that performed the measurements did not know which demographic data belonged to which data obtained with MSU. To preclude possible bias, the researcher that performed the measurements was not the author of this article.

### Clinical relevance

Health professionals in their daily practice can now successfully use MSU to provide patients with a visual impression of the FHL and its tendon. This could serve as biofeedback and will primarily be useful for patients that need to regain their motor control and strength of the FHL or its tendon. In the future, MSU on the FHL tendon could be used as an extra addition to the clinical examination for different pathologies which involve the FHL tendon such as the TTS. MSU is able to increase specificity when there is symptomatic correlation, however the accuracy of the diagnosis is operator and experience related (26). Considering that this study was done by a student who had no experience with MSU prior to this study was able to master the technique of MSU, a health professional who does have experience with MSU should have even more reliable results.

### Recommendations

In order to improve measurements within the sample population several recommendations should be considered. The first recommendation is excluding participants with a BMI of 30 kg/m<sup>2</sup> or higher as this negatively influences the images on the MSU device. Secondly, the range in age of the sample population has to be representative of those who are most commonly susceptible to pathologies that occur as a result of influence of the FHL or its tendon. Doing so would result in increased validity of the results obtained from this study for those in this age range. It is also recommended to evaluate the positioning of the transducer for the CSA measurements to improve the measurement protocol. If possible it would be better to take the most dorsal part of the medial malleolus as a landmark instead of the most prominent part, since the anatomical variance in the most prominent part is limiting the standardization. Besides positioning the transducer the correct way, standardizing the position of the researcher would be an improvement to the measurement protocol as well.

Further research with MSU has to be done for the FHL, preferably using the same measurement protocol but with the suggested improvements. A study with two sample populations can be recommended, where one sample represents the population with TTS or similar symptoms and the other sample population representing people with no history of ankle pathology. When these two groups have similar demographic data, the difference in the images can be compared so that it would be more applicable for TTS patients. Besides this goal, the different anatomic variances and anomalies within the TTS group should be documented as well. The documented anatomic variances and anomalies can then be used to determine if MSU is reliable tool to successfully include or exclude TTS.

## 5. Conclusion

This study has concluded that the use of MSU on the FHL tendon has excellent test-retest reliability for the CSA and fair to excellent test-retest reliability for the thickness. Nevertheless further research for the use of MSU on the FHL tendon is recommended. Likewise for the involvement of the FHL tendon in TTS.

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# Appendices

## Appendix I : Informatiebrief

### **Informatiebrief**

Het betreft een multidisciplinair onderzoek over echografie van de flexor hallucis longus pees en testen voor het meten van de kracht van flexor hallucis longus spier.

Geachte heer/mevrouw,

Gaarne vragen wij u deel te nemen aan een onderzoek van twee vierdejaars podotherapie studenten, vier vierdejaars fysiotherapie studenten en één vierdejaars medisch beeldvormende en radiotherapeutische technieken (MBRT) student in opleiding aan de Fontys Paramedische Hogeschool Eindhoven.

In deze brief kunt u uitleg vinden over het doel, de locatie en de verwachtingen van het onderzoek. Tevens wordt er besproken wat er met de gegevens gedaan zal worden. Heeft u nog vragen na het lezen van de informatiebrief, neem dan gerust contact op met één van de onderzoekers. De contactgegevens staan onderaan deze brief vermeld.

### **Doel van het onderzoek**

Fysio- en podotherapeuten bieden hulp aan mensen met onder andere voet en/of enkelklachten. Aan de binnenzijde van de enkel bevindt zich de tarsale tunnel. Dit is een ruimte waar twee bloedvaten, één zenuw en 3 pezen doorheen lopen, waaronder de flexor hallucis longus pees. In de tarsale tunnel kan een zenuwbeklemming ontstaan, welke diverse oorzaken kan hebben. De beklemming zorgt ervoor de zenuw zijn functie, welke onder andere de aansturing van de flexor hallucis longus spier is, niet naar behoren kan uitvoeren. De tarsale tunnel kan in beeld gebracht worden door middel van echografie. Daarnaast kan een krachtmeting van de spier uitsluiten of er al sprake is van problemen in deze ruimte. Het is nog onzeker hoe betrouwbaar het gebruik van echografie en de krachtmeting is. In dit onderzoek wordt de betrouwbaarheid hiervan onderzocht, zodat de waarde van echografie en de krachtmeting bepaald kan worden voor in de praktijk.

### **Voorwaarde voor deelname aan het onderzoek**

- ✓ U heeft een leeftijd tussen de 18 en 30 jaar
- ✓ U beheerst de Nederlandse en/of Engelse taal
- ✓ U heeft in de afgelopen 6 maanden geen klachten gehad aan de grote tenen, de voeten, de enkels en/of de onderbenen
- ✓ U heeft in het verleden geen operaties gehad aan de grote tenen, de voeten, de enkels en/of de onderbenen
- ✓ U heeft geen diabetes mellitus, reuma of neuromusculaire aandoeningen aan de grote tenen, de voeten, de enkels en/of de onderbenen.

### **Samenstelling van het onderzoek**

Het onderzoek bestaat uit diverse onderdelen, welke gezamenlijk ongeveer 60 minuten duren. Eén onderdeel zal bestaan uit het meten van lengte, gewicht en het beantwoorden van een vragenlijst betreffende geslacht, mogelijke sportactiviteiten en tijdsbesteding van die activiteiten. Een ander onderdeel is het observeren van de binnenzijde van uw enkels met behulp van echografische apparatuur. Hiervoor wordt een laagje gel aangebracht op uw voet, waarna een scanner op de enkel wordt geplaatst. Er zullen een aantal screenshots worden gemaakt van het beeld tijdens het echografieonderzoek. De beelden worden gebruikt voor diktemetingen van een aantal structuren en het markeren ervan.



De scanners worden na iedere deelnemer gedesinfecteerd. Bij een volgend onderdeel wordt de kracht van de flexor hallucis longus spier gemeten met behulp van een hand held dynamometer. De deelnemer wordt in de juiste positie gezet en daarna wordt gevraagd om de grote teen te buigen. De meting zal in totaal twee keer herhaald worden. De hand held dynamometer wordt na elke deelnemer gedesinfecteerd.

#### **Wat wordt er van u verwacht?**

Indien u wilt deelnemen aan het multidisciplinaire onderzoek kunt u contact opnemen met een van de onderzoekers. U wordt dan gevraagd om naar het Fontys Paramedische Hogeschool gebouw in Eindhoven te komen. Daar bestaat de mogelijkheid tot het stellen van eventuele vragen en zal een toestemmingsformulier worden getekend. Vervolgens zal het onderzoek gestart worden. Gedurende het onderzoek bent u vrij om wat te eten en/of te drinken of wat te lezen indien u dit zou willen.

#### **Mogelijk voor- en nadelen van het onderzoek**

Deelname aan het onderzoek levert geen fysieke voor- en/of nadelen voor u op. De metingen zullen tevens pijnvrij worden uitgevoerd. De podotherapie, fysiotherapie en MBRT studenten van dit onderzoek hebben voordeel van de verzamelde gegevens en kunnen hiermee mogelijk de toekomstige gezondheidszorg verbeteren ten aanzien van de behandeling van klachten in de tarsale tunnel.

#### **Wat gebeurt er als u wenst geen deelname te hebben aan dit onderzoek?**

Deelname aan dit onderzoek is geheel vrijwillig en wordt door u bepaalt. Bij weigering van deelname of bij tussentijds terugtrekken van deelname hoeft u geen verklaring te geven en het zal op geen enkele wijze consequenties voor u hebben.

#### **Wat gebeurt er met uw gegevens?**

De verzamelde gegevens worden anoniem verwerkt en alleen gebruikt voor medisch wetenschappelijke doeleinden. De anonieme gegevens worden veilig bewaard voor een periode van 15 jaar op de Fontys Paramedische Hogeschool te Eindhoven. Er bestaat een mogelijkheid dat de gegevens gebruikt worden voor herziening van het huidige onderzoek of voor verder onderzoek. Wanneer u de resultaten van het huidige onderzoek wilt ontvangen, kunt u dit aangeven bij het tekenen van het toestemmingsformulier.

#### **Krijgt u een vergoeding voor deelname aan dit onderzoek?**

Aan het einde van het onderzoek zal er een klein presentje worden uitgedeeld voor deelname aan het onderzoek.

#### **Goedkeuring ethische commissie**

Dit onderzoek is goedgekeurd door de ethische commissie van de Fontys Paramedische Hogeschool te Eindhoven.

Indien u nog vragen heeft kunt u contact opnemen met onderstaand mailadres.

Met vriendelijke groet,

De onderzoekers:

Fysiotherapeuten in opleiding: Broos Spijkers, Vincent Kluitmans, Gard Patursson en Remon Vijfvinkel.

Podotherapeuten in opleiding: Yannick Warnier en Ayra Bekker – de Waard.

MBRT'er in opleiding: Younes Elhamdaoui.

Emailadres: bestFHLresearch@outlook.com

## Appendix II : Information letter (English version)

### **Information letter**

This letter contains information concerning the multidisciplinary research about echography of the flexor hallucis longus tendon and tests for measuring the force of the flexor hallucis longus muscle.

Dear Sir or Madam,

We would like to ask you to participate in a research project of two podiatry, four physiotherapy students and one medical imaging and radio-therapeutic techniques (MIRT) student at Fontys University of Applied Sciences Eindhoven.

In this letter we will inform you about the purpose, the location and the expectations of the research. The way the data will be handled is also mentioned. If you have any questions after reading this letter please feel free to contact one of the researchers. Contact information is listed below.

### **Research purpose**

Physiotherapists and podiatrists offer help to people with foot and/or ankle issues. On the inside of your ankle you can find the tarsal tunnel. It is a space that contains two blood vessels, one nerve and three tendons, for instance the flexor hallucis longus tendon. A nerve compression can occur in the tarsal tunnel, which can have different causes. The compression causes the nerve to fail at performing his assigned job, which include innervation of the flexor hallucis longus muscle. The tarsal tunnel can be framed using echography. In addition to the image a force measurement can determine if there already are any issues with the tarsal tunnel. It is uncertain if the usage of echography and the force measurement are reliable. In this research the reliability is examined, so that the value of echography and the force measurement can be determined for practical use.

### **Requirements for participating in the research**

- ✓ You have an age between 18 and 30 years.
- ✓ You master the Dutch and/or English language.
- ✓ You did not have any issues concerning your big toes, feet, ankles and/or lower legs for the past six months
- ✓ You did not have any surgery concerning your big toes, feet, ankles and/or lower legs for the past six months
- ✓ You do not have diabetes mellitus, rheumatism or neuromuscular disorder concerning your big toes, feet, ankles and/or lower legs.

### **Construction of the research**

The research contains multiple parts and the whole research will take approximately 60 minutes. One part will be the measurement of your body height, weight and answering a questionnaire with regards to gender, possible sporting activities and approximate time use on these sporting activities. A different part will be observing the insides of your ankles with echography. A layer of gel will be applied on your skin after which the probe will be placed on your ankle. A couple of screenshots will be made of the frame during the echography examination. The frames will be used for measuring the thickness and cross sectional area of the flexor hallucis longus. The probes will be disinfected after each participant. Another part will be the force measurement of the flexor hallucis longus muscle with a hand held dynamometer. The participant will be placed in the right position after which they will be asked to bend the big toe.

The measurement will be repeated two times. The hand held dynamometer will be disinfected after each participant.

**What will be expected from you?**

If you want to participate in this multidisciplinary research you can contact one of the researchers. You will be asked to come to the Fontys University of Applied Sciences in Eindhoven. You have the opportunity to ask questions and will sign an informed consent form after which the examination will be started. During the examination you are free to eat and drink something or read if you would like.

**Possible advantages and disadvantages of the research**

Participating in this research will bring no physical advantages and/or disadvantages. The measurements will be performed in a pain free matter. The podiatry, physiotherapy and MIRT students of this research will benefit from the collected data and possibly improve the future healthcare concerning treatment of issues in the tarsal tunnel.

**What will happen if I wish not to participate in this research?**

Participating in this research is completely voluntarily and will be decided by you. If you refuse to participate or withdraw during the examination you do not have to give an explanation and it will have no consequences for you what so ever.

**What will happen with your data?**

The collected data will be processed anonymously and will be used only for medical scientific purposes. The anonymous data is stored safely for a period of 15 years at the Fontys University of Applied Sciences in Eindhoven. There is a possibility that the data is used for revision of the current research or for further research. When you want to receive results of the current research you can mention this in the informed consent form.

**Is there any compensation for participating in this research?**

At the end of the examination you will receive a small gift for participating in the research.

**Approval ethical commission**

The ethical commission of the Fontys University of Applied Sciences approves this research.

If you have further questions you can contact by the email address listed below.

Kind regards,

The researchers:

Physiotherapist in training: Broos Spijkers, Vincent Kluitmans, Gard Patursson and Remon Vijfvinkel.

Podiatrists in training: Yannick Warnier and Ayra Bekker – de Waard.

MIRTer in training: Younes Elhamdaoui.

Email address: [bestFHLresearch@outlook.com](mailto:bestFHLresearch@outlook.com)

### Appendix III: Toestemmingsformulier (Dutch version)

#### **Toestemmingsverklaring**

Het betreft een multidisciplinair onderzoek over echografie van de flexor hallucis longus pees en testen voor het meten van de kracht van flexor hallucis longus spier.

Bij deze verklaar ik zowel schriftelijk als verbaal op de hoogte te zijn gebracht over het onderzoek en zijn mijn vragen naar tevredenheid beantwoord. Gedurende het onderzoek ben ik altijd in de mogelijkheid tot het stellen van vragen.

Ik ben bekend met het feit dat deelname aan het onderzoek op geheel vrijwillige basis is en ik het recht heb om zelf te beslissen over deelname aan het onderzoek. Gedurende het onderzoek bestaat de mogelijkheid om op elk moment tijdens het onderzoek te stoppen zonder hiervoor een verklaring te moeten geven. Mijn beslissing zal op geen enkele wijze consequenties voor mij hebben.

Ik ben bekend dat de verkregen gegevens uit het onderzoek geanonimiseerd worden, zodat informatie niet terug te herleiden is naar mij als persoon. Ik ga er akkoord mee dat de onderzoekers de verkregen gegevens mogen verzamelen, analyseren, documenten en/of presenteren voor wetenschappelijke doeleinden. Tevens ga ik er akkoord mee, dat persoonlijke gegevens na het huidige onderzoek vernietigd zullen worden en dat overige anonieme gegevens veilig worden opgeborgen voor een tijd van 15 jaar.

Naam deelnemer:

---

Handtekening deelnemer:

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Datum:

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Plaats:

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Gelieve de volgende vragen beantwoorden:

Ik wil graag op de hoogte worden gebracht van de uitkomsten van het onderzoek

- ☐ Ja
- ☐ Nee

U mag foto's van mijn voeten maken als onderdeel van het onderzoek en deze mogen gebruikt worden voor visuele doeleinden in de verslaglegging en/of presentatie.

- ☐ Ja
- ☐ Nee

Indien u op de hoogte gebracht wilt worden van de uitkomsten graag uw e-mailadres noteren:

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## Appendix IV: Informed consent (English version)

### **Informed consent form**

This informed consent contains information concerning the multidisciplinary research about ultrasonography of the flexor hallucis longus tendon and tests for measuring the force of the flexor hallucis longus muscle.

I declare to have been notified about the research both verbally and in writing. My questions have been answered to my content and I am aware that it is possible to ask questions during the research.

I am familiar that participating in this research is fully voluntarily and I have the right to decide about taking part in the research. During any time of the examination I am allowed to quit without giving an explanation. My decision will have no consequences for me what so ever.

I am aware that the obtained information will be anonymous to make sure that it will not be traced back to me as a person. I agree that the researchers can collect, analyse, document and/or present the data obtained from the research for scientific purposes. I also agree that personal information is destroyed after the current research and anonymous data are stored safely for 15 years.

Name participant: \_\_\_\_\_

Signature participant: \_\_\_\_\_

Date: \_\_\_\_\_

Place: \_\_\_\_\_

Please answer the following questions:

I would like to receive information of the research outcomes.

- ☐ Yes
- ☐ No

You can take pictures of my feet during the examination and they can be used for visual purposes in the research paper or presentation.

- ☐ Yes
- ☐ No

In case you want to receive information about the research outcomes please fill in your email address below:

\_\_\_\_\_

## Appendix V : Measurement protocol

During this research the MSU imaging will be done using the Hitachi Prosound Alpha 7. The transducer that will be used is a linear transducer from Hitachi Aloka, type UST-5412 36 mm high frequency. The imaging will be done with a frequency of 27Hz and a depth of 3 cm (27). The researcher is allowed to use the gain within the range of 65% - 75% for optimizing the image. This range of the gain will be beneficial for capturing the most optimal image according to the researchers of this study and their supervisor, based on pre-testing with the MSU.

### **Step 1. Position of the participant**

It is important that the participant is comfortable since the imaging will take ten minutes at each station. For this reason the researchers chose to adjust the back support in an angle of 110°. The art.coxae will be externally rotated and in a slight flexion. The art. genus also has to be positioned in a slight flexion so the lateral edge of the foot lies on the surface. A pillow will be placed under the art. genus for the relaxation of the participant. Subsequently the art.talocruralis will be positioned in maximal dorsal flexion using a towel that the participant will be holding during the imaging (Figure 1). The positioning that is done in this research is important in order to prevent artefacts and anisotropy (27,28).



**Figure 2. Positioning of the participant**

### **Step 2. Transversal view of the left foot ('Cross Sectional Area' or 'CSA')**

Before the researcher starts imaging the transducer will be provided with ultrasound gel (28). After the gel has been applied on the transducer, the researcher can start localizing the flexor hallucis longus (FHL) in the left ankle. The localization will be done using the transducer which will be placed transversally on the tarsal tunnel. The most prominent point of the medial malleolus will be used as the initiating landmark. The indicator side of the transducer will be placed on this landmark pointing ventrally. Studying the anatomy in the literature has shown that the FHL does not descend in a straight line but in a slight angle around the medial malleolus. Knowing this, the transducer has to be in an angle as well to be measuring the FHL perpendicular. The researcher will rotate the transducer in between a range of five and ten degrees towards the heel with the indicating part of the transducer fixated.(Figure 2) (28,29). When the participant and the transducer are both rightfully positioned, the researcher will hold the hallux and passively move it into flexion and extension of the first metatarsophalangeal joint (MTP1). The movement in the MTP1 will lengthen and shorten the FHL, which helps to ensure that the researcher has the FHL on the screen instead of a different anatomical structure. When this is ensured, the researcher will aim to get the tendon of the FHL in the middle of the image, followed by saving this image for step five.



**Figure 1. Positioning of the transducer (transversal view)**

### **Step 3. Longitudinal view of the left foot ('thickness')**

The participant will stay in the same position as described in step two. When step two is done the researcher will rotate the transducer 90° with the indicator of the transducer pointing cranially. The middle of the transducer should be dorsally from the level of the most prominent part of the medial malleolus and ventral to the Achilles tendon if positioned right (Figure 3). The FHL lies nearby the talotibial joint (30). The researcher will hold the transducer perpendicular to the FHL to prevent any artefacts or anisotrophy (27,28). When the transducer is rightfully positioned again, the researcher will hold the hallux and passively move it into flexion and extension of the MTP1. The movement in the MTP1 will lengthen and shorten the FHL which helps to ensure that the researcher has the FHL on the screen instead of a different anatomical structure. When this is ensured, the researcher will aim to get the tendon of the FHL in the middle of the image, followed by saving this image for step five.



**Figure 3. Positioning of the transducer (longitudinal view)**

### **Step 4. Transversal view ('Cross Sectional Area' or 'CSA') and Longitudinal view ('thickness')**

When the imaging at step two and step three are finished on the left ankle, the researcher will perform the exact same imaging on the right ankle.

### **Step 5. Repeating the imaging at station two and second time at station one.**

At the second station a different researcher will follow the same steps as the researcher at station one. When both the ankles have been imaged by the second researcher the participant will move to the first station for the second time. At the second visit at station one, the same researcher as with the first visit will follow the same steps as in the first visit. In total there will be 8 images on each visit, which means that each participant will have 24 images taken from his/her ankles. The images will be saved on the MSU equipment with the anonymous code that the participant received at the entry station. The images will be exported from the MSU equipment onto a USB-stick every hour. At the end of one day of research the images will be transferred from the USB-stick onto a laptop or computer. There will also be a back-up of the data that was collected in a secured Microsoft Onedrive group.



## Step 6. Measuring the images

The program that will be used for the measurements is 'ImageJ'. Firstly the scale will have to be set at 30 mm. This can be done by drawing a line from the top to the bottom of the image. When this line is drawn, the scale can be set by clicking on 'Set Scale' which can be found under 'Analyze'. Then the 'Known distance' should be set at 30 and the 'Unit of length' at mm as can be seen in figure 4. The thickness measurements of the FHL in the images will be done using the 'straight tool' or 'line' in ImageJ followed by 'Measure' which can be found under 'Analyze'. The exact point for measuring the thickness will be located 10 mm from the point where the Talus and FHL tendon cross each other on the MSU screen (Figure 5). The measuring landmarks will be at the top and the bottom part of epitenon of the FHL tendon (Figure 6). For the CSA measurements, the 'Freehand selections' will be used. The measuring landmark will be the epitenon of the FHL tendon which will be shown circularly on the MSU screen followed by 'Measure'. Because the lack of time during the research the researchers chose to do the measurements in the week after the data collection is done. The CSA will be measured in  $\text{mm}^2$  and the thickness will be measured in mm.

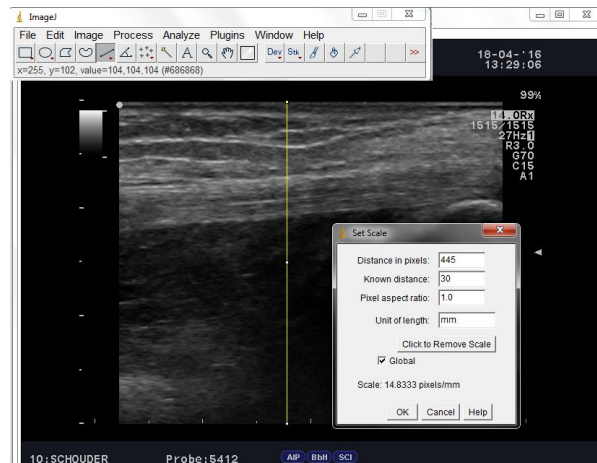


Figure 4. Setting up the scale

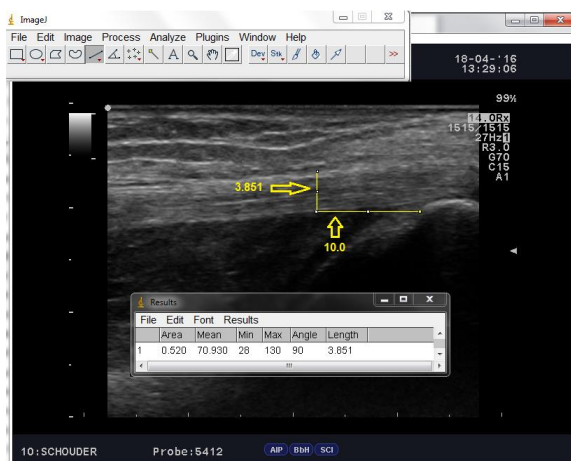


Figure 5. Thickness measurement in the longitudinal image of the FHL tendon.

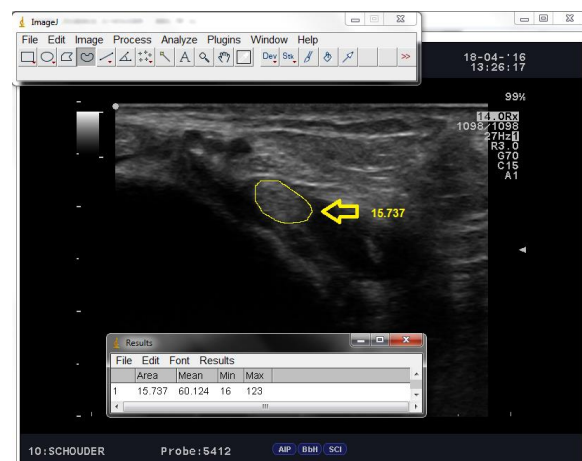


Figure 6. CSA measurements in the transversal image of the FHL tendon.

## Step 7. Processing

The data that was collected by following this protocol as described above will be processed in Windows Excel.