Structural characteristics and functional consequences of lateral ankle sprains

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Submitted in Partial Fulfilment of the Requirement

of the Degree of Doctor of Philosophy (PhD)

2018

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Table of contents

List of table

List of figures

Publication, conferences paper and poster

- Presented a poster at SPARC (Salford Postgraduate Annual Research Conference) at University of Salford on 15-14/06/2016.
- Demonstrated an ultrasound workshop at congress of a EUROPEAN network of Podiatry Schools on 22/03/2018
- Presented a poster at Annual conference of American Institute of Ultrasound in Medicine in the United States of America (USA) on 24/03/2018.
- Presented a presentation at International Foot and Ankle Biomechanics (i-FAB2018) Meeting in the USA on 09/04/2018.
- Published an article in Journal of Ultrasound in Medicine on 12/09/2018.

OBJECTIVE: Ankle sprains constitute approximately 85% of all ankle injuries, and up to 70% of people experience residual symptoms. While the injury to ligaments is well understood, the potential role of other foot and ankle structures has not been explored. The objective was to characterize and compare selected ankle structures in participants with and without a history of lateral ankle sprain.

METHODS: A total of 71 participants were divided into 31 healthy, 20 coper, and 20 chronic ankle instability groups. Ultrasound images of the anterior talofibular and calcaneofibular ligaments, fibularis tendons and muscles, tibialis posterior, and Achilles tendon were obtained. Thickness, length, and cross-sectional areas were measured and compared among groups.

RESULTS: When under tension, the anterior talofibular ligament (ATFL) was longer in copers and chronic ankle instability groups compared to healthy participants ($P < .001$ and $P = .001$, respectively). The chronic ankle instability group had the thickest ATFL and calcaneofibular ligament among the 3 groups ($p < 0.001$). No significant differences ($P > .05$) in tendons and muscles were observed among the 3 groups.

CONCLUSIONS: The ultrasound protocol proved reliable and was used to evaluate the length, thickness, and cross-sectional areas of selected ankle structures. The length of the ATFL and the thickness of the ATFL and calcaneofibular ligament were longer and thicker in injured groups compared to healthy.

Trainings undertaken during the course of the PhD

Date	Title of training course	Key learning aim
11-11-2015	Excel: Formulas and function	How to use the formula and the function and applied that in your work.
17-11-2015	Musculoskeletal ultrasound lectures1	Lecture was given about Musculoskeletal ultrasound
18-11-2015	Musculoskeletal ultrasound lectures2	Lecture was given about Musculoskeletal ultrasound
24-11-2015	Tackling literature review	-structuring a review. -Critically assessing literature.
30-11-2015	Critical and Analytical Skills	-how to be a critical student.
30-11-2015	Organising and synthesising your work	-research practices. -how knowledge is used to construct an academic argument.
01-12-2015	Doing a literature review	-help the student get started with literature review. -explain what it is, what it is not.
10-12-2015	Becoming a researcher: realizing your potential and raising your profile	This session help you to describe characteristics of excellent researchers and map your own current performance.
16-12-2015	PGR seminar series that spans the research themes of: Rehab, Gait, Knee, Foot & Ankle biomech, and Activity Monitoring	PGR seminar session.
05-02-2016	The Seven Secrets of Highly Successful Research Students	This workshop describes the key habits that our research and experience with thousands of student's shows will make a difference to how quickly and easily you complete your PhD. Just as importantly, these habits can greatly reduce the stress and increase the pleasure involved in completing a PhD.
08-02-2016	Advanced Search: Health & Social Care Databases	Locating databases for Health and Social Care
11-02-2016	Using Other People's Work in Your	-Understand the basics of copyright law

	Research	and how it affects academic use. -Be able to assess the risks involved in breaching copyright.
16-02-2016	Googlescholar for research	It is a Hands-on session on making effective academic use of Google scholar, aimed at Postgraduate Researchers.
18-02-2016	Research Ethics for PGR's	This session will discuss the issues and procedures around statutory requirements and professional codes for maintaining the highest possible ethical standards.
26-02-2016	PHD students meeting	In this meeting there will be a discussion on critical reading/writing and the use of a data synthesis matrix
01-03-2016	LEAP higher writing session	This session is designed to help PGRS in academic writing
02-03-2016	Understanding how skin contributing to balance control	PGR seminar session.
08-03-2016	LEAP higher writing session	This course designed to give support with academic writing, including grammar, vocabulary and how to organize this information for an assessment.
10-03-2016	Creating academic poster	This workshop provides an introduction to presenting at academic conferences
18-03-2016	Research Ethics for PGR's	This session will discuss the issues and procedures around statutory requirements and professional codes for maintaining the highest possible ethical standards.
23-03-2016	Intro to Endnote X7	How to use the EndNote X7 (bibliographic software) to organise and manage my reference.
19-04-2016	Locating and using historical archives for researchers	To learn how to find unique historical materials for your research
20-04-2016	Introduction to SPSS	-This introductory session to SPSS will provide participants with an overview of the capabilities of this common statistical package. -Understand how to enter primary data, import Excel data into SPSS.

25-04-2016	Presenting at Academic Conferences	This workshop provides an introduction to presenting your research at academic conferences
26-04-2016	Ultrasound scanning session	Practice the new ultrasound protocol
29-04-2016	PGR monthly meeting	Discusses about the IA and IE.
03-05-2016	Ultrasound scanning session	Practice the new ultrasound protocol
05-05-2016	LEAP (academic writing)	This session focuses on PhD students to help them writing in academic way.
10-05-2016	LEAP (critical writing)	To help PhD student to be critique in their writing.
18-05-2016	Critical Thinking and Critical Writing at Doctoral Level	This session focuses on research practices and how knowledge is used to construct an academic argument
20-05-2016	Rehearsal and coaching session for poster	Practise before shooting the film next week, and the facilitator will be able to go through some tips with us and show us how the autocue works in the studio.
27-05-2016	PGR monthly meeting	
15-06-2016	SPARC	Present my poster in the poster session of SPARC.
07-10-2016	Word: formatting your thesis	To learn more about the specific feature of word in design the writing.
02-11-2016	A Survival Guide to Doing a PhD	An essential guide to surviving your PhD
09-11-2016	The Interview: its place in social scientific research strategies	The session will explore both the theoretical and practical issues associated with interviewing as a data gathering technique
10-11-2016	Being critical	Bases for critique throughout the thesis
17-11-2016	Excel: Analysing Data	-To learn how to sort and filter the data. -To construct a pivot table and pivot chart from a table of data.
17-11-2016	Building the argument	-Structure of an argument. -keeping the thread. -Gaps in the literature.
18-01-2017	Doctoral Training seminar	-Getting your first paper published
25-01-2017	Electronic Resources for Researchers	
27-01-2017	Pathway to Professional: Time management	
01-02-2017	Foot/Knee research programme meeting	

15-02-2017	Doctoral Training seminar	-Statistics and data analysis of kinematic/kinetic data
15-03-2017	Doctoral Training seminar	- Critical/peer review
19-04-2017	Doctoral Training seminar	- Translating research into industry
26-04-2017	Ankle sprain webinar	
17-05-2017	Doctoral Training seminar	-Research governance
02-11-2017	Building resilience and bouncebackability	-Improve your resilience to 'knockbacks' and criticism.
02-11-2017	Goal setting and staying on tack	-To set effective and realistic goals to progress your research.
02-11-2017	How to submit a conference paper	-Strategies to approaching writing abstracts for conferences.
03-11-2017	Your thesis: structure	-Introduction to different types of thesis structure - Planning your own thesis structure
03-11-2017	Your thesis: the thesis of your thesis	A practical session where the researchers will produce their own clear thesis statement to help us focus our PhD work around a central proposition.
03-11-2017	Formatting and submitting your thesis- getting it done	What a thesis should look like and how to format it - Supervisory role and getting other sources of feedback - Submission process - Staying motivated in the late stages
30-01-2018	Publishing during your PhD	The quick guide to publishing whilst doing your PhD – navigating the process, publishers, peer review and procrastination.
15-02-2018	Intensive data SPSS	-This is a practical workshop using SPSS - -This session aims to explore the experimental design of your research.
14-05-2018	Radiation dose and image quality in digital radiography seminar	

Acknowledgement

The opportunity to live in Manchester and to study at the University of Salford have been one of the most exciting experiences of my life, which I will treasure its memories forever. I would like to express my sincere gratitude to my supervisor Prof. Chris Nester for giving me the chance to undertake this research and for his unwavering belief in my ability. It was my pleasure to have supervisor like you with your kindness attitude and your great experience in biomechanics and research. I always appreciate your guidance, your support, your critical comments and your time. I would like also to extend a huge thanks to my co-supervisors, Dr. Paul Comfort and Dr. Chelsea Starbuck for their guidance, support, assistance, and helpful suggestions. Thank you my supervisory team to whom I owe the completion of this thesis.

Great thanks to my friends and postgraduates colleagues especially Basmah Allarakia and Kholoud Alzyoud who have supported me through my PhD journey. Thanks to everyone who took part in the studies of this thesis, your time was much appreciated. This thesis could not completed without you.

Special thanks go to my wonderful family, in particular my loving **parents**, thank you for supporting me to pursue what I loved, and thank you for your endless motivation and your unconditional love. You are always the source of my power and strength and I am forever grateful to you. I wish you always feel proud of me.

My husband, **Suhail Almansour**, has been my rock, and I can't thank him enough for his supporting and understanding throughout my PhD years. Even we were miles away, he has been with me at every turn and deeply appreciate all of his motivation during my hard times. Thank you for your care assisted me to overcome setbacks and stay focused to complete this thesis. My acknowledgement will never be complete without the special thanks to my daughters, **Ramah and Talyah**, for wait patiently for my return. I thank God for all the miracles that got me where I am today.

List of abbreviation

ACL Anterior cruciate ligament

AD Anterior drawer

AFO Ankle and foot orthosis

AL Anterolateral

ALARA As low as reasonably achievable

ALS Amyotrophic lateral sclerosis

AII Ankle Instability Instrument

AM Anteromedial

AMTI Advanced Mechanical Technology Incorporation

ANOVA Analysis of variance

ANT Anterior

ATFL anterior talofiblar ligament

AT Achilles tendon

B-mode Brightness mode

BMI Body mass index

C Celsius

CAI Chronic ankle instability

CAIT Cumberland Ankle Instability Tool

CAST Calibration anatomical system technique

CFL Calcenofibular Ligament

CINAL Cumulative Index to Nursing and Allied Health Literature

CLAHE Contrast Limited Adoptive Histogram Equalization

cm centimetre

CNS Central Nervous System

CoM Centre of Mass

CPP contrast per pixel

CSA Cross sectional area

C3D Coordinate 3 dimensional

DAQ Data acquisition

DICOM Digital imaging and communication in medicine

DLT Direct-linear transformation

EDL Extensor digitorum longus

EHL Extensor halluces longus

EMG Electromyography

FAAM Foot and Ankle Ability Measure

FADI Foot and Ankle Disability Index

FAI Functional ankle instability

FAOS Foot and Ankle Outcome Score

FI Functional instability

FCM Fuzzy c-mean

FFCM Fast fuzzy c-mean

FDL Flexor digitorum longus

FHL Flexor halluces longus

GE General electric

GPPAQ The general practice physical activity questionnaire

GRFs Ground Reaction Forces

ICC Intraclass correlation coefficient

IdFAI Identification of Functional Ankle Instability

IL Illinois

JPEG Joint Photographic Experts Group

Kg Kilogram

kHz Kilohertz

K-S Kolmogorov-Smirnov

L Lateral

LAS Lateral ankle sprain

LM Lateral malleolus

LoM Limit of agreement

LT Left

M Medial

m meter mm millimeter

MAI Mechanical ankle instability

MATLAB Matrix laboratory

MBIM Model-based image-matching (MBIM)

MEDLINE Medical Literature Analysis and Retrieval System Online

MD Maryland

MHz Megahertz

MI Mechanical instability

MM Medial malleolus

MRI Magnetic resonance image

MSKUS Musculoskeletal ultrasound

N Neutral

NHS National Health Service

NWB Non-weight bearing

PAI Physical Activity Index

PBT peroneal brevis tendon

PBM Peroneal brevis muscle

PF Plantar fascia

PhD Doctor of philosophy

PL Posterolateral

PLT peroneal longus tendon

PLM Peroneal longus muscle

PM Posteromedial

POST Posterior

PubMed Public/Publisher Medical Literature Analysis and Retrieval System Online

QI Quetelet Index

QTM Qualisys Track Manager

RBF-NN Radial Basic Function Neural Network

RT Right

RNA Ribonucleic acid

ROI Region of interest

ROM Range of Motion

SD Standard deviation

SEBT Star Excursion Balance Test

SPARC Salford Postgraduate Annual Research Conference

SPSS Statistical Package for the Social Sciences

SRAD Speckle Reducing Anisotropic Diffusion

T Tension

TAT Tibialis anterior tendon

TAB Tibialis anterior muscle

TPT Tibialis posterior tendon

TPM Tibialis posterior muscle

TT Talar tilt

TTB time-to-boundary

TTBMM time-to-boundary mean minima

UK United kingdom

US Ultrasound

USA United states of America

WA Washington

WB Weight bearing

WBLT Weight bearing lung test

Y Year

2D Two dimensional

3D Three dimensional

µ mu

Abstract

Ankles injuries account for 8% of health care consultations and ankle sprains constitute about 85% of all ankle injuries. Lateral ankle sprain is a type of injury that affects both the general and the sporting population. Lateral ankle sprain has a high rate of recurrence which can often lead to individual developing chronic ankle instability. This chronicity contributes to continue deficits of sensorimotor and constrained functioning) which could have a decrease effect on the health-related quality of life, the level of physical activity, and absence from training or competition for athletes, thus create a substantial global healthcare burden. With such negative consequences and related financial burden associated with LAS and CAI enhanced effort to understand structure and function differences between those that develop CAI and those that do not following an initial acute LAS is needed.

Understanding the relationships between the integrity of the ankle structures pre and post sprain, and functional ability of the ankle is important to inform our understanding of the long-term effects of sprains and consider better targeting of interventions. This thesis aims to study the structural characterisation and functional consequences of lateral ankle sprain. Three studies were undertaken.

The first study used ultrasound to characterise and compare selected ankle structures between healthy (n=48), coper (n=22) and chronic ankle instability (n=32) groups. Participants with prior injury had significantly longer anterior talofibular ligament when the ligament was under tension (by 6% when compared CAI to healthy participants), and thicker anterior talofibular ligament and calcaneofibular ligament compared to healthy participants (by 54.21% and 8.3% respectively when compared CAI to healthy participants). These gross structural differences are evidence of residual structural damage. However, they do not indicate whether the quality and nature of the ligament tissue is similarly affected.

In study 2 image analysis techniques were used to provide a quantitative measure of the echogenicity of the anterior talofibular ligament by computer-aided greyscale analysis. Echogenicity was used as an indicator of ligament quality. The result showed that the echo intensity of anterior taloibular ligament was lowest intensity in chronic ankle instability (40 % and 18.8% lower than the intensity of healthy and coper respectively) followed by copers and healthy respectively. The echogenicity of the anterior talofibular ligament in copers was significantly different from chronic ankle instability and from healthy participants. Characterisation of these further structural changes reveals the extent of residual tissue

damage. However, it does not provide any insight into any functional consequences of these changes.

In a third study, the dynamic balance was evaluated in healthy (n=28), coper (n=18) and chronic ankle instability (n=22) ankles during the star excursion balance tests, using force plate and ankle kinematic analysis. This sought to investigate the functional consequences of the structural changes identified in studies 1 and 2. Participants with chronic ankle instability demonstrated poorer dynamic balance and altered ankle kinematics compared to healthy and coper participants, and copers also had altered kinematics. There was a significant negative relationship between the thickness of the ligament and the distance achieved when reaching in the anterolateral direction of the balance test ($r = -0.53$, $p<0.001$and $r = -0.40$, $p<0.001$ respectively). Characterisation of normal and injured ligaments appears to differentiate post sprain functionally. Balance tests reveal functional balance deficits and altered kinematic strategies that relate to the lateral ankle structures previously injured.

Lateral ankle sprain causes damage to lateral ankle ligaments and impaired sensory pathway to the CNS. Then the initial consequences lead to structural alteration (increased the laxity of ATFL and increased the thickness of ATFL and CFL). Joint loading could be altered and changes in normal movement patters occur as demonstrated in decrease reach distance and alter the kinematics of the ankle joint in injured participants compared to healthy participants. This is the first study that combined both structural changes and functional consequences of lateral ankle sprain and investigate any relationship between them to provide an overall understanding of how these two factors are related.

Chapter one: Introduction

This PhD thesis is focussed on changes in foot and ankle structures that occur due to lateral ankle sprains and the functional consequences of these. This first chapter will introduce the background to the PhD thesis and set out the various chapters, including the individual contributions made by each study to the overarching purpose of the PhD.

1.1 Overview of the problem of lateral ankle sprains

In the United States of America (USA) there are more than three million emergency room visits each year for foot and ankle injuries and the highest portion of self-reported musculoskeletal injuries concern the ankle (Gribble et al., 2014). More than 628,000 injuries to the ankle are treated annually in emergency rooms, accounting for approximately 20% of the total treated injuries in the USA's emergency departments (Gribble et al., 2014). Furthermore, ankle sprains account for approximately 3% to 5% of the United Kingdom (UK emergency room visits, consuming a considerable amount of healthcare resources (Gribble et al., 2014). More generally, ankle sprains represent one of the most common sources of musculoskeletal joint pain and disability faced in primary care (Doherty, Bleakley, Delahunt, & Holden, 2017).

Ankle sprains have a high rate of incidence, posing a substantial risk for people who participate in numerous physical activities and sports (Doherty et al., 2017). Although ankle sprains are often considered benign injuries, they have poor long-term prognosis with a high rate of recurrence, with up to 70% experiencing residual symptoms (Thompson et al., 2018). As a result, ankle sprains are associated with significant economic losses resulting from medical care and secondary disability (Lobo et al., 2016). However, it has been reported that around 55% of people who sprain their ankles do not call for evaluation or treatment from medical professionals (Gribble et al., 2014). Inadequate management to ankle sprain injury could lead to many consequences and problems such as ankle instability and osteoarthritis (Fong, Chan, Mok, Yung, & Chan, 2009a). Osteoarthritis is one possible long-term consequence of a ligament injury and considered the most prevalent joint disorder universally (Hauser & Dolan, 2011). Researcher reported a link between chronic ankle instability (CAI), that is repeated sprains, and post-traumatic ankle osteoarthritis; almost 68% to 78% of CAI patients developing ankle osteoarthritis (Hirose, Murakami, & Minowa, 2004; Wikstrom &

Brown, 2014). Thus, it seems important to fully understand and seek to prevent CAI in order to decrease the burden of disease and the cost of healthcare.

1.2 The research problem

There are three aspects to the research problem that this thesis contributes to.

1- we do not have a complete understanding of structural differences between healthy ankle and those injured by a lateral ankle sprain;
2- we do not have quantitative measure of tissue quality for the damaged ligament structures;
3- we do not know whether the structural changes at the ankle relate to functional changes.

Lateral ankle sprains (LAS) are the most common form of musculoskeletal injuries encountered both in clinical practice and in the sporting community (Fong et al., 2007). Radiography is part of the initial diagnostic test in many cases of apparent ankle sprain, but ligaments do not show up clearly on radiographs. This can lead to ligament tears being missed and false diagnoses of ankle sprains (Hauser et al., 2013). A number of authors have shown that Magnetic Resonance Imaging (MRI) offers a high level of sensitivity in diagnosing a ligament sprains or rupture (Hauser et al., 2013; Polzer et al., 2012; Slimmon & Brukner, 2010). However, in a systematic review evaluating MRI versus arthroscopy, the authors demonstrated that MRI does not have the ability to reveal ligament damage when it is stretched or lax (Crawford, Walley, Bridgman, & Maffulli, 2007). In other words, there is no difference in appearance between an injured ligament that has been stretched many times and an uninjured ligament in MRI (Hauser et al., 2013).

Musculoskeletal ultrasound (MSKUS) on the other hand can provide more detailed images of the structure of ankle ligaments (Hauser et al., 2013), and has been shown to be reliable in scanning foot-related structures (Crofts, Angin, Mickle, Hill, & Nester, 2014). However, little is known about ultrasound's characterisation of the structures relevant to ankle sprains.

Moreover, previous research has demonstrated that ligamentous injury could disturb the normal echogenicity of the ligament (Agut, Martínez, Sánchez-Valverde, Soler, & Rodríguez, 2009). The echogenicity of ultrasound (US) has been defined as the intensity of the returning ultrasound sound waves (Das, 2016). Most of the prior literature has demonstrated that subjective measurement of echogenicity is sensitive to the sonographer's experience and may not be appropriate for a study of ankle structures involving different sonographers. Therefore, there is a need to evaluate the structural integrity of the ligaments quantitatively and provide more objective evaluation of echogenicity such that changes in structure can be better understood.

In addition to the structural changes associated with ankle sprains, functional deficits in postural stability are believe to occur (Mettler, Chinn, Saliba, McKeon, & Hertel, 2015). Meehan, Martinez-Salazar, and Tprriani (2017) reported that injury to lateral ankle ligaments was linked to lateral ankle instability and thus functional ankle instability can result from unbalanced loading of the ankle joint. These deficits may decrease health-related quality of life and impact on an individual's long-term mobility and thereafter health. Continuing to improve our understanding of ankle sprains is important in the evaluation of treatment strategies to assist people with sprained ankle to overcome the related health difficulties (Hoch, Gaven, & Weinhandl, 2016).

1.3 Overview and structure of the Thesis

The focus of this PhD thesis is to investigate the characterisation of ankle sprain that could explain the differences between people in their structural and biomechanical response to a dynamic balance test. Therefore, the thesis is comprised of six chapters. **Chapter one**, the introduction chapter, is to introduce an overview of the problem of ankle sprains and its effect on patients and the healthcare support provided. The research problem is also discussed which introduces the research being conducted and places the ankle sprain issue in the radiology and biomechanics context, and why it is important to study the structural and functional of people with lateral ankle sprain.

In **chapter two**, the search strategy for the conducting review is discussed. Previous studies are reviewed covering the prevalence of lateral ankle sprains and the structural and functional anatomy of selected ankle structures. Critical reviews of the mechanism, risk factors, and classification of ankle injury follow, as well as appraisal of radiographic evaluation of ankle injury and the potential role of ultrasound imaging. Prior work investigating potential association between ankle injury, structural changes and functional impairment in maintaining balance is reviewed.

Chapter three builds on prior use of ultrasound to evaluate selected ankle structures and presents the method, results, and discussion of the first study. The study begins with a pilot study followed by an investigation of the reliability of the ultrasound measurement. Statistics are used to compares the length, thickness, and cross sectional area of selected ankle structures between healthy vs injured participants (coper and chronic ankle instability), chronic ankle instability vs coper participants, neutral vs tension positions of the ankle. Part of this chapter was accepted as journal paper in Journal of Ultrasound in Medicine.

In **chapter four** quantitative analysis is conducted on ultrasound images of anterior talofibular ligament in healthy, coper, and chronic ankle instability participants. A computer-aided grayscale analysis was used to provide a numerical value of the echo intensity of the ATFL, and compare this between healthy, coper, and chronic ankle instability participants.

Work in **Chapter five** investigates the functional consequences of lateral ankle sprain using force plate and ankle kinematics to explain changes in strategies to maintain balance during a dynamic balance test. The star excursion balance test is used to challenge the maintenance of balance using ankle strategies and ankle kinematics measured whilst balance is maintained. The performance of the balance tests is compared between healthy, coper, and chronic ankle instability participants, and any correlation between structural changes in the ATFL and CFL and balance.

Finally, **chapter six** provides an overall summary of the thesis. The limitations of the thesis and the implications for future research studies and clinical practice are presented.

2 Chapter two: Background/Literature review

In this chapter, the results of a literature search on the prevalence of lateral ankle sprain, mechanism and classification of ankle injury, the role of ultrasound in evaluation the ankle injury as well as measuring the postural stability of people with injured ankles will be presented. The literature will be reviewed and critiqued for the risk factors for lateral ankle sprain, methods of radiographic assessment, the subjective and objective evaluation of ultrasound images of ankle injury, and use of the star excursion balance test (SEBT) and kinematic assessment to investigate the effects of sprain on measuring the postural stability. The chapter will conclude with a discussion on the potential effectiveness of using ultrasound as a screening tool for evaluating the structural part of ankle sprain, and using SEBT as functional test to evaluate the function consequences of ankle sprain. The gap in the radiographic and biomechanics literature on ankle sprain, which leads to setting the rationale for the subsequent studies.

2.1 Search strategy

In order to find literature relevant to this thesis, a literature search was conducted using scientific online databases utilising the following search engines: Pub-med, Google Scholar, CINAHL (Cumulative Index to Nursing and Allied Health Literature), ScienceDirect, Wiley Online Library, SPORTDiscus and Ovid-Medline. Moreover, books, magazines, and leaflets were searched for literature related to the aim of this study. In addition, publications with unrestricted accessibility to their full-text were included. To obtain scientific literature on the prevalence, structural and functional anatomy, risk factors of ankle sprains. Search terms and key words were used: lateral ankle sprain, ankle injury, sprained ankle, coper, and chronic ankle instability, combined with the following words: aetiology, epidemiology, anatomy, physiology, and risk factors. To acquire related literature on the radiographic, subjective and objective evaluation of ankle sprain, the following search words were used: diagnosis, foot and ankle radiography, ankle x-ray, ultrasound, sonography, stress sonography, musculoskeletal ultrasound, echogenicity, echogenic, hypoechoic, hyperechoic, ligaments medical images, ultrasound quantification, echo intensity, greyscale intensity, and greyscale histogram. For the relevant literature of ankle sprain in postural stability, the keywords were used as balance test, dynamic postural control, SEBT, Y-balance test, kinematics, ankle dorsiflexion, stability, and balance control. There was no time limit on the search boundaries to ensure that significant early seminal studies were also included in the search results. The search used Boolean operators (OR, AND & NOT) to further narrow the results. To ensure that the knowledge and information obtained in the literature review chapter is accurate, only submissions from peer-reviewed journals were included. Moreover, only texts related to ankle ultrasound and SEBT were included. The search was limited to English language articles and undertaken iteratively between October 2015 and September 2018.

2.2 Prevalence of ankle injury and lateral ankle sprain

Lower limb disorders are common in developed countries and foot and ankles injuries account for 8% of all health care consultations (Lobo et al., 2016). Whilst few detailed epidemiological studies are available, the evidence suggests that a great number of people experience ankle sprains and ankle instability.

Ankle injuries rank among the most frequent musculoskeletal injuries that affect both athletes and the general population (Fong et al., 2007; Meehan et al. 2017; Sun et al., 2018; Wade, Mok, & Fong, 2018). A systematic review on ankle injury and ankle sprain in sports found that among 70 different types of sport, ankle classified as the first injured body site in most studies (Fong et al., 2007), with ankle sprains accounting for approximately 85% of all ankle injuries even as much as 100% of ankle injury obtained in some sports such as soccer, volleyball, rugby, and handball (Fong et al., 2007). Verhagen and cholleagues (2004a) found that ankle sprain was the most common injury accounting for 41% of all volleyball related injuries. Critically, 80% to 90% of all ankle sprains are injuries to the lateral ligament complex and the result of an inversion injury (Funk, 2011; Jain, 2014; Meehan et al., 2017; Rein, Hagert, Schneiders, Fieguth, & Zwipp, 2015).

Recently, it has been reported that about 20% of athletes suffer from sport’s injuries each year and ankle injuries constitute about 14% of these; 80% of those ankle injuries are ligamentous in nature (Singh et al., 2016). A longitudinal 2 year study among a military academy at the United States reported that ankle sprains were the most common injury and account for 78% of ankle injures (Waterman, Belmont, Cameron, DeBerardino, & Owens, 2010). Fong and colleagues (2008) conducted a study in China to investigate the sport-related ankle injuries attending the Accident and Emergency unit during one-year period. The authors found that ligamentous sprain (81.3%) was the most prevalent types of the sport-related ankle injury.

Ankle sprains demonstrated a high incidence also in general population attending to the emergency departments. Three studies in the United States conducted in the emergency department. Waterman, Owens, Davey, Zacchilli, & Belmont (2010) found that during four years study period, the incidence rate of 2.15 per 1000 person-years occur in general population attending to the emergency departments. Lambers, Ootes, & Ring (2012) found that ankle sprain was the greatest incidence of lower extremity injuries (36%) with the incidence rate of 2.06 per 1000 person-year during one year. Moreover, Shah and colleagues (2016) reported that ankle sprains demonstrated a common injury in the emergency departments with incidence rate of 3.02 lateral ankle sprains per 1000 person-year. In the United Kingdom there are approximately 5600 ankle sprains per day, which account for almost 3% - 5% of all admissions to hospital emergency departments with a significant draw upon healthcare resources (Cooke, Lamb, Marsh, & Dale, 2003). A study has been done to investigate the incidence of ankle sprains in four health districts in the West Midlands of England found that attendance rate of patients with ankle sprains to the Accident and Emergency units was 52.7 per 10,000 person yearly (Bridgman, Clement, Wallety, Phair, & Maffulli, 2003).

These numbers are getting higher each year; with population growth, the active life style and with the increasing number of people taking part in sporting activities (Jung, 2016). Up to 55% of individuals who experience an ankle sprain do not ask for assessment from healthcare professional or hospital treatment for ankle sprain, the accurate prevalence is probably higher (Gribble et al., 2014; Hiller et al., 2012). Ankle sprains have a high rate of reoccurrence, resulting in a high incidence of persistent chronic ankle problems (Pourkazemi et al., 2016).

2.3 Ankle sprain in health care

Lateral ankle sprain (LAS) is defined as traumatic injury that affects the ligaments of the lateral side of the ankle as a result of foot supination (Delahunt et al., 2010; Gribble et al., 2016b). Many authors define the word “sprain” as a morphologic situation that demonstrates a variety of pathologic conditions, starting with overstretching of one or more of the stabilising ligaments, to complete tear and rupture of the ligament with significant instability of the ankle joint (Al-Mohrej & Al-Kenani, 2016). Lateral ankle ligamentous sprains are classed as inversion injuries and involve the anterior talofibular ligament (ATFL) and the calcaneofibular ligament (CFL) (Rein et al., 2015). Medial ankle ligament sprains are classed as eversion injuries and are far less common, accounting for approximately 5% -15% of ankle sprain injuries; these involve damage to the deltoid ligament (McCriskin et al., 2015). A third type of ankle sprain is syndesmosis sprains which occurs in the ligaments above the ankle joint between fibula and tibia which involves the anterior tibiofibular, posterior tibiofibular, and transverse tibiofibular ligaments (high ankle sprain). Syndesmosis sprains account for around 10% of all ankle sprains (Rein et al., 2015).

Following the first lateral ankle sprain, residual symptoms occur in up to 73% of people (Pionnier, Découfour, Barbier, Popineau, & Simoneau-Buessinger, 2016). In addition, it has been reported that the risk of re-injury increases by twofold in the following year of a LAS (Gribble et al., 2016b). Several previous studies have evaluated the consequences of ankle sprains in various population settings. Braun (1999) reported that almost 73% of patients in general practice had residual symptoms six to 18 months after the ankle sprain. In a prospective study, Konradsen and colleagues (2002) observed that 32% of individuals who had been seen in the emergency rooms and diagnosed with sprained ankle suffer from recurrent and chronic ankle pain seven years post ankle injury.

On the other hand, several prospective studies have been done on athletic population. Gerber et al. (1998) founded residual symptoms in 40% of young military recruits six months after the ankle sprain. Anandacoomarasamy and Barnsley (2005) deduced that 74% of individuals suffered from ankle sprains had persistent symptoms and recurrence of ankle sprains up to four years following the first injury. It could be that different population in each study produce different percentage of residual symptoms and CAI. Pozzi, Moffat, & Gutierrez (2015) reported that long-term sequels of CAI are still unknown. However, it has been assumed that the sprain reoccurrence and instability of the ankle joint could damage the articular surface of the joint, therefore, increasing the risk of developing osteoarthritis. A study conducted in 2012 sought to define the spread of chronic ankle disorders in Australia using a computer assisted telephone survey of individuals aged 18-65 years (Hiller et al., 2012). The authors found that 45.5 % had a history of ankle injury but not any chronic ankle conditions, and 23.7% had chronic ankle disorders which was classified as recurrent ankle sprains, and the feeling of giving way (Hiller et al., 2012). “Giving way” has been defined as “the regular occurrence of uncontrolled and unpredictable episodes of excessive inversion of the rear foot (usually experienced during initial contact during walking or running), which do not result in an acute lateral ankle sprain” (Delahunt et al., 2010).

Ankle sprains could have an effect on an athlete's performance, and cause their absence from training or competition, and thereafter impact their quality of life (Doherty et al., 2014). LAS accounts for almost one-sixth of all the time people are unable to be involved in their sport activities (Jain, 2014). Indeed, Audenaert et al. (2010) reported that 29 days was found to be the average period of being away from normal work duties as a consequence of an ankle sprain.

It follows that ankle sprains have a major negative financial effect on communities. Gribble et al. (2016b) reported in his evidence review for 2016 International Ankle Consortium, that societal cost of ankle sprains in British population to be around £940, and in the Netherlands the costs of ankle sprains attending at an emergency departments are €823 with an estimated cost of €208 million per year due to impact on sport alone. Furthermore, it has been reported that the cost of treatment and rehabilitation of LAS is about $2 billion annually in the USA (McCriskin et al., 2015). A study in 2016 reported that the average cost of lateral ankle sprain per emergency room visit was $1,008 per incident in the United States (Shah, Thomas, Noone, Blamchette, & Wikstrom, 2016).

2.4 Structural and functional anatomy of selected ankle structures related to the ankle joint

The ankle joint is where the leg and foot segments meet (Fong et al., 2009a). It has been reported that the ankle joint is considered to be a very stable joint because of its design (Pal, 2014) which tolerates more weight per unit area than any other joint in the body (Gu, Ren, Ruan, Zeng, & Li, 2011) and is able to resist 1.5 times an individual's body weight during walking, and up to 8 times body weight during running at high speed or jumping (Pal, 2014). The ankle joint can be divided structurally into: bones and joints, ligaments and tendons, muscles, nerves, and blood vessels (Pal, 2014).

2.4.1 Bones and joints

The ankle joint or talocrural joint is synovial hinge joint which is formed by articulation of tibial plafond, dome of talus, the lateral malleolus and medial malleolus (Fong et al., 2009a). The primary ankle bone is the talus and its upper part articulates in a socket made by the distal part of the tibia and fibula. The lower part of the talus sits on the heel bone (calcaneus) (Figure 2.1) (Smith, 2006).

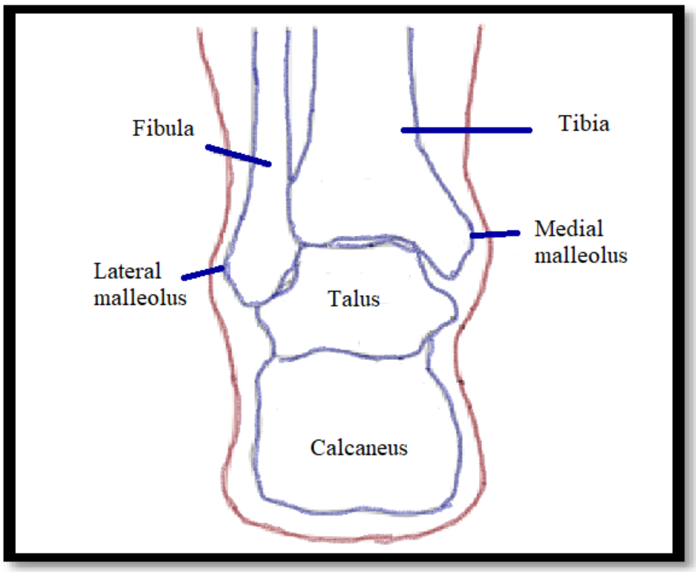


Figure 2-1: The bones of ankle joints

The talus acts similar to hinge inside the socket to permit the foot to move up toward the chest (dorsiflexion) and down (plantar flexion) (Figure 2.2) (Parkin, Logan, & McCarthy, 2007).

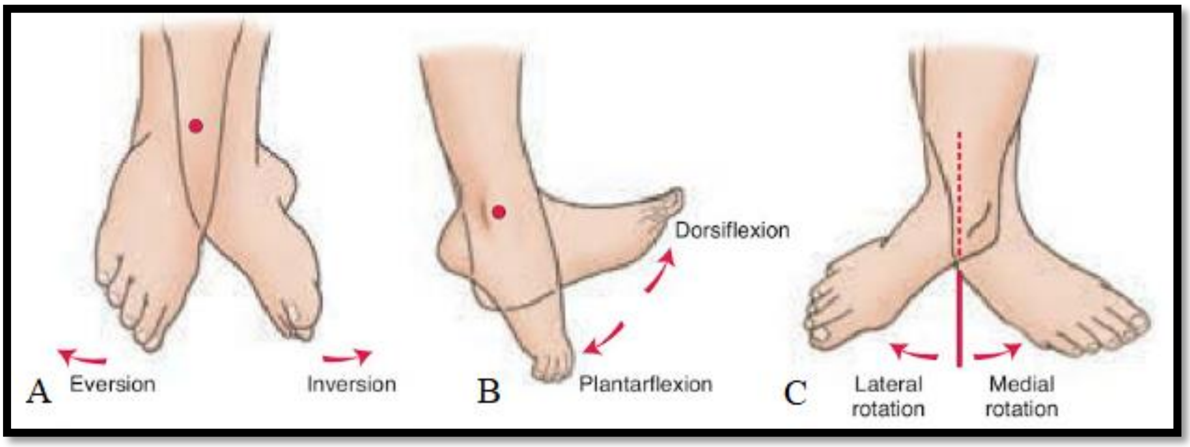


Figure 2-2: The movement of foot and ankle joint. A: Frontal plane components of inversion/eversion. B: Sagittal plane components of dorsiflexion/plantarflexion. C: Transverse plane components of lateral rotation/medial rotation. The red dot and tube demonstrates the axis for that motion (Muscolino, 2016)

The surface of the bones inside the joint are wrapped with a durable slick material known as articular cartilage (Figure 2.3) (Vangsness, 2013). This creates a smooth, lubricated surface for articulation and eases the transmission of loads with a frictionless coefficient that assists joints to perform throughout a lifetime of repetitive use (Fox, Bedi, & Rodeo, 2009; Vangsness, 2013).

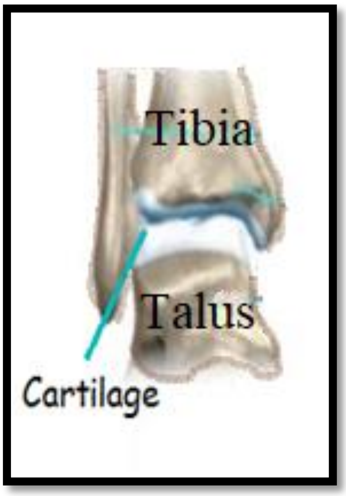


Figure 2-3: Example of articular cartilage at the ankle joint Ligaments and tendons

The main function of the ankle ligaments is force transmission from bone to bone and to provide joint stability, and tendons enable load transmission from muscle to bone. Ligaments and tendons consist of small collagen fibres which are bundled together to create a stiff but flexible structure (Figure 2.4) (Pal, 2014).

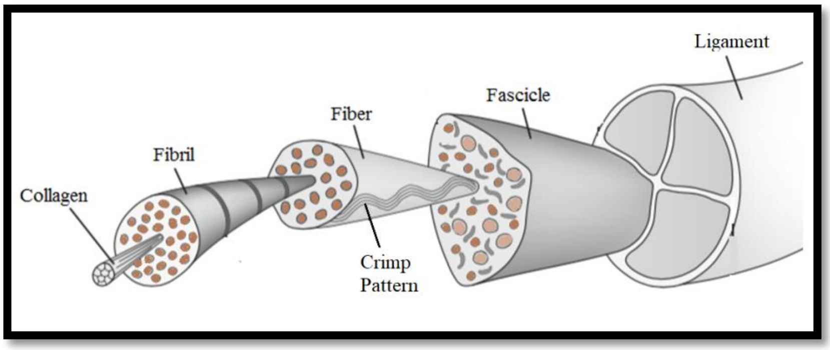


Figure 2-4: Schematic diagram presenting hierarchical structure of ligament in cross section

The ankle joint has ligaments on both sides to assist maintaining the bones together. The lateral collateral ligaments of the ankle joint consist of: ATFL, CFL, and PTFL (posterior talofibular ligament) which resist inversion and internal rotation stress (Figure 2.5) (Yildizgoren, Velioglu, Demetgul, & Turhanoglu, 2017). The medial ankle is supported by the thick deltoid ligaments, which resist eversion and external rotation stress (Liu et al., 2015).

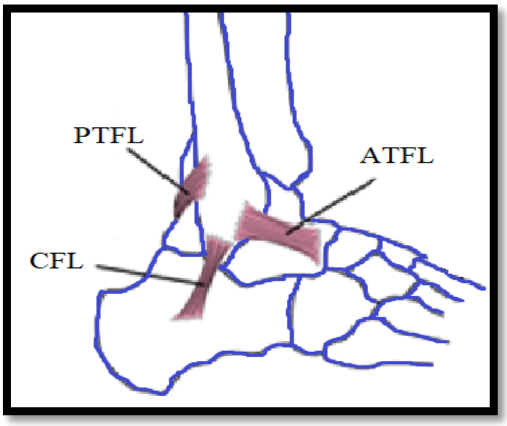


Figure 2-5: Lateral view demonstrates the lateral ligaments

The ATFL controls the anterior displacement and the plantar flexion of the talus (Al-Mohrej & Al-Kenani, 2016). ATFL is quadrilateral and flat in structure (Yildizgoren et al., 2017) and originates from the fibula at the anterior edge of the lateral malleolus (LM) (Meehan et al., 2017). It runs anteromedially and slightly downwards from its origin to insert into the articular cartilage of talar dome (Figure 2.5) (Saxena 2012; Yildizgoren et al. 2017). The ATFL has 16-20 mm in length and 2 mm in thickness (Al-Mohrej & Al-Kenani, 2016; de Asla, Kozánek, Wan, Rubash, & Li 2009). In a neutral position (i.e. 90 degree between foot and leg) the ATFL is almost horizontal to the ankle; however, the ATFL inclines downward and upward in plantar flexion and dorsiflexion position respectively (Golanó et al., 2010). It remodels based on the applied stress and motion (Yildizgoren et al., 2017). The ATFL comes under strain in plantar flexion position and susceptible to injury, especially when the foot is inverted (Golanó et al., 2010).

The CFL is a strong flat oval or cord-like structure (Rein et al., 2015) originating from the anterior part of LM, often with a partial connection to ATFL at the fibula just distal to the ATFL (Meehan et al., 2017). The CFL has about 30 mm in length and 2 mm in thickness (Al-Mohrej & Al-Kenani, 2016; Dimmick, Kennedy & Daunt, 2009), it is very occasionally has a fan shape (around 2% of the population) (Martin, Davenport, Paulseth, Wukich, & Godges, 2013). It runs posteromedially, downward and backward, to insert into the calcaneus, distal to the subtalar joint (Figure 2.5) (Martin et al., 2013; Meehan et al., 2017). The CFL stretches, and become vertical when under strain in the dorsiflexion position and becomes more horizontal in plantar flexion, as demonstrated in Figure 2.6. (Croy, Saliba, Saliba, Anderson, & Hertel, 2012; Golanó et al., 2010).

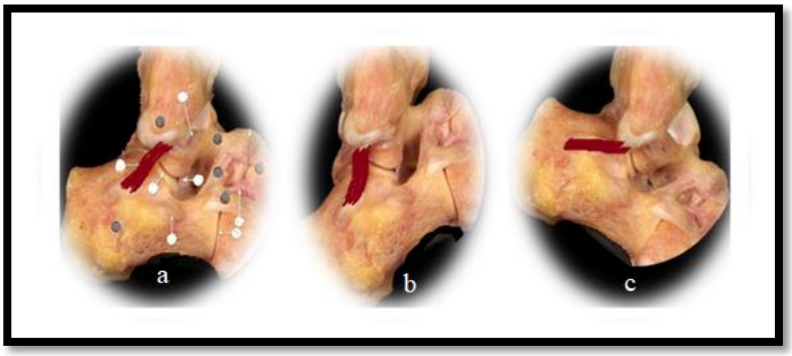


Figure 2-6: CFL throughout the movements of ankle. a: neutral position. b: Dorsal flexion c: Plantar flexion

The PTFL is the strongest of the lateral ankle ligaments (Martin et al., 2013) and originates from the back of the lateral malleolus. It runs horizontally from the posteromedial part of the fibula to the posterior aspect of the talus (Figure 2.5) (Bonnel, Toullec, Mabit, & Tournne, 2010). The PTFL comes under strain in extreme dorsiflexion and external rotation of the foot (Martin et al., 2013). The PTFL acts primary to produce rotatory stability in the transverse plane (Martin et al., 2013).

The medial part of the ankle contains the deltoid ligament, which consists of four ligaments (Figure 2.7) (Al-Mohrej & Al-Kenani, 2016). The ligament has a triangular or a fan shape (Silvestri, Muda, & Sconfienza, 2012) and originates from the distal end of the medial malleolus before dividing into four bundles. Two ligaments run anteriorly; the tibionavicular ligament, which is the more superficial of the two and inserts into the dorsal surface of the scaphoid, and the anterior tibiotalar ligament, which is deeper and inserts into the medial aspect of the talus (Silvestri et al., 2012). Medially, tibiocalcaneal ligament inserts into the substentaculum tali of the calcaneus. The posterior tibiotalar ligament runs posteriorly and inserts into the medial aspect of the talus (Silvestri et al., 2012).

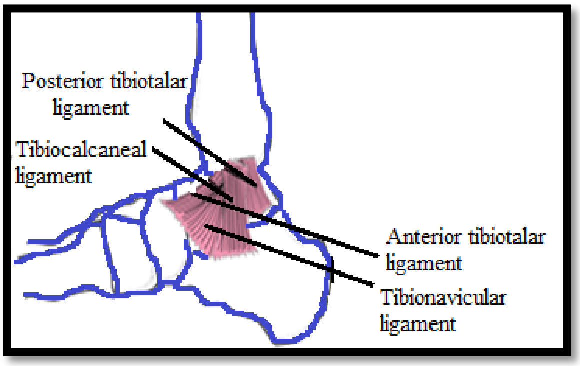


Figure 2-7: Medial side of the ankle demonstrating deltoid ligament

In addition to the ligaments, tendons around the ankle play a significant part in supporting the ankle joint. Two peroneal tendons are sited on the lateral aspect of the ankle; these are the peroneus longus tendon (PLT) and peroneus brevis tendon (PBT), which lie directly superior to the CFL (Dimmick et al., 2009). The PLT originates from the peroneus longus muscle (Silvestri et al., 2012). It runs diagonally beneath the foot (inferior to the cuboid bone) to insert into the plantar aspect of the first cuneiform and the base of the first metatarsal (Figure 2.8) (Dubin, Comeau, McClelland, Dubin, & Ferrel, 2011; Hodgson, O'Connor, & Grainger, 2012). The PBT, meanwhile, originates from the peroneus brevis muscle (Silvestri et al., 2012). It runs anteriorly to insert into the dorsal aspect of the lateral aspect of the fifth metatarsal base (Figure 2.8) (Hodgson et al., 2012). The main function of the peroneus longus and peroneus brevis tendons is to provide dynamic stabilisation of the lateral ankle and to play a role in eversion of the foot. Furthermore, both tendons allow plantarflexion of the ankle joint as well as abduction and pronation of the foot (Neustadter, Raikin & Nazarian, 2004).

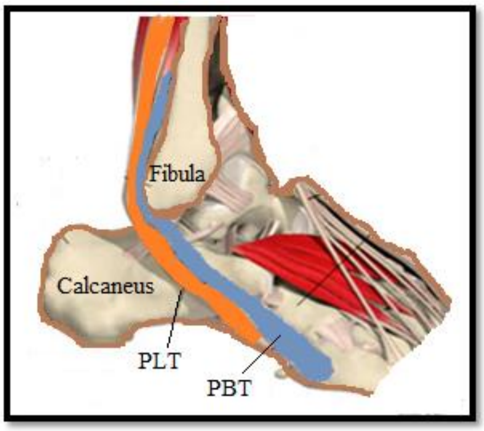


Figure 2-8: The peroneus longus and brevis tendons

There are three tendons located on the medial aspect of the ankle: the tibialis posterior tendon (TPT), the flexor digitorum longus (FDL), and the flexor halluces longus (FHL). Among these three tendons, the TPT is the largest and most anterior (Precerutti, Bonardi, Ferrozzi, & Draghi, 2014). It passes over a specific sulcus along the posterior surface of the medial malleolus, and then goes underneath the malleolus, encircling it distally, superficial to the deltoid ligament (Figure 2.9) (Precerutti et al., 2014). Subsequently it splits into many slips that attach to the tuberosity of the navicular bone, after which the major attachment splits into a fan-shaped array of slips which spread to all tarsus bones except the talus and the second, third, and fourth metatarsals (Precerutti et al., 2014). The main function of the TPT is to support the arch and to permit the plantarflexion and supination of the ankle (Pal, 2014).

The FDL tendon is more slender and is located posterior to the TPT (Precerutti et al., 2014). It passes underneath the medial malleolus, over the internal part of the sustentaculum tali, and continues into plantar surface, reaching the plantar fascia (Figure 2.9). The distal part of the FDL terminates with insertions into the plantar parts of the distal phalanges of the second, third, fourth, and fifth toes (Precerutti et al., 2014).

The FHL tendon is the deepest and most posterior among the three medial tendons (Precerutti et al., 2014). It is passes between the medial and lateral tuberosities along the posterior part of the talus. It continues into the plantar surface, intersecting the FDL tendon underneath the sustentaculum tali, and inserting into the distal phalanx of the big toe (Figure 2.9) (Precerutti et al., 2014).

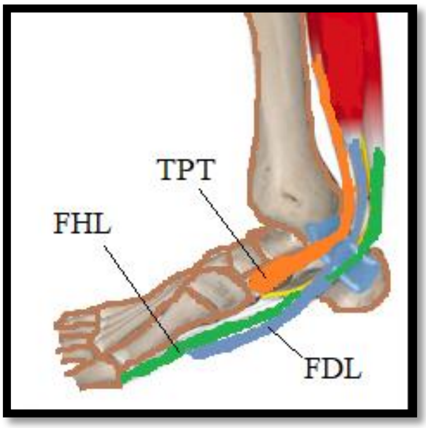


Figure 2-9: Medial tendons of the ankle; TPT (tibialis posterior tendon), FDL (flexor digitorum longus), and FHL (flexor halluces longus)

The anterior ankle includes three tendons arranged in the following order from medial to lateral: tibialis anterior tendon (TAT), extensor halluces longus (EHL), and extensor digitorum longus (EDL) tendons (Demetracopulos & Deland, 2013). TAT runs obliquely and inferomedially to insert on the medial surface of the first cuneiform (Figure 2.10) (Precerutti et al., 2014). The main function of TAT is to dorsiflex the ankle and it also inverts the foot (Demetracopulos & Deland, 2013).

The EHL is the thinner tendon that locates lateral to TAT (Precerutti et al., 2014). It runs through the dorsal surface of the foot and inserts into the base of the distal phalanx of the hallux (Figure 2.10). The EHL acts to dorsiflex the metatarsophalangeal and interphalangeal joints of the great toe. Furthermore, EHL helps in ankle dorsiflexion (Demetracopulos & Deland, 2013).

EDL is the most lateral tendon of the anterior component. The proximal part of the ligament is thin and broad (Precerutti et al., 2014). Furthermore, EDL splits into four distinct tendons just inferior to the neck of the talus, which insert on the dorsum of the distal phalanges of the second, third, fourth, and fifth toes (Figure 2.10) (Precerutti et al., 2014). The EDL tendon works to assess the ankle dorsiflexion (Demetracopulos & Deland, 2013).

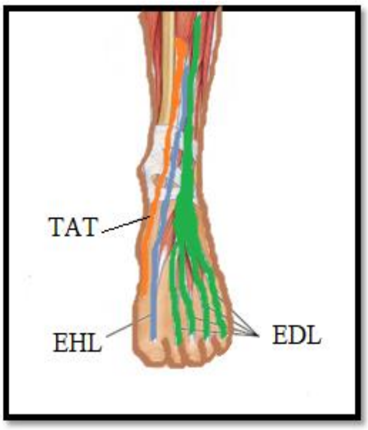


Figure 2-10: Anterior ankle tendons

On the other hand, the posterior part of the ankle has the largest and strongest tendon in the body, which is known as the Achilles tendon (AT) (Doral et al., 2010). It originates in the middle part of the calf, from the medial and lateral gastrocnemius muscles, and merges with the soleus muscle in the distal part of the calf (Doral et al., 2010). The AT inserts into the posterosuperior aspect of the calcaneus (Figure 2.11) (Jane & Marriott, 2009). It allows individuals to stand up on their toes (Pal, 2014).

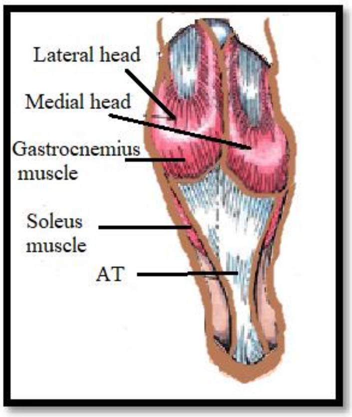


Figure 2-11: Achilles tendon

2.4.2 Muscles

Movement of the ankle during walking, running, and jumping is due to muscle contraction in response to forces from the ground (Pal, 2014). The peroneal (longus and brevis) muscles are located on the lateral aspect of the leg and have a significant role in the lateral stability of the ankle and foot (Mansfield & Neumann, 2014). The peroneal longus muscle (PLM) is the more superficial of the two muscles, originating from the upper two-thirds of the lateral aspect of the fibula and the lateral condyle of the tibia (Figure 2.12) (Taljanovic et al., 2015). The PLM acts in eversion and plantarflexion of the foot (Bisschops & Lavallee, 2016). The peroneal brevis muscle (PBM) is located just anterior to the longus (Bisschops & Lavallee, 2016). It originates from the distal two-thirds of the lateral aspect of the fibula (Figure 2.12) (Jung, 2016). The primary function is to evert the foot, with a secondary role in stabilisation of the lateral ankle (Taljanovic et al., 2015).

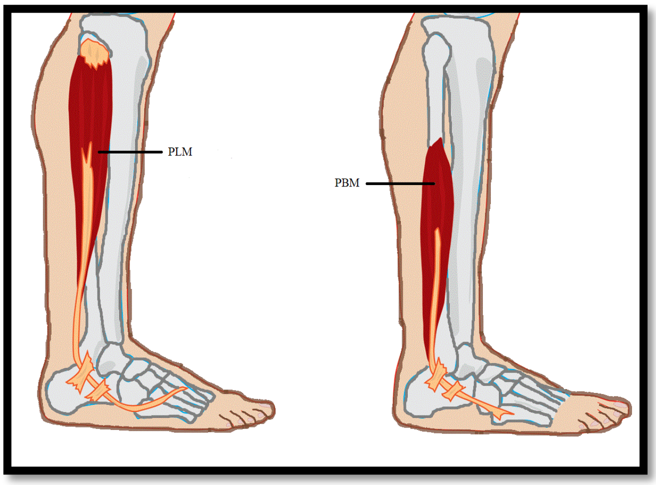


Figure 2-12: Peroneal tendons and muscles

The tibialis posterior muscle (TPM) is found in the posterior compartment of the leg (Silverstri, Muda & Orlandi, 2014) and is the most powerful supinator of the hind foot (Marder & Lian, 2012). It arises from the inner posterior part of the fibula and tibia, just underneath the superior part of tibiofibular joint (Figure 2.13) (Silverstri et al., 2014). The belly of the muscle runs posteriorly between the FDL and FHL muscles (Silverstri et al., 2014). The primary functions are inversion and plantarflexion of the foot. Moreover, the tibialis posterior muscle has a vital part in dynamic stabilisation of the medial longitudinal arch of the foot (Silverstri et al., 2014).

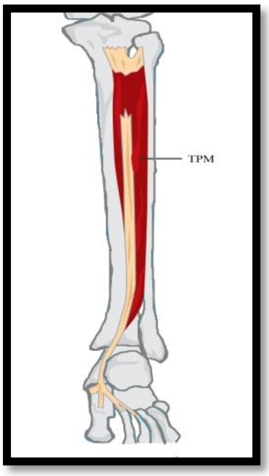


Figure 2-13: Tibialis posterior muscle

The calf (gastrocnemius and soleus) muscles are situated in the posterior aspect of the leg (Doral et al., 2010). The gastrocnemius muscle has medial and lateral heads which provide the bulging shape of the calf (Figure 2.14) (Doral et al., 2010). The medial head arises from the popliteal aspect of the femur, whereas the lateral head originates from the posterolateral surface of the lateral femoral condyle and shorter than the medial head (Figure 2.14) (Doral et al., 2010). Both heads extend to the posterior calcaneus through the Achilles tendon (Doral et al., 2010). The gastrocnemius muscle generates the plantarflexion of the ankle (Mansfield & Neumann, 2014). The soleus muscle is located under the gastrocnemius muscle, and arises from the posterior part of the head of the fibula and the middle third of the medial boundary of the tibia (Figure 2.14) (Doral et al., 2010). It unites with the gastrocnemius muscle to insert into the calcaneus via the Achilles tendon. Along with the gastrocnemius muscle, it makes the three-headed triceps surae which works to provide the plantarflexion movement of the ankle joint through its conjoint tendon (Achilles tendon) (Doral et al., 2010).

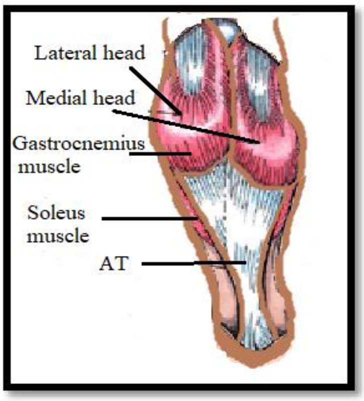


Figure 2-14: Lateral and medial head of the gastrocnemius and soleus muscles

2.5 Ligament injury

Ligament injuries are one of the predominant causes of musculoskeletal joint pain in acute care (Hauser et al., 2013). Injury to the ligaments often creates an imbalance between mobility and stability of the joint and abnormal loading on the joint may disrupt other skeletal or soft tissues in and around a joint, resulting in pain (Jung, Fisher, & Woo, 2009). In 2012, Bartlett and Bussey identified three different types of ligament failures that are based on the loading rate of the ligament, which represents the speed at which load is applied to the ligament (Puddle & Maulder, 2013):

- Mid-substance tears.
- Bony avulsion.
- Cleavage at the ligament-bone interface.

Mid-substance tears of the ligamentous tissues which occur in the mid length of the ligaments are the most common mechanism of ankle ligament injury (Bartlett & Bussey, 2012). This injury is characteristic of fast loading rate failures where the bundle of ligament fibres fails through shear and tension mechanisms, and is typical in sports such as football. This leads to a partial or complete rupture of the ankle ligaments, which represents Grade II and Grade III ankle sprains respectively (Myrick, 2014).

By contrast, bony avulsion (rupture of the bony attachment of the ligament) failure is characteristic of slow loading rate failures. The failure takes place underneath the insertion site of the spongy bone. This type is more prominent in young athletes, whose ligaments are stronger than their bones (Bartlett & Bussey, 2012).

The third type of failure is cleavage at the ligament-bone interface. The failure takes place in the mineralised fibrocartilage. This injury is less common because of the efficient force dissipation that takes place on the ligament insertion (Bartlett & Bussey, 2012). However, biomechanical analysis of lateral ankle ligaments in animals, demonstrated that the stiffness distribution between the pole (close to the fibular insertion) and the centre of the ATFL, is significantly different. In other words, in case of low loading, the pole of the ligament deforms and elongated more easily than the centre (Takebayashi et al., 2002). Based on a cadaver study (Pierre et al., 1984), Martin et al. (2013) stated that 50% of ATFL sprains occur as mid-substance tears, while the other half is bony avulsion from the fibula.

2.5.1 Aetiology of ankle sprain

It has been proposed that most ankle sprain injuries were occurred because of increased supination moment at subtalar joint, which was often as consequence of the extent and the position of the vertically projected ground reaction force (GRF) at early foot contact (Fong et al., 2009a). An ankle sprain is possibly caused by the deviation of such GRF vector from the centre of the ankle joint, commonly at the lateral plantar edge acting to the medial aspect during a sideway cutting movement, creating a large moment arm and therefore an explosive ankle supination torque (Fong et al., 2011). This torque would quickly trigger joint twisting motion and stretch the lateral ligaments in robust way (Fong et al., 2011).

It has been suggested that incorrect foot positioning at landing is one of the common aetiology of ankle sprain. Wright and colleagues (2000) reported that increased touchdown plantar flexion caused increased in the occurrence of ankle sprain. In other words, if the foot was plantarflexed at touchdown, the foot contact the ground with the forefoot, therefor the ground reaction force moment arm about the subtalar joint axis might increase (Wright, Neptune, van den Bogert, & Nigg, 2000) and increase the resultant joint torque to create abrupt explosive twisting motion and thus ankle sprain injury (Fong et al., 2009a).

It has been proposed that a deficit in feedforward and feedback neuromuscular response in peroneal might contribute to incorrect foot position prior to and at initial contact (Fong, Wang, Chu, Chan, 2013). Delayed reaction time of the peroneal muscles at the lateral aspect of the ankle is also reported as aetiology of ankle sprain injury (Fong et al., 2013). Ashton-Miller and colleagues (1996) proposed that a lateral ankle sprain happened in 40 milliseconds. The peroneal muscles (peroneal brevis and longus) work to pronate the ankle which opposes supinate motion of ankle joint. Several previous studies found that the reaction time of the peroneal muscles in healthy participants to be from 58 to 69 milliseconds (Hopkins, McLoda, & McCaw, 2007; Vaes, Duquet, & Van Gheluwe, 2002). However, the reaction time found to be longer in participants with ankle instability (98 milliseconds) (Méndez-Rebolledo, Guzmán-Muñoz, Gatica-Rojas, & Zbinden-Foncea, 2015). Thus, it is assumed that the response of human reflex is not sufficient quick to accommodate the abrupt explosive motion in a sprain injury (Fong et al., 2009a).

2.5.2 Mechanism of ankle ligamentous sprain

Lateral ankle ligamentous sprain is the most common sports injuries, however the mechanism of the sprain is not clear (Fong, Ha, Mok, Chan, & Chan, 2012b). Acute ankle sprain has been defined by Delahunt et al. (2010) and endorsed by the International Ankle Consortium (Gribble et al., 2016) as "an acute traumatic injury to the lateral ligament complex of the ankle joint as a result of excessive inversion of the rear foot or a combined plantarflexion and adduction of the foot. This usually results in some initial deficits in function and disability". Understanding the mechanism of ankle injury with quantitative analysis of ankle biomechanics is important for the design of protective tool and the improvement of injury prevention protocols (Fong et al., 2012).

Ankle sprain does not happen at only one single plane; however, it is accompanied by the other two planes. It is commonly known that the most prominent mechanism of lateral ankle sprain is supination (Seah & Mani-Babu, 2011) accounting for 84% of all sport-related ankle injuries (Fong et al., 2007). The supination is defined as a combination of ankle inversion and forefoot adduction in plantar flexion, resulting in a lateral inversion sprain (Seah & Mani-Babu, 2011). Excessive inversion of the ankle shifts the centre of gravity to the lateral edge of the weight-bearing leg, resulting in a twisting of the ankle at high velocity (Figure 2.15) (Myrick, 2014). Damage to the ligaments is dependent on the foot and ankle positions at the time of injury and the velocity of the mechanism of injury determine the ligament's injury (Martin et al., 2013). The supination mechanism of an ankle sprain causes a partial tear or complete rupture of the lateral ankle ligaments (Martin et al., 2013).

Figure 2-15: Mechanism of inversion ankle sprain (Al-Mohrej & Al-Kenani, 2016).

Several methods were published in the literature to quantitatively evaluate the mechanism of ankle sprain such as motion analysis of non-injury simulations, injuries during biomechanical experiments, cadaver studies, video analysis, and athlete interviews (Ha, Fong, & Chan, 2015). Kinematics of sprained ankles have been investigated through simulated sub-injury or close-to-injury where an inversion perturbation device abruptly inverts the ankle to simulate the mechanism of LAS (Chan, Fong, Yung, Fung, & Chan, 2008; Myers, Riemann, Hwang, Fu, & Lephart, 2003). Fong et al. (2009b) mentioned that since these experiments did not create real injury, they might only represent the kinematics to a limited degree.

The most appropriate method to evaluate the mechanism of the injury is to investigate actual injury incidents; however, it is unethical and impracticable to make tests that generate a high risk of injury for the subjects (Ha et al., 2015). Real life mechanisms of ankle sprain have been described based on video recordings of injuries from Norwegian and Icelandic elite football (Andersen et al., 2004). They concluded that about half of all ankle injuries was due to player to player contact, with collision with an opponent on the medial side of the leg, just prior to or at the moment of foot strike, creates a laterally directed force and the player lands with the ankle in a vulnerable inverted position (Figure 2.16) (Andersen et al., 2004). In addition, authors observed some cases where the injured player hits the opponent's foot when trying to shoot or clear the ball, positioning the ankle in forced plantar flexion. It seems that these mechanisms are specific to football injury. Moreover, the study was done on elite male football players. The mechanism of injury could be different for different players, such as female or different types of sport.

Figure 2-16: Classical mechanism for lateral ligament injury in football (Andersen et al., 2004)

Very occasionally, sprains occur during biomechanical testing (Fong et al., 2009b; Gehring et al., 2013; Kristianslund et al., 2011). Fong et al., (2009b) published the first kinematic analysis of inversion ankle sprain, which occurred accidentally on one male athlete who was performing a sequence of cutting motion trials in the laboratory. The motion of the injury was videotaped by 3 synchronized and calibrated high-speed cameras. Fong et al. (2009b) analysed the video sequences of the injury utilising model-based image-matching (MBIM) technique described by Krosshaug and Bahr (2005). This motion analysis technique was introduced to reconstruct 3D (three dimensional) human motion from uncalibrated video sequences and applied to define the mechanism of anterior cruciate ligament (ACL) ruptures that occur during real world events (Krosshaug, Slauterbeck, Engebretsen, & Bahr, 2007). However, this technique was only validated for knee and hip joints. Therefore, Mok et al. (2011a) conducted a study evaluate the validity and reproducibility of the MBIM method to estimate the kinematic of ankle joint. The authors concluded the validity, inter-rater, and intra-rater reliability was excellent and MBIM motion analysis method could give excellent estimates of ankle joint kinematics (Mok et al., 2011a).

The results of Fong's study (2009b) indicated that at the time of injury, the ankle was inverted to 48°, internally rotated to 10º and about 18° dorsiflexed (Fong et al., 2009b). Moreover, Kristianslund et al. (2011) described the kinematic of accidental lateral ankle sprain that happened in a female elite handball player during sidestep cutting in a motion analysis laboratory. The sprain occurred at 23° ankle inversion, 46° internal rotation, and 22° dorsiflexion. There was a substantial degree of ankle dorsiflexion in both studies which indicated that plantar flexion is not required for sprain to occur (Kristianslund et al., 2011) and there could be several possible mechanisms that create an inversion sprain injury (Fong et al., 2012b).

There are several cases of injuries captured through televised sports events (Fong et al., 2012b; Mok et al., 2011b). Mok et al. (2011b) followed Fong et al. (2009b) procedures and used MBIM to study the kinematics of two ankle sprains during the 2008 Beijing Olympics. Again, the ankles were not planter flexed (high jump case and field hockey case) and the mechanism of injury was concluded as inversion and internal rotation at the ankle joint (Mok et al., 2011b). In 2012b, Fong et al. analysed five inversion sprain incidents from tennis competitions. The result again demonstrated a sudden inversion and internal rotation at the ankle joint was the most likely mechanism. The peak inversion/internal rotation were 48°-126° and 35°- 99°, respectively. The ankle joint fluctuated in the sagittal plane between dorsiflexion and plantar flexion within the first 0.50 second after foot strike. This confirms the observation that plantar flexion was not a prerequisite for a lateral ankle sprain. The peak inversion velocity ranged from 509 to 1488 degree/sec (Fong et al., 2012b).

These findings (Fong et al., 2009b, Fong et al., 2012b; Kristianslund et al., 2011; Mok et al., 2011b) contrast with the previous hypothesis which believed that planter flexion position of the ankle is essential in sprain injury. The classical mechanism of ligamentous ankle sprain was a combination motion of internal rotation, inversion, and plantar flexion (Garrick, 1977). Another study proposed that an ankle sprain was the result of plantar flexion with the subtalar joint adducting and inverting (Vitale & Fallat, 1988). Gehring et al. (2013) reported the mechanism of ankle sprain that occurred accidentally in a soccer male player during a run and cut movement in the laboratory. The ankle during the ground contact was rapidly plantar flexed (1240 degree/sec), inverted (1290 degree/sec) and internally rotated (580 degree/sec) reaching it maximum displacement within the first 150 millisecond after heel strike (Gehring et al., 2013). In addition, Chan et al. (2014) reported on an ankle sprain during a basketball game, observing plantar flexion movement only during the maximum inversion (Chan et al., 2014).

As a result from previous studies it seems that great variation in inversion with peak values 48º-126º, internal rotation with peak values 26º-99º with the possibility of the absent of plantar flexion and thus supination could not be the only one possible mechanism. Most previous studies were based on athlete case studies, and it remains an open question if the observed mechanism is specific for an athletes (Gehring et al., 2013). Even though most of the studies demonstrated that the combination of inversion and internal rotation at the ankle joint are occurred during the lateral ankle sprain, there are contradictory results regarding the occurrence of planter flexion. Different sports may create different mechanisms of ankle injury. The mechanism during landing from a jump in basketball and volleyball could be inversion and planter flexion, since the position of ankle joint is most likely to be plantar flexed before landing (Fong et al., 2012b). However, in a sport that required horizontal sideward movements such as tennis, instead of planter flexion, the internal rotation might be a critical factor in the ankle sprain occurring (Fong et al., 2012b).

2.5.3 Three grades of ankle sprain

Depending on the severity of the ligament damage, anatomical injury, and clinical system, ligamentous sprain falls into three grades (Myrick, 2014; Al-Mohrej & Al-Kenani, 2016). Grade I sprains are characterised by stretching of the ligament with microscopic tears, while Grade II sprains have a partial tear of the ligamentous fibres. Grade III sprains involve complete ligament rupture (Figure 2.20) (Myrick, 2014). Specific characteristics of each grade are demonstrated in Table 2.1.

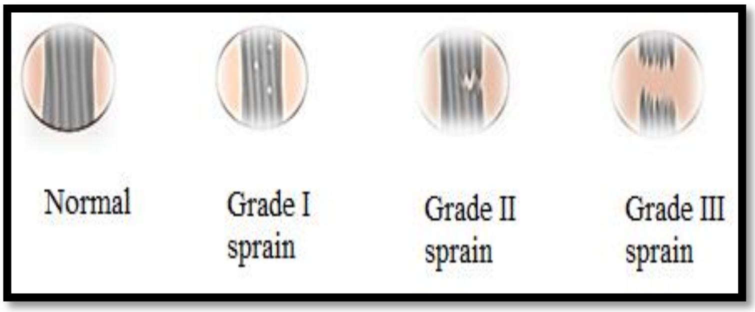


Figure 2-17: Grading of lateral ligaments sprain

Table 2.1: Summary of grading ankle sprains

Severity	Swelling	Pain	Joint instability	Weight bearing	Damage to ligament
Grade I	minimal	minimal	none	fully / Partial	stretching or small tear
Grade II	moderate	moderate	mild to moderate	difficulty	partial
Grade III	severe	severe	severe	unable	complete

2.5.4 Structures associated with ankle sprain

Among the three lateral ankle ligaments, the ATFL is the most vulnerable and the main ligament to be affected with inversion sprain since it has the lowest strength and it tolerates the maximum strain when the foot in plantar flexion position (Ha et al., 2015). ATFL is the first ligament to be affected because the primary function of the ATFL is to restrain the inversion motion during the plantar flexion position of the ankle, therefore, the ATFL is particularly susceptible in the combined inversion and plantarflexion position (Liu et al., 2015). In addition, Fong et al. (2011) proposed that internal rotation and plantarflexion, both with inversion, could have greatly strained and torn the ATFL during the reported injury event. In case of inversion and internal rotation, the ATFL and CFL were strained to about 14 -16% while in case of inversion, plantar flexion, and internal rotation, the ATFL was strained to 20%. Moreover, ATFL is the weakest of the collateral ligaments and contains the lowest modulus of elasticity followed by CFL in more severe ankle sprains (Figure 2.21) (Martin et al., 2013).

Apoorva, Lalitha, and Patil (2014) stated that CFL is strong and is involved in 50 - 75% of acute lateral ankle sprains. Interestingly, a recent histological study revealed that the three lateral ankle ligaments have the same structural composition, which rules out histological reasons for the ATFL being the most likely lateral ligament to be injured (Rein et al., 2015).

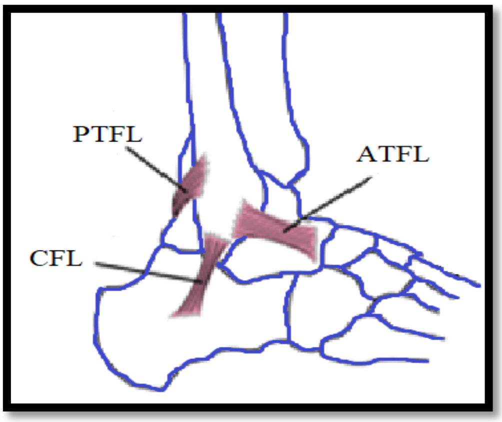


Figure 2-18: Lateral ankle ligaments

In addition to ligament injuries, tenderness along the peroneal tendons is very common for people with an acute ankle sprain, often detected in presentations at an emergency departments (Nelson & Rottman, 2007). In contrast, Van Zoest, Janssen, and Tseng (2007) concluded that injury to the peroneal longus tendon is uncommon and sometimes overlooked in the diagnosis. While Dubin et al. (2011) supported the notion that peroneal tendons are involved in lateral ankle sprains. Furthermore, the clinical signs of isolated tendon injuries may mimic ankle sprains (Yammine & Fathi, 2011) and are often misdiagnosed as lateral ankle sprains (DiDomenico & Anania, 2013). An MRI study performed by Yammine and Fathi (2011) looking at the incidence of associated bone and tendon injuries for athletes with ankle sprains exhibiting normal radiographs, revealed that the incidence of tendon injuries was 38.8%. Quantitative analysis of ultrasound measurements of the thickness and cross sectional area (CSA) of peroneal tendons among healthy and injured ankles have not been reported previously in the literature. This could be important in terms of signs of injury or post healing consequences in peroneal tendon use.

Attached to peroneal tendons are the peroneus longus and brevis muscles which are located in the lateral compartment of the leg (Bisschops & Lavallee, 2016). Both muscles play a significant role in lateral ankle and foot stabilisation (Mansfield & Neumann, 2014). Fong and colleagues (2007, 2009a) reported that the inadequate reaction time of the peroneal muscles in response to an improper foot contact case is consider as one of the aetiological factors contributing to ankle joint inversion injury. Furthermore, weakness of the peroneal muscles predispose the foot to the inversion position, which could result in a lateral ankle sprain (Mansfield & Neumann, 2014). Alongside the confusion over involvement of peroneal muscle, it might be pertinent to evaluate the structural characteristics of these muscles and compare between injured and healthy ankles. Moreover, measurement of CSA of the muscles has been shown to be related to the strength of the muscles (Kurihara et al., 2014).

The Achilles tendon is the primary source of plantar flexion force around the ankle and is thus critical in sports involving jumping and landing, running and cutting (Olsson, 2013). An accurate diagnosis of acute ankle sprains should include examination for concomitant injuries. A recent case study concerned on unrecognised the Achilles tendon rupture associated with severe lateral ankle sprain (Lam & Lui, 205). An MRI examination of a 27-year-old male with ankle inversion injury found a complete tear of the Achilles tendon at its calcaneal insertion. However, the patient had been suffering from heel cord pain a couple of months before the sprain, which could suggest Achilles tendinopathy, predisposing him to the rupture (Lam & Lui, 2015). Moreover, another study has reported that 12.2% of ankle sprains involve Achilles tendinopathy (Fallat, Grimm, & Saracco, 1998). These reports suggested a possible association between Achilles function and ankle sprains.

Even though, tibialis posterior tendon (TPT) rupture is rarely associated with traumatic injuries such as ankle sprains (Kohls-Gatzoulis et al., 2004), Lhoste-Trouilloud (2012) stated that an acute tear of TPT could occur in athletic people who frequently have an ankle sprain. TPT is the largest tendon on the medial side of ankle. Tibialis posterior is the most powerful supinator of the ankle due to the inverter moment arm at the ankle and subtalar joint and its relatively large muscle mass (Lhoste-Trouilloud, 2012). A considerable amount of literature has been published on evaluation TPT using ultrasound, where it is thought to have an important role in evaluation of TPT integrity (El-Liethy & Kamal, 2016; Lhoste-Trouilloud, 2012).

Like the AT, the TPT and muscle play an important role in ankle movement. Little attention has been paid to these structures in understanding mechanisms of lateral ankle sprain and also in post injury ankle. Although the primary site of injury is the lateral ankle ligaments, Lam and Lui, (2015) have recognised the potential for involvement of other structures and suggested that patients should be assessed for associated injuries. This seems consistent with the lateral ankle sprain literature that refers to concurrent injuries to peroneal muscles or tendons, Achilles tendon, and tibialis posterior tendon too. Looking too narrowly at only the lateral ankle ligament structures might lead to incomplete definition of the injury and fail to inform any rehabilitation strategy. Since the function of associated muscles and tendons might affect the task of the lateral ankle structures, ongoing deficit in their structure or function might be linked to risk of further recurrent sprains.

2.5.5 Classification of ankle injury

Musculoskeletal injury is generally classified into three main stages: acute, subacute, and chronic. Although this classification is largely created according to the biological healing process, there is no clear-cut definition to establish the ending of one specific stage and the starting of another. Furthermore, there is much disagreement on the duration period of one stage and the exact beginning of the next stage (Shultz, Houglum, & Perrin, 2016).

An acute ankle ligament injury has a sudden onset of macro-trauma but this only lasts for a short period (Shultz et al., 2016). Daugherty, Manske, and Brotzman (2011) claimed that an acute injury is experienced instantly, as soon as the injury occurs and lasted for up to 3 to 5 days. Diaz (2014) claimed that the signs and symptoms of the acute stage could continue for up to one month. This duration of acute injury is similar to that recently pointed out by Shultz et al. (2016), who suggested that an ankle ligament injury can be classified as acute for up to 4 weeks from the onset of injury. It follows that, a subacute injury can begin around 4 weeks after the onset of injury, and might be shorter duration, perhaps 2-3 weeks (Daugherty et al., 2011; Shultz et al., 2016).

An ankle ligament injury is classified as chronic from six to eight weeks following onset and can continue for many months (Shultz et al., 2016). The long-term affect in individual with ankle sprain such as swelling, pain, and recurrent ankle sprain is known as chronic ankle instability (Cao et al., 2018). The outcomes of a systematic review highlight the lack of agreement regarding the definition of chronic ankle instability (Delahunt et al., 2010). Terms that seems to be applied interchangeably involve ankle instability, chronic instability, chronic lateral instability, functional ankle instability (FAI), recurrent ankle sprain, and multiple ankle sprain (Delahunt et al., 2010). Most researchers utilise various inclusion criteria that leads to heterogeneous sample of participants which limits our ability to compare the results across different studies (Delahunt et al., 2010). CAI is an encompassing term utilised to categorise an individual with both mechanical ankle instability (MAI) and FAI (Dallinga, van der Does, Benjaminse, & Lemmink, 2016). Residual symptoms such as "giving away" and feeling of ankle joint instability must be present at least 1 year after first sprain for individuals to be classified as having CAI (Delahunt et al., 2010). MAI is defined as excessive anterior laxity of the ankle joint as measured by equipment (stress radiography or arthrometry) or manual stress testing. Joint ROM is beyond the normal expected physiological ROM expected for that joint (Delahunt et al., 2010). It involves insufficient arthrokinematics, ligamentous laxity, synovial changes, and degenerative changes (Khuman, Surbala, & Kamlesh, 2014).While functional ankle instability refers to "a situation whereby a subject reports experiencing frequent episodes of ''giving way'' of the ankle joint and feelings of ankle joint instability". It involves impaired proprioception, alterations in sensation and neuromuscular control (Needle et al., 2013), strength deficits and improper postural control (Khuman, Surbala, & Kamlesh, 2014). Even though functional and mechanical ankle instability can occur separately, it has been hypothesised that a combination of both functional and mechanical ankle instabilities are likely to contribute to CAI (Figure 2.22) (Khuman et al., 2014). The National Centre for Biotechnology Information (2014) has report that several authors have concluded that CAI is a condition characterised by a feeling of "giving way", six months or more following the initial ankle sprain. In addition, following the initial ankle sprain, symptoms can present in up to 73% of cases (Pionnier et al., 2016).

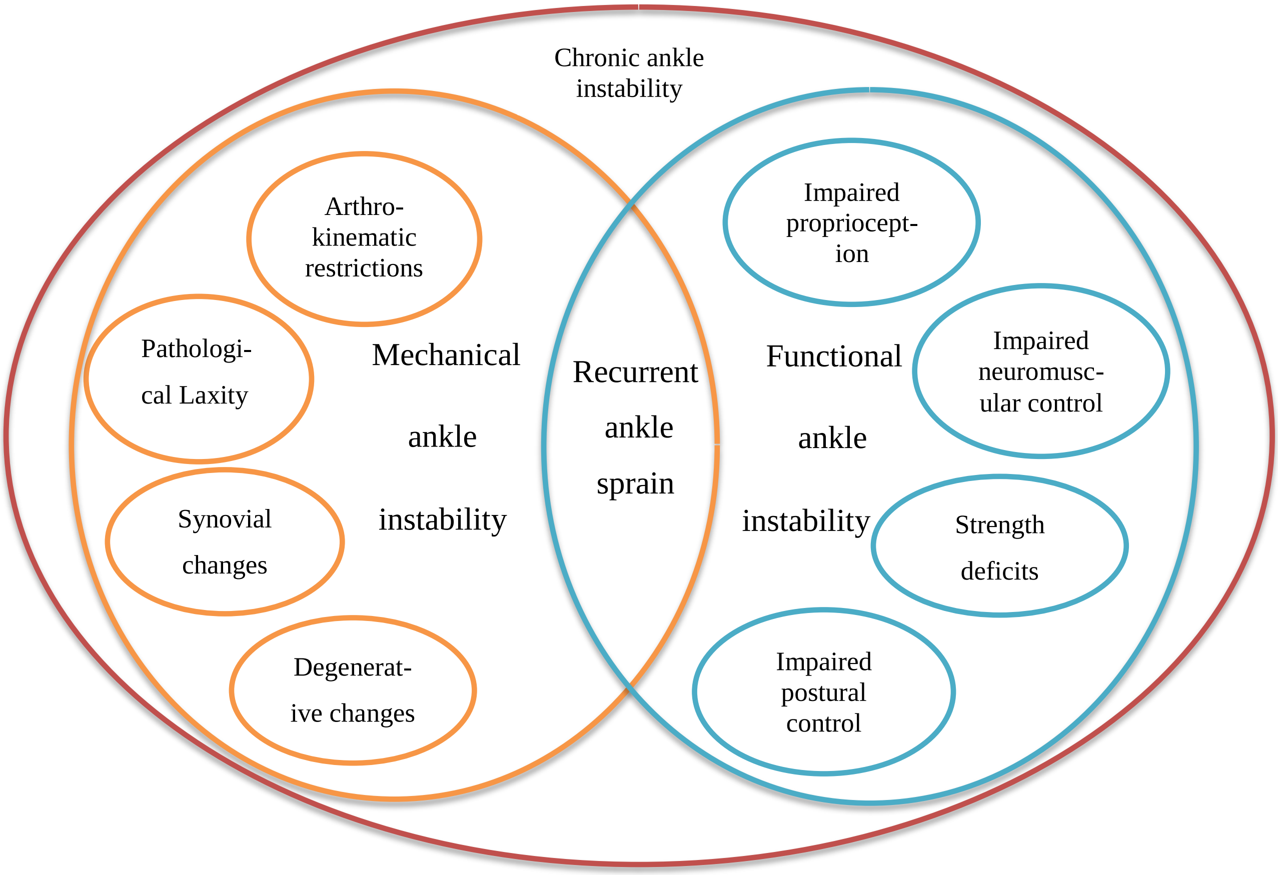


Figure 2-19: Diagram of mechanical and functional ankle instability that contributes to chronic ankle instability

A systematic review has found that the ankle joint "giving way" and "recurrent sprains" are the most frequently applied criteria for describing the consequences of ankle instability (Delahunt et al., 2010). Recurrent sprain has been defined as, "a minimum of two acute lateral ankle sprains on the same lower limb which are not associated with frequent episodes of ''giving way'' and feelings of ankle joint instability" (Delahunt et al., 2010).

However, some believe the absence of a gold standard and universally accepted inclusion criteria leads to use samples that are too heterogeneous, mixing people with FAI and CAI, and potentially explaining the variable outcomes found in the FAI and CAI literature (Donahue et al., 2011). A systematic review including 118 studies has recognised almost 90 inclusion criteria, some of these studies are summarised in Table 2.2 (Delahunt et al., 2010).

Table 2.2: Summary of previous studies in the literature. A blank cell indicates that the data were not provided. FI: functional instability, FAI: functional ankle instability, MI: mechanical instability, MAI: mechanical ankle instability, WB: weight bearing, ROM: range of motion, AJFAT: Ankle Joint Functional Assessment Tool, CAIT: Cumberland Ankle Instability Tool; CAI: chronic ankle instability, AD: anterior drawer, TT: talar tilt, AII: Ankle Instability Instrument, NWB: non-weight bearing, FADI: Foot and Ankle Disability Index.

Author/ Year	Ankle Instability Definition	Indicate CAI, MAI, FAI, or Other	Inclusion Criteria
Arnold & Docherty (2006)		FI	1) One unilateral ankle sprain 2) Episodes of giving way
Bernier, Perrin, & Rijke (1997)	A feeling of giving way	FI	1) History of multiple sprains 2) Repeated episodes of giving way
Brown & Mynark (2007)	CAI = subjective repeated episodes of giving way and sprains MAI= physiological laxity at the ankle	CAI	1) MAI and FAI groups: inversion sprain requiring immobilization or NWB for at least 3 days 2) FAI = two or more episodes of giving way 3) MAI = positive manual AD or TT
Brown, Ross, Mynark, & Guskiewicz (2004)		FAI	1) Two or more recurrent ankle sprains 2) A feeling of giving way on activity 3) A score of ≤20 on AJFAT
Buchanan, Docherty, & Schrader (2008)	1) Disabling loss of reliable static and dynamic support of a joint 2) Tendency for the foot to give way	FAI	1) History of moderate to severe ankle sprain 2) Episodes of giving way or instability
Caulfield, Crammond, Sullivan, Reynolds, & Ward (2004)	1) FAI = motion beyond voluntary control yet within physiological limits 2) MAI = motion beyond the physiologic ROM	FI	1) Two or more unilateral inversion injuries requiring , WB or immobilization 2) Chronically weak since injury 3) More painful since injury 4) Less functional since injury
Caulfield and Garrett (2002)	1) A condition characterized by a tendency of the foot to repeatedly	FI	1) History of at least two sprains to their lateral ligament complex

	sprain or give way 2) Ankle joint motion is beyond voluntary control yet within physiological limits 3) MAI = ankle motion beyond physiological limits		2) Subjective reporting of a tendency of the ankle to give way during sporting activities
Caulfield and Garrett (2004)	Joint motion that is beyond voluntary control yet within physiological limits	FAI	1) Two or more unilateral inversion injuries requiring , WB or immobilization 2) Chronically weak since injury 3) More painful since injury 4) Less functional since injury
Clark and Burden (2005)	1) FI = a feeling of giving way in the ankle 2) Subjective weakness in the absence of MI	FI	1) Three or more lateral sprains in two years 2) Subjective weakness 3) Negative anterior drawer
Dayakidis and Boudolos (2006)	1) FI = tendency of the foot to ‘‘giving way’’ without exceeding the normal ROM 2) MI = motion beyond physiological limits	FI	1) Five or more sprains requiring , WB or immobilization 2) Reported instability 3) Tendency to give way during sporting activities
Delahunt, Monaghan, & Caulfield (2007)	1) CAI = repeated inversion injury with residual symptoms (pain, swelling and ‘‘giving way’’) 2) MI = ligamentous damage resulting in joint motion exceeding normal physiological limits 3) FI = joint motion not exceeding normal physiological limits but beyond voluntary control	FI	1) Two or more unilateral inversion injuries requiring , WB or immobilization 2) Chronically weak since injury 3) More painful since injury 4) Less functional since injury
Delahunt, Monaghan, & Caulfield (2006)	Multiple inversion sprains resulting from slight or no external provocation	FI	1) Two or more unilateral inversion injuries requiring , WB or immobilization 2) Chronically weak since injury

			3) More painful since injury 4) Less functional since injury 5) Gives way during sports
Demeritt, Shultz, Docherty, Gansneder, & Perrin (2002)	1) Tendency to give way during normal activity 2) Disabling loss of reliable static and dynamic used support of a joint	CAI	1) One significant lateral sprain unable to WB or used crutches 2) Episodes of one or more repeated lateral ankle injury 3) Feelings of instability 4) Giving way
De Noronha, Refshauge, Kilbreath & Crosbie (2007)	Description of symptoms of giving way, weakness, pain, and decreased function	FAI	1) History of ankle sprain 2) CAIT score 23 for the affected ankle
Docherty, Valovich McLeod, & Shultz (2006)	Residual feeling of instability after a lateral ankle sprain	FAI	Instability as based on the AII
Eechaute, Vaes, & Duquet (2008a)		CAI	1) History of lateral ankle sprain followed by pain, swelling, or stiffness hampering activity 2) Two or more medical visits 3) Repetitive sprains 4) Fear of giving way 5) Decreased function on activities
Eils and Rosenbaum (2001)		CAI	1) Recurrent inversion ankle sprains 2) Self-reported feeling of instability or giving way
Freeman, Dean, & Hanham (1965)	Tendency of the foot to give way	FAI	One year history of FI after rupture or simple lateral ligament sprain
Høiness, Glott, & Ingjer (2003)		MI	1) History of major sprain without fracture 2) Unilateral recurrent sprains 3) MI on stress x-ray and manual tests
Karlsson and Andreasson, (1992)	AI is a complex syndrome where functional, mechanical and neuromuscular factors are all at fault	FAI	1) Unilateral supination injury 2) Radiological MI 3) Self-reported , sports activity
Monaghan, Delahunt, & Caulfield (2006)	Subjectively reported phenomenon defined as a tendency of the foot to	CAI	1) Two or more unilateral inversion injuries requiring , WB or immobilization

	give way during normal activity		2) Chronically weak since injury 3) More painful since injury 4) Less functional since injury
Munn, Beard, Refshauge, & Lee (2003)		FAI	1) One or more sprain ankle 2) Chronically weak since injury 3) More painful since injury 4) Less functional since injury
Santilli et al. (2005)	Recurrent ankle sprains and a feeling of the ankle giving way without structural alterations in the ankle articular complex	FAI	1) At least one significant inversion injury requiring , WB 2) Repeated sprains 3) Feeling of the ankle giving way 4) Minimum of two successive sprains to the initial episode
Santos and Liu (2008)	Recurrent ankle sprains	FAI	1) Two or more sprains 2) Sensations of ankle instability or giving way
Sawkins, Refshauge, Kilbreath, & Raymond (2007)		Ankle instability	Score ≤24/30 on CAIT
Sedory, McVey, Cross, Ingersoll, & Hertel (2007)	Frequent giving way of the ankle	CAI	1) History of ankle injury 2) ‘‘Yes’’ to four other ankle question symptoms on the AII 3) Disability on at least two items on the FADI
Vase, Duquet, Casteleyn, Handelberg, & Opdecam (1998)	FI = disabling loss of reliable static and dynamic support of a joint	FAI	1) Minimum of one traumatic ankle sprain needing immobilization 2) Two recurring sprains with complaints of pain or swelling for a minimum of 5 days 3) A feeling of instability after each sprain
van Cingel et al. (2006)		CAI	Sought medical attention for at least one sprain that caused ecchymosis with pain and swelling requiring immobilization, minimum of three sprains over the last 6 months that occurred in daily life or during sporting activity

Gribble et al. (2014) reported the minimum inclusion criteria advised by the International Ankle Consortium for participants involved in research into CAI. These include:

(1) A history of at least one significant ankle sprain with the first sprain occurring 12 months, as a minimum previously, had some degree of inflammatory symptoms such as swelling and pain, and failed to participate in the desired physical activity on at least one day at the time of injury. The most recent ankle sprain should have occurred more than 3 months before conducting the study.

(2) A history of the previously injured ankle joint ‘giving way’, and/or recurrent sprain and/or feelings of instability of the injured ankle joint. Subjects must have a minimum of two episodes of giving way in the previous six months before conducting the study. Feeling of ankle joint instability is defined as the condition in which the individual feels that the ankle joint is unstable during daily living and sporting activities and is often associated with the fear of sustaining an acute ligament sprain. In addition, subjects must self-reported ankle instability using a specific validated ankle instability questionnaire with related cut-off score, such as:

a- Ankle Instability Instrument (AII): “yes” answer should be provided to a minimum of five yes/no questions (must contain question 1, plus four others).

b- Cumberland Ankle Instability Tool (CAIT): the total score should be less than 24.

c- Identification of Functional Ankle Instability (IdFAI): the total score should be greater than 11.

(3) A low score on specific self-reported questionnaire which is recommended to rate the overall function of the foot and ankle, and to determine the level of disability. This includes:

a- Foot and Ankle Ability Measure (FAAM): the scale of activities of daily living less than 90%, scale of sport less than 80%.

b- Foot and Ankle Outcome Score (FAOS): the score must be less than 75% in three or more categories. However, this last inclusion criterion should only apply if the nature of the research questions relates to the level of function of participants.

Over the past several years, healthy or uninjured control subjects have been chosen to compare with CAI subjects in biomechanical and clinical studies (Wikstrom & Brown, 2014).

However, they could not be the perfect sample if researchers want to define why group of people experiencing a lateral ankle sprain develop CAI. Rather than choose a control subjects that have never been exposed to sprain, a more suitable comparison could be people who had a lateral ankle sprain but did not develop CAI. These people seem to cope successfully with damage occurred by the first ankle sprain and often known as a “coper group” which has been adopted in ankle instability research (Liu et al., 2015). Wikstrom and Brown (2014) reviewed 21 studies to report standard criteria and definition of people who “cope” with their chronic ankle instability. The authors recommended that three key components included in operational definitions of coper: (1) a first lateral ankle sprain; (2) lack of CAI symptoms such as no giving away or suffering of ankle disability; (3) a time component (Wikstrom & Brown, 2014). People who have their first ankle sprain in less than 12 months ago must be defined as “potential copers” since it is still unclear if they will develop the disability related to CAI. 12 months was chosen as a cut off for three reasons: (1) the risk of recurrent of ankle sprain after 12 months is similar to risk of first-time ankle sprain; (2) to guarantee that “potential coper” do not adjust their physical activity after the ankle sprain to the level that they avoid the reoccurrence of the injury; (3) 12 studies requisite that the previous history of LAS occur at least 12 months before conducting the test (Wikstrom & Brown, 2014).

Wikstrom and Brown (2014) reported minimum standards for copers in ankle instability research as individual had a severe LAS that led to immobilization and/or no weight bearing ability for a minimum of three days at the time of injury or utilise a protective devise such as ankle brace for a minimum of one week. Also, and most distinctively, copers have had no episodes of re-injury, episodes of giving way, and returned to at least moderate level of physical activities without any limitation for at least 12 months (Wikstrom et al., 2012). Lastly, they recommended that copers have a minimal, if any, level of self-reported functional disability.

2.6 Self-reported functional ankle instability measures

Evaluative self-reported tools use the response of participants to measure changes in health conditions over time (Martin, Irrgang, Burdett, Conti, & Swearingen, 2005). Self-reported tools are extensively applied in the lateral ankle sprain literature to characterise participants and create sub groups according to structural or functional impairments post the ankle injury (Donahue, Simon, & Docherty, 2011).

Most of the criteria that are used are based on some form of self-reported questionnaire to identify ankle stability status (Donahue et al., 2011). The original aims and designs of these questionnaires differ greatly; some of them have been created to determine people with FAI, and others are established mainly to measure ankle pain (Docherty, Gansneder, Arnold, & Hurwitz, 2006; Eechaute, Vaes, & Duquet, 2008b; Hiller, Refshauge, Bundy, Herbert, & Kilbreath, 2006; Martin et al., 2005; Roos & Karlsson, 2001).

Henderson (2015) reported that people define the word "injury" differently when using self-reported questionnaires. This can lead to the argument that validated hospital record is more appropriate. Contrary to this idea, and as mentioned previously, about 55% of people having an ankle sprain do not seek assessment or treatment from healthcare professionals (Hiller et al., 2012). Even though an validated hospital record method appears more precise, numerous injuries are probably missed because of the great number of under-reported cases (Henderson, 2015). Therefore, utilising a self-reported questionnaire would document a high number of ankle injuries.

Martin et al. (2005) stated that if the questionnaire is created properly and has obtained evidence of validity, the collected information can consequently be used to explicate the impact of pathology and subsequent deficiency on physical function (Martin et al., 2005). Information acquired from this questionnaire can also be utilised to compare and evaluate the effectiveness of treatment interventions. Validity evidence for the self-reported questionnaire is required, thus that score could be meaningfully interpreted.

Donahue et al. (2011) critically reviewed self-reported functional ankle instability questionnaires that have been published in the past decade to support standardisation of inclusion criteria for research in lateral ankle sprain. These include: Ankle Joint Functional Assessment Tool (AJFAT), Ankle Instability Instrument (AII), FAAM, FAOS and CAIT (Donahue, Simon, & Docherty, 2012). These questionnaires present in various designs and layouts (Donahue et al., 2011). Some were created to differentiate individuals with FAI and CAI, and others for measuring pain in the ankle (Docherty et al., 2006; Eechaute et al., 2008b; Martin et al., 2005).

Based on the different aims set, some questionnaires were longer than others: AJFAT contains two pages, CAIT contains one page, while FAOS contains four pages. Some items in the questionnaires have complex layouts, which may need additional clarification to some participants, although this is not proper and feasible for each study design (Donahue et al., 2011). As a consequence of these factors, there arose number of incidents in which it is obvious the participants have incorrectly or incompletely filled in the questionnaire and thus the outcomes from these participants' data had to be excluded (Donahue et al., 2011).

Each questionnaire deals differently with limbs; AII, FAOS and FAAM ask subjects to fill in the same form for each limb, taking longer to complete, while AJFAT askes subjects for their responses depending on a comparison of their limbs. As a result, this type of questionnaire cannot be used for people with bilateral ankle symptoms (Donahue et al., 2011). Each self-reported questionnaire has a specific number of items. AII contains 12 items; FAOS contains 42 items divided into five subscales (9 items for pain, 7 items for other symptom, 17 items for activities of daily living, 5 items for sport/recreation, and 4 items related to quality of life); FAAM contains 31 items; AJFAT contains 12 items. CAIT contains only 9 items, and is designed not to require comparison between limbs like AJFAT, decreasing patient burden and increasing reliability. It is believed that the accuracy of the questionnaires is increased for those that have a multiple answer option (Vuurberg, Kluit, & van Dijk, 2016).

Moreover, the response to the AII questionnaire cannot added together to produce a score to define the functional ankle instability. Instead, researchers have to see the participants' responses and define which questions will better reflect the level of instability concerning them the most (Docherty et al., 2006). For FAOS, the score of each question is based on a 5-point Likert scale from 0-4 (none, mild, moderate, severe, and extreme problems). Scores are calculated by adding the total score of each subscale and dividing it by the possible maximum score. The result of normalised score is interpreted as score from 0 to 100 with 0 equals to extreme problems and 100 equals to no problems (Golightly et al., 2015).

On the other hand, CAIT asks subjects to answer each question for each limb at the same time which making it easier for participants to fill in and for the researcher to evaluate both ankles individually (Donahue et al., 2011).

FAOS and FAAM have been demonstrated to be valid for the Dutch language. However, they are not specified for symptoms of instability. These questionnaires do not take into account feelings of giving way and reoccurrence of ankle sprains, which are proposed to be the main cause of disability (Vuurberg et al., 2016). Wright et al. (2014) have reported that the CAIT has been commonly applied in the literature relating to ankle instability, being translated into several languages (e.g. Portuguese, Spanish, Korean), and plays a significant role in predicting ankle instability. Authors have reported that CAIT was the first questionnaire to be presented as a reliable and valid measure to be utilised in categorising those with CAI (Hiller et al., 2006; Wright et al., 2014). Vuurberg et al. (2016) tested validity to evaluate whether FAOS or CAIT is more appropriate to utilise for individuals with ankle instability using Spearman’s correlation coefficients. The CAIT demonstrated a significant correlation with self-reported ankle instability, while no significant correlation was found between FAOS and self-reported ankle instability. This result may point to CAIT being more appropriate for assessing ankle instability than the FAOS.

Before using the questionnaire, it is necessary to know that many studies have already tested the reliability of most questionnaires. A high reliability score demonstrates that a questionnaire will be a valuable instrument in both research and clinical settings, giving clinicians and researchers confidence that participants will answer the questions consistently and in a similar manner. Intraclass correlation coefficient (ICC) of AII ranged from 0.70 to 0.89, which demonstrated good reliability (Hiller et al., 2006), FAOS ranged from 0.85 to 0.96 (Roos & Karlsson, 2001), FAAM ranged from 0.87 to 0.89 (Martin et al., 2005), and the ICC of CAIT was 0.96, which demonstrated excellent reliability (Hiller et al., 2006).

Copers demonstrate better self-reported function of the ankle than people with CAI, in some situations this was equal to those who never experienced an ankle sprain (Wikstrom & Brown, 2014). However, there are some variability in the findings related to the self-reported questionnaires and how it was used. When AJFAT was utilised as measuring tool, copers demonstrated significantly better self-reported function than CAI, but not always better than healthy groups. When CAIT was applied as a measuring tool, the scores for copers were greater than those for people with CAI, indicating a significantly better function, with scores that were probably equal to those for healthy people (Wikstrom & Brown, 2014). Multiple studies have reported similar outcomes when using questionnaires, such as the Foot and Ankle Ability Measure Activities of Daily Living (FAAM-ADL) and sports subscale (FAAM-S), independently of the inclusion criteria. Wikstrom and Brown (2014) have concluded from systematic reviews that copers demonstrate different functional status from those with CAI, even though they have had similar injury characteristics. For instance, no differences were observed in the severity of the first ankle sprain between coper and CAI groups (Hubbard, 2008; Wright et al., 2013), number of individuals who sought a medical diagnosis for the first lateral ankle sprain (Wright et al., 2013), and acute treatment for the first lateral ankle sprain (Hubbard, 2008). A systematic review was conducted of 21 studies to report on the standards for copers in the CAI literature. The authors found that CAIT was the most common questionnaire utilised to quantify self-reported functional ankle instability (Wikstrom & Brown, 2014).

CAIT was first published in 2006 by Hiller. It is an objective pencil-and-paper questionnaire that contains nine questions which cover 30 points in order to identify and evaluate the severity of functional instability of the ankle joint (De La Motte, Arnold, and Ross (2015); Henderson, 2015; Pourkazemi et al., 2016). Eight of the nine questions are designed to evaluate ankle instability in the participants through their daily and sports activities, while one question is designed to determine when the participants feel pain (Martin et al., 2013). The questionnaire scores range from 0 to 30, with a higher score representing a high degree of stability of the ankle joint, and a lower score representing instability of the ankle joint (Henderson, 2015). In their report testing the validity and reliability of CAIT, Hiller et al. (2006) concluded that scores from 28 to 30 indicate a stable ankle, while scores equal to or less than 27 indicate significant or severe ankle instability. Wright, Arnold, Ross, & Linens (2014) believed that the cut-off score of ≤ 27 seemed to be too high and could be suboptimal for applying to people with chronic ankle instability. Moreover, people who have experienced a history of ankle sprain but who have subjectively stated that they did not complain about their ankle, were sometimes categorised as CAI according to the cut-off score of ≤ 27 (Wright et al., 2014). It could be because of this concern that Hiller and colleagues (2007) and De Noronha, Refshauge, Kilbreath, and Crosbie (2007) have independently decreased the cut-off score to ≤ 23 and ≤ 24 respectively. The International Ankle Consortium has recommended using a cut-off score of ≤ 24 to classify people with CAI (Gribble et al., 2014). In other words, Wright et al. (2014) recalibrate and revalidate the CAIT cut-off score for CAI populations. The data reported in their study appear to support the recommendation to lower the cut-off score to ≤ 24, boosting the usefulness of the CAIT in differentiating between people with and without CAI (Wright et al., 2014).

The ability of the CAIT to distinguish between the healthy population and the CAI population enhances its utility in both the clinical and research fields. In the clinical setting, the CAIT allows the practitioner to evaluate the severity of functional ankle instability, and to monitor the outcome of treatment and rehabilitation of CAI. In the research field, the CAIT allows researchers to more precisely define, identify, and objectively compare groups of participants (Hiller et al., 2006). The CAIT score has the possibility of predicting future sprain in people with FAI. It could be that individuals with previous ankle sprain with a low

CAIT score are more likely to re-sprain, while those with a high CAIT score are less likely to re-sprain (Hiller et al., 2006; Pourkazemi et al., 2016).

2.7 Risk factors of lateral ankle sprain

Risk factors were generally categorised as extrinsic or intrinsic (Lysens et al., 1984). Extrinsic risk factors are defined as those factors that are external to the body and are known to be environmentally related (Fong et al., 2009a; Vereijken, 2012) such as level of play, position played, shoe type, and landing surface (Martin et al., 2013). On the other hand, intrinsic factors are defined as those factors internal to the body, known as personal characteristics (Fong et al., 2009a; Vereijken, 2012), such as body size, previous ankle injury, age, gender, and joint laxity (Martin et al., 2013).

It has been recognised that several factors, such as physical activity level, gender, body mass index (BMI), the number of the previous sprains, and balance are considered to be significant risk factors for ankle sprains (Gribble et al., 2016). Physical activity level plays a vital role in increasing the incidence of ankle sprain (Cameron et al., 2010). Cameron et al. (2010) concluded that the incidence of ankle sprains among the military population was five times greater that of civilian populations. However, McManus, Stevenson, and Finch (2006) found that athletes who trained for more than four hours each week had a 39% reduced risk of injury in comparison with athletes who trained less than four hours per week. A possible explanation is that, as the athletes spent more time on the activity, they improved their techniques and skills in terms of strength, balance, and proprioception, therefore decreasing the risk of injury (McManus, 2006). Contradicting this result, Naja, Naja, and Hassan (2017) found that male cadets in military school who spent four or more times per week in training are at a high risk of ankle sprain that could be associated with increased exposure to ankle injury (Naja et al., 2017).

It is well documented in the literature that female athletes are at a higher risk of sustaining knee injuries than male athletes, especially anterior cruciate ligament (ACL) sprains. Voskanian (2013) reported that female athletes have 3.5 times higher risk of ACL injury compared with males. Moreover, Mandelbaum and Mora (2013) mentioned that the ACL injury rate for female basketball players was four times higher than for males, whilst the rate in female soccer players was more than twice that of males. Peck et al. (2013) have also reported that females are at a high risk of injury and concluded that the rate of ACL injury among female intercollegiate rugby players was 5.3 times higher compared to males. The impact of gender on ankle injuries is less clear than the relation between knee ligament injuries and gender (Murphy, Connolly, & Beynnon, 2003). Cameron et al. (2010) conducted a retrospective cohort study to describe the gender among members of the USA armed services with ankle injury. They concluded that female members were 21% more likely to sustain ankle sprains than male members (Cameron et al., 2010). While these studies have reported that females are at the high risk of sustaining ankle sprain, one study demonstrated that ankle ligament injuries among male soccer players had a significantly greater rate, almost three times more, than among female players (Lindenfeld, Schmitt, Hendy, Mangine, & Noyes, 1994). In contrast to these studies, Beynnon et al. (2001) reported the incidence of ankle sprains to be similar among 118 male and female athletes. Even though, studies have shown that there is an association between gender and the likelihood of ankle injury, there is a lack of evidence of the effect of gender on ankle injury. Furthermore, differences in results could be due to differences of inclusion criteria and the types of sport involved in each study.

Males tend to have larger muscles and greater absolute strength than females (Chow et al., 2000). Developments in the technology utilised to evaluate body composition, such as ultrasound and MRI, have allowed measurement of the size (thickness, CSA and angle of pennation) of tendons and muscles of the lower extremity (Chow et al., 2000; Kubo, Kanehisa, & Fukunaga, 2003; Ying et al., 2003). Studies in the literature concerning the influence of gender on the mechanical properties of ligaments, tendons and muscles, such as structure stiffness and thickness, seem to demonstrate different results. Onambe and colleagues (2007) found that the patellar tendon was thicker in males compared to females, while Taş et al. (2017) found no statistically significant difference in the thickness of patellar tendon between males and females. Chow et al. (2000) also reported that males had thicker gastrocnemius and soleus muscles than females. The main weakness of this study is a lack of demographic data for the participants; height, weight and body mass index of participants were not mentioned in the study, which could affect the results.

Body mass index (BMI) has been mentioned previously as a risk factor for ankle sprains. BMI is designed to deliver an indication if an individual is overweight by adjusting body weight for height (Tyler, Mchugh, Mirabella, Mullaney, & Nicholas, 2006). BMI has been valuable in population based studies by virtue of its wide acceptance in determining particular classification of body mass as a health issue (Nuttall, 2015). A study of 390 military recruits deduced a statistically significant (P=0.004) relationship between BMI and the occurrence of ankle sprain (Milgrom, Shlamkovitch, Danon, Wosk, & Simkin, 1991). McHugh and colleagues (2006) agreed with Milgrom that athletes with higher BMI were at higher risk of sustaining ankle sprain. Furthermore, Tyler et al. (2006) studied the role of previous ankle sprains and BMI as risk factors for ankle sprain in high school football players. While the rate of injury for players with normal BMI was 0.52 per 1000 exposures (defined as a player's participation in a game or practice), this rate increased in players at risk of being overweight to 1.05 per 1000 exposure and to 2.03 per 1000 exposure for overweight players (Tyler et al., 2006). In addition, they reported that overweight players who had experienced an ankle sprain previously were 19 times more likely to have a noncontact ankle sprain compared to players with normal weight and no ankle sprain previously (Tyler et al., 2006). Furthermore, a study conducted on 100 professional soccer players demonstrated that heavier players (more than 72.6 kilogram) and with higher BMI (more than 23.1 kg/m²) are at a high risk of ankle sprain (Fousekis, Tsepis, & Vagenas, 2012). The findings of a recent study done by Hartley, Hoch, & Boling (2018) aligned well with the previous literature that demonstrated that higher BMI could be a predictor of ankle sprain occurrence in male collegiate athletes; participants with a BMI $\geq$30.2 kg/m² were 3.85 times sustaining ankle sprain than participants with BMI <30.2 kg/m². On the other hand, several studies have reported that BMI, height and weight are not risk factors for ankle sprain (Beynnon et al., 2001; McKay, Goldie, Payne, & Oakes, 2001).

The effect of high BMI on the occurrence of noncontact ankle sprain could be associated with inability of the individual to alter momentum efficiently and rapidly (Tyler et al., 2006). Since mass times velocity generates momentum, at any given velocity of movement, a larger force is needed to alter the momentum of a heavier individual. Because the foot is the pivot around which the body alters momentum, the ankle joint should be adequately stable to effectively transmit ground reaction forces during an alteration of momentum (Tyler et al., 2006). An ankle sprain could happen when the required forces for altering momentum surpass the dynamic stability of the ankle joint. If these forces produce an inversion moment at the ankle, dynamic stabilisation could be inadequate for stability of the ankle joint (Tyler et al., 2006). In this case, both previous ankle sprain (which probably reduces the stability of the ankle joint) and a high BMI could increase the effective risk of injury (McHugh et al., 2006; Tyler et al., 2006).

A number of studies have tested the association between BMI and the size of some ankle structures (Abate, 2014; Klein et al., 2013). Mirza (2016) conducted an ultrasound study to measure the thickness of the Achilles tendon in three different groups (underweight, normal, and overweight) of healthy participants. They found that weight of the players was the more significant role in determining BMI, whereas height was not significantly different among the three groups even though it has an important part in determining BMI (Mirza, 2016). They concluded that the thickness of the AT is BMI-dependent, as overweight participants have a thinner Achilles tendon. A serious weakness with this result is the very small sample size, involving five participants in each group. On the other hand, Abate and colleagues (2012) pointed out that there was a positive relationship between BMI and Achilles tendon thickness. Most studies on the effect of BMI on the size of ankle structures have been conducted on Achilles tendons. There is a lack of studies on the other selected ankle structures.

Balance control is negatively related to ankle injury, this relationship between poor balance control and increase the risk of injury was determined 30 years ago. Tropp et al. in 1984 found that soccer players with poor balance were almost four times sustaining ankle injuries than those with normal balance ability. The literature on risk factors has highlighted balance as a risk factor for ankle sprain. A prospective study on 230 male and female athletes found a significant correlation between the positive single limb balance (unable to maintain balanced) and future ankle sprains ($p=0.02$) (Trojian & McKeag, 2006). The study was conducted on athletes with specific sports (men's American football, men's and women's soccer, and women's volleyball). Another study conducted on high school basketball players found a higher postural sway being predictive for ankle sprains (Wang, Chen, Shiang, Jan, & Lin, 2006). Inability to control postural sway could indicate inadequate performance and a functional instability, leading to ankle injury. In addition, De Noronha et al. (2013) conducted study on active university students to investigate if the postural control could predict ankle sprains. The postural control was tested in a dynamic test by SEBT (anterior, posteromedial, and posterolateral directions) and found that participants with star excursion balance test under 80% of their limb length in posterolateral direction was 48% greater risk of having ankle sprain while participants who reached 90% or higher had a significantly lower occurrence of sprains. A recent study found that male collegiate athletes with ankle sprain injury achieved significantly worse on the anterior reach direction of the YBT (Y-balance test) (Hartley et al., 2018); participants with a normalised anterior reach of ≤54.4% were 3.64 times sustaining ankle sprain than participants with a normalised anterior reach of >54.4%.

The majority of prospective cohort studies in the literature have reported that an individual with previous ankle sprain injury has high risk of a recurrent ankle sprain and the risk of injury increases as the number of previous injuries increases (Engebretsen, Myklebust, Holme, Engebretsen, & Bahr, 2009). Furthermore, several studies have demonstrated that there is a relationship between history of previous ankle sprains and higher risk of future sprains (Arnason, Sigurdsson, Gudmundsson, & Holme, 2004; Engebretsen et al., 2009; Hiller, Herbert, & Kilbreath, 2008; Kofotolis, Kellis, & Vlachopoulos, 2006; Naja et al., 2017; Steffen, Myklebust, Andersen, Holme, & Bahr, 2008; Tyler et al., 2006). On the other hand, some studies have found no relationship between previous ankle injury and the rate of ankle injury. Baumhauer and colleagues (1995) reported that a history of a previous mild grade I ankle sprain among collegiate athletes was not found to increase the risk of having a further inversion ankle sprain. Athletes with severe grade II or III ankle sprain were excluded from the study; thus the result was restricted to grade I only. Consistent with these findings, Hartley et al. (2018) found no association between a previous ankle sprain and future ankle sprains in male collegiate athletes. Some treatment methods such as ankle bracing and balance training that used after the first ankle sprain showed to be an effective in preventing future ankle sprains (Hupperets, Verhagen, & Van Mechelen, 2009; Janssen, Mechelen, & Verhagen, 2014; . It could be clinicians are using these treatment methods and rehabilitating ankle sprains more efficiency to avoid the reoccurrence of ankle sprains (Hartley et al., 2018).

The lack of consistency in the results of studies on risk factors studies could be due to the disparities in the measurement methods, differences in the baseline risks related to various sports, dissimilar statistical analyses, variation in the methods of data collection, and disparities in the definition and severity of ankle injury.

One interesting finding from Hiller et al.'s (2008) prospective cohort study of 115 adolescent dancers is that previous ankle sprain can be a predictor for future ankle sprain, not only at the same injury site, but also on the contralateral side. They reported that this result is likely to be related to the alteration in central motor processing which happens in subjects after sustaining ankle sprain, making both sides of the ankle susceptible to sprain (Hiller et al., 2008). It is worth noting that 32% to 74% of people with a history of previous lateral ankle sprains have some kind of residual symptoms and can suffer further ankle sprains which can lead to chronic ankle instability (Gribble et al., 2014). One possible explanation is that almost half of individuals with sprained ankles do not seek any initial assessment or care from a medical practitioner, which probably reflects underestimation of the severity of ankle sprain (Gribble et al., 2014). Hertel (2002) has argued that the management of lateral ankle sprains seems to be ineffective in avoiding recurrent sprains. Aslan, Sofu, and Kirdemir (2014) agreed with Hertel that a consensus on optimal treatment process for prevention of acute ankle sprain is not established. This may be due to the fact that most patients with ankle injury are referred for foot and ankle x-ray examination, which is not good for diagnosing soft tissue injury. It would therefore be more appropriate to use an imaging technique, such as ultrasound imaging, which is more suited for assessing soft tissue than radiography.

2.8 Diagnosis and evaluation of ankle injury

Many researchers hold the view that a delayed physical examination (four to seven days after injury) is the gold standard in diagnosing an acute lateral ankle sprain and it provides a more accurate diagnosis than a physical examination within the first 48 hours of injury (Kerkhoffs et al., 2012; Lin et al., 2013; Van den Bekerom, Kerkhoffs, McCollum, Calder, & Van Dijk, 2013). A possible explanation is that the swelling, tenderness and diffuse location of pain at the time of injury make it difficult for the examiner to differentiate between haematoma and oedema (Van den Bekerom et al., 2013). However, this assumption is based upon data from a single study involving 160 participants that was conducted over 20 years ago by van Dijk, Mol, Lim, Marti, & Bossuyt in 1996. Delayed physical examination has a higher sensitivity and specificity than arthrography, stress radiography and sonography, but only when practised by an experienced investigator. However, when delayed physical examination was performed by an inexperienced investigator, in the same study, the sensitivity and specificity were lower than the other modalities. Therefore, the reliability of the study is questionable since part of the diagnostic strategy depends so much on the expertise of the examiner, for example the outcomes of the anterior drawer test. Delayed physical examination is thus considered to be a subjective test, rather than an objective test, so it cannot be considered as a gold standard for lateral ankle sprains.

Fong et al. (2009a) reported that it is essential to include differential diagnosis method for each acute ankle sprain injury because it is not uncommon for clinicians to misdiagnose many ankle issues such as simple ankle sprain. Lynam (2006) provided a strategy to help nurses to evaluate and categorise acute foot and ankle sprain. Fracture injuries diagnosed first since these patients usually require admission for emergency treatment (Fong et al., 2009a; Mak, Chan, & Leung, 1985). Diagnosis of ankle fracture injuries is usually conducted with radiography, or the Ottawa Ankle Rules, which determine if an x-ray is required for patients with acute ankle sprain (Hedelin, Goksör, Karlsson, & Stjernström, 2013). Bachmann and colleagues (2003) found that Ottawa Ankle Rules decreased the number of x-rays by 30% to 40% only on patients without a fracture, despite the fact that 85% of the cases do not have a fracture (Hedelin et al., 2013). Furthermore, Fong et al (2009a) reported palpating the ligamentous structures to determine which ligament was injured. This could be performed by testing a range of motion, particularly in terms of voluntary dorsiflexion and plantarflexion movement. Once fracture is ruled out, particular tests such as the anterior drawer test and the talar tilt test must be carried to accurately diagnose if the issue is a ligamentous injury (Fong et al., 2009a). On the other hand, a meta-analysis was conducted by Schneiders and Karas (2016) to discuss the accuracy of clinical tests in diagnosing ankle ligament injury and found that clinicians are unable to rule out ligamentous injury in case of negative clinical test, and thus further imaging is needed. Thus, imaging is a helpful diagnostic method.

A considerable amount of literature has been published on different imaging modalities to be used in the assessment and diagnosis of lateral ankle sprains, such as arthrography (Van Dijk et al., 1998; Oae, Takao, Uchio, & Ochi, 2010), MRI (Campbell, 2006; Chu et al., 2017; Tan, Jing, Teh, & Chee, 2017), 3D computed tomography (Nakasa, Fukuhara, Adachi, & Ochi, 2006), and sonography (Lee & Yun, 2017; Sanjay, Babulreddy, & Umamahesh, 2018; Milz et al., 1998). Traditionally, foot and ankle x-ray imaging is the initial diagnostic test that has been used to exclude bony injury (Hauser et al., 2013). However, most cases of ankle trauma involve ligaments and soft tissue injury and thus do not clearly show up on radiographs, which can lead to missed ligament tears, false positive, and false negative results due to inaccurate readings (Hauser et al., 2013). It is plausible that ultrasound could provide an alternative diagnostic option for ankle sprain assessments.

2.9 Ultrasound as diagnostic image modality

2.9.1 Ultrasound history and physics

An Austrian psychiatrist and neurologist, Dr. Karl Dussik was the first physician to utilise ultrasound as a diagnostic technique in medicine when he tried to scan brain tumours in 1942 (Soni et al., 2014). Musculoskeletal ultrasound (MSKUS) was applied in the world of radiology more than 50 years ago, following the foundation of the American Institute of Ultrasound in Medicine in 1951 (Pinzon & Moore, 2009). The initial study of the application of musculoskeletal ultrasound was done by McDonald and Leopold (1972) to distinguish between Baker's cysts and thrombophlebitis (inflammation of the vein). Just a few years later, the uses of ultrasound had gradually increased. In 1978, Cooperberg and colleagues used ultrasound to evaluate and follow up patients with rheumatoid arthritis. One case reported by Maner and Marsh (1981) used ultrasound as a diagnostic method to evaluate a rupture in the Achilles tendon. Dillehay and colleagues (1984) proposed that the ultrasound method must be considered as a valuable technique for the assessment of ligaments and tendons due to the progress made in higher megahertz transducers at that time which allowed these superficial structures to be visualised. As the technology progressed in the early 1990s, a great improvement was made in the resolution of ultrasound images and the enhancement of soft tissue contrasts, which gave opportunities to use ultrasound to evaluate small joints (Wakefield et al., 2005). The applications of MSKUS have been constantly expanding (Patil & Dasgupta, 2012) and nowadays it has the potential to detect a wide spectrum of musculoskeletal pathologies (Artul & Habib, 2014; Cai, Li, Chen, Hua, & Shan, 2017; Radwan et al., 2016). MSKUS is a real time modality that can directly visualise and describe the smallest inflammation and any initial structural changes. These abilities can assist in monitoring disease progression and help in providing adequate treatment (Artul & Habib, 2014). In addition, Cho and Wansaicheong (2012) have stated that ultrasound is considered the second line of imaging modality after x-rays for the evaluation of foot and ankle pathology. On the other hand, Artul and Habib (2014) have stated that MSKUS should be considered the first diagnostic imaging modality for the evaluation of foot and ankle pain because most cases of ankle trauma involve ligaments and soft tissue injury and thus do not clearly show up on radiographs.

Ultrasound is defined as sound waves with a frequency that exceeds the natural range of human hearing (20 Hz to 20 kHz) (Goyal, 2018; Merritt, 2017). The range of frequencies that is utilised in diagnostic medical ultrasound is from 2 to 20 MHz, which is about 500 to 1000 times greater than the normal limit of human hearing (Goyal, 2018). Ultrasound images are tomographic, presenting the anatomical structures in a cross-sectional (slice) view. Several ultrasound imaging modes have been established to improve image acquisition such as 2D (two dimensions), M-mode (motion-mode), and Doppler mode (Soni et al., 2014). Most diagnostic ultrasound imaging is done in the 2D mode (Figure 2.20A). This mode is also known as B-mode, which stands for "brightness", because the echogenicity or brightness of the reflected signals is displayed as bright points based on the intensity of the signals

(Shetty & Moiyadi, 2016). M-mode or "motion" mode, is an old mode of imaging; however, it is utilised most frequently nowadays to evaluate range of motion (Figure 2.20B), such as the heart rate (Carovac, Smajlovic, & Junuzovic, 2011; Goyal, 2018). In contrast to natural US imaging, in which sound waves reflect from the soft tissue, the sound waves in Doppler imaging are reflected from moving structures such as the blood (Figure 2.20C) (Mohamed, 2015). The Doppler mode is commonly utilised to measure the speed and flow of the blood (Mohamed, 2015).

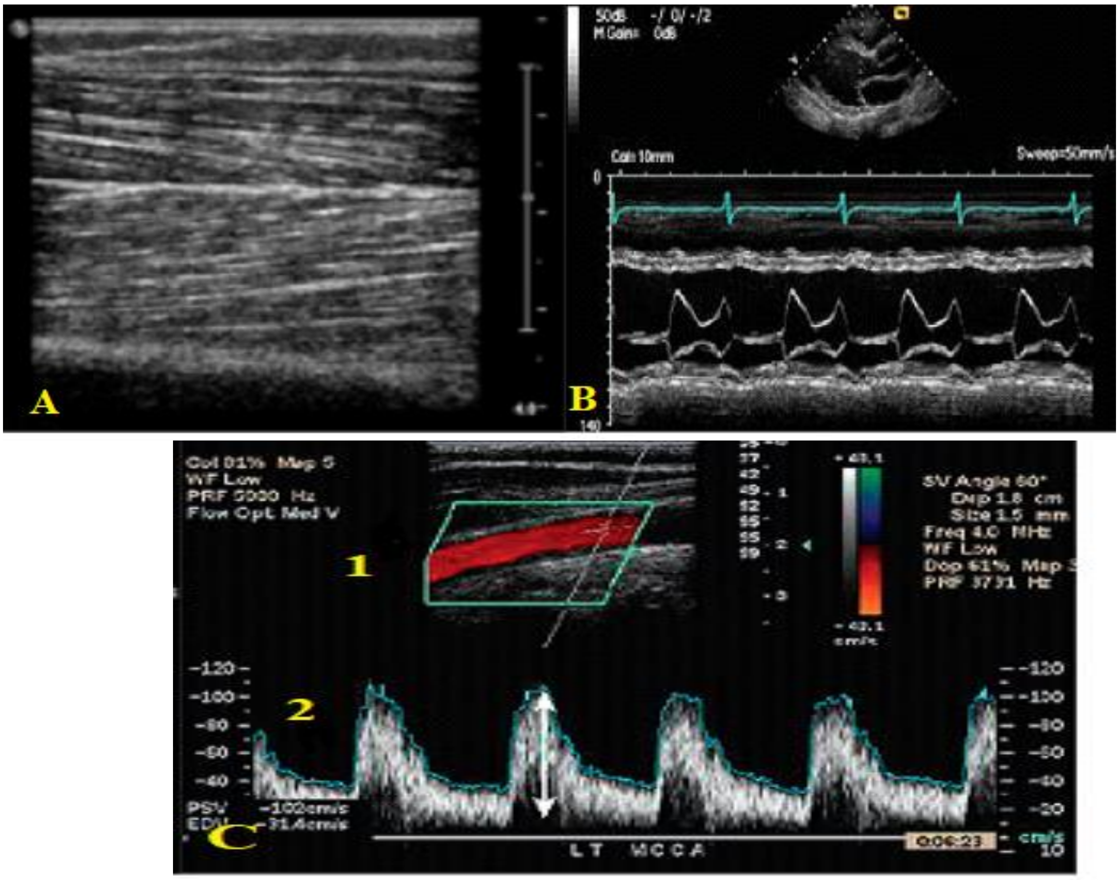


Figure 2-20: Three different modes of ultrasound. (A) 2D of tibialis anterior muscles (Pillen, 2010). (B) M-mode presenting the mitral valve leaflets of the heart (Gill, 2012). (C) Doppler mode of carotid artery: (1) colour Doppler and (2) pulsed Doppler (Merritt, 2017).

The principle of current medical sonograms is a pulse-echo approach with a B-mode display (Narouze, 2011). The B-mode ultrasound image is created in three different planes (sagittal, frontal and transverse) based on the anatomical position of the human body (Figure 2.21) (Mohamed, 2015). Therefore, ultrasound images appear as anterior view (sagittal), lateral view (frontal) or cross sectional view (transverse) (Mohamed, 2015).

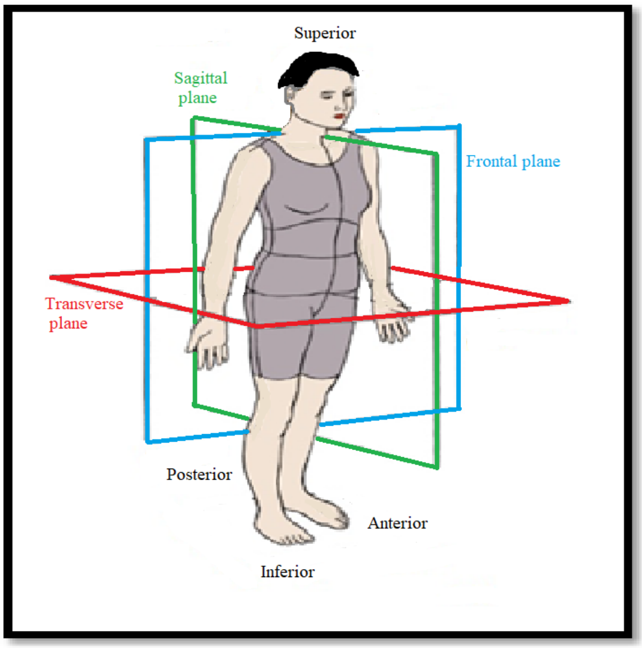


Figure 2-21: Anatomical position of human body with the three planes. Sagittal plane divides the body into left and right. Frontal plane divides the body into front and back. Transverse plane divides the body into superior and inferior

Ultrasound machines apply the piezoelectric effect to create an image (Jacobson, 2017). The piezoelectric effect involves the generation of electrical energy by using another type of energy, such as that produced by exerting pressure on a crystal (Strakowski, 2015). In the case of ultrasound, this results from the production of sound waves, which are released from a crystal attached to a transducer by applying electrical signals causing the crystal vibrate (Strakowski, 2015). The sound waves released by the probe are also referred to as a pulse. This method is called the reverse piezoelectric effect (Strakowski, 2015). The direct piezoelectric effect happens when electrical signals are produced due to the impact on the crystals from the sound waves reflected back from the soft tissue to the probe; this is the echo (Strakowski, 2015). The special pattern of electrical signals produced by the echo is utilised to generate the image on the ultrasound screen (Strakowski, 2015). Specific computer software is used to produce a two-dimensional, black and white image of the anatomical structure. As the sound waves penetrate the tissue, some of them interact with the soft tissue interface, while some are reflected back to the probe where they are converted to electrical signals to be utilised to generate the ultrasound image (Figure 2.22) (Jacobson, 2017). The echo is based on acoustic impedance (Chan & Perlas, 2011), which is defined as a specific property of tissue that allows sound waves to propagate. It is basically the product of the density and velocity (Merritt, 2017; Strakowski, 2015). The reflection of sound energy back to the probe is in direct proportion to the difference in acoustic impedance between tissues. In other words, the larger the difference in impedance, the brighter (hyperechoic) the returning signals appear on the ultrasound screen (Jacobson, 2017). The reflected signals are processed and combined to create the ultrasound image (Figure 2.22) (Ruas, Pinto, Lima, Costa, & Brown, 2017). Therefore, the probe acts to both generate and receive sound waves (Chan & Perlas, 2011). For superficial structures, a higher frequency transducer provides higher resolution (Jacobson, 2017) and good depth penetration (O'Neill, 2008). Moreover, the ultrasound beam should be perpendicular to the structure so that as much as possible of the ultrasound wave returns to the probe, thus creating the best possible visualisation for the ultrasound image (Jacobson, 2017) and to eliminate anisotropy (changes in the normal echogenicity of a specific structure when the ultrasound beam is not perpendicular to the plane of the structure being scanned) (O'Neill, 2008). An ultrasound gel is used to facilitate the transmission of the sound beam between the transducer and the skin and to reduce the risk of misinterpretation of images due to pressure of the transducer (Goyal, 2018; Ruas et al., 2017).

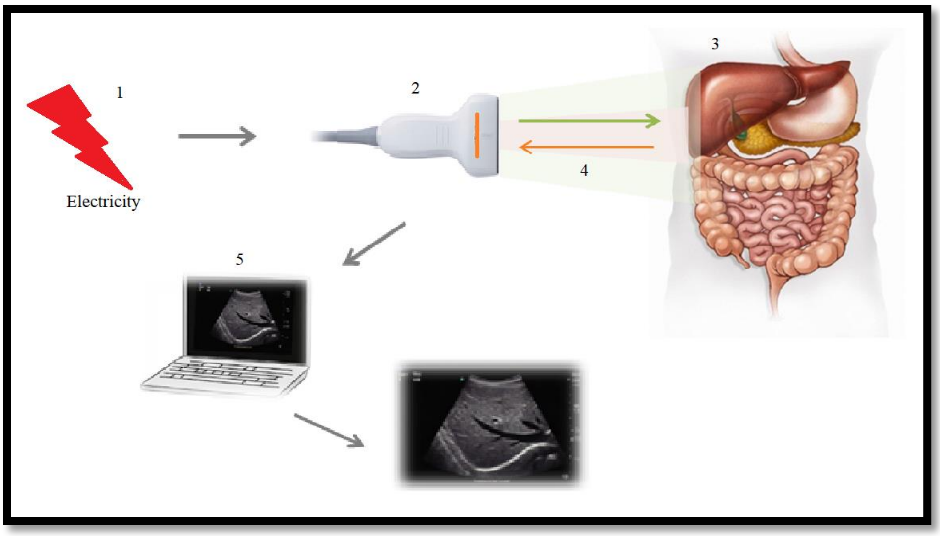


Figure 2-22: Creation of ultrasound images. (1) Electricity is applied to the probe. (2) Piezoelectric crystals vibrate quickly, creating sound waves. (3) Ultrasound beam penetrates tissues. (4) Sound waves reflected (echo) and returned to the probe. (5) Echoes are turn into electrical signals which are processed into grey-scale image

Ultrasound scanning is counted as a very safe diagnostic image modality; however, some limitations should be considered. The intensity of the ultrasound beams can cause two possible kinds of injuries: thermal (heat generation) and mechanical (cavitation) from contrast-enhanced ultrasound (Mayatte & Mohabir, 2014). The current ultrasound systems produce intensities from 10 to 430 mW/cm^2. The American Institute of Ultrasound in

Medicine currently recommend that exposure to intensities less than 1 W/cm^2, which are equivalent to raising the temperature of soft tissue by almost 1° C above the baseline. It is difficult to precisely measure temperature rises inside the human body (Mayatte & Mohabir, 2014). The raised temperature disperses quickly, particularly in blood vessels; however, it can theoretically be as high as 4° C in cases of prolonged exposure at the focal point. Due to this theoretical risk, societies advocate the As Low As Reasonably Achievable (ALARA) principle, with reduction of exposure time at any one point being the most adaptable significant risk factor. These principles are significant particularly during the scanning of sensitive structures such as the eye and foetus (Mayatte & Mohabir, 2014).

2.9.2 Role of ultrasound in evaluation of ankle injury

US imaging has become critical in evaluating foot and ankle injury (Latting & Spritzer, 2017). It has been reported that US has the ability to provide more detailed images of the structure of the ankle ligaments than x-ray and MRI (Hauser et al., 2013) and unlike an x-ray, US is considered a non-ionising radiation (Latting & Spritzer, 2017; Thomason & Cooke, 2012). In addition, Van Den Bekerom et al. (2013) pointed out that US and MRI plays a role in diagnosing injuries related to tendon and are considered as routine examinations for lateral ankle injuries in professional athletes. Others have agreed with Van Den Bekerom et al. (2013) that when injuries related to tendon are suspected, US and MRI are valuable diagnostic imaging modalities. Despite this, US and MRI are not routinely used for acute ankle ligament sprains (Lin et al., 2013). Thus, there is disagreement about using US and MRI in a routine examinations. The limitation of this support for US and MRI is that it provides no clear evidence or explanation of why they should or should not be used in routine examinations. Furthermore, Kerkhoffs et al. (2012) reported that understanding the use and the diagnostic performance of US and MRI needed further research.

A number of authors have shown that MRI has been used as a standard with high sensitivity in diagnosing ligament sprain or rupture (Hauser et al., 2013; Polzer et al., 2012; Slimmon & Brukner, 2010). Oae and colleagues (2010) conducted a study to clarify the efficacy of stress radiography, MRI, and US in detecting the ATFL injury compared to the arthroscopy as the gold standard. They reported that the accuracy of the stress x-ray, MRI, and US was 67, 91, and 97% respectively in diagnosing the ATFL injury. Hauser et al. (2013) reported that the accuracy and sensitivity of MRI could differ between ligaments themselves, which makes it impractical to depend only on MRI or to apply it as a gold standard.

Furthermore, Latting and Spritzer (2017) have stated that a high frequency ultrasound transducer offers resolution at least equal to MRI. Based on a systematic review that was conducted to evaluate MRI versus arthroscopy in the diagnosis of ligaments in the knee, it has been demonstrated that MRI does not have the ability to reveal the ligament when it is stretched or lax (Crawford et al., 2007). In other words, since MRI demonstrates only soft tissue contrast, there is no difference in appearance in terms of length between an uninjured ligament and one that has become stretched many times (Hauser et al., 2013).

In addition, US is considered a low cost modality with shorter examination time when compared to MRI (Ekinci, Polat, Günalp, Demirkan, & Koca, 2013; Latting & Spritzer, 2017). The cost of ultrasound compared to MRI, when examining the same body parts, is almost 80% less (Fessell & Jacobson, 2009). Moreover, it has been reported that in one institution, the total cost of professional and technical musculoskeletal ultrasound examination is 19% of that for MRI study (Grant, Kelikian, Jereb, & McCarthy, 2005). Ultrasound has another advantage over MRI it can be used to scan patients who are claustrophobic or obese or unable to undergo MRI due to metal artefacts such as cochlear implants and pacemakers (Fessell & Jacobson, 2009). Because of the high occurrence of ankle sprain, and the availability and cost of ultrasound, it is more favourable for diagnosing ankle sprain (Artul & Habib 2014; Polzer et al., 2012).

MSKUS can be used in the routine investigation of lateral ankle sprain for several reasons. The advantage of having a portable type of ultrasound machine has made it more likely to deliver a diagnostic service in public, in sports clubs or even in emergency departments (Patil & Dasgupta, 2012). According to the American College of Emergency Physicians in its "*Emergency Ultrasound Guidelines*" published in 2009, portable ultrasound has been considered for use in diagnosing acute life threatening conditions such as soft tissue, trauma and musculoskeletal injuries for emergency medical practitioners. Furthermore, because ultrasound is a real time dynamic examination, it allows a quick contralateral comparison in a brief period of time, which provides additional history (Fessell & Jacobson, 2009) and can answer several musculoskeletal issues (Patil & Dasgupta, 2012). In other words, US allows the physician to associate the images with the patient's clinical condition giving a rapid interpretation and making an immediate decision possible (Ekinci et al., 2013). It has also been reported that patients feel more comfortable with ultrasound assessment since it can be done by the same doctor who evaluates the patient before the scan (Ekinci et al., 2013). In other words, ultrasound is considered the only imaging modality that allows direct human contact between patients and healthcare professionals and allows patients to talk easily with a physician about the location of the area of greatest tenderness (Rockett, 1999). For MRI, meanwhile, studies rely completely on single position of the foot and ankle for every scanning plane. A vital part of the ligament or tendon could be poorly visualised if the foot is not placed in the ideal position (Rockett, 1999). It seems that all of these advantages give ultrasound the opportunity to be used routinely to exam lateral ankle sprains. In addition, a recent study conducted by Sanjay et al. (2018) assessed the use of ultrasound in delineating the grade of sprain. They concluded that ultrasound was effectively able to differentiate between grade I and grade II and provided a clue for further management. The authors recommended routine use of ultrasound for every ankle sprain. A recent systemic review was done to investigate the accuracy of imaging for diagnosing chronic lateral ankle ligament injury (Cao et al., 2018). The study found that ultrasound manifested high diagnostic accuracy in diagnosing chronic lateral ankle ligament injury. However, US is considered as an operator-dependent imaging modality (Patil & Dasgupta, 2012).

In addition, Hua and colleagues (2012) assessed 83 patients with chronic ATFL injury with ultrasound and used the ankle arthroscopy as a standard reference (Hua, Yang, Chen, & Cai, 2012). They concluded that the accuracy and sensitivity of ultrasound in identifying the ATFL injury were 95.2% and 97.7% respectively (Hua et al., 2012). The specificity, positive predictive value, and negative predictive value were 92.3%, 93.5%, and 97.3% respectively. Based on these results, the authors concluded that US imaging is an accurate tool for assessing chronic ATFL injury (Hua et al., 2012). Numerous authors have also studied the role of US and MRI for evaluating tears of the foot and ankle tendons. When compared with surgery findings, ultrasound had an accuracy of 92% in discriminating between full and partial thickness tears of Achilles tendon (Hartgerink, Fessell, Jacobson, & Van Holsbeeck, 2001). For peroneal tendons, US had 90% accuracy, 100% sensitivity, and 85% specificity (Grant et al., 2005). The researchers concluded that dynamic ultrasound is efficient for detecting peroneal tendon tears and must be considered a first line diagnostic method (Grant et al., 2005). When comparing MRI with surgical findings, MRI also demonstrated a high sensitivity in detecting tears of the Achilles and posterior tibialis tendons (Kuwada, 2008). However, the sensitivity reduces in determining small tears in peroneal tendons (Kuwada, 2008; O'Neill, Aman, & Guyton, 2010). In other words, Lamm et al. (2004) found that MRI had 83% sensitivity and 75% specificity in determining the tears of the peroneal brevis when compared to intraoperative findings. Cao et al. (2018) reported that clinicians should be aware that MRI is limited in detecting chronic CFL injuries. Accurate diagnosis of chronic lateral ankle ligament injury is important and critical for surgical intervention of CAI.

Even though US has several advantages over other imaging modalities, it is not able to image an area behind the cortex of bone (Ihnatsenka & Boezaart, 2010). It has been stated, that US is considered as an operator-dependent imaging modality with poor repeatability (Patil & Dasgupta, 2012). Latting and Spritzer (2017) reported that many physicians who are not formally trained are, in fact, utilising ultrasound as an essential evaluation in their clinics. Moreover, the experience of the investigator plays a significant role in musculoskeletal ultrasound studies (Pinzon & Moore, 2009; Polzer et al., 2012; Van den Bekerom et al., 2013). Several studies have tested the inter-rater reliability of experts in musculoskeletal ultrasonography, rating its reliability as moderate to high (Naredo et al., 2006; Scheel et al., 2005). Moderate to high reliability of inter-rater was also demonstrated between experienced radiologist and rheumatologist with limited ultrasound training (Szkudlarek et al., 2003). Moreover, Backhaus et al. (2010) also conducted a study to assess inter-rater agreement between three sonographers (senior 10 years, junior 10 months, and beginner one month). They concluded that there was substantial agreement between the junior and the senior sonographers. Agreement between the two levels was fair at the outset, but had substantially improved within two months. Therefore, they suggested that a relatively short period of training has the ability to allow sonographers to provide adequate US imaging (Backhaus et al., 2010). Gun et al. (2013) reported that after a six hour training program, emergency physicians could utilise bedside ultrasonography to assess patients with suspected ATFL injury. They found that sensitivity and specificity of bedside ultrasonography, when compared to MRI, were 93.8 % and 100% respectively. Thus, the major criticisms of US, that it is a difficult imaging modality to learn and that it is considered to be the most operator-dependent technique, might be somewhat lessened (Backhaus et al., 2010).

2.9.3 Ultrasound imaging of healthy ankle

It has been reported that each musculoskeletal structure has its own unique normal appearance (Ahmed & Nazarian, 2010); therefore, it is essential to recognise these appearances in order to simplify the detection of abnormalities (Ahmed & Nazarian, 2010). These unique appearances of ligaments, tendons, and muscles are described in depth in chapter four. Several studies of the unique sonographic appearance of musculoskeletal structures, including tendons, muscles, and ligaments have been published (Pillen, 2010) .

For example, muscle has a special sonographic appearance and can be easily distinguished from the structures around it such as blood vessels, nerves, bone, and subcutaneous fat (Pillen, 2010). Muscle has a speckled appearance in the transverse plane due to the reflections of the perimysium connective tissue, while the fascicular architecture appears in the longitudinal plane as a pinnate structure for peroneal muscles on the ultrasound image (Figure 2.23) (Pillen, 2010). Ahmed and Nazarian (2010) concluded that MSKUS is a valuable method for depicting normal ankle structures in the musculoskeletal system (Ahmed & Nazarian, 2010).

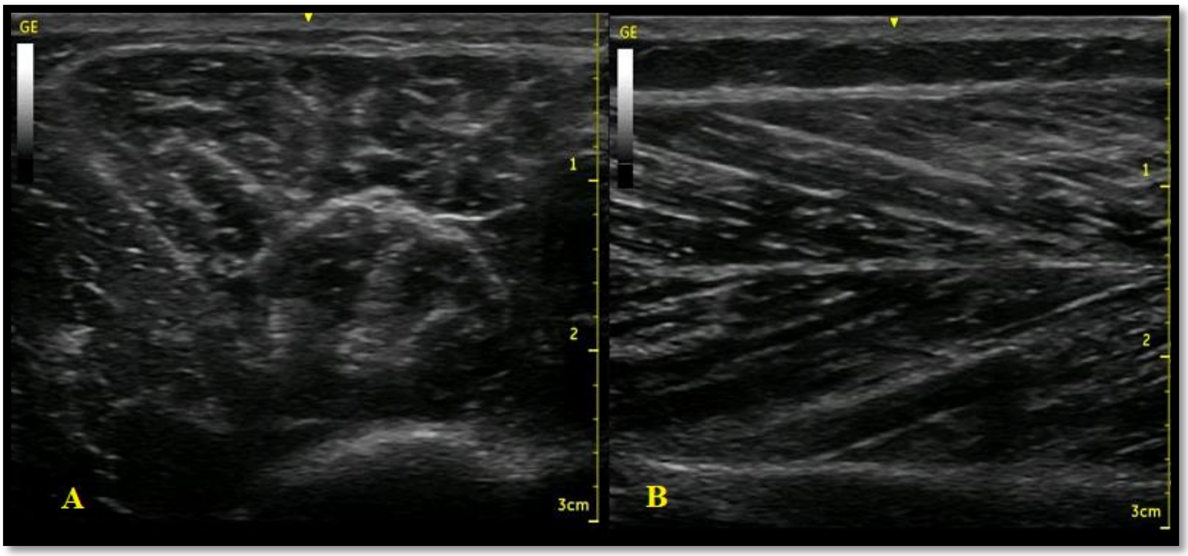


Figure 2-23: Normal appearance of peroneal muscles. (A) Cross sectional plane. (B) Longitudinal plane.

Furthermore, Precerutti et al. (2014) stated that ultrasound imaging of the ankle is a very common examination in the field of osteoarticular imaging, which needs a great knowledge of the normal anatomical appearance of ankle structures (Precerutti et al., 2014). In addition to this normal appearance, an effective sonographic protocol relies on a comprehensive knowledge of normal ankle anatomy, which was explained previously in section 2.4, and on the particular positioning of the transducer at different skeletal landmarks to obtain the best ultrasound image. It is important to determine first the standard reference values for musculoskeletal ultrasonography for ankle structures among healthy participants before scanning injured ankles for comparison in order to define abnormality. Therefore, the current study will provide normative ultrasonography data for selected ligaments, tendons, as well as selected muscle thickness and cross sectional area for right and left, male and female, normal and overweight, in order to give a better understanding and knowledge of healthy ankle structures.

2.9.4 Ultrasound imaging of injured ankle

Cao et al. (2018) conducted a systematic review on imaging diagnosis for chronic lateral ankle ligament injury and stated that ultrasound can precisely distinguish various ligament conditions such as lax, torn, or thickened ligaments. Yildizgoren et al. (2017) reported an increase in the thickness of ATFL reveals morphologic changes which happen secondary to injury of the ankle joint. Several studies have assessed the reliability of MSKUS (Black, Cook, Kiss, & Smith, 2004; Cheng, Tsai, Yu, & Huang, 2012; Crofts et al., 2014; Drolet, Martineau, Lacroix, & Roy, 2016; Iagnocco, Naredo, & Bijlsma, 2013), however, there is a lack of a reliability test for US in diagnosing selected ankle structures that related to lateral ankle sprains.

Numerous studies have described some of the US criteria that are used to evaluate ankle injury. Oae et al. (2010) reported that discontinuity of bundles and a hypoechoic (different shades of darkness) lesion within the ligament are the diagnostic criteria for ligament injury. On the other hand, Langer (2011) described that the sonographic characteristics of ankle sprains include an anechoic (completely dark) area following the superior border of the ligament or across the ligament, and thickening of the ligament. In addition, when assessed using ultrasound, the ATFL and peroneal tendons become thickened and hypoechoic in the case of injury (Bass & Marriott, 2009). El-Liethy and Kamal (2016) also reported that the ligament in a chronically injured ankle could be thickened or wavy with low fibrotic signal appearance. Based on the literature, it seems that the thickness and the echogenicity of the structure are important sonographic criteria to be evaluated. A MSKUS study on chronic groups found that thickness of the ligament increased by 16% in people with a previous sprained ankle, compared with healthy ATFL (Liu et al., 2015). Utilising ultrasound could assist in early identification of the affected ligaments, therefore, assisting in avoiding chronic instability (Yildizgoren et al., 2017). The capability of ultrasound to categorise the grade of injury reveals the severity of ankle damage, providing a more certain diagnosis (Radwan et al., 2016). With more accurate diagnosis, healthcare professionals are able to set more adequate goals and treatment plans, and can predict the prognosis for the patient more accurately (Radwan et al., 2016).

Ultrasound not only defines ATFL injury, it is also able to categorise the degree of ligament injury. The ultrasound classification of ATFL injury, based on Hua et al. (2012), is: "(i) ligament tear: a partial or total interruption of the ligament fibres at the fibular side, talar side or in the mid stance; (ii) lax ligament: the ligament remained curved when the ankle was in the maximum inversion and plantar flexion; (iii) thick ligament: the width of the ligament was > 24 mm or > 20% of the contralateral normal ligament".

2.10 Subjective and objective evaluation of ankle injury using ultrasound

It has been stated that variations in the size, contour, and echogenicity of ligaments or tendons after injury are the most significant criteria during ultrasound evaluation (Agut et al., 2009; Spinella et al., 2015). Echogenicity is determined by the brightness of the reflected echo intensity from the structure in the greyscale image (Kremkau, 2016). Varanoske et al. (2016) stated that echo intensity of skeletal muscle emulates the quality of the muscle. It has been shown to be sensitive to neurological and/or pathological disorders, and the level of intramuscular fat, fibrous and connective tissue (Pillen et al., 2009). There is some evidence to suggest a strong correlation between the interstitial fibrous tissue and echo intensity, and structural changes in muscle to determine the severity of muscle pathology (Pillen et al., 2009).

Changes in echogenicity can also be due to changes that occur during the healing process, such changes in the amount of collagen fibres, muscle fibres, nerves, and fat in the ligament or tendon (Agut et al., 2009). The initial phase of healing process, known as the acute inflammatory phase starts within minutes of an injury and lasts up to 72 hours. In this phase, the blood begins to collect where the injury occurred and the process of clot formation is initiated which increased the vascularity in the area (Buschmann & Burgisser, 2017). Therefore, the injured area will appear anechoic due to the vascularity.

A second phase known as proliferative starts when immune cells produces many growth factors and cytokines. This initiates fibroblast proliferation signals to rebuild the ligament tissue matrix (Hauser et al., 2013). The tissue in this phase appears as disorganised scar tissue having more fat cells, fibroblasts, blood vessels, and inflammatory cells than healthy normal tissue.

Over the next several weeks, the proliferative phase merges into the remodeling phase (third phase), in which collagen maturation starts, often lasting for months to years after the initial injury (Hauser et al., 2013). With time, the tissue matrix begins to resemble normal ligament tissue; though, variation in matrix structure persists such as collagen disorganised and type III collagen becomes more prevalent (Hauser et al., 2013). It seems possible that an anechoic to hypoechoic appearance of injured ligaments could occur due to the different time in healing process. However, previous studies just mentioned that the echogenicity became hypoechoic after injury without stating the time after injury or the stage of healing process. The three phases of healing process is demonstrated in Figure 2.24.

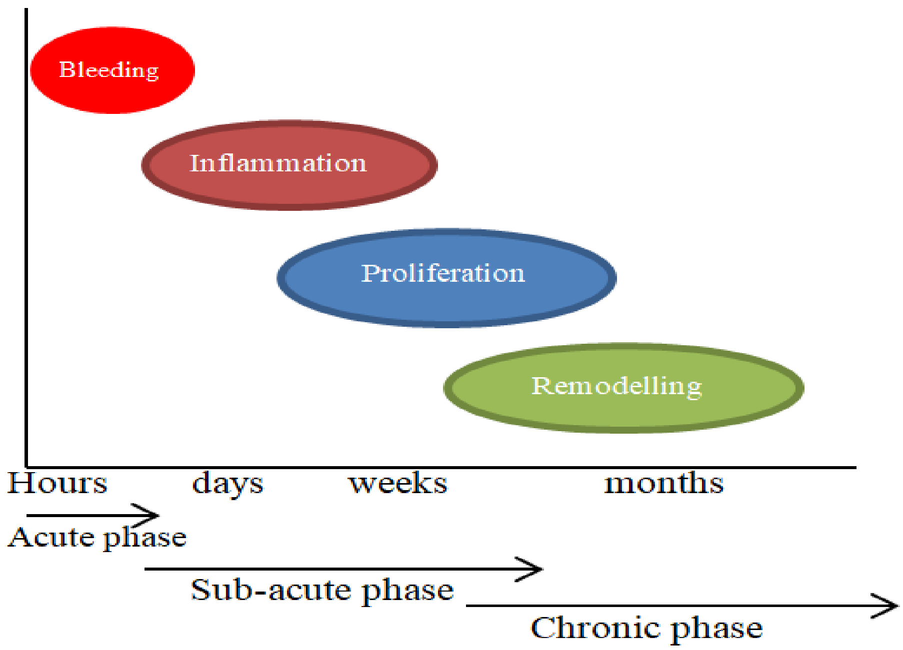


Figure 2-24: Three phases of healing process (inflammation, proliferation, and remodelling) during acute, sub-acute, and chronic phases

Structural elements in tissues create a reflection and scattering of sound waves which influence the acoustic impedance of the ultrasonographic image (Agut et al., 2009). The reflected sound waves (ultrasound echoes) can be transformed to greyscale (Das, 2016) and the intensity of the returning waves defines the echogenicity of the tissue being scanned. The echogenicity of ultrasound is generally described by four specific terms (Figure 2.25):

1. Echogenic (or hyperechoic), which involves an increased reflected echo when compared to adjacent tissue and is appears as white (bright echo) on US images.
2. Hypoechoic, which is a reduced reflection of the echo, appearing as different shades of darkness (weak echo) on US images.
3. Isoechoic, the echogenicity of the reflected echo is similar to the adjacent tissue.

4. Anechoic, also known as sonolucent, whereby no reflected echo occurs and this appears as completely dark areas on US images (Das, 2016; Goyal, 2018).

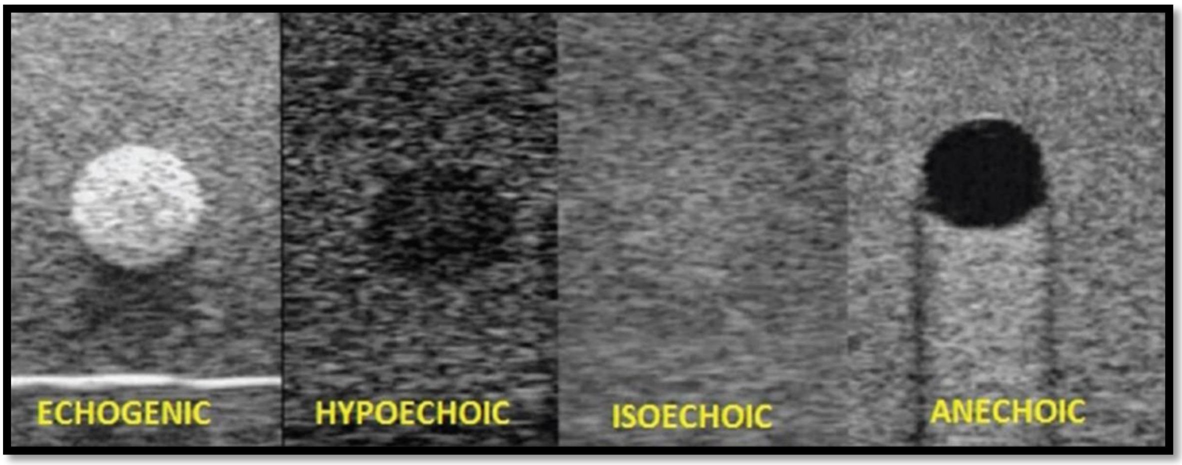


Figure 2-25: Glossary of US echogenicity terms (Das, 2016)

Assessment of echogenicity of a structure can be quantitative or qualitative (Spinella et al., 2015). Qualitative methods rely on an estimation of the intensity of ultrasound echoes and correspond to the image brightness (Spinella et al., 2015). Much of the ultrasound echogenicity measured in the literature is based on subjective evaluation as it is clearer, faster and simple to conduct. Several authors have described the normal echogenicity of tendon and ligament as hyperechoic, with ligament being less echogenic than tendon (Hodgson, O'Connor, & Grainger, 2012; Pinzon & Moore, 2009). Moreover, the echogenicity of injured ankle ligaments has been evaluated subjectively and described as hypoechoic (Langer, 2011; McNally, 2014; Oae et al., 2010). So, by subjective evaluation, both healthy and injured ligaments are hypoechoic compared to tendon and there is a lack of evidence that quantitatively compared healthy and injured ankles. Therefore, using qualitative criteria, there is a potential problem differentiating healthy and injured ligaments when compared to tendon.

Çekiç et al. (2017) was concerned that the term "hypoechogenicity" is qualitative, and it does not give any absolute quantitative information related to the degree of echogenicity. Furthermore, subjective evaluation depends on the sonographer's experience and thus it is not ideal for systematic study of ankle structures where many sonographers may be included. Therefore, there is a need to evaluate the structural integrity of the ligaments quantitatively and provide more objective evaluation of echogenicity, such that changes in structure can be better understood.

Many researchers hold the view that quantitative analysis of echo intensity can be performed through computer-aided greyscale analysis (Arts, Pillen, Schelhaas, Overeem, & Zwarts, 2010; Çekiç et al., 2017). This method is easily accessible, cheap, safe, more objective than visual evaluation, less dependent on the examiner's experience, and allows statistical analysis (Arts., 2010; Cadore et al., 2012).

Several authors have introduced quantitative methods including mean echogenicity to objectively evaluate musculoskeletal ultrasound images. Prior reports have focused on digital flexor ligaments and tendons of metacarpal area in horses, through analysis of greyscale areas within images (Agut et al., 2009; Spinella et al., 2015; Vergari et al., 2012). Cheng and colleagues (2012) studied the reliability of measuring the echogenicity of the human foot plantar fascia quantitatively by measuring the mean greyscale of their specific area of interest. They demonstrated that the reliability of greyscale assessments of echogenicity was high to very high (ICC ranged from 0.76 to 0.94). It was concluded that calculating the mean greyscale could reduce inconsistencies in the interpretation of echogenicity among different sonographers (Cheng et al., 2012). It has been reported recently that quantitative ultrasound techniques have the ability to provide quantitative evaluation of the structure and function of musculoskeletal tissues (Wang, Huang, Yeow, Pickering, & Saarakkala, 2017) and computer-aided image analysis software has been widely used for ultrasound images.

ImageJ is Java-based computer-aided image analysis public domain software developed by the National Institutes of Health (Bethesda, MD, USA). The effectiveness of using ImageJ has been shown with thyroid, breast, uterus, and muscles imaging (Arts et al., 2012; Çekiç et al., 2017; Chou et al., 2011; Grani et al., 2015; Watanabe et al., 2013). The quantification of the echo intesity in ImageJ has been made using grey-level histograms, which demonstrates the distribution of the pixels in the ultrasound image by plotting the number of pixels at each level of colour intensity (Figure 2.26). Santos and Armada-da-Silva (2017) found moderate to very high reliability of measuring the echo intensity of the quadriceps femoris muscle by using histogram analysis in ImageJ.

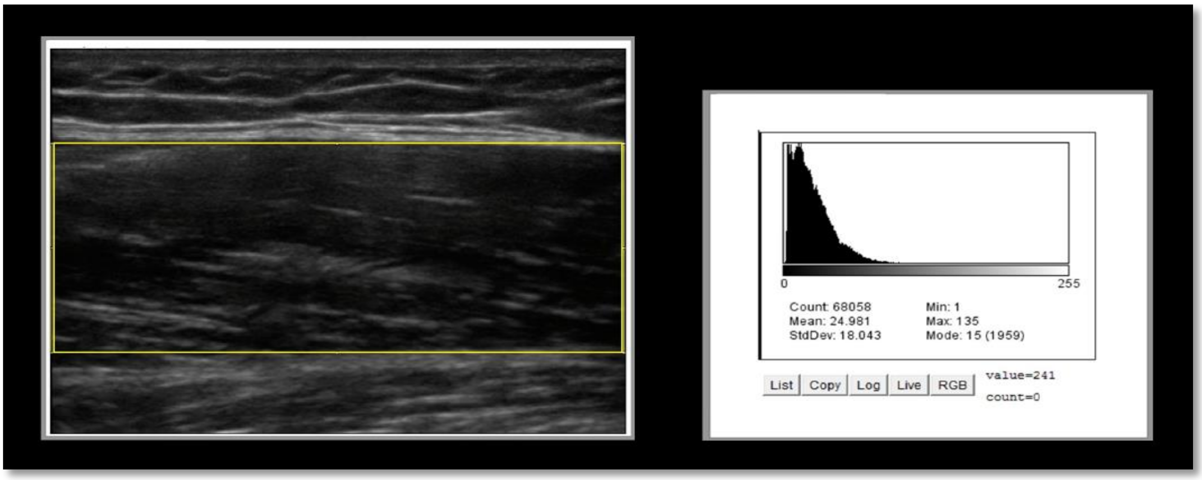


Figure 2-26: Image analysis region of interest selections and the corresponding greyscale histogram values. Yellow rectangular represented the region of interest of the longitudinal image of rectus femoris. The corresponding greyscale histogram showed the mean echo (Harris-Love, Seamon, Teixeira, & Ismail, 2016)

Hsu and colleagues found that grey-level histogram of ultrasound images is a promising method which was sensitive to differences in participants with tendinopathy. Moreover, Erol et al. (2013) reported that the histogram analysis provides quantitative information regarding the echogenicity of the breast tissue. Chou and colleagues (2011) showed that grey-level histograms were useful in evaluating the physiological condition of the endometrium (Chou et al., 2011).

Çekiç et al. (2017) studied echo intensity in patients with Hashimoto thyroiditis and concluded that greyscale histograms provide objective and quantitative information regarding the echogenicity of the thyroid parenchymal. Histogram analysis was also able to differentiate between normal and heterogeneous parenchyma. Moreover, Grani and colleagues (2015) used histogram analysis to evaluate the echogenicity of thyroid nodules, finding they were able to quantify the degree of hypoechogenicity (Grani et al., 2015). Erol et al. (2013) concluded that lesion echogenicity ratio measured by greyscale histogram can be utilised as an adjunct ultrasound parameter to discriminate between malignant and benign breast lesions.

A number of previous studies have quantified echo intensity to describe the quality of muscle (Arts et al., 2012; Cadore et al., 2012; Mangine et al., 2014; Watanabe et al., 2013). Arts et al. (2012) conducted an ultrasound study on nine different muscles in a patient with amyotrophic lateral sclerosis (ALS). The echogenicity was measured as the mean greyscale value of the muscle, expressed as a value between 0 (black) and 255 (white). They concluded that the echogenicity of all muscles (range 52-91) in ALS patients was almost twice the normal value (28-42). They compared their findings with the post-mortem histopathological examination of an older woman who had had ALS and found that fibrous tissue was the main contributing factor to increase the echogenicity of the muscle (Arts et al., 2012). Furthermore, Watanabe et al. (2013) conducted a study to investigate if the quality of the muscle, based on echogenicity, is related to muscle strength independently of muscle size in elderly men. Echogenicity of the rectus femoris muscle was measured by greyscale analysis, utilising the standard histogram function and a significant negative correlation was found between echogenicity and muscle strength (Watanabe et al., 2013).

However, the existing literature on echo intensity in musculoskeletal ultrasound focused particularly on plantar fascia and muscles. There has been little quantitative analysis of the specific echogenicity of the tendons and ligaments around the foot and ankle. To the author's best knowledge, no study has quantitatively evaluated the echo intensity of the ATFL in healthy, coper, and CAI participants. It is believed that a precise knowledge of tendon and ligament echogenicity reference values for specific structures may serve as a tool to distinguish between physiological and abnormal cases (Agut et al., 2009; Spinella et al., 2015).

Whilst the measurement of foot and ankle structures can characterise structural changes, it does not provide any insight into the functional consequences of any such changes. People with CAI present with impairment such as pain, loss of function, and restricted motion that affect the daily living activity in general population and athletic participation (Pope et al., 2011). It has been thought that CAI is related to ligamentous and soft tissue damage to the lateral ankle structures and is further confounded by changed muscle activation, limited range of motion, and balance deficits (Arnold, De La Motte, Linens, & Ross, 2009; Hertel, 2008; Hoch et al., 2016).

2.11 Ankle injury and postural control

Several researchers hold the view that individuals with a history of ankle sprains are at a high risk having another ankle sprains or CAI (Engebretsen et al., 2009; Trojian & McKeag, 2006; Naja et al., 2017; Steffen et al., 2008), with 47% of recurrent ankle sprain occurring in the same ankle that had been already sprained (Trojian & McKeag, 2006). Many factors are thought to contribute to the reoccurrence of an ankle sprain such as: limited mobility of the ankle joint, weakness of the ankle muscles, and damage to the proprioceptors in the ankle ligaments (Akbari, Karimi, Farahini, & Faghihzadeh, 2006). Proprioceptors exist through ligaments, tendons, and muscles and provide sensory information for the control of balance (Chae, Kim, & Lee, 2017). Balance is the ability to maintain the body's centre of mass within the base of support (Greenfield, 2014) and postural sway is the movement of the centre of mass when standing, and reflects the control of balance (Cho, Lee, Lee, Lee, & Lee, 2014).

A systematic review found that postural control is impaired following an acute lateral ankle sprain (McKeon & Hertel, 2008b), specially for those with CAI (Arnold, De La Motte, Linens, & Ross, 2009; Gribble, Hertel, & Denegar, 2007; Hiller, Kilbreath, & Refshauge, 2011; Mettler et al., 2015). Deficiencies in postural control are thought to arise because of a combination of diminished proprioception (the cumulative neural input to the CNS from mechanoreceptors in the joint capsules, ligaments, tendons, muscles, and skin) and neuromuscular control (the efferent response that is generated as the consequence of the proprioceptive afferent input) (Hertel, 2002; Voight & Cook, 2014).

Deficits in the pattern of lower extremity muscle activation have been assume to be contributing factor to impairments in postural stability and perceived function in people with CAI (Webster, Pietrosimone, & Gribble, 2016). Delahunt et al. (2006), for example, found that CAI participants had less anticipatory activation of peroneus longus during a unipedal drop jump compared to control participants. They suggested that this alteration in motor control could contribute to the risk of further inversion injuries in cases of CAI. Previous studies comparing neuromuscular control between coper and CAI participants found that copers demonstrated increased activation of peroneus muscle during jump landing (Gutierrez et al., 2012), and tibialis anterior during pre- and post- touchdown phases of gait (Dundas, Gutierrez, & Pozzi, 2014). The researchers concluded that copers may have developed these adaptive strategies as a protective mechanism to prevent further injury. However, more researches need to support this conclusion. This again points to cases of CAI having a neuromuscular control basis to the risk of further injury.

Damage in any part of afferent or efferent controls could create postural control deficiency. Proprioception is defined in the literature as one's ability to integrate the sensory signals from many mechanoreceptors to thereby set the body's movement as well as its position, in space (Goble, 2010; Han, Waddington, Adams, Anson, & Liu, 2016). It has been stated that ankle proprioception is probably one of the most significant contributor to control of posture, particularly in sport, since the ankle-foot complex is the only segment of the body touching the ground in most of sports activities (Han, Anson, Waddington, Adams, & Liu, 2015). Furthermore, ankle proprioception and control give vital information and stability that allows modification of the upper body in order to effectively achieve the complex motor tasks needed in many sports (Han et al., 2015). A systematic review conducted on balance ability and the performance of athletes found that balancing abilities of ice hockey players demonstrates a significant association with extreme skating speed (Hrysomallis, 2011). Furthermore, a study in male athletes from various sports involving jumping and landing (basketball, handball, volleyball and soccer) found balancing ability was significantly associated with the athletes' agility (Sekulic, Spasic, Mirkov, Cavar, & Sattler, 2013). This evidence supports a hypothesis that controlling balance is essential for the performance of sport activities (Han et al., 2015) and thus the role of the ankle in postural control, and how ankle injury impacts this role, is pertinent to understanding risk of lateral ankle sprains.

2.11.1 Strategies of postural control

The strategy of postural control can be either compensatory (reactive) or anticipatory (predictive) or a combination of both (Pollock, Durward, & Rowe, 2000). An anticipatory postural control strategy could include a voluntary movement, or increase in the activity of the muscle in anticipation of a predicted disturbance (Pollock et al., 2000). A movement or muscular response after unpredicted/unexpected disturbance is known as compensatory postural control strategy. These responses may involve a "fixed-support", in which the base of support remains unchanged but the location of the centre of mass is moved, or "change-in-support" in which the base of support is altered so that the centre of mass remains within it (Pollock et al., 2000).

Postural control is based on continues integration of sensory information from visual, vestibular, and somatosensory receptors (mechanoreceptor and proprioceptors). The CNS gathers and processes this sensory information from the receptors in the joints, tendons, ligaments, and muscles to gauge the motion and the position of limbs (Mills, 2018). Modig (2013) reported that postural control as a constantly ongoing process which cycles through corrective feedforward (predictive) and feedback (reactive) regulative movement control systems (Figure 2.30). For instance, when the body sway from upright position, it generates a passive torque around the ankle joint because of the acceleration of the body due to gravity.

This information is transmitted to the brain from the joint and muscles in the form of sensory feedback, thereafter a corrective, active torque is applied to maintain balance (Cnyrim et al., 2009). Disturbance of any of these sensory inputs due to injury create a change in the control of balance, reflected experimentally in postural sway (Clark, Treleaven, & Ulrik, 2015; Serra-AÑó, López-Bueno, García-Massó, Pellicer-Chenoll, & González, 2015).

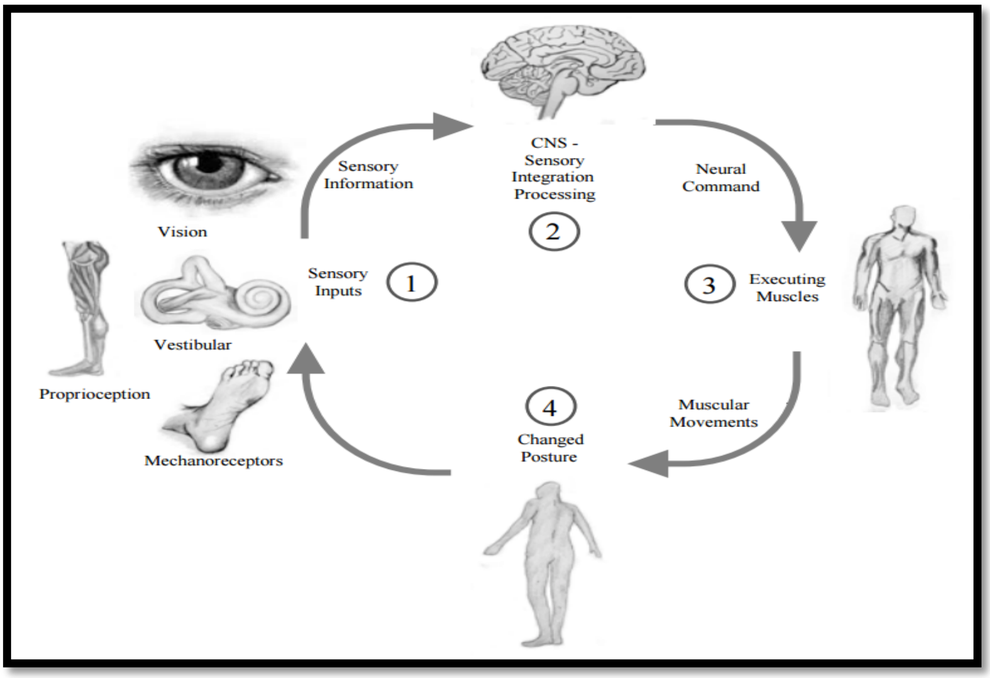


Figure 2-27: Human postural control

In case of single-leg balance, the participants foot's supinate and pronates to maintain the body centre of mass above the base of support which defined as 'ankle strategy' of postural control (Hertel, 2002). CAI participants on the other hand have been shown to rely more on 'hip strategy' to maintain single limb balance than healthy participants, this strategy assumed to be less efficient than ankle strategy (Hertel, 2002; Pintsaar, Brynhildsen, & Tropp, 1996).

2.11.2 Measuring postural stability

Postural control can be categorised into three states: static with the goal of maintaining position with an insignificant level of movement; semi-dynamic with the goal of maintaining position by moving the base of support; or dynamic with the goal of maintaining a fixed base of support by performing a given movement (Hosseinimehr, Daneshmandi, & Norasteh, 2010; Pionnier et al., 2016).

Evaluation of static postural control can be measured with instrumented tool such as force plate or reliable and valid clinical scale such as Balance Error Scoring System (Gribble, Hertel, & Plisky, 2012). Even though measuring the postural control through static test provides valuable clinical information, the original task of maintain standing may not translate certainly to movement tasks that occur in physical activity (Gribble et al., 2012). On the other hand, dynamic types could include performing an action such as jumping or hopping to a different place then instantly trying to stay as still as possible, or trying to perform decided segment movements (reaching) without compromising the established base of support (Gribble et al., 2012). Dynamic single-leg jump-landing test and the SEBT are alternative evaluation methods that might challenge balance more than single-leg static balance test (Arnold et al., 2009). Even though performing these dynamic actions to evaluate postural stability do not precisely replicate participation in daily activity and sport, they more closely imitate the demands of physical activity than evaluating postural stability in static way (Gribble et al., 2012). A meta-analysis study demonstrated that all of dynamic measuring outcomes are able to exhibit the balance deficits in CAI individuals (Arnold et al., 2009) while de Vries and colleages (2010) found that there was no statistical significant difference in the measurements of static balances between healthy, participants shortly after acute ankle sprain and participants with CAI. Dynamic balance tests could provide an overall evaluation of joint stability, strength, sensorimotor function, which could assist clinicians detect balance deficits that would not be identify with static tests. Time to stabilisation is an another dynamic task that evaluates how long the participant takes to return the ground reaction force to a stable range. It measured during landing from a single-leg jump (Brown & Mynark, 2007; Ross, Guskiewicz, Yu, Carolina, & Hill, 2005; Dallinga et al., 2016). Time to stabilisation test needs force plates and specific processing software to produce the measures. This makes time to stabilisation less convenient to use clinically than SEBT (Arnold et al., 2009).

Dynamic evaluation such as SEBT may be preferable and able to provide a more precise evaluation of the function of lower extremities in people with CAI than static evaluation which involves only quiet standing, the SEBT needs greater demand from an individual and therefore more sensitive to picking up differences in balance compared to the static (Razeghi, Rahnama, & Shokri, 2016). Two systematic reviews have revealed that stability differences could not be detected through static balance tasks because they may not be sensitive enough to detect minor variances in postural control (McKeon & Hertel, 2008b; Wikstrom, Naik, Lodha, & Cauraugh, 2010).

2.11.2.1 Star excursion balance test (SEBT)

SEBT has been known as the extent to which the participant can reach without lifting their feet and staying stable. SEBT was first used by Gray as a clinical treatment method (Gray, 1995), since then it has been applied as an objective measurements for dynamic postural control, and utilised as a diagnostic task to determine risk of injury, and to discriminate pathological conditions (Anderson, 2016). The performance of the SEBT provides a quantitative evaluation, with the reach distance in centimetres is the main outcome measure for clinical and research setting. This outcome is normalised to leg length to facilitate the comparison between people (Gribble & Hertel, 2003). The quantitative evaluation of the reach distance by either absolute value (cm) or normalisation (%) has many valuable purposes as it produces normative data, which will permit for ranking for statistical means, determines risks of injury, and monitors consequence of interventions (Anderson, 2016; Gribble, Hertle, & Plisky, 2012). Moreover, it has been thought that SEBT is an excellent representor for functional activity among other postural control tests options since it integrates a combination of flexibility, strength, and neuromuscular control while challenging the limits of postural stability (Hoch et al., 2016).

Several authors have proposed that by performing SEBT, participants challenge their limits of stability while trying to reach as far as they can, thereby revealing to some degree of their dynamic postural stability (Dallinga et al., 2016; Gribble et al., 2012; Hoch et al., 2016). First, performing SEBT requires greater strength than quiet standing tasks. A recent study found that isometric hip strength in participants with CAI significantly influenced the performance of dynamic balance, with abduction and external rotation explained almost 25% of the SEBT score during the posterolateral and posteromedial directions respectively (McCann et al., 2017). Moreover, in order to achieve the goal of SEBT, closed kinetic motion at the ankle, knee, and hip should effectively controlled by the musculature of the lower limb (Olmsted, Carcia, Hertel, & Shultz, 2002). On the other hand, keeping a single-leg stance while standing-up on a steady platform puts a quite a small strength demand on the lower extremity musculature (Olmsted et al., 2002). Second, performing SEBT requires a greater range of motion than static tasks do. Keeping one leg stable while reaching a specific point with the other leg requires the stable leg to be capable of adequate motion of the ankle, knee, and hip (Olmsted et al., 2002). Anderson (2016) used SEBT to measure the functional joint mobility and found that ankle dorsiflexion of healthy participants was correlated with normalised reach distance in the SEBT. Moreover, Kang et al. (2015) hypothesised that kinematics of the lower extremity would be strong predictors for the reach distance of the SEBT (anterior, posteromedial, and posterolateral). They found that ankle dorsiflexion was the best predictor of normalised reach in the anterior direction.

The SEBT is a simple, low-cost, reliable (ICC was 0.95 and 0.98 for intra-rater and inter-rater respectively) alternative to sophisticated instruments that measure the functional performance of a lower limb (Hyong & Kim , 2014; Khuman et al., 2014). SEBT is designed to challenge postural stability during various leg reaching tasks and has recently gained increased attention in research and clinical areas (Doherty et al., 2015a). It is theorised that SEBT may predict lower extremity injuries. Plisky, Rauh, Kaminski, and Underwood (2006) demonstrated that the SEBT was able to predict a 2.5 times greater chance sustaining a lower extremity injury if the difference between right and left anterior reach distances greater than 4 cm. De Noronha et al. (2013) reported that participants with SEBT posterolateral under 80% were at 48% greater risk of having ankle sprain. One possible explanation the only posterolateral direction may predict the incidence of ankle sprains is rely on the variation of muscle activation patterns that my impose for each direction.

The reliability of SEBT in assessing dynamic balance was first established by Kinzey and Armstrong in 1998. Furthermore, four studies have since shown the high reliability of the SEBT in measuring dynamic balance capability in participants with and without chronic ankle instability (Hertel, Miller, & Denegar, 2000; Hoch, Staton, & McKeon, 2010; Hyong & Kim, 2014; Munro & Herrington, 2010). Hertel et al. (2000) evaluated the intra-rater and inter-rater reliability of SEBT in 16 healthy athletes. The ICC of the intra-rater was up to 0.96 on both days and the ICC of inter-rater was up to 0.84 on day 1 and up to 0.93 on day 2. Hyong and Kim (2014) also evaluated the inter-rater and intra-rater reliability of SEBT in 67 healthy participants. The ICC of inter-rater was up to 0.95 and the ICC of intra-rater reliability was up to 0.98. They concluded that SEBT is a highly reliable test that can be used to measure dynamic balance (Hyong & Kim, 2014). In addition, Olmsted et al. (2002) argued that SEBT is the first non-instrumented functional test that has been demonstrated to be greatly sensitive and reliable in determining the deficits between healthy participants and CAI participants. The test-retest reliability of modified SEBT (known as Y-balance test) was determined by Hoch et al. in 2010.The researchers concluded that the test has good reliability to evaluate the dynamic postural control in a CAI group, and the anterior direction has the highest reliability followed by posteromedial and posterolateral (ICC = 0.92, 0.86, 0.74 respectively).

Plisky et al. (2006) assessed the between-session reliability of the SEBT on basketball players and found ICC values ranging from 0.84 to 0.87. However, they used a Y-balance test containing only anterior, posterolateral and posteromedial directions. Therefore, Munro and Herrington (2010) assessed the between-session reliability of the eight directions of the SEBT. The ICC ranged from 0.84 to 0.94 and the authors concluded that SEBT is a reliable measure in healthy recreational athletes to determine their lower limb function (Munro & Herrington, 2010). There is a lack of reliability of the eight directions of the SEBT in people with CAI since most of the reliability studies that have been done previously were on healthy participants.

In addition to the positive reporting regarding the reliability of SEBT discussed above, it has also been reported that SEBT is effective in detecting the dynamic type of postural control in both healthy and CAI participants (Khuman et al., 2014). The process of performing the test is easy to understand and easy to apply, with minimal equipment required (Khuman et al., 2014). Furthermore, the body of evidence reveals that SEBT is a functional clinical test that is widely used in ankle disorders such as acute lateral ankle sprain (Doherty et al., 2015b).

However, the SEBT can often be time-consuming and take approximately 45 minutes to complete (Munro & Herrington, 2010). It is recommended that subjects complete six trials in each direction (eight directions in total) and then taking another three recorded trials (Hertel et al., 2000). Hertel et al. (2000) recommended applying six practice trials due to a significant learning effect across trials one to six, with the most consistent scores and longest reach distances from trials seven onwards. Significant learning effects were seen between trials seven-nine and one-six ($p<0.05$). Moreover, Hertel et al. (2000) protocol allowed participants to use their arms for balance, which is not in line with the most common SEBT protocol which is that hands should remain on hips. These two factors could increase the learning time for the tasks (Munro & Herrington, 2010). Khuman et al. (2014) followed Hertel’s recommendation and applied six practice trials in all eight directions. Even though six practice trials were recommended, several authors considered the problems associated with Hertel’s protocol and reduced the number of trials to five (Akbari et al., 2006). Robinson and Gribble (2008b) conducted a study of the longest normalised reach distances to define the precise numbers of trials required for stabilising scores. The results of the study demonstrated that the lateral direction was the only direction which required more than four practice trials before constancy was achieved on the fifth trial. The researchers established that only four practice trials are required before the baseline measuring reaches distances suitable for research or clinical purposes. Hoch and colleagues (2016) also asked their subjects to perform four practice trials and then three recorded trials, taking the average of the three recorded ones for analysis. Similarly, Pionnier et al. (2016) asked their participants to get familiar with the test by performing four practice trials and then one recorded trial. However, Payne, Mccabe, and Pulliam (2016) reduced the trials in performing SEBT to three trials, and averaged these for data analysis. Their study had the ability to identify the differences in postural control between healthy and CAI participants. Therefore, it appears that applying SEBT can be simplified by using fewer practice trials (Munro & Herrington, 2010).

In addition to reduce the number of trials to minimise the time required to do the SEBT, researchers built the Y-balance test (YBT) which based on previous research suggesting redundancy in the eight directions of the SEBT in order to reduce the time required to do the SEBT and to decrease the development of fatigue. Pilsky et al. (2006) applied Y or “peace sign” including anterior, posteromedial, and posterolateral directions as a predictor of lower extremity injury in high school basketball players, which in turn led to the development of the YBT.

It has been demonstrated in the literature that individuals with acute ankle sprain and CAI achieved shorter reach distance on the SEBT than individuals without sprain (Brown, Bowser, & Orellana, 2010; Doherty et al., 2015a; Gribble et al., 2007; Jaber et al., 2018; Hertel, Braham, Hale, & Olmsted-Kramer, 2006; Pionnier et al., 2016; Plante & Wikstrom, 2013). Olmsted et al. (2002) was the first to conducted a study on 20 healthy participants and 20 with unilateral CAI, and found that participants with CAI revealed significantly shorter reach distances from healthy when standing on the injured limb (78.6 cm versus 82.8 cm). In their analysis, the eight reach distances from all SEBT directions were averaged together. The lateral direction had the shortest reach distances in comparison to the other seven directions, followed by the anterolateral direction, which was shorter than the other directions except the lateral. Furthermore, no joint kinematics were tested, leaving the explanation of the result difficult. In addition, Olmsted et al. (2002) did not normalise the reach distances to the subjects’ leg lengths with average leg length of CAI participants was 2 cm lower than healthy participants; instead, they matched the height and leg length for both healthy and CAI groups as closely as possible. A study by Gribble & Hertel (2003) which recruited 30 recreationally active subjects was designed to test the role of leg length and height on reach distances while performing the SEBT. The findings were that height and leg length were strongly correlated with reach distances, with leg length displaying a stronger correlation than height (Gribble & Hertel, 2003). Therefore, the authors recommended that the result for reach distance should be normalised to leg length (Khuman et al., 2014; Payne et al., 2016).

Akbari et al. (2006) examined the differences in reach distance in 30 athletes with unilateral acute lateral ankle sprains. Even though their results showed a significant difference in reach distance in injured limb and uninjured limb (84.97±10.26 cm and 86.80±9.34 cm respectively), they fail to specify which reach directions they applied. In addition, authors did not compare the result with healthy participants as they assumed that the uninjured limbs are healthy. However, Doherty et al. (2015a) found decrease normalised reach distance on the injured and noninjured limbs of people with 6 months after acute lateral ankle sprain during the performance of the YBT. In addition, Hiller et al. (2008) reported that previous ankle sprain could predispose both ankles for sustaining an ankle sprain, therefore, it seems important to compare the injured ankle of CAI participants with a healthy ankle in participants who never expose to ankle sprain.

Applying normalised reach distances, Gribble and colleagues (2004) found a decreases reach distance in CAI on their injured side in the three directions of the SEBT (anterior, medial, and posterior). Khuman et al. (2014) also used SEBT to compare dynamic postural control between 30 healthy and 30 CAI participants. The results of their study agreed with Olmsted's results, as they found that the reach distance of CAI participants was significantly lower than that of the control participants, and lateral and anterolateral directions had the shortest reach distances. In addition, the injured limb in CAI participants has the lower reach distance compared to the uninjured limb ($p<0.01$), and no significant differences were reported between dominant and non-dominant injured limbs ($p>0.05$) (Khuman et al., 2014). Recently, Pionnier et al. (2016) applied a new approach to SEBT which aimed to evaluate dynamic postural control in 17 healthy and 17 participants with CAI. They used an optoelectronic cameras system as a more accurate method to assess the reach distances. In addition to measure the lower limb range of motion (ROM), they computed new relevant variables, such as the absolute time required to reach each distance. The main outcome was a significant difference in reach distances between the two groups; that participants complaining of CAI reached 80% of leg length while the healthy participants reached 85% of leg length. However, the authors reported the average of the reach distance for all 8 directions (Pionnier et al., 2016). Even though the authors used a different approach to evaluate postural control, the results of the three studies were consistent. Pionnier et al. (2016) also found that a shorter time was needed to perform the test in CAI participants even there was a lack of statistically significant difference between CAI and healthy participants. Furthermore, the authors demonstrated a significant decrease in the ROM of lower limbs joint (ankle, knee, and hip) and the velocity of the centre of pressure ($p<0.05$) in the CAI participants compared to healthy participants. These decreased quantitative performance highlighted a deficit in dynamic postural control.

In contrast to earlier findings, Hertel et al. (2006) evaluated the SEBT in 48 adults with CAI and 39 controls and suggested that the reach distances in the posteromedial, medial, and anteromedial directions were significantly lower in CAI compared to the controls, and that the posteromedial direction was the highly associated with the performance of the eight directions in the CAI group. In addition, they recommended that these three directions may be clinically utilised to evaluate the functional deficits associated with CAI. However, the clinical implications of these three specific medial reach directions are not clear and needs additional study.

Following the recommendation of Herterl et al. (2006), Sefton et al. (2009) used the three medial directions of the SEBT as a measurement tool to understand the role of sensorimotor impairment in participant with chronic ankle instability. Their result showed no statistically significant differences in the three reach distances between control and CAI participants. It seems possible that this result is due to the variety of activity level between the groups. CAI participants spend more years on sport competition than healthy participants. Many of CAI participants had a high level of activity which indicates these participants are promoting coping mechanisms to deal with their disability (Sefton et al., 2009). Conducting a study on very homogeneous people might be more possible to create significant differences between healthy and chronically unstable ankles. In addition, De La Motte et al. (2015) found no differences between CAI and healthy participants. Researcher only examined the three medial directions. It seems that the three medial directions could not be the most sensitive to CAI related achievement deficits.

Several authors used YBT which contains only three reach directions (anterior, posteromedial, and posterolateral) instead of the eight directions of the SEBT in their studies.

Payne et al. (2016) studied the effect of CAI on the Y-balance test among soccer players and found that the differences in reach direction between healthy and CAI participants was only demonstrated in the anterior direction. The study compared only 15 injured subjects to 59 healthy ones; the sample sizes were therefore not equal, which may have affected the results of the study. Plante and Wikstrom (2013) found a significant decreased normalised reach distance in only posteromedial direction in CAI participants compared to coper and healthy, no differences were found between coper and healthy participants. A recent study demonstrated a poorer dynamic balance performance mostly during the anterior direction of YBT between the three groups (healthy, coper, and CAI) with CAI group had the lowest reach distance during the anterior direction and no significant differences was observed between copers and healthy groups (Jaber et al., 2018).

A possible explanation of the conflicting results between the studies regarding the affected reach direction is that each reach direction could activate the stance lower extremity muscles to different extent. Earl and Hertel (2001) measured the EMG (electromyography) activity of different muscles (gastrocnemius, anterior tibialis, biceps femoris, medial hamstring, vastus lateralis, and vastus medialis oblique) during the performance of all eight directions of the SEBT. The authors found that all muscle activity is reach dependant except the gastrocnemius. Feger and cholleagues (2014) found a significantly less anterior tibialis muscle activity duting the anterior and posteromedial reach directions in the CAI participants. Jaber et al. (2018) found less anterior tibias and gluteus maximus activation in CAI participants compared to coper and healthy participants during the anterior and posterolateral direction of the YBT, which could demonstrate a relationship between altered neuromuscular control at the hip and ankle and the performance of the YBT. Posterolateral is challenging reaching direction, as participants need to maintain a level pelvis on the stance leg. As participants reach backward across the stance leg, they shift their trunk anteriorly to keep the centre of mass within the base of support. Trunk flexion creates hip flexion moment that is controlled by the contraction of hip extensors. Therefore, increased gluteus maximus activation could be required in this case to counteract the sagittal plane flexion of the hip and trunk. It seems that CAI participants did not fire the gluteus maximus as it needs to counteract this motion, resulting in overcompensation to maintain the body's centre of mass within the base of support, which could lead to create a higher sway in the posterolateral direction (Jaber et al., 2018).

Furthermore, Razeghi et al. (2016) examined the ability of the YBT to discriminate between female athletes with and without CAI. Their findings contradicted the previous findings, as no statistically significant differences were found in any of the reach distances between the CAI and control groups. They concluded that the YBT may not be sensitive enough to determine balance deficiency between athletes with and without CAI (Razeghi et al., 2016). However, the study was conducted on female athletes and from inhomogeneous population from various types of sport such as: basketball, volleyball, and handball, which may affect the finding.

There is a lack of consistency in inclusion and exclusion criteria, and methods within the literature therefore making it difficult to compare studies. Subjects exist possibly along a continuum of instability and may display more or less disability than participants in other studies. This is evidenced by the difference in self-reported number of sprains and scores on functional questionnaires. Each study used different types of questionnaires to evaluate the level of ankle dysfunction such as CAIT, FADI, and AJFAT. Moreover, there are various level of function with different activities and differences in the number of episodes of giving way (Brown et al., 2010). These differences are likely to produce different definitions of groups.

A study conducted by Coughlan and colleagues (2012) debated that different postural control strategies are employed during the traditional YBT compared to the commercial YBT. In other words, variations in selected reach directions and the way the test was performed may also play a role in the differences among the findings. This notion is supported by Coughlan et al. (2012) and Fullam, Caulfield, Coughlan & Delahunt (2014), who found differences in the anterior reach distance between the SEBT and YBT well as angular displacement in sagittal plane of the hip joint at the point of maximum reach. Coughlan et al. (2012) measured the SEBT reach directions by attaching three measuring tape to the floor, one placed anteriorly toward the apex and the remaining two placed at 135º to the one line in the posteromedial and posterolateral directions. The Y-balance test was measured by commercially available device (Y Balance Test, Move2Perform, Evansville, IN, USA). The differences could be that each test presents different mechanism of feedback and feedforward control. During the SEBT, participants place downward pressure through the reach foot at the end of the reach excursion only, thus, do not receive same level of afferent information throughout the movement, possibility depend on feedforward control technique till the toe touched the measuring tape. While participants in Y-balance test, need to stand in an elevated position on a central footplate and pushing a sliding block. Therefore, participants receive constant proprioceptive feedback through the reach excursion from the plantar surface of the reach foot (Coughlan et al., 2012). Thus, researcher should be careful when interpreting the reach distance from both tests specially the anterior direction.

It seems important to exam the eight directions of the SEBT not only YBT because of the different findings in reaching directions which have been found between using the eight directions of the SEBT and YBT. In addition, Plisky et al. (2006) did not mention as to why they selected the posterolateral as one of the three, and not any other reach directions. Furthermore, several studies have found significant difference in reach distance in CAI compared to healthy in directions other than YBT (Hertel et al., 2006; Khuman et al., 2014; Pionnier et al., 2016).

Authors suggested future research of measuring dynamic postural stability should include kinematics to assist define mechanisms which could give an idea about the strategy of the movement and the original of deficiency which cause the poor performance in the SEBT in particular individuals (Olmsted et al., 2002). Recognising those mechanisms can assist in improving rehabilitation programs.

2.11.2.2 Ankle kinematics

Although the reliability and validity of the SEBT have been recognised previously, this test is remarkable in that a hypothetically several types of strategies may be utilised to achieve the movement goal and produce a similar result (Gribble et al., 2013). It has been believed that biomechanical assessment of dynamic balance in individuals with lateral ankle sprain while performing the SEBT may provide insight into the strategies and mechanisms by which individual progress to fully recover or develop another sprain (Hoch et al., 2016). This aspect of the SEBT has sparked many researchers with the aim of testing the kinematics that being a part to an efficacious achievement of this task (Hoch et al., 2016).

Biomechanical measurement of lower limb kinematics during the SEBT has been limited mostly to sagittal plane. Robinson and Gribble (2008a) tested the lower limb kinematics of 20 healthy participants while performing the SEBT by using an electromagnetic tracking system. They found that hip and knee flexion account for 62-95% of the difference in the reach distance. However, they did not test the dorsiflexion of the ankle joint, which has been shown to be the greatest sagittal plane contributor to maximal anterior reach distance in healthy participants (Fullam, 2014). During maintain balance in a close kinetic chain, the ankle goes into dorsiflexion when the proximal part of tibia moves forward, forcing the knee into flexion (Hoch, Staton, & McKeon, 2011). This could overestimate the results presented by Robinson and Gribble (2008a) about the strong prediction of the hip and knee flexion on the reach distance, by not including ankle motion in the equation. In addition, Hoch and colleagues (2011) found a significant correlation between the dorsiflexion ROM of the ankle joint (measured during a WBLT; weight-bearing lung test) and reach distance of YBT only on anterior direction among healthy participants. They proposed that dorsiflexion ROM accounted for approximately 28% of the alteration in anterior direction (Hoch et al., 2011) and concluded that WBLT elucidated a significant percentage of the alteration in the anterior reach distance. Thus, the anterior reach distance of the SEBT can be a good clinical test to evaluate the impacts of dorsiflexion limitations on dynamic balance and sagittal plane kinematics of lower limb seems to be the most influence to the performance of SEBT. However, the previous mentioned studies conducted on healthy participants only, and most of them measured only three out of eight directions of the SEBT, therefore it is unclear the contribution of ankle kinematics on other directions of the SEBT.

Wikstrom and Hubbard (2010) found that talar position was significantly more anterior in injured CAI participants compared to healthy participants which could explained the restrictions in posterior talar glide in participants with previous ankle sprain. Changed joint arthrokinematics could contribute to observed decrease in ankle dorsiflexion ROM (Wikstrom & Hubbard, 2010).

The degree of ankle dorsiflexion plays a role in the risk of lower extremity injuries (Backman & Danielson, 2011; Drewes, McKeon, Casey Kerrigan, & Hertel, 2009). The limitation of ankle dorsiflexion ROM after lateral ankle sprain has been considered a predisposing factor for ankle sprain reoccurrence because decreased dorsiflexion prevents the ankle from reaching its closed-pack position by holding the ankle in a hyper-supinated position (Terada, Harkey, Pietrosimone, & Gribble, 2014). A systematic review by De Noronha et al. (2006) supported the relationship between dorsiflexion and ankle sprain. There are several techniques have been used in the research and clinical settings to measure the dorsiflexion range of motion associated to ankle injury such as goniometers and inclinometers. However, these approaches depends on the technique which used by the examiner during the test (Almansour, 2015). A WBLT has also been used to measure the dorsiflexion and demonstrated high reliability with an ICC of 0.99 (Konor, Morton, Eckerson, & Grindstaff, 2012). Doherty et al. (2015a) measured the ankle dorsiflexion ROM between participants six months after their acute lateral ankle sprain and healthy participants by using the WBLT and found no differences between the two groups. However, they found significant decrease of ankle dorsiflexion in the acute lateral ankle sprain groups while the performance reach directions of YBT by using 2D video analysis. Since the WBLT demonstrated a similar ankle dorsiflexion between the two groups and the YBT demonstrated differences in ankle dorsiflexion between the groups, the authors speculated that the compensatory movement of the motor control in other planes of motion could be used more in healthy participants, causing the increase in reach distance (Doherty et al., 2015a). Pourkazemi et al. (2016) conducted a cross-sectional study to determine balance impairments between healthy participants with no history of ankle sprain and participants with acute ankle sprain. They found that the anterior reach distance was the only direction with a significant difference in reach distance between the two groups. They concluded that participants with acute ankle sprain also demonstrated decreased dorsiflexion ROM, which was moderately associated with achieving the anterior direction in the YBT (Pourkazemi et al., 2016). Both studies measured only the sagittal plane in only three directions of the SEBT and were conducted on acute phase of ankle sprain.

Several authors have reported decrease ankle dorsiflexion in people with CAI (Basnett et al., 2013; Delahunt et al., 2006; Drewes et al., 2009; Grindstaff, Dolan, & Morton, 2017; Vicenzino, Branjerdporn, Teys, & Jordan, 2006). Researchers measured the dorsiflexion ROM through WBLT and the maximum reach distances through YBT in healthy and CAI participants (Hoch, Staton, McKeon, Mattacola, & McKeon, 2012). They found a decreased dorsiflexion range of motion along with shorter distances in balance test in CAI participants. Basnett et al. (2013) conducted a study to find the relationship between the ankle dorsiflexion ROM measured in a weight-bearing position and dynamic balance utilising the SEBT in CAI participants. The authors found a significant positive relationship between ankle dorsiflexion ROM and reach distance in the SEBT. Ankle dorsiflexion ROM had the greatest relationship with the anterior reach direction (r =0.55) and interpreted 31% of the difference in reach distance. This demonstrates that mechanical impairments in ankle motion can affect dynamic function during a balance task. Clinically, this shade the lights the significance of adequate ROM for dynamic tests in people with ankle pathology (Basnett et al., 2013). In addition, it seems that WBLT was able to identify the decrease in ankle dorsiflexion in CAI people, however, the test may not be sensitive to detect the ankle dorsiflexion in people in acute stage of ankle injury.

Two previous studies have been done by Gribble et al. in 2004 and 2007 studied the effect of CAI and fatigue on the performance of the SEBT. Gribble et al. (2004) firstly quantified 2D kinematics (sagittal plane joint angles) of the ankle, knee, and hip of the stance leg at the maximum reach distance during a SEBT (anterior, medial, and posterior). CAI participants demonstrated a less degree of knee and hip flexion than healthy participants (Gribble et al., 2004). These decreases in the joint motions were hypothesised to contribute to the reduction of dynamic balance observed (CAI individuals demonstrated reduced reach distances compared to healthy individuals). Regression analyses were conducted to evaluate the impact of kinematic differences between CAI and healthy individuals could have had on the several reach distances when performing the SEBT (Gribble et al., 2004). The hip and knee flexion angles corresponded to approximately 49% of the differences in reach distance in the anterior direction of SEBT. Therefore, the authors stated that the positioning of the knee and hip of the stance limb have a significant part in shorter reach distance and consequently resulting in reduced dynamic balance in CAI participants. In 2007, Gribble et al. evaluated sagittal kinematics of the ankle, knee, and hip joints and reach distance difference in CAI and healthy participants. Findings from their stepwise regression analysis demonstrated that a decrease of sagittal motion in the hip and knee after a fatigue protocol clarified the decrease in the reach distance during the anterior, medial, and posterior reach directions of the SEBT. The joint angles were measured by using a digital video camera (Panasonic Electronics) at the maximum reach distance, and then calculated by SMART video analysis system. Even though video analysis method has been used previously in many studies, laboratory based 3D motion capture systems are considered the "gold standard" to analyse body motion and evaluate biomechanical factors (Fullam et al. 2014).

3D motion analysis systems have commonly been used in previous studies in order to quantify lower limb kinetics and kinematics (De Ridder, Willems, Vanrenterghem, Robinson, & Roosen, 2015; Delahunt et al., 2013; Fullam et al. 2014; Hewett, Myer, Ford, Heidt , & Colosimo, 2005; Milner, Westlake, & Tate, 2011). This type of system permits the researcher to quantitatively measure three motion planes while performing dynamic tasks. Reflective markers attached to specific anatomical area in the human body create a skeletal model which allows the researcher to track human movements, record and measure the biomechanical features during functional tests.

De La Motte et al. (2015) investigated the joint angle of lower extremity at the point of maximum reach distance in medial directions (medial, anteromedial, and posteromedial) of the SEBT using 3D optical motion-capture system. The authors did not find change in the kinematic of ankle and knee, instead that CAI participants rely more on trunk rotation, pelvic rotation, and hip flexion at the point of maximal reach distance even though no significant differences of reach distance were found between healthy and CAI participants in any medial directions. The author revealed that CAI participants may use alternative movement strategies that could be depend more on non-sagittal plane motion to achieve the movement goal (De la Motte et al., 2015). One possible justification why CAI participants could adopt transverse or frontal plane strategies is due to the limitation of dorsiflexion range of motion at ankle joint which is frequently recognised in people with CAI (Hoch et al., 2012) as discussed previously. One weakness of this study is that authors only examined the medial directions of the SEBT which could not be the utmost sensitive to CAI related achievement deficits. In addition, their findings obtained from recreationally active groups, which could not be apply to another active or nonactive groups.

In agreement with De La Motte's study (2015), Doherty et al. (2016) studied sagittal plane lower extremity kinematics during a YBT in CAI participants. They found no differences in dorsiflexion in anterior direction between CAI and healthy participants, despite CAI participants presenting with shorter reach distances. Thus, sagittal kinematic data does not fully explain the poorer anterior reach distances by CAI participants. It has been stated that progressive ranges of sagittal plane lower extremity motion are most probably never develop without some degree of frontal and/or transverse plane motion (Doherty et al., 2016). Therefore, the required movement for any specific reach distance is most likely predicted by movement of flexion-dorsiflexion of the whole lower extremity (Robinson & Gribble, 2008a), and a combination of some degree of frontal and transverse motion (Davids, Glazier, Araújo, & Bartlett, 2003). CAI participants could have eventually poorer achievement compared to healthy, and this could be elucidated by transverse and frontal plane motion patterns not obtained in Doherty's study.

Hoch et al. (2016) investigated the relationship between the kinematics (sagittal, frontal, and transverse), maximal reach distance only in anterior direction, and weight-bearing dorsiflexion in 15 CAI participants. The authors found that maximum reach distance was significantly related to weight bearing dorsiflexion. A combination of frontal plane motion of trunk, hip, and ankle was also predictors of maximal anterior reach distance on SEBT. However, the study was done on the anterior direction only. In addition, participant was wearing shoes during the performance of the test. One possible limitation to wearing shoes is that difficulty to detect heel lift within the shoe which could overestimate the result of maximum reach distance.

There are limited studies examining kinematics during the performance of the eight directions of the SEBT in coper and CAI individuals. Pozzi et al. (2015) compared the lower leg kinetic and kinematic during the performance of the SEBT in healthy, coper, and CAI participants. They found no significant differences in reach distance or kinetics and kinematics between the groups. However, the study was done on the posteromedial direction only and further kinematic studies are needed to include coper and perform the SEBT during the eight directions.

Possible explanations for the differences in the results of previous studies may be associated to the number of average measurements taken for the each reach distances. Furthermore, baseline group mean differences among healthy and CAI participants prior to fatigue protocol in Gribble's study were not documented, therefore, a straight comparisons between the findings of the studies are difficult to draw. Furthermore, the differences in methodology to capture and process the kinematic data may also play a role. De La Motte et al (2015) calculated the joint kinematics data by utilizing a 3D optical motion-capture system with 35 retroreflective markers and Plug-in Gait software. While Gribble et al. (2007) utilized digital video camera and the markerless SMART system. The congruence among these two systems has not been assessed and could affected the measurements of the angles (De La Motte et al., 2015).

Without the kinematics and kinetic analyses, no one would know the motion of the ankle joint. The quantified data can serve as a development base from which to explore the motion of the ankle joint in injured ankles (coper and CAI), thus making it easier for the researcher to compare between coper and CAI ankles which improves our understanding of ankle motion in coper and CAI ankles.

2.12 Rationale for the study

a) Ankle injuries are an important problem

Musculoskeletal injures are on the rise, with foot and ankles injuries accounting for 8% of health care consultations (Lobo et al., 2016). Furthermore, ankle sprains constitute about 85% of all ankle injuries (Jain, 2014). A lateral ankle sprain is a type of injury that is prevalent in both the general and physically active populations (Fong et al., 2007; Hiller et al., 2011; Gribble et al., 2016b). LAS has a high rate of recurrence which can often lead to an individual developing chronic ankle instability. This chronicity contributes to continue deficits of sensorimotor and constrained functioning (Gribble et al., 2016a) which could have a decrease effect on the health-related quality of life, the level of physical activity, and absence from training or competition for athletes, thus create a substantial global healthcare burden (Gribble et al., 2016a). Moreover, CAI is highly associated with post-traumatic ankle osteoarthritis, creating a larger than assumed financial, with billions spent yearly on first treatment and follow-up care (Gribble et al., 2016b). With such negative consequences and related financial burden associated with LAS and CAI enhanced effort to understand structure and function differences between those that develop CAI and those that do not following an initial acute LAS is needed.

b) Ankle injuries are common and often reoccur and we would like to understand why

Hoch et al. (2016) stated that almost one in every three people with acute ankle sprain develop CAI. Suboptimal diagnosis of the specific ligament injury could lead to inadequate treatment and increased risk of long-term problems (recurrent sprains, swelling and foot/ankle pain) (Lam & Lui, 2015). In order to provide an adequate treatment for a lateral ankle sprains, a high quality diagnosis is therefore required. However, this is not possible unless the characteristics of healthy and injured lateral ankle soft tissue structures can be characterised and these characteristics associated with impaired ankle function.

As mentioned previously, 32-74% of people with a previous history of LAS had persistent symptoms (Gribble et al., 2016b). It is unknown whether these persistent complaints are related to structural changes in selected ankle structures occurred due to the trauma. Identification of structural changes that likely related to persistent complaints might assist in prognosis and treatment for people with previous ankle sprain. Almost all patients complaint of pain in foot or ankle in the emergency department will refer to radiographic tests. Despite the extensive utilise of Ottawa Ankle Rules, less than 15% of these patients had a fracture (Baezegari, Amedfar, Moezzi, Kohandel, & Rafiei, 2017). In addition, radiography is not appropriate for evaluation of soft tissue and ligaments (Van Ochten et al., 2014). These can be evaluated more directly and accurately using ultrasound.

Ultrasound is a cost effective and reliable method that could be used to characterise the soft tissues of the ankle. Ultrasound provides a dynamic greyscale image that can show a range of soft tissue features that could provide valuable data to help better understand the nature and extent of structural injury that occurs in LAS. Whilst MRI offers non-ionising high contrast imaging of soft tissue, it has limited availability and is expensive. Several studies have evaluated the reliability of ultrasound of foot tissues and shown a high reliability when measuring the thickness of plantar fascia (Cheng et al., 2012; Rathleff, Molgaard, & Olesen, 2011), and selected foot structures that are relevant to foot and ankle function (Crofts et al., 2014).

A comprehensive characterisation of what is normal and thereafter abnormal ankle structures and function will help develop future diagnosis tools. Measuring structure and function across a population that varies with gender and body mass index will improve understanding normal and abnormal ankles structures and function. In addition, it has been suggested that the number of previous ankle injuries can affect the integrity of the ankle structures and function, which leads to muscle imbalance and reduced the proprioception (McManus et al., 2006). Several studies have demonstrated that there is an association between history of previous ankle sprains and the high risk of future sprains (Engebretsen et al., 2009; Hiller et al., 2007; Kofotolis et al., 2006; Tyler et al., 2006). However, despite a history of ankle sprain, some people known as copers could return to their pre-injury condition without any residual symptoms of CAI such as giving away and/or recurrent ankle sprains. The reason of developing CAI in some people but not in others has not been fully understood. Therefore, an investigation of whether and how healthy and injured ankles differ in different groups (coper and CAI) and how any structural differences related to impaired ankle function is needed.

There are few studies in the literatures concerning about characterisation of the ligaments tissue quantitatively using the computer-aided greyscale analysis. The knowledge of reference values for ligament echogenicity and their changes in people with previous ankle sprain could function, in the researcher’s opinion, as a tool to distinguish the normal and abnormal tissue conditions. Therefore, in addition to measuring the thickness and the length of selected ankle structures, quantitative analysis of echo intensity needs to be measured to provide further data to overcome the limitation of subjective and qualitative definitions which is commonly used in ultrasound field. Echo intensity value can be used as objective quantitative measure in differentiation of ankle with and without ankle sprains and especially between coper and CAI ankles.

The ankle sprain must be diagnosed based on accurate evidence which is more economical and for understanding of grade of sprain. The goal of achieving uniformity of diagnosis is to prevent recurrences and chronicity. Knowledge about the use of ultrasound examination and the diagnostic performance is hampered by lack of research. Gribble et al. (2016a) also recommended that there is a need to use structural examination for people with LAS with imaging which could simplify precise diagnosis and accurate treatment.

c) The functional consequences of injuries to ankle structures is not fully understood

Gribble et al. (2016b) stated that there could be many functional and sensorimotor deficits that persist in the months after ankle sprain. Shaffer et al. (2013) proposed a hypothesis that individuals with a history of ankle sprains and chronic ankle instability have deficit in their postural control (Mettler et al., 2015; Shaffer et al., 2013;). Using only imaging will not be enough to understand the effect of the ankle sprains on selected ankle structures. Whilst the measurement of foot and ankle structures can characterise structural changes, it does not provide any insight into the functional consequences of any changes. To achieve this, functional deficits of postural control involving ankle structures will be compared between different groups of people who have different ankle structures (i.e. due to injury). Functional deficits will be measured using SEBT which is a simple, reliable alternative to sophisticated instruments that measure functional performance of lower limb (Khuman et al., 2014). SEBT is designed to challenge postural stability during various leg reaching tasks and it has gained more attention in research and clinical areas (Doherty et al., 2015a; Gribble & Hertel, 2003). Four studies have shown the high reliability of SEBT in measuring dynamic balance capability in healthy people and in people with chronic ankle instability (Hertel, Miller, & Denegar, 2000; Hoch et al, 2010; Hyong & Kim, 2014; Munro & Herrington, 2010). In addition, it has been reported that SEBT is effective in determining the dynamic postural control in both healthy and CAI participants (Khuman et al., 2014). By measuring the 3D kinematics of ankle joint in addition to SEBT, the study will have a great functional understanding of ankle sprain by studying the response of the postural-control system to a specific postural disturbance combined with lateral ankle injury.

While most of previous studies have applied healthy groups to compare with CAI groups, a few recent studies have applied copers to compare with CAI groups. Comparing multiplanar different movement strategies between CAI and copers group will deliver signs as to which joint position and muscle activation strategies may contribute to CAI (Son, Kim, Seeley, & Hopkins, 2017). Enhancing the in-depth knowledge of the structural characterisation of the ankle joint and functional biomechanics assists greatly in determining the treatments and rehabilitation options. In addition, Gribble et al. (2016a) reported that to minimise the high incidence of LAS recurrence and CAI, clinicians and researchers must promote appropriate follow-up with standard rehabilitation which identify the arthokinematic and sensorimotor deficits while permitting for best tissue restoration simultaneously. Authors recommended that research is needed to determine the optimal treatments and rehabilitations protocols that address the serious deficits that expose people to develop CAI (Gribble et al., 2016). This could not be happened unless we understand the functional consequences of lateral ankle sprain.

To the researchers' knowledge, this is the first research that study the structural characterisation and functional consequences of selected ankle structures in healthy, coper and CAI participants. Understanding the relationships between the integrity of the ankle structures post sprain, and functional ability of the ankle is important to inform our understanding of the long-term effects of sprains and consider better targeting of interventions. Therefore, the overarching aim of the work in this thesis is to:

(1) Investigate differences between healthy and injured ankles (coper and CAI) in terms of soft tissues associated with ankle function.
(2) Investigate the differences in controlling the balance between healthy and injured ankles (coper and CAI) and find any relationship between ankle structures and function.

To achieve (1) research will be undertaken to quantify a range of relevant foot and ankle soft tissues in healthy and injured ankles. This will be done in various cohorts to allow the effects of gender, body mass index, and number of injuries to be investigated. This study will provide a better understanding of whether (and how) factors such as, gender, body mass index, and prior ankle sprain affect ankle structures.

To achieve (2), balance will be measured using SEBT with kinematics and compared between those with and without ankle injuries. The nature of these injuries and changes in ankle structures will be informed by the result of (1).

2.13 Aim of the PhD

The aims of this thesis are to:

1. Characterise selected ankle structures in people with and without a history of ankle sprain. The five specific questions are:
   - What are the specific structural ankle characteristics in normal healthy, coper, and chronic ankle instability participants (chapter 3)?
   - Do specific structural ankle characteristics differ between participant with one injury (coper) and with multiple lateral ankle sprains (chronic ankle instability) (chapter 3)?
   - Do specific structural ankle characteristic differ between neutral and tension position during ultrasound scanning (chapter 3)?
   - Do specific structural ankle characteristics differ between males and females (chapter 3)?
   - Do specific structural ankle characteristics differ between people who are normal weight and those overweight participants (chapter 3)?
2. Provide a quantitative analysis of echogenicity of the anterior talofibular ligament (ATFL). The two specific questions are:
   - What is the quantitative method to objectively evaluate the structural integrity of ATFL (chapter 4)?
   - What are the differences in echo intensity between healthy, coper and chronic ankle instability participants (chapter 4)?
3. Investigate the dynamic balance in people with and without a history of ankle sprain. The four specific questions are:
   - How does the dynamic balance differ in terms of the reach distance and the ankle motion during the SEBT between healthy, coper and CAI participants (chapter 5)?
   - How does the dynamic balance in terms of the reach distance and the ankle motion during the SEBT differ between participant with one injury and with multiple injuries (chapter 5)?

- How does the strategy of the reach distances of SEBT differ in the sagittal, frontal, and transverse planes of the ankle between healthy, coper, and chronic ankle instability (chapter 5)?
- How does the structural changes at the ankle relate to functional changes (chapter 5)?

3 Chapter three: Ultrasound characteristic of selected ankle structures in healthy, coper and chronic ankle instability

3.1 Chapter overview

Lateral ankle sprains are one of the most common musculoskeletal injuries encountered both in clinical practice and in the sporting community (Fong et al., 2007; Meehan et al., 2017). Radiography is part of the initial diagnostic test in many cases of apparent ankle sprain to rule out the fracture. However, ligaments are soft tissue and do not clearly show up on radiographs. This can lead to missed ligament tears, false diagnoses of ankle sprains (Hauser et al., 2013) and poor matching of structural damage to treatment strategies. Ultrasound has the ability to provide more detailed images of ankle ligament structures and structural damage (Hauser et al., 2013). It therefore offers a diagnostic advantage over traditional radiographic techniques.

Results of systematic review revealed that ultrasound is effective, reliable and accurate in the diagnosis of CAI (Radwan et al., 2016). An understanding of ligament morphology has become even more important to provide an accurate interpretation of the injury mechanism and therefore an accurate diagnosis of ankle sprains. Furthermore, since the ankle ligaments are part of a complex interaction between related muscle, tendon and fascia tissues, all acting to control foot and ankle joint motion, it is also possible that damage to an ATFL or CFL could lead to changes in other soft tissue structures. It is therefore important to understand whether the selected soft tissue ankle structures can be measured reliably, whether and how they differ between injured and non-injured ankles, and the extent of any normal variation in ankle structures, such as between left and right, male and female, and normal weight and overweight groups.

This chapter describes the first study in this thesis. To achieve the aims, a set or research objectives have been formulated. The chapter starts with a pilot study which conducted in order to evaluate the feasibility of the method and to make any changes that might need before start the main study. A reliability study also conducted to test the reliability of the researcher on ultrasound scanning. The next section of the chapter focuses on the details of the method which includes: the study design, ethical considerations, recruitment strategies, the inclusion and exclusion criteria, and the ultrasound techniques for scanning selected ankle structures. A discussion builds on the finding of this study and the chapter concludes with information on the comparison of the ultrasound measurements of selected ankle structures between healthy, coper (people who had a history of one LAS at least 12 months before conducting the study, returned to all pre-injury levels of activities for at least 12 months, and have not got CAI yet) and CAI participants. An overview of this chapter is demonstrated in Figure 3.1.

The difference in the selected ankle structures between healthy, coper and CAI participants has been published in Journal of Ultrasound in Medicine (Abdeen, Comfort, Starbuck, & Nester, 2018).

3.2 Aims, objectives, and hypothesis of the study

The primary aim of this study is to characterise and compare selected ankle structures in healthy, coper and CAI groups by using ultrasound. The outcome of the study will determine if there are differences in length, thickness, and CSA of selected ankle structures among the three different groups. The secondary aims are to investigate whether there are differences in measurements of selected ankle structures when scanning in neutral (90 degree) or tension position. And if there are differences among healthy participants in terms of symmetry, gender and BMI, and whether there are differences in selected ankle structures between participants with different numbers of past ankle ligaments injuries. To achieve the aims of the study, the following objectives were established:

- Evaluate the length, thickness and CSA of selected ankle structures in healthy, coper and CAI participants.
- Evaluate the differences in selected ankle structures between the three groups.
- Evaluate the differences in selected ankle structures between neutral and tension position during ultrasound scanning.
- Evaluate the differences in selected ankle structures between right and left limbs among the healthy participants.
- Evaluate the differences in selected ankle structures between males and females among the healthy participants.
- Evaluate the differences in selected ankle structures between normal weight and overweight participants among the healthy participants.

Hypothesis: There will be statistically significant differences in the length and thickness of selected lateral ankle ligaments between healthy, coper, and CAI participants.

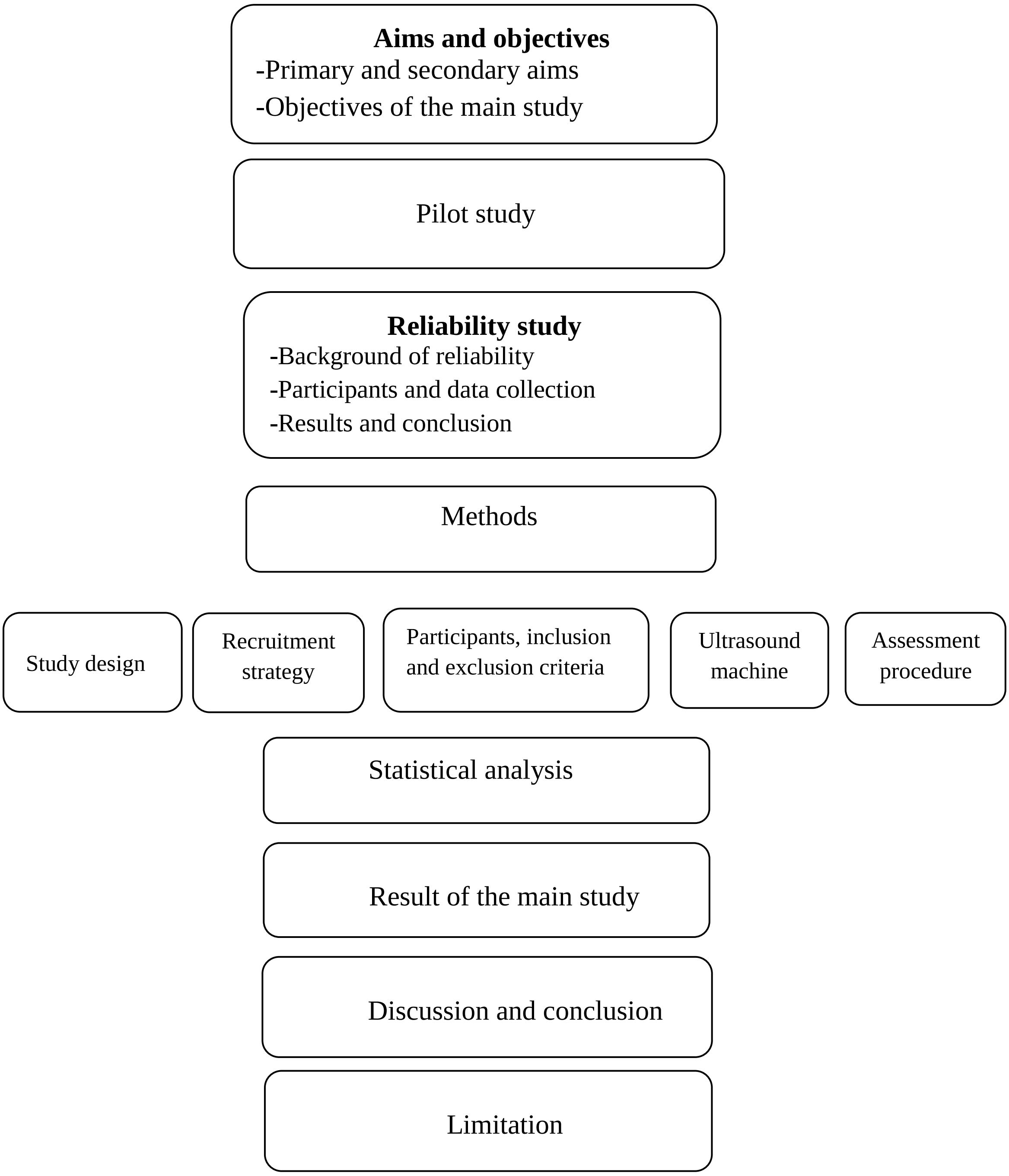


Figure 3-1: Flowchart demonstrating the structural of this study

3.3 Pilot study

Pilot studies are trial runs or mini-versions of a full-scale study (Doody & Doody, 2015). There is agreement in the literature that pilot studies are essential for several reasons. They are important in developing and testing the adequacy of research instruments, designing the protocol for research, evaluating if the protocol developed is realistic and workable, ensuring that the researcher fully understands the protocol and is consistent in the process of data collection, developing the research plan, estimating variability in the results to assess the determination of sample size, and collecting preliminary data (Gardner, Gardner, MacLellan, & Osbornea, 2003; Teijlingen & Hundley, 2001). For example, in this study the pilot work was required to find the optimal position for ultrasound scanning and the appropriate places for measurements on ultrasound images. Pilot studies are usually conducted on small groups of participants who are similar to those to be recruited for the main study. Finally, it is important to conduct a pilot study to identify any problems that could occur in applying the proposed methods (Teijlingen & Hundley, 2001), thus allowing the problem to be modified or changed before starting to conduct the main study (Doody & Doody, 2015).

The researcher wanted to investigate the most appropriate position for each selected ankle structure to clearly display the ligaments, tendons, muscles and their adjacent tissues on ultrasound image, as well as to make the scanning easier for the patients. Different position of the foot could affect the measurement of the structures. For instance, previous study found that the length of the ATFL has increased from neutral to inversion position (Croy, Saliba, Saliba, Anderson, & Hertel, 2012). Thus, it is important to study the measurements of selected ankle structures under tension position and to also find if other selected ankle structures will affected with tension position. According to Bautista (2016), a small sample size (about 10) could be appropriate for pilot testing. Therefore, a pilot study was conducted on 10 (5 healthy and 5 injured) participants who were university students and had the inclusion and exclusion criteria which will be described later in section 3.5.5; the study was conducted in the ultrasound room at the Allerton Building, in the Department of Radiology at University of Salford. The participants mean age of 32.00 ± 3.59 years, mean height 1.64 ± 0.09 m, mean weight 62.20 ± 11.83 kg, and mean BMI 22.92 ± 2.40 kg/m^2.

A portable ultrasound machine with high frequency transducer was used to test the study protocol. This started with measuring the selected ankle structures when the ankle was in a 90 degrees position while the participants were supine, and then placing each structure in tension position. To achieve tension in CFL and Achilles tendon, a wedge (7 cm at the toe end and 5 cm wide across/under the foot seen in Figure 3.2) was made and inserted on the plantar aspect of the foot to dorsiflex the foot 15° (details of the protocol and the procedure of the study are described later in section 3.5.8). Several measurements were made on ultrasound images for each structure to find the most appropriate place for measurement. Examples of the peroneal tendon measurements on ultrasound images are demonstrated in Appendix 1. The reliability of the proposed ultrasound protocol is discussed in detail in the next section 3.4.

Figure 3-2: designed wedge to dorsiflexed the foot to 15°

Shapiro-Wilk test showed a normal distribution of the data and paired samples t-tests were used to compare the length, thickness and CSA of selected ankle structures between two positions. The results showed no significant differences between the neutral and tension positions ($p>0.05$) for PLT, PBT, AT, TPT, PLM and PBM. Moreover, dependent t-test demonstrated a significant increase in the length of ATFL in tension position compared to the neutral position ($p=0.04$, Cohen's $d= 1.53$), and a significant thicker in the thickness of ATFL and CFL in injured participants compared to healthy participants ($p< 0.05$, $d>0.8$).

3.4 Reliability study

3.4.1 Aim of the reliability study

Whittaker (2011) recommended that researchers should demonstrate the reliability of their measurements. Knowing the reliability of measurements is obviously important for allowing the researcher to decide whether a specific measurement is of any value. The present study used an intra-tester research design to determine reliability of ultrasound in measuring length, thickness and cross sectional area of selected ankle structures (ATFL, CFL, PLT, PBT, TPT, AT, PLM, and PBM). The participants were tested on two occasions, one week apart, by the same sonographer under the same test conditions.

3.4.2 Background to reliability studies

Prior to applying the developed protocol to address the research questions outlined previously in chapter two, a reliability study was conducted. Reliability indicates the degree to which measurements are consistent (Ruas et al., 2017) over time and operators, and is an important requirement for measurements related to clinical decisions (Whittaker, 2011). Any measurement identified as reliable will produce similar outcomes, regardless of the investigator, time, or other potentially influencing factor (Lee et al., 2012). Therefore, the present study measured the reliability of soft tissue measurements based on ultrasound images. Specifically, the study measured the reliability between two independent measures of the same structure using intraclass correlation coefficient (ICC) and the limits of agreement (LoA) using the Bland-Altman method.

Fisher (1954) was the first to introduce the ICC as a modification of the Pearson correlation coefficient. Today, the ICC is extensively applied in conservative care medicine to assess intra-tester, inter-tester, and test-retest reliability (Koo & Li, 2016). The ICC is a common method used to measure the reliability of a measurement in several studies (Crofts et al., 2014; Golriz et al., 2012; Palmer, Akehi, Thiele, Smith, & Thompson, 2015; Zaki, Bulgiba, Nordin, & Ismail, 2013). Evaluation of reliability is essential to clinical and research evaluation because it gives confidence about measurements so that researchers can draw rational conclusions from these measurements more confidently (Koo & Li, 2016).

There has been considerable debate regarding the utmost suitable type of ICC to be applied in calculating reliability; sometimes researchers are hesitant and confused about which type of ICC to apply.

Statistically there are two common types of ICC are consistency and absolute agreement with three different models: two-ways mixed, two-way random, and one-way random. The two-way fixed model with absolute agreement types was the appropriate type to use in this study because the tester (researcher) is fixed and the participants was chosen randomly and the researcher wants the measurements to be agree absolutely in the two days.

The ICC is defined as the proportion of variances derived from a statistical test. It is estimated from the proportion of variance in a mean or set of scores (true score variance) and variance of error between scores (Weir, 2005). It has no unit and the values of measurements are set between 0 and 1. It has been stated that there are no standard ICC values for acceptable reliability (Koo & Li, 2016). The higher reliability, the closer the ratio of ICC is to 1, whereas there is lower reliability, the closer the ratio is to 0 (Ruas et al., 2017; Zaki et al., 2013). Moreover, there is no ordering of the repeated measures in the ICC, and it can be applied to more than two repeated measurements.

Different theories exist in the literature regarding the measurement of ICC and, to date, there is no definite agreement about the exact appropriate or acceptable level of agreement when reporting ICC. Therefore, there are several interpretations of the value of ICC (Golriz et al., 2012). Portney and Watkins (2009) deduced that good reliability is greater than 0.75, whereas poor to moderate reliability is less than 0.75. Rosner (2010) provided more detail and suggested that an excellent reliability is ICC ≥ 0.75, fair to good reliability is $0.4 \leq$ ICC < 0.75 whereas poor reliability is ICC < 0.4. Cheng and his colleagues (2012) suggested that ICC is very high (0.9 – 1.0), high (0.7 – 0.89), moderate (0.50 – 0.69), low (0.26 – 0.49), or little (0.00 – 0.25). Koo and Mae (2016) suggested in their guidelines for selecting and reporting ICC for reliability research that an ICC value is considered as poor when it is less than 0.5, moderate from 0.50 to 0.75, good from 0.76 to 0.90, and excellent when the value is greater than 0.90. This study has followed this most recent guideline for ICC interpretation.

Another approach quantifying reliability is to use the Bland-Altman LoA. This method was originally proposed for the analysis of agreement in test-retest situations. Giavarina (2015) reported that the Bland-Altman method has been established to define agreement between two numerical measurements through creating limits of agreement. These limits of agreement demonstrate the limits of how different the measurements from two tests could plausibly be for an individual observer (Jones, Dobson, & O’Brian, 2011). The Bland-Altman plot does not tell whether the agreement is adequate or appropriate for use of the protocol. It basically measures the bias and a range of agreement within which 95% of the differences between one measurement and the other are included (Giavarina, 2015). The limits are calculated statistically by applying the mean and the standard deviation (SD) of the differences between the two measurements. In the Bland-Altman method, 95% limits of agreement are used which are calculated as the mean difference between day 1 and day 2 ±

1.96 SD of the difference which are plotted in a simple diagram. This graph displays the size and the extent of the differences and whether the differences are constant among the range of measurements (Bland & Altman, 1986). The advantages of this method are that the data can be visually interpreted quickly by using the scatterplots. Any bias, outliers, or relationship between the mean and variance in measures can be easily seen (Bruton et al., 2000). Examples of Bland-Altman method is demonstrated in Figures 3.3 and 3.4.

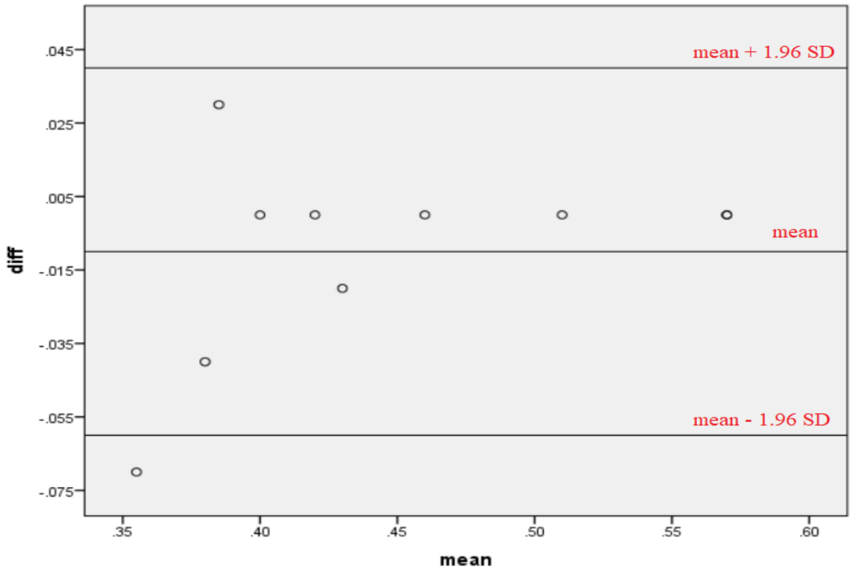


Figure 3-3: Bland and Altman plot for CSA of AT in normal position with representation of limit of agreements. The middle line demonstrates the mean of the differences between the day 1 and day 2 and the side lines demonstrate mean differences ± 1.96 times the SD of the difference between the two.

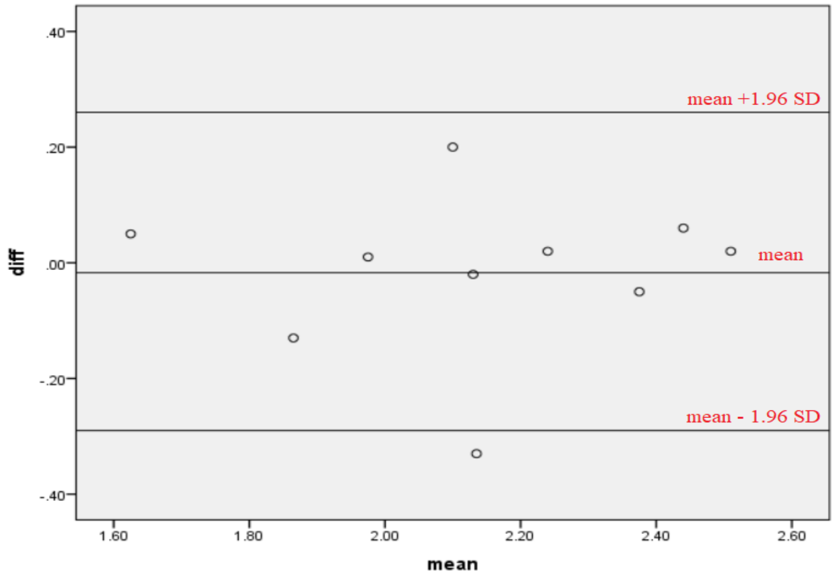


Figure 3-4: Bland and Altman plot for length of ATFL in tension position with representation of limit of agreement. The middle line demonstrates the mean of the differences between the day 1 and day 2 and the side lines demonstrate mean differences ± 1.96 times the SD of the difference between the two.

It is believed that measuring agreement is an important aspect in evaluating the quality of a new protocol (Zaki et al., 2013). Agreement signifies lack of error in the measurements. It evaluates the closeness of the findings of repeated measurements to the true or standard value. Bruton et al. (2000) and Zaki et al. (2013) proposed that a combination of methods is more likely to provide a true picture of reliability. Thus, LoA was also used in the reliability study, which permits the differences between the two days to be put into the context of the measurements being taken, and the changing in the measures are likely due to ageing, disease, or injury (Crofts et al., 2014). A summary of the definition of reliability and agreement is in Table 3.1.

Table 3.1: Summary of reliability and agreement

Reliability	Limit of Agreement
- It quantifies the degree of consistency or reproducibility of a measurement made by same or different observers measuring the same quantity at different time. - Measured by using Intra class correlation coefficient.	- It quantifies the bias and a range of agreement, within which 95% of the differences between one measurement and the other are included. - Measured by using Bland and Altman method.

3.4.3 Reliability study participants

A total of 20 participants were recruited from students and staff of the University of Salford (13 males and 7 females) with a mean age of 31 ± 6.15 years and mean BMI of 24.06 ± 2.81 kg/m^2 to participate in the reliability study. Ethical approval was granted by the University of Salford (Appendix 2). Each participant read the participants information sheet and signed the written informed consent (Appendix 3). Each participant completed questionnaires for demographic data and to identify any ankle injury (Appendix 4) and filled in the CAIT questionnaire (Appendix 5). The participants were classified as 10 healthy participants with no history of any lower limb or musculoskeletal disorder (5 males and 5 females with mean age of 32 ± 3.59 years, mean height 1.64 ± 0.09 m, mean weight 62.20 ± 11.83 kg, and mean BMI 22.92 ± 2.40 kg/m^2) and 10 injured (coper and CAI) participants according to the inclusion criteria of the study (8 males and 2 females with mean age of 30 ± 8.71 years, mean height 1.66 ± 0.09 m, mean weight 69.60 ± 8.92 kg, and mean BMI 25.21 ± 3.22 kg/m^2). Coper and CAI participants were at the same group in this reliability study because the aim of this reliability study is to test the consistency of the researcher for scanning the ankle structures rather than a comparison between the groups. According to Bautista (2016), a small sample size (about 10) could be appropriate for pilot testing.

3.4.4 Reliability study data collection

Ultrasound scanning was performed using a portable Venue 40 musculoskeletal ultrasound system (GE Healthcare, UK) with a 12 MHz linear array transducer with a 12.7 x 47.1 mm footprint area. The scans were made twice, a week apart, by a sonographer who is the author of this thesis. The sonographer had an experience about one year in Ultrasound department. Good contact was maintained between the skin and the transducer without applying too much pressure using B-mode ultrasound. Measurements of each ankle structure were achieved in both the longitudinal and transverse planes.

Participants were in a supine position with their feet extended on the examination table. Details of the participants' position and the scanning techniques are described later in section (3.5.8.1). Each saved ultrasound image was decoded and measured by the same sonographer. Measurements of each structure were taken from still imaging utilising ImageJ software (National Institute for Health, Bethesda, MD, USA) and compiled in a Microsoft Excel spreadsheet. Thickness was measured utilising the straight line function of ImageJ, while the CSA was measured utilising the oval or freehand selection.

3.4.5 Reliability statistical analyses

ICC and LoA was calculated using the Statistical Package for the Social Sciences (SPSS) software version 23.0 (SPSS Inc, Chicago, IL) for intra-tester reliability. Descriptive information of the length, thickness and CSA for each day in normal and tension positions for healthy participants and injured participants are listed in Tables 3.2, 3.3, and 3.4 respectively.

3.4.6 Reliability study results

Different theories exist in the literature regarding the measurement of ICC and, to date, there is no definite agreement about the exact appropriate or acceptable level of agreement when reporting ICC.

Table 3.2: Intra-tester reliability for selected ankle structures in neutral position for healthy participants. L: Length, T: Thickness, CSA: Cross sectional area

Tissue	Day 1 mean(SD)	Day 2 mean (SD)	ICC	95%LoA		LoA %
				Lower	Upper	
ATFL L (cm)	1.93(0.13)	1.94(0.16)	0.94	-0.14	0.13	14
ATFL T (cm)	0.18(0.01)	0.18(0.02)	0.97	-0.01	0.01	11
CFL T (cm)	0.16(0.01)	0.16(0.01)	0.95	-0.01	0.01	12.5
PLT T (cm)	0.22(0.03)	0.22(0.03)	0.94	-0.03	0.03	27.5
PBT T (cm)	0.16(0.01)	0.16(0.01)	0.98	-0.01	0.01	12.5
TPT T (cm)	0.24(0.03)	0.24(0.03)	1.00	-0.01	0.01	8.3
AT T (cm)	0.30(0.02)	0.30(0.03)	0.98	-0.01	0.01	6.6
PLT CSA (cm²)	0.20(0.06)	0.19(0.06)	0.99	-0.02	0.03	22.5
PBT CSA (cm²)	0.14(0.04)	0.13(0.04)	0.96	-0.02	0.02	30
TPT CSA (cm²)	0.18(0.03)	0.18(0.03)	0.95	-0.03	0.03	33.3
AT CSA (cm²)	0.44(0.08)	0.45(0.07)	0.97	-0.06	0.04	23
PLM T (cm)	0.60(0.05)	0.60(0.06)	0.94	-0.05	0.06	17
PBM T (cm)	0.85(0.17)	0.85(0.18)	0.98	-0.10	0.10	12
PLM CSA (cm²)	0.95(0.28)	0.94(0.24)	0.99	-0.10	0.10	22
PBM CSA (cm²)	2.16(0.67)	2.18(0.73)	0.95	-0.32	0.34	30

Table 3.3: Intra-tester reliability for selected ankle structures in tension position for healthy participants. L: Length, T: Thickness, CSA: Cross sectional area

Tissue	Day 1 Mean(SD)	Day2 Mean(SD)	ICC	95%LoA		LoA%
				Lower	Upper	
ATFL L (cm)	2.13(0.28)	2.15(0.28)	0.98	-0.29	0.26	25
ATFL T (cm)	0.18(0.01)	0.18(0.02)	0.96	-0.01	0.01	11
CFL T (cm)	0.16(0.01)	0.16(0.01)	0.94	-0.01	0.01	12.5
PLT T (cm)	0.21(0.03)	0.22(0.03)	0.95	-0.03	0.02	23
PBT T (cm)	0.16(0.01)	0.16(0.01)	0.94	-0.01	0.01	11
TPT T (cm)	0.23(0.02)	0.23(0.02)	0.99	-0.01	0.01	7
AT T (cm)	0.29(0.03)	0.29(0.02)	0.99	-0.01	0.01	6.5
PLT CSA (cm²)	0.19(0.04)	0.18(0.04)	0.98	-0.02	0.03	23
PBT CSA (cm²)	0.14(0.03)	0.14(0.01)	0.94	-0.02	0.03	30
TPT CSA (cm²)	0.18(0.04)	0.18(0.03)	0.97	-0.03	0.02	26
AT CSA (cm²)	0.41(0.07)	0.42(0.07)	0.98	-0.04	0.02	14
PLM T (cm)	0.59(0.06)	0.59(0.05)	0.96	-0.05	0.04	15

PBM T (cm)	0.83(0.17)	0.86(0.18)	0.95	-0.06	0.04	12
PLM CSA(cm²)	0.91(0.19)	0.90(0.22)	0.95	-0.02	0.04	7.5
PBM CSA (cm²)	2.03(0.76)	2.12(0.82)	0.95	-0.25	0.27	26

Table 3.4: Intra-tester reliability for selected ankle structures for injured participants in tension. L: Length, T: Thickness, CSA: Cross sectional area

Tissue	Day 1 Mean(SD)	Day 2 Mean(SD)	ICC	95%LoA		LoA%
				Lower	Upper	
Neutral position						
ATFL L (cm)	1.90(0.17)	1.91(0.23)	0.92	-0.22	0.21	22
ATFL T (cm)	0.27(0.02)	0.28(0.02)	0.89	-0.03	0.02	18
CFL T (cm)	0.17(0.02)	0.18(0.01)	0.85	-0.03	0.02	23
PL T (cm)	0.26(0.03)	0.27(0.02)	0.92	-0.03	0.02	17
PB T (cm)	0.18(0.03)	0.18(0.03)	0.85	-0.02	0.02	23
PT T (cm)	0.26(0.02)	0.26(0.02)	0.91	-0.03	0.02	17
AT T (cm)	0.31(0.02)	0.32(0.03)	0.90	-0.03	0.02	13
PLT CSA (cm²)	0.22(0.02)	0.22(0.03)	0.93	0.03	0.03	25
PBT CSA (cm²)	0.16(0.05)	0.16(0.03)	0.94	-0.02	0.03	25
TPT CSA (cm²)	0.15(0.02)	0.15(0.02)	0.93	-0.02	0.02	26
AT CSA (cm²)	0.51(0.03)	0.51(0.03)	0.95	-0.03	0.03	12
PLM T (cm)	0.76(0.07)	0.76(0.08)	0.97	-0.05	0.05	12
PBM T (cm)	0.95(0.09)	0.99(0.09)	0.89	-0.13	0.06	19
PLM CSA (cm²)	0.75(0.05)	0.75(0.05)	0.98	-0.03	0.03	8
PBM CSA (cm²)	2.59(0.36)	2.58(0.40)	0.95	-0.30	0.32	23
Tension position						
ATFL L (cm)	2.35 (0.20)	2.27 (0.28)	0.92	-0.15	0.29	19
ATFL T (cm)	0.30 (0.04)	0.31 (0.03)	0.96	-0.03	0.02	16.5

The ICC of length and thickness of healthy ATFL and CFL in neutral and a tension position was 0.94 - 0.98 which is graded as excellent reliability; the limits of agreement were between 11% and 25% of the relative sizes of the ligaments. The length of ATFL in tension position had the highest ICC (0.98), whereas the thickness of CFL in tension position had the lowest ICC (0.94). On the other hand, the ICC of length and thickness of injured ATFL and CFL in neutral and tension position was 0.85 - 0.96 which is graded as good to excellent reliability. The thickness of ATFL in tension position had the highest ICC (0.96), whereas the thickness of CFL in neutral position had the lowest ICC (0.85).

Liu et al. (2015) calculated the ICC of ATFL thickness among two examiners to be 0.91, which indicates excellent reliability. Moreover, it has been established that measurements of ATFL length under inversion and plantar flexion stress showed good to excellent intra-tester reliability (0.68 – 0.94) (Croy et al., 2012). This study was in line with Sisson et al. (2011) who found a high inter-tester and intra-terster reliability for ultrasound in measuring the length of ATFL under inversion; ICC was 0.91 and 0.94 respectively (it was 0.77 and 0.96 when in a neutral position).

3.4.7 Reliability study conclusion

Based on the results of the reliability study, the ultrasound protocol applied in this study has been shown to be a reliable method to measure the morphology of ATFL, CFL, PLT, PBT, TPT, AT, PLM, and PBM.

3.5 Method

3.5.1 Study design

This study was an observational study to investigate individuals with and without previous incidence of ankle sprains. A cross sectional study design was employed to compare individuals across the different groups. This type of design is usually utilised to evaluate the differences between groups in terms of the outcomes investigated (De Vaus, 2001). The ultrasound study was conducted in the ultrasound room located within the Allerton Building or in the gait laboratory in the podiatry clinic, both at the University of Salford in Greater Manchester, United Kingdom.

3.5.2 Ethical considerations

The study had received ethical approval by the University of Salford College of Health and Social Care Ethics Committee (Appendix 6). Several days were given for all interested participants to carefully review the participant information sheet (Appendix 7) that explained the aim of the study, the safety of ultrasound, the scanning techniques, and the rights of the participants before they made their decision to be involved. On the day of the study, the study protocol was explained verbally to each participant and the researcher gave the participants the opportunity to ask questions or raise any concerns. Those participants who agreed to take part in the study were then asked to sign the consent form (Appendix 8). It was clearly explained to the participants that they could withdraw from the study at any time without providing any reason and that they had the right to ask for any of their data already collected to be deleted from the study records. Participants were also reimbursed for their time. All data gathered were coded to a specific number for each participant in order to keep their data anonymous. The participants' information on the ultrasound images was also anonymised by giving each participant an identifier code which was used later in the analysis. The names of participants were not identified in any situation, such as on academic posters, conference presentations, and journal publications.

The risk assessment form (Appendix 9) was approved by the Ethics Committee. The study had very little risk to any of the participants, since ultrasound is considered as a safe imaging modality and does not use ionising radiation. Although the ultrasound transducer gel used in the study is hypoallergenic and non-hazardous, there was still a risk of an allergic reaction occurring with participants. Therefore, a patch test was performed prior to testing to make sure the participants had no reaction to the gel.

3.5.3 Sample size for the main study

G power is a computer software programme that has been utilised in many studies to calculate sample size. It has been shown to have excellent accuracy. One way ANOVA has been run on the result of the pilot study to calculate the effect size. G power with alpha set to 0.05 and the calculation power 0.95 provided 15 participants in each group are need to conduct the study. To detect a small difference between groups, a higher power (0.95) has been chose. This means there is a 95 percent chance of detecting a difference as statistically significant, if in fact a true difference exists.

3.5.4 Recruitment strategy

Participants were recruited based on the inclusion and exclusion criteria mentioned later in section 3.5.5. As demonstrated previously the calculation of initial power estimated that 15 subjects should be recruited for each group. To obtain this number of participants, recruitment was carried out from January 2017 to April 2018. Many recruitment strategies were used to conduct the study, such as poster/flyer (Appendix 10) placed across different notice boards in different buildings of the university. Interested volunteers were asked to email or text the researcher for additional information and a copy of the participants' information sheet. In addition, a radio advertisement was made through Salford City Radio to encourage people to take part in the study. The flyer was also posted in different social media, such as Facebook and Twitter. My supervisors, staff at the University of Salford, and the postgraduate research support officer also emailed announcements to the students and staff of the University of Salford to participate in the study. The researcher was responsible for contacting all participants to arrange an appointment to attend the session, and to text or email a reminder to each participant 24 hours before the appointment day.

3.5.5 Inclusion and exclusion criteria

Recruited participants were placed into groups based on the inclusion criteria given below. Inclusion criteria for injured participants were similar to those in Koshino et al., (2016) which were partially based on the recommendations of the International Ankle Consortium (Gribble et al., 2014). The authors provide an agreed set of selection criteria for coper and CAI participants which rely on the best available evidence to be utilised in future research (Gribble et al., 2014).

1. Healthy group:

- Self-reported good health.
- No previous history of lateral ankle sprain or any lower extremity injuries.
- Being physically active based on general practice physical activity questionnaire (GPPAQ) (Appendix 11).
- Had a score of ≥ 25 on CAIT questionnaire.

2. Coper group:

- Self-reported good health.
- Self-reported lateral ankle sprain diagnosed by health care professionals.
- A history of one LAS and this should be at least 12 months before conducting the study.
- Coper participants should have returned to all pre-injury levels of activities for at least 12 months.
- Had a score of ≥ 25 on CAIT questionnaire.

3. CAI group:

- Self-reported good health.
- Self-reported lateral ankle sprain diagnosed by health care professionals.
- A history of two or more sprains to the ankle in the last two years.

- Failed to return to the pre-injury level of activities, the re-injury occurring between 3 and 24 months ago, with several episodes of the ankle “giving way”, and not currently undergoing active treatment for LAS injury.
- Had a score of ≤ 24 on CAIT questionnaire.

The exclusion criteria were:

- A history of previous surgeries on the lower limb extremity.
- A history of fracture in the lower limb extremity.
- Acute injury in the last six weeks.
- A history of injury with residual symptoms in the lower extremities in the previous six months.

3.5.6 Participants information

A data collection sheet was used to collect the participants’ demographic data and history of the ankle injury at the end of the study. Using data on each participant’s height and weight, the researcher obtained their BMI, also often known as Quetelet Index (QI), by dividing the weight in kilograms by the height squared in meters [BMI= (weight in kilogram) / (height in meters)^2] (Kolimechkov, 2016). The BMI score was divided into four groups: (1) lower than 18.5 (underweight); (2) 18.5 – 24.9 (normal); (3) 25 – 29.9 (overweight); and (4) over 30 (obese) (Nuttall, 2015). This is necessary to test the question of whether there is a difference in the measurements of selected ankle structures between normal weight and overweight participants.

After completion of the demographic data, the participants were asked to complete a physical activity questionnaire, followed by self-reported functional assessment. Physical activity was measured by using the GPPAQ because it is a quick questionnaire used in the National Health Service (NHS) as a validated screening tool to evaluate the level of physical activity within primary care (National Health Service, 2009). The GPPAQ was used to evaluate the physical activity level of adults (16 - 74 years). It gives a straightforward simple four-level physical activity index (PAI) (Table 3.5), which categorises individual as follows (National Health Service, 2009, p. 13):

Table 3.5: Summary of physical activity index (National Health Service, 2009,p. 13)

	Occupation			
Physical exercise and / or cycling (hr/wk)	Sedentary	Standing	Physical	Heavy Manual
0	Inactive	Moderately Inactive	Moderately Active	Active
Some but < 1	Moderately Inactive	Moderately Active	Active	Active
1-2.9	Moderately Active	Active	Active	Active
≥ 3	Active	Active	Active	Active

The participants categorise as following:

- "Inactive
  - Sedentary job and no physical exercise or cycling
- Moderately inactive:
  - Sedentary job and some but < 1 hour physical exercise and/or cycling per week
  - Standing job and no physical exercise or cycling.
- Moderately active:
  - Sedentary job and 1-2.9 hours physical exercise and / or cycling per week
  - Standing job and some but < 1 hour physical exercise and / or cycling per week
  - Physical job and no physical exercise or cycling
- Active:
  - Sedentary job and ≥ 3 hours physical exercise and / or cycling per week
  - Standing job and 1-2.9 hours physical exercise and / or cycling per week
  - Physical job and some, but < 1 hour, 1-2.9 hours, ≥ 3 hours physical exercise and / or cycling per week
  - Heavy manual job."

After completing the physical activity questionnaire, the self-reported functional assessment for ankle instability was measured using the CAIT questionnaire which was explained in depth in the literature review.

3.5.6.1 Demographic data for comparison between healthy, coper and CAI participants

	Healthy	Coper	CAI	p-value	Effect size
Number of participants	48	22	32		
Sex male/ female	25/23	13/9	17/15		
Age (y)	28.87 ± 6.10	28.65 ± 05.65	27.70 ± 07.99	>0.05	0.07
Weight (kg)	67.86 ± 9.23	69.90 ± 10.06	69.94 ± 15.38	>0.05	0.08
Height (m)	1.69 ± 0.10	1.67 ± 0.10	1.68 ± 0.10	>0.05	0.08
BMI (kg/m²)	23.76 ± 1.24	24.57 ± 2.36	24.54 ± 3.85	>0.05	0.14
CAIT score	28.75 ± 1.65	27.90 ± 1.86	18.24 ± 4.42 [a]	<0.01	1.62
Time since last injury (months)	0.0 ± 0.0	18.60 ± 04.73 [a,b]	7.10 ± 2.57 [a]	<0.01	2.30

Abbreviations: CAIT, Cumberland Ankle Instability Tool; BMI, Body Mass Index.
*Values are mean ± SD.
[a] Indicates statistical differences between CAI and coper, and between CAI and healthy.
[b] Indicates statistical differences between coper and healthy.

3.5.6.2 Demographic data for comparison between right and left limbs among healthy participants

Variable	
Number of participants	20
Sex male /female	10/10
Age (y)	29.60 ± 2.63
Weight (kg)	69.40 ± 10.79
Height (m)	1.70 ± 0.09
BMI (kg/m²)	23.95 ± 1.90

*Values are mean ± SD.

3.5.6.3 Demographic data for comparison between male and female healthy participants

Variable	Male	Female	P-value	Cohen's d
Number of participants	23	22		
Age (y)	29.80 ± 3.94	29.52 ± 8.74	0.59	0.18
Weight (kg)	66.06 ± 5.04	65.12 ± 3.89	0.46	0.19
Height (m)	1.67 ± 0.05	1.66 ± 0.04	0.67	0.17
BMI (kg/m²)	23.58 ± 1.28	23.56 ± 0.87	0.85	0.02

*Values are mean ± SD.

3.5.6.4 Demographic data for comparison between normal weight and overweight healthy participants

Variable	Normal	Overweight	P-value	Cohen's d
Number of participants	22	23		
Sex male/female	9/13	13/10		
Age (y)	28.71 ± 5.67	28.43 ± 4.74	0.205	0.05
Weight (kg)	61.95 ± 6.25	77.55 ± 10.09	<0.001[a]	1.86[a]
Height (m)	1.66 ± 0.07	1.72 ± 0.08	<0.001[a]	0.80[a]
BMI (kg/m²)	22.48 ± 1.09	26.07 ± 1.87	<0.001[a]	2.3[a]

* Values are mean ± SD.
[a] Indicates statistical and meaningful differences between normal weight and overweight participants.

3.5.7 Medical ultrasound machine

A portable Venue 40 US system (GE Healthcare, UK) was used for scanning (Figure 3.5). The ultrasound transducer is considered the most significant part of an ultrasound machine. The characteristics of the transducer define the frequency and the resolution of the image (Strakowski, 2015). The transducer usually receives sound waves during 80% of the active scanning time and transmits sound waves during the other 20% of the time. Various different types of transducers are utilised in ultrasound. A linear transducer is utilised for most musculoskeletal applications (Strakowski, 2015). A 12 MHz linear array transducer, with a 12.7 x 47.1 mm footprint area, was used for scanning (Figure 3.6). The 10-13 MHz frequency range is suited for the assessment of ankle structures, because higher frequencies produces shorter wavelengths which provide better image resolution for most musculoskeletal structures (Patil & Dasgupta, 2012).

This portable ultrasound machine was checked by Christie Medical Physics & Engineering Company annually. The department performs acceptance tests are based on Institute of Physics and Engineering in Medicine and the British Medical Ultrasound Society guidelines. These form a comprehensive check on the system operating characteristics and acts as a baseline for future testing. A Gammex RMI test object was used for most of the imaging and measurement checks. The process of quality control should be done to ensure that the machine primarily operates at its expected level of performance and is suitable for clinical and/or research use. In addition, sonographer and researcher seek assurance that the information acquired in the ultrasound procedure is accurate and the machine is safe to use. Based on the test report (June 2018), the overall greyscale, axial and lateral resolution and background noise was checked and compared with similar systems used in the North West. The overall image quality was good.

Figure 3-5: portable ultrasound machine

Figure 3-6: Linear array transducer

3.5.8 Assessment procedure

Once the participants had signed the consent form, they were asked to wear shorts and take off their shoes and socks in order to leave the lower limbs exposed. The participants were then asked to sit gently on the examination table in supine position. The researcher made sure that the participants were comfortable and asked them if they had any questions or concerns before starting the test. In addition, the researcher locked the testing room to ensure participants' privacy and to avoid any distraction. Prior to starting the scanning, the researcher checked the ultrasound machine and its settings which will be demonstrated in the next section (3.5.8.1). Once everything was fine, the test started, with the foot is in neutral position (90 degrees) and then in tension position (the details of the scanning techniques and measurements will be discussed in the next section). Each structure was imaged in both longitudinal and transverse scans. The CSA of the structures was measured in transverse plane. McCreesh and Sinead (2011) reported that no significant difference in the measurements of the thickness from longitudinal and transverse images and thus either method could be used. Measuring the thickness on longitudinal image could allow several areas to be measured on a single image. In addition, longitudinal scanning provides more ease of transducer alignment on the lower leg (McCreesh & Sinead, 2011). It has been reported in the literature that measurements of the thickness of tendon should be the same in both longitudinal and transverse scans. Regardless of the fact that transverse and longitudinal scanning are both appropriate techniques to measure the thickness of tendon, the longitudinal plane has some minor advantages. During dynamic scanning, it can be easier to distinguish between epitenon and the paratenon in the longitudinal plane. Moreover, a longitudinal scan is an absolute necessity for evaluating the thickness of tendon because it is the only technique allowing the sonographer to properly record the distance from the bony attachment to the point where the thickness is measured. Thus, all the measurements are made at the same point exactly for all participants and measuring the thickness on longitudinal image would be the method of choice for this study.

The ultrasound images were saved onto a memory card. Once the ultrasound scanning was completed, the participants had a rest for three to five minutes before starting the second test (which is described in details in chapter five). Once they had finished both tests, they changed their shorts and put their socks and shoes back on in the changing room; the researcher then gave them their vouchers and thanked them for taking part in the study. The researcher was then responsible for cleaning the ultrasound transducer and preparing the examination bed between participants by applying a new section of paper bed roll.

3.5.8.1 Ultrasound techniques and measurements

The settings for the scanning were as recommended by Liffen (2014) for grey scale diagnostic ultrasound images to achieve the best resolution and clarity:

- Linear transducer (rectangular shape), most useful for superficial parts and musculoskeletal (Goyal, 2018), with high frequency ≥ 12 MHz.
- Depth adjusted to 3 cm was applicable for the region of interest to be clearly visualised (Figure 3.7), however it changed sometimes to 4 cm in scanning the muscles for some participants. Controlling the depth changes the size of the scanning area. The measurement of the depth appears in most ultrasound machines to simplify measurement of the size of the structure and the depth of the field.
- Focus was automatically placed in the middle of the image as a triangular symbol (Figure 3.7).
- Gain of the image was optimised to adjust overall brightness of the image, which is in part determined by ambient light in the examination room (Jacobson, 2012). The gain must be ideally adjusted so that one can recognise the ultrasound characteristics of normal soft tissue (as described later in chapter four) (Figure 3.7).
- During the scanning, for the Mechanical Index and Thermal Index, the “as low as reasonably achievable” (ALARS) principle was applied.

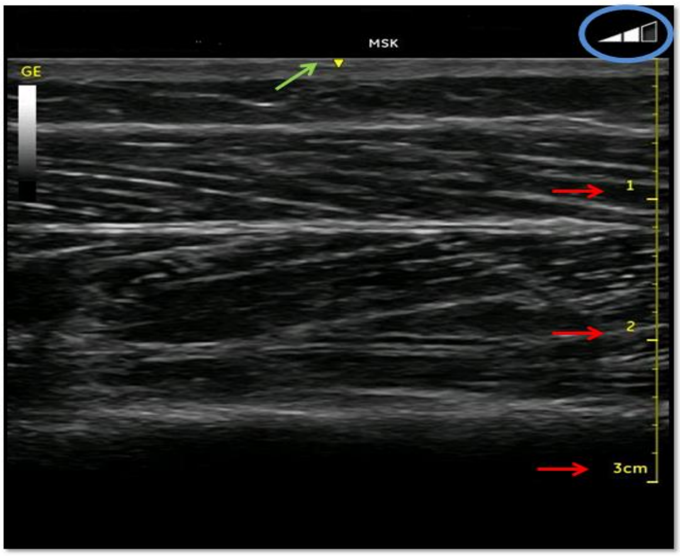


Figure 3-7: An ultrasound image demonstrating the depth scale to the right of the screen which define by red arrows; Green arrow defines the focus of the image; Blue circle defines the gain

The structures of interest were ATFL, CFL, PBT, PLT, TPT, AT, PBM and PLM. These were to be scanned in two different foot positions, so that they were measured in a common position for all and when under tension. Since different structures are orientated differently and resist different motions, the under tension position varied for different structures (Table 3.6). The neutral position was performed by asking the participant to sit on the examination table with their leg fully extended. The examined leg was held in an ankle-foot orthosis (AFO), with the strap placed around the forefoot, and rested on a sand bag to maintain the foot in neutral position (0° inversion/eversion and 0° dorsi-/plantar flexion) (Figure 3.8).

Figure 3-8: Right feet hold on AFO for neutral position

Table 3.6: Summarise the neutral and tension positions for each structure:

	Neutral position	Tension position			
		Dorsiflexion	Plantarflexion	Eversion	Inversion
ATFL	✓		✓		✓
CFL	✓	✓			
PBT	✓		✓		✓
PLT	✓		✓		✓
TPT	✓		✓	✓	
AT	✓	✓			
PBM	✓		✓		✓
PLM	✓		✓		✓

To create an ultrasound image, the transducer is held on the skin surface to image the structures underneath the transducer. An ultrasound gel must be applied to allow the transmission of the sound beam from and to the transducer by removing the air between the skin surface and the transducer (more than 99.9% of the sound beam is reflected at a tissue-air interface, thus no sound beam would be available for imaging) (Goyal, 2018; Jacobson, 2012). The gel used in this study was a thick gel, which is better than a more liquid gel because it tends to remain localised at the image site. Aquasonic 100 ultrasound transmission gel has a hypoallergenic, bacteriostatic, non-sensitising, and non-irritating formula. It is the most widely used gel for diagnostic and therapeutic medical ultrasound (Universal Medical, 2019).

The transducer must be grasped by the examiner's dominant hand between the thumb and the fingers with the distal part of the transducer close to the ulnar aspect of the hand (Figure 3.9A). The marker on the transducer must pointed, in a longitudinal scan, towards the patient's head and towards the right during transverse scanning (Goyal, 2018). The transducer must be stabilised on the skin by either the little finger or the heel of the imaging hand (Figure 3.9B). This method is necessary to retain appropriate pressure of the transducer on the skin, to reduce muscle fatigue in the examining arm, to keep the transducer in position and to avoid involuntary movement of the transducer (Jacobson, 2012).

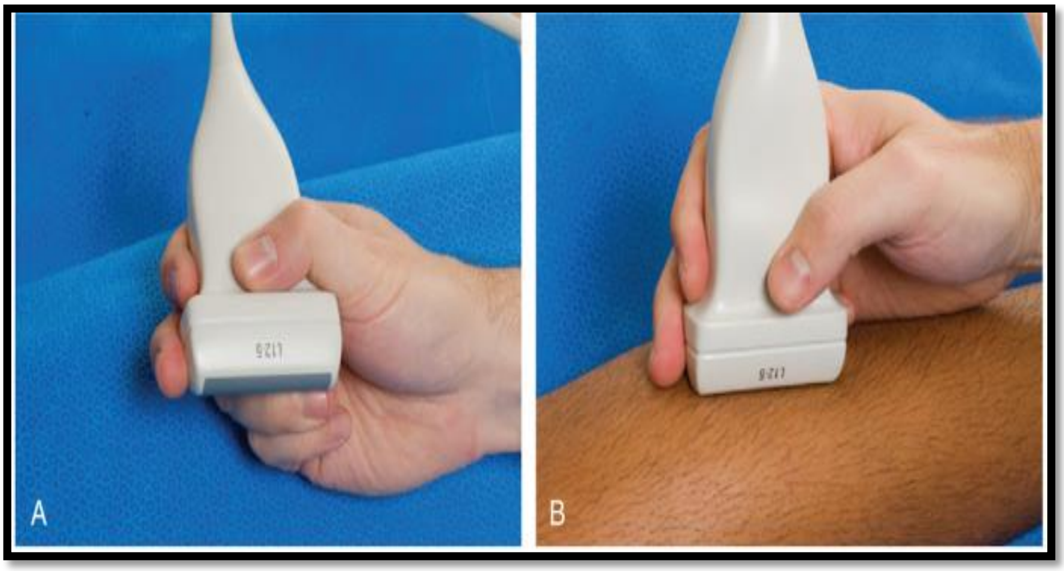


Figure 3-9: Transducer held and positioning (Jacobson, 2012).

3.5.8.1.1 Anterior Talofibular Ligament (ATFL)

With the foot in the neutral position, the lateral malleolus was palpated by the examiner and the proximal edge of the transducer was placed over the anterior border of the LM bone while distal edge was placed over the talus bone (Figure 3.10).

Figure 3-10: Transducer position to scan ATFL

The origin and insertion points of the ATFL were used as bony landmarks; the anterolateral aspect of LM was identified as the origin of the ATFL while the peak of the talus was identified as the insertion point [similar to previous protocols (Lee et al., 2014; Sisson, Croy, Saliba, & Hertel, 2011)]. These bony landmarks were clearly identifiable in ultrasound images due to their hyper-echogenicity (bright white area) and identification of bony landmarks is also helpful for orientation (Figure 3.11) (Yildizgoren et al., 2017).

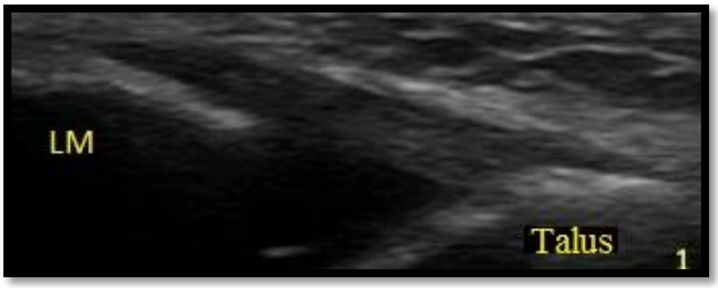


Figure 3-11: US image for ATFL in neutral position

For the tension position, the AFO was taken off and the participants instructed to relax the leg muscles while the ankle was passively and manually moved to the end of the ankle plantar-flexion and inverted range. The sand bags were replaced on the medial and lateral sides of the participant's leg. A strap was placed around the lateral forefoot and wrapped around the sand bag to maintain the foot in its plantar-flexed and inverted position (Figure 3.12). Khawaji and Soames (2015) reported that measurements of ligament lengths are made for the longest fibres of the ligaments because of the differences in the maximum range of movement in each position which create differences in ligament length.

Figure 3-12: Tension position for ATFL

The full length of ATFL was measured from the origin (LM) to the insertion point (talus) in the longitudinal plane (Figure 3.13A) while the thickness was measured halfway between LM and talus [as per Dimmick et al.'s (2009) protocol] (Figure 3.13B).

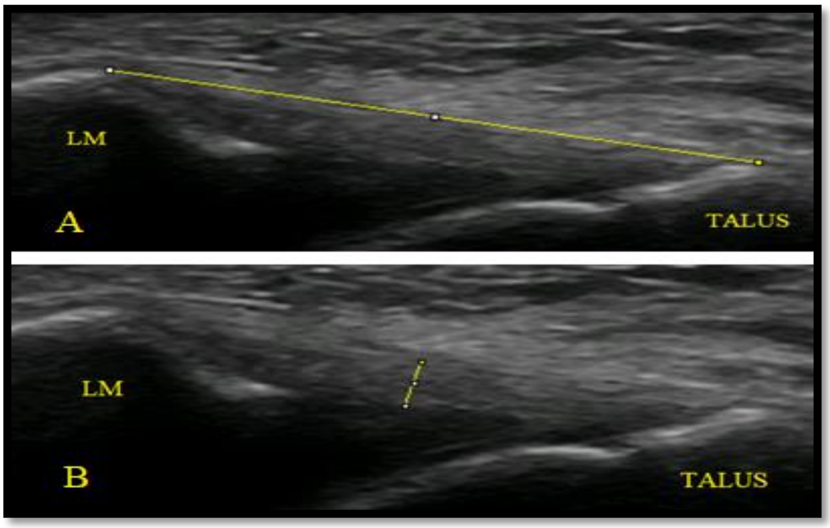


Figure 3-13: Longitudinal measurement of ATFL in tension position. A: The length. B: The thickness

3.5.8.1.2 Calcaneofibular Ligament (CFL)

With the foot in the neutral position, the head of the transducer was placed anterior to the tip of the lateral malleolus in an oblique coronal plane which positioned the distal part of the probe towards the heel [as described by De Maeseneer et al. (2009)] (Figure 3.14). This oblique position is required in order to find the exact location of CFL (De Maeseneer et al., 2009). The peroneal tendons can help in precisely finding the ligament which is located proximal to the CFL (Figure 3.15B).

Figure 3-14: Transducer position to scan CFL in neutral position

For the tension position, a wedge was inserted between the plantar surface of the foot and the AFO. This helped to dorsiflex the foot (15°) and thus placed the CFL under tension. The foot was placed on an orthotic insole on the top of the wedge to ensure a smooth foot contact with the wedge (Figure 3.15A). The measurements of the CFL were taken from longitudinal plane. The full length of the CFL is rarely visible because the origin underlies the LM. However, the thickness was measured 1 cm from the insertion point (calcaneus) [as per the protocol used by Dimmick et al. (2009)] (Figure 3.15B).

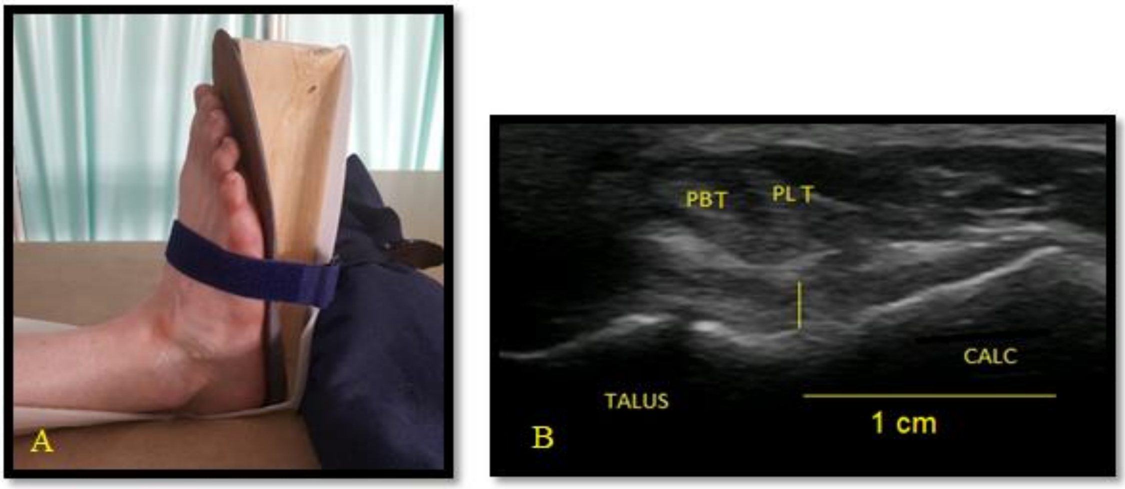


Figure 3-15: A: Tension position for CFL. B: US measurement for CFL in tension position. PBT: peroneal brevis tendon, PLT: peroneal longus tendon, and CALC: calcaneus

3.5.8.1.3 Peroneal Tendons

With the foot in the neutral position PLT and PBT were scanned in both longitudinal and transverse planes. US examination of peroneal tendons commonly starts with transverse scanning (Taljanovic et al., 2015). For a transverse image, the transducer was placed slightly inferior to the distal part of the LM in the transverse plane and at the posterolateral ankle (De Maeseneer et al., 2009) (Figure 3.16A). The peroneus brevis is located near to the LM while the peroneus longus is seen more superficially (Figure 3.16B) (De Maeseneer et al., 2009).

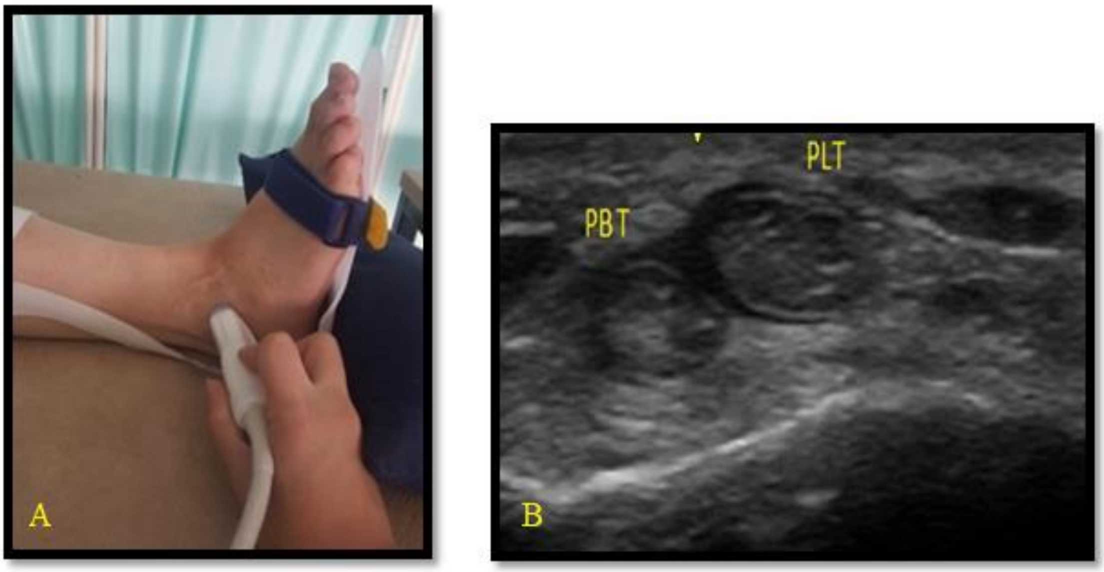


Figure 3-16: A: Transducer position to scan peroneal tendons in neutral position. B: Transverse US image of peroneal tendons.

Having confirmed the PLT and PBT locations, the transducer was rotated 90º to obtain the longitudinal image of the tendons. The transducer was moved slightly up (towards the dorsum of the foot) to scan the PBT and slightly down (towards the plantar of the foot) to scan the PLT.

For the tension position, the foot was plantar-flexed and inverted as per the tension position for ATFL. The transducer position is demonstrated in Figure 3.17A and 3.17B.

Figure 3-17: Transducer position to scan peroneal tendons in tension position. A: Scanning PBT. B: Scanning PLT

The measurements of the peroneal tendons were taken 1 cm below LM [as pilot work has shown that the measurements are the same at the three different locations (at LM, above LM, below LM)] (Appendix 1). The cross sectional area of the tendons was measured in transverse plane in the same image for both peroneal tendons (Figure 3.18). However, the thickness was measured separately for each tendon in the longitudinal plane (Figure 3.19).

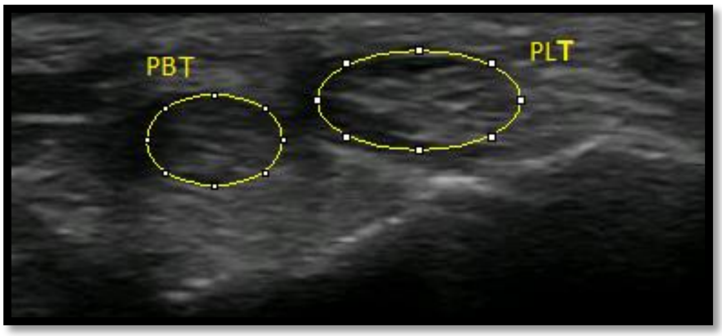


Figure 3-18: CSA measurement of peroneal tendons

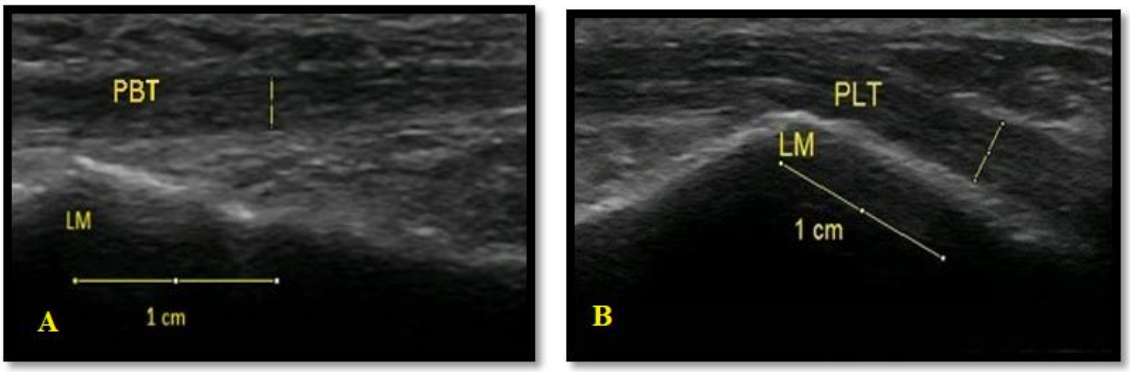


Figure 3-19: Thickness measurement of peroneal tendons in tension position. A:PBT (peroneal brevis tendon). B:PLT (peroneal longus tendon)

3.5.8.1.4 Tibialis Posterior Tendon (TPT)

With the foot in the neutral position, the transducer was placed above the medial malleolus (MM) in an oblique transverse plane (transverse with an angle) (Figure 3.20A) to allow a transverse image. The TPT is close to the MM and twice the size of the flexor digitorum tendon which is located lateral to it (Jacobson, 2012; Lhoste-Trouilloud, 2012). The transducer was then rotated 90° (Figure 3.20B) to obtain the longitudinal image of the TPT from which its thickness was measured.

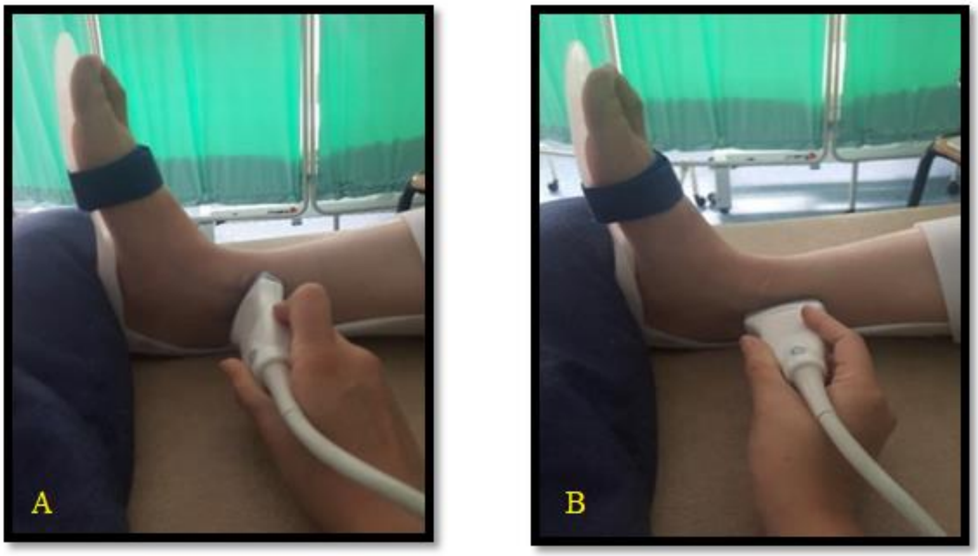


Figure 3-20: Transducer position to scan the TPT. A: Transverse plane. B: Longitudinal plane

For the tension position, the same procedure as for the ATFL was followed, but with the foot in a plantar-flexed and everted (rather than inverted) position and the strap on the medial, not the lateral, side of the forefoot (Figure 3.21A). The measurements of the TPT were taken 2 cm above the MM. The cross sectional area of the tendon was measured in the transverse plane while the thickness was measured in the longitudinal plane. An example of measuring the thickness is demonstrated in Figure 3.21B.

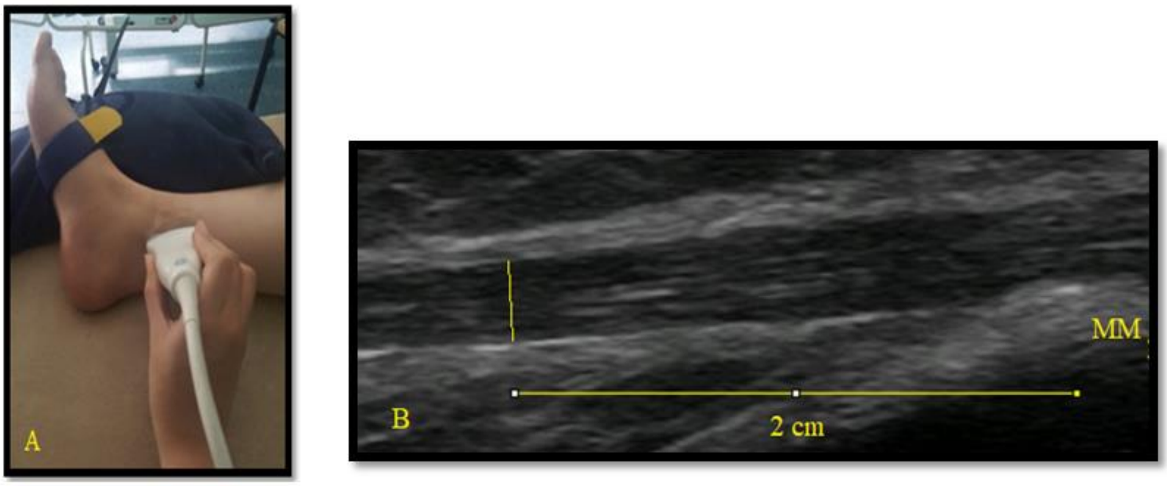


Figure 3-21: A: Transducer position to scan longitudinal plane tibialis posterior tendon (TPT) in tension position. B: Thickness measurement of TPT. MM: Medial malleolus

3.5.8.1.5 Achilles Tendon (AT)

The participants were asked to move down the assessment bed to allow the foot to hang off the end, giving access to the Achilles in neutral position (Figure 3.22A). For the tension position, the wedge was inserted between the plantar surface of the foot and the AFO (Figure 3.22B). This kept the foot in a dorsiflexed position. The transducer was placed onto the palpable Achilles tendon at the posterior ankle in a sagittal plane (Figure 3.22C) to scan the AT in the longitudinal plane (Figure 3.23A); the transducer was then rotated 90º to obtain the transverse image of the AT (Figure 3.23B) (Dong & Fessell, 2009).

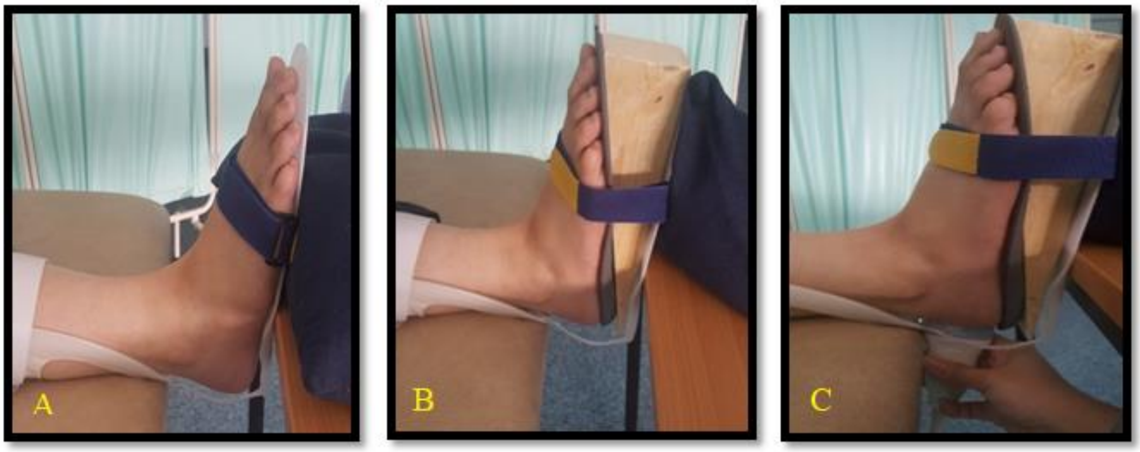
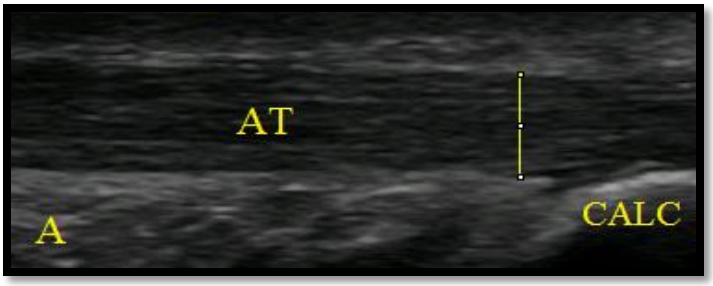


Figure 3-22: Scanning the AT. A: Neutral position. B: Tension position. C: Transducer position to scan longitudinal plane of AT.

The measurement was taken at the point where the Achilles tendon separates from the calcanei, which is considered to be the maximum thickness of the tendon (Figure 3.23A) (Bjordal, Demmink, & Ljunggren, 2003). Furthermore, according to Kharate and Chance-Larsen (2012), this area is the most common place for tendon pathology with a relative lack of blood supply (Asplund & Best, 2013). Due to the hypovascularity, this area is more susceptible to injury. The CSA was measured in the same area (Figure 3.23B).



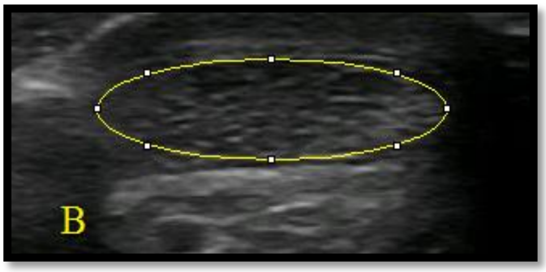


Figure 3-23: A: Thickness measurements of AT in longitudinal plane. B: CSA measure of AT in transverse plane

3.5.8.1.6 Peroneal Muscles

With the foot in the neutral position, the PLM and PBM were scanned in both longitudinal and transverse planes. For the transverse image, the transducer was placed perpendicular to the calf bone (fibula), halfway (50%) between the head of the fibula and the inferior border of the LM (Angin et al., 2014). The transducer was then rotated 90° to scan the muscles in the longitudinal plane. For the tension position, the position of the leg and foot was the same as for the tension position of the peroneal tendons (Figures 3.24A – 3.25A).

The measurements of the muscles were taken at the midway point between the head of the fibula and the inferior border of the LM. The cross sectional area of the muscles was measured in the transverse plane in the same image (Figure 3.24B), as in the protocol used by Lobo et al. (2016). The thickness was measured in the longitudinal plane in the same image (Figure 3.25B).

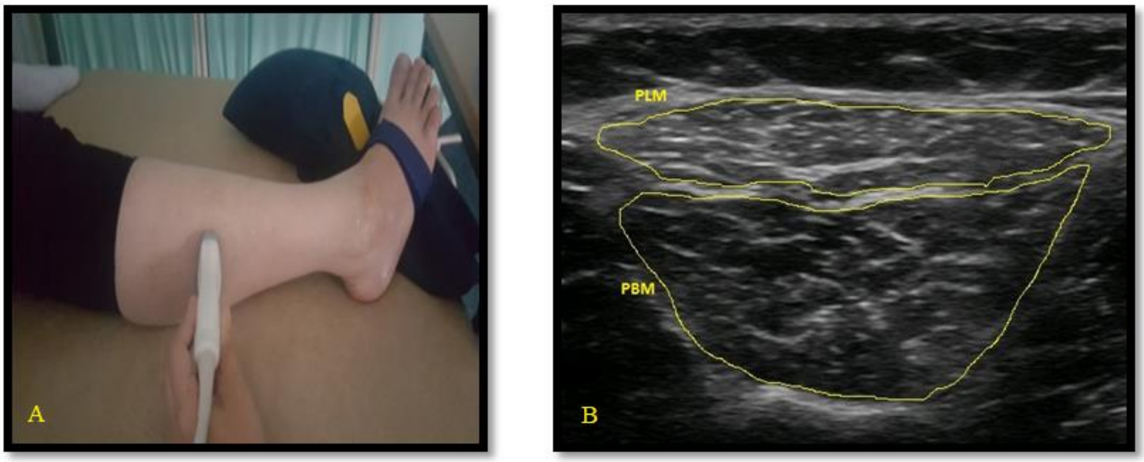


Figure 3-24: A: Transducer position to scan transverse plane peroneal muscles in tension position. B: CSA measurement of peroneal muscles.

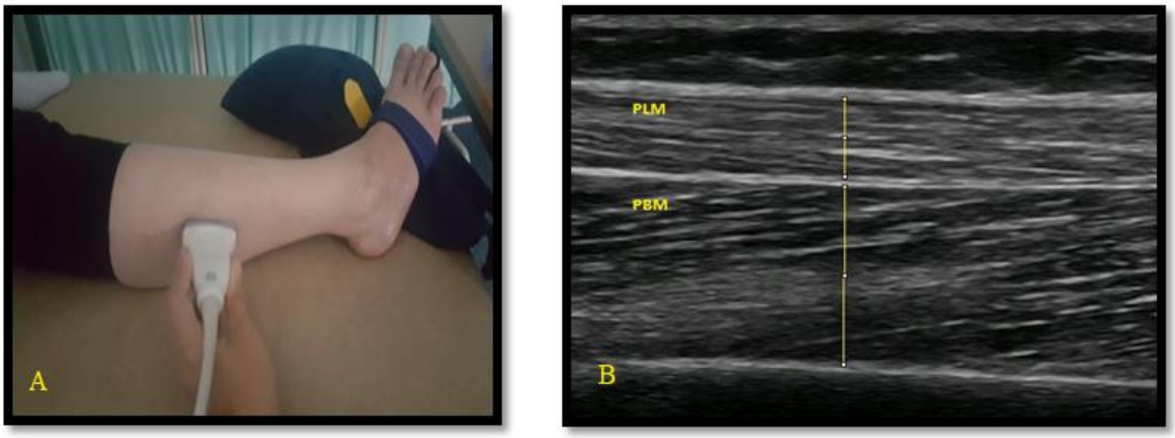


Figure 3-25: A: Transducer position to scan longitudinal plane peroneal muscles in tension position. B: Thickness measurement of peroneal muscles.

The ultrasound images were copied to memory drive for later measurement; each image was coded to a specific number. The computer monitor was a 5 mega-pixel DOME E5 (NDSsi, Santa Rosa, CA,USA; 2048 by 2560 pixels) calibrated to the DICOM Grey Scale Display Function Standard (The Royal College of Radiologists, 2012). The ambient room lighting was < 8 lux. The measurements were performed many days later using ImageJ software, a public-domain, Java-based image processing program developed by the National Institute of Health (Bethesda, MD). ImageJ software has been shown to have an excellent inter-rater reliability when measuring the thickness of muscle from ultrasound images (McCreesh & Sinead, 2011). By the time the measurements were made, the researcher was blinded to the classification of the groups. The field of view for each ultrasound image was 13.3 x 10.8 cm containing 600 x 655 pixels. A digital caliper was calibrated to 156 pixels per cm for all images with 3 cm deep and 150 pixels per cm for 4 cm deep images. Data were imported from ImageJ into an Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA). All of the data were then analysed with SPSS version 23.00 (SPSS Inc, Chicago, IL, USA). This method is similar to those used by Croy et al. (2013).

3.6 Image analysis

Length (mm), thickness (mm), and CSA (mm²) of selected ankle structures were measured using ImageJ software (National Institute for Health, Bethesda, MD, USA) from ultrasound images, with the researcher (the PhD student) blinded to the participant groups; the data were then compiled into a Microsoft Excel spreadsheet. The thickness of the structure was the perpendicular linear distance between aponeuroses, while CSA of the tendons and muscles was measured by trace ellipse method with an electronic marker in the software, so that ellipse just surround the echogenic boundary of the tendons and muscles.

3.7 Statistical analyses

A common mistake in the interpretation of the statistical significance of results is equating statistical significance (i.e. observing a result with a p-value less than the pre-established significance level, typically 0.05) with clinical significance. A p-value is the probability that the results are due to chance alone, or in other words, the probability of incorrectly rejecting the null hypothesis. It does not, however, provide any information about the magnitude of the effect or the clinical or practical importance of the findings. Furthermore, p-values are sensitive to sample size and many studies are underpowered (often reflecting difficulties in recruitment). This means that a small effect could be statistically significant if the sample size is very large and, conversely, a large effect may be observed in a sample of small size with the corresponding p-value not supporting statistical significance (Berben, Sereika, & Engberg, 2012).

To complement the traditional null hypothesis testing approach, additional approaches have been proposed regarding the interpretation of the extent of effects or differences in the data. This is termed "effect size" (Sawilowsky, 2009) and focuses on the magnitude of the differences, not just their statistical significance (Coe, 2004). Effect size is ''a statistical expression of the magnitude of the relationship between two variables, or the magnitude of the difference between groups with regard to some attribute of interest'' (Blessing & Forrister, 2015). Effect size estimates have the added value of not being sensitive to sample size (Berben et al., 2012).

The common measure of effect size is *d*, known as Cohen's *d,* also known as the standardised mean difference (McGough & Faraone, 2009). This can be used when comparing two means, such as when a *t*-test is used, and is the difference in the means of the two groups divided by the average of their standard deviations. McGough and Faraone (2009) suggested that d=0.2 is considered a "small" effect size, 0.5 represents a "medium" effect size and 0.8 a "large" effect size.

The normality tests are supplementary to the graphical evaluation of normality (Ghasemi & Zahediasl, 2012). Evaluating the normality is a significant statistical step to determine the appropriate statistical tests (parametric or non-parametric) that must be used.

The Kolmogorov-Smirnov (K-S) test seems to be the most common test for normality. However, researchers have proposed that this test should no longer be applied because of its low power (Ghasemi & Zahediasl, 2012). It is highly recommended to use the Shapiro-Wilk test to assess normality in addition to visual evaluation (Ghasemi & Zahediasl, 2012; Thode, 2002).

Since the data was normally distributed, a series of one-way analyses of variance (ANOVA) tests were performed to investigate significant differences in demographics, length, thickness and CSA for the selected ankle structures between groups (healthy vs coper vs CAI), as well as for the different foot positions (neutral and tension) between the three groups. Post-hoc Bonferroni tests were performed to provide pairwise comparisons with an *a priori* alpha level set at $p < 0.05$. Cohen's d effect sizes were calculated, with $d = 0.20 - 0.49$ considered as a 'small' effect size, 0.50 - 0.79 as 'medium' and > 0.80 as 'large'.

According to Kent State University (2019), dependent *t*-test compares two means that are from the same individual. Thus, dependent sample *t*-tests were done to evaluate for significant differences between right and left limbs. On the other hand, independent sample *t*-tests were used to evaluate the significant differences between male and female, and between normal weight and overweight participants. The difference between equivalent measures was considered to be statistically significant if the corresponding p value was less than 0.05. Data analyses were performed using SPSS software version 23.0.

3.8 Results

3.8.1 Participants

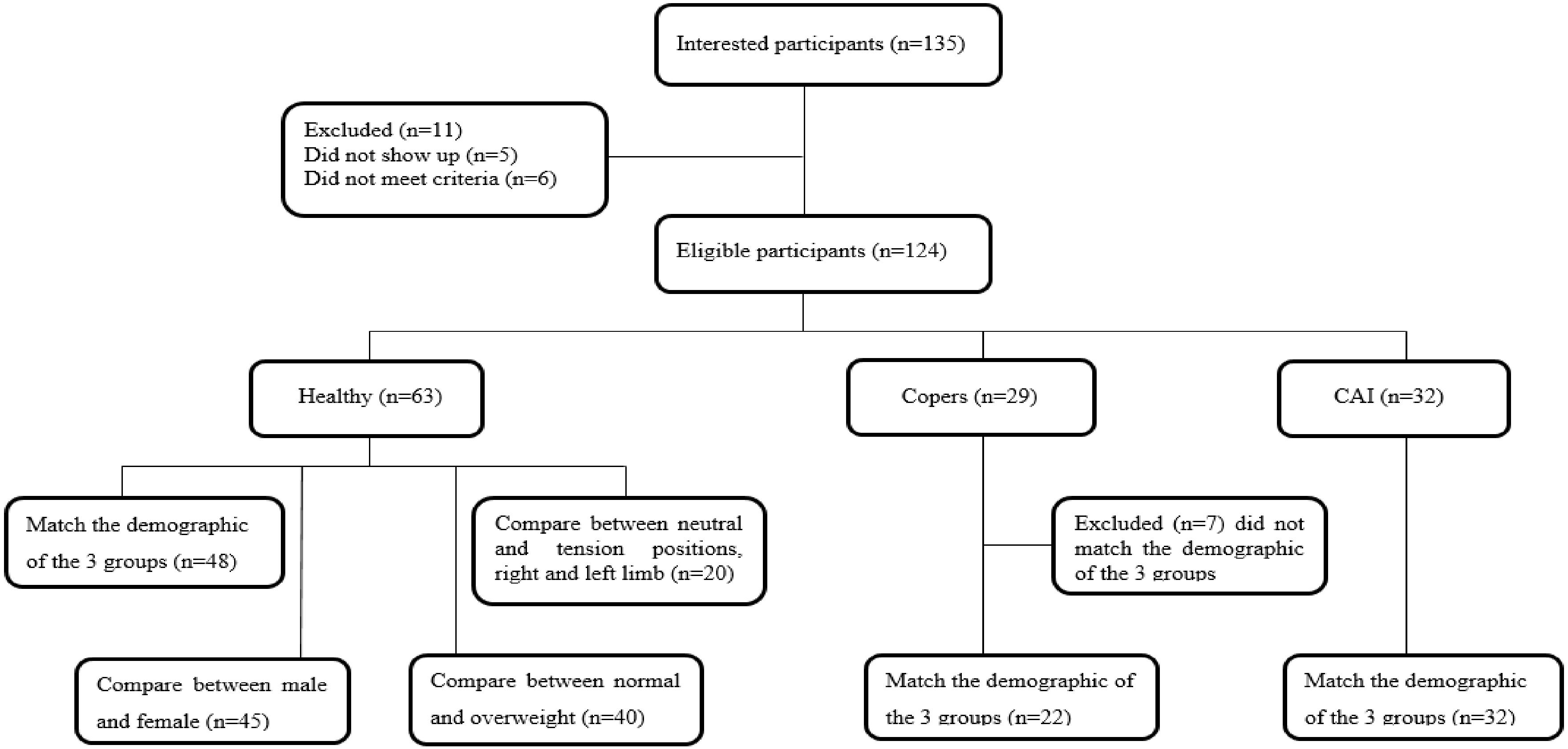


Figure 3-26: Flowchart demonstrating the number of participants in this study

3.8.2 Comparison of length, thickness and CSA of selected ankle structures between healthy, coper and CAI

There was no statistically significant difference in the length of ATFL between the three groups when the ankle was in a neutral position (p=0.57) (Figure 3.27). The ATFL was a large significantly longer when under tension in CAI (23.61 ± 1.10 mm) and coper (23.48 ± 0.82) compared to healthy participants (22.22 ± 1.47 mm) (Figure 3.27). In other words, a statistically large significant difference was found with the ankle under tension ($p<0.001$, $d=1.10$) for healthy versus coper participants, and p-value was equal to 0.001 and Cohen's d was equal to 1.06 for healthy versus CAI participants, but not for coper versus CAI participants (p=0.98, d=0.13) (Figure 3.27). The change from neutral length to tension length in the coper (4.72 mm) and chronically unstable groups (4.79 mm) was greater than that of the healthy group (3.07 mm).

The ATFL was significantly thicker in copers (2.45 ± 0.38 mm) and CAI (2.93 ± 0.31 mm) compared to healthy participants (1.90 ± 0.16 mm), with $p<0.001$ and $d=1.81$, and $p<0.001$ and $d=4.17$ respectively (Figure 3.28). Interestingly, there was a large significant difference in thickness of ATFL between coper and CAI participants ($p<0.001$, $d=1.40$). The CFL was significantly thicker in CAI (1.82 ± 0.12 mm) compared to healthy participants (1.68 ± 0.15 mm) ($p=0.003$, d =1.03) (Figure 3.28). Whilst not statistically significant, the thickness of CFL had a large effect size when comparing copers to healthy participants ($p=0.87$, $d=1.03$) and copers to CAI participants ($p=0.08$, $d=0.90$). There were no meaningful or significant differences in thickness and CSA of the tendons and muscles between healthy, coper and CAI participants ($p>0.05$ and $d<0.2$) (Figures 3.28 – 3.29). The descriptive numerical values of length, thickness, and CSA of selected ankle structures between the three groups are presented in detail in the Appendix 12.

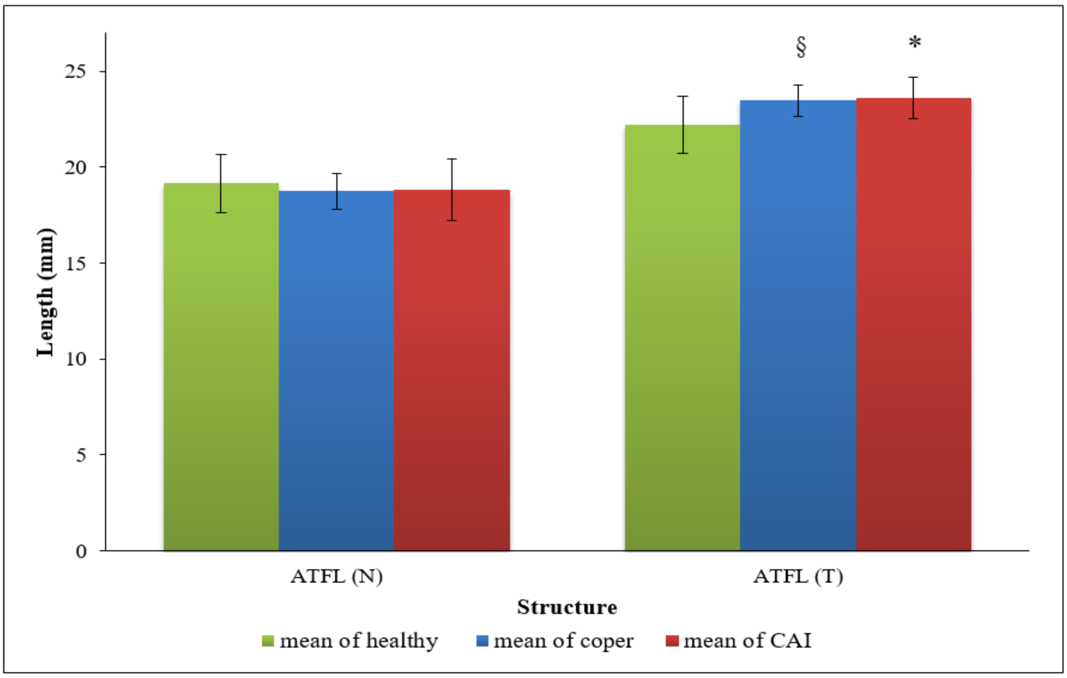


Figure 3-27: The mean and SD of the length (mm) of ATFL in neutral and tension positions. (N) Neutral, (T) Tension between the three groups

** CAI is statistically significant longer than healthy participants*

§ Coper is statistically significant longer than healthy participants

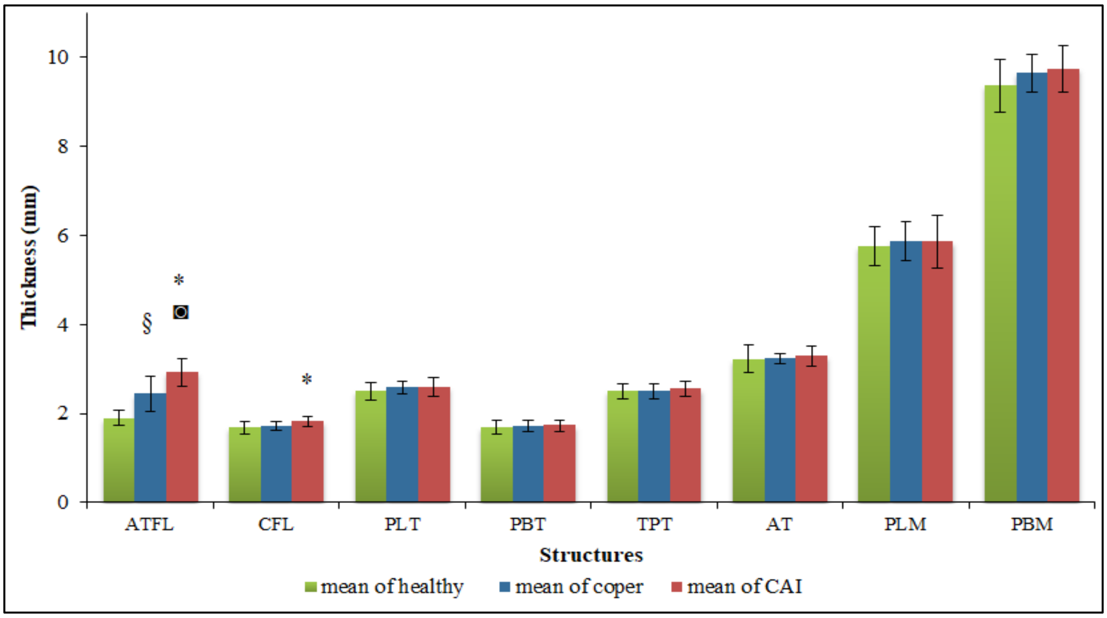


Figure 3-28: The mean and SD of thickness (mm) of selected ankle structures between the three groups.

** CAI is statistically significant thicker than healthy participants.*

§ Coper is statistically significant thicker than healthy participants.

◙ *CAI is statistically significant thicker than coper participants.*

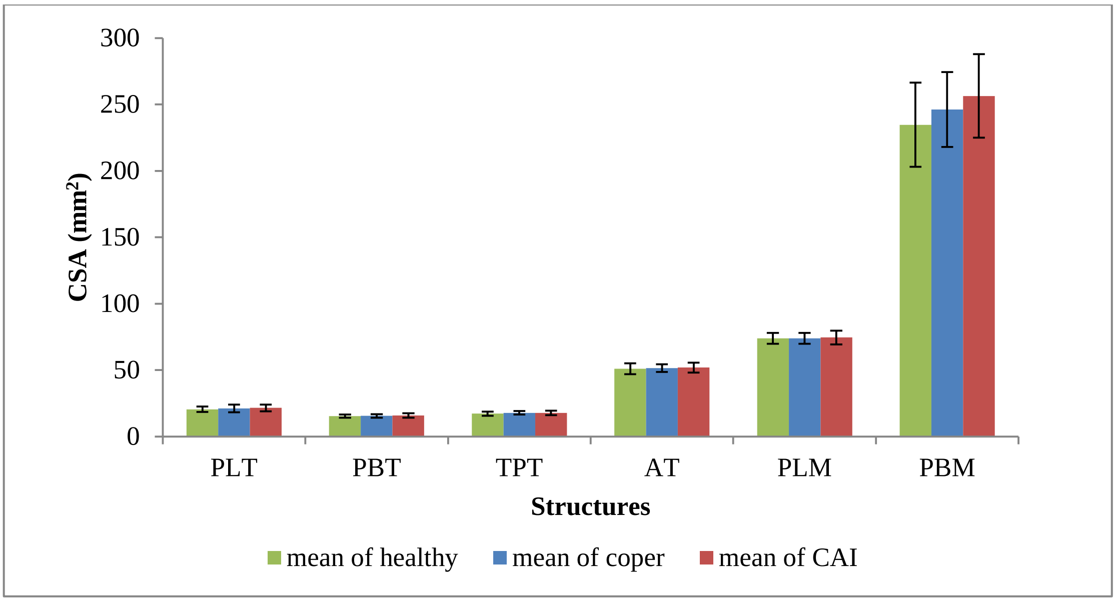


Figure 3-29: The mean and SD of the CSA (mm^2) of selected ankle structures between healthy, coper and CAI participants.

3.8.3 Comparison between neutral and tension position among healthy participants

The descriptive values for selected ankle structures for neutral and tension position are presented in detail in Appendix 13.

The length of ATFL in two positions (neutral (N) and tension (T)) is represented in Figure 3.30. The length of ATFL was significantly longer in tension (21.36 mm ± 2.74) compared to neutral position (18.74 mm ± 1.34).

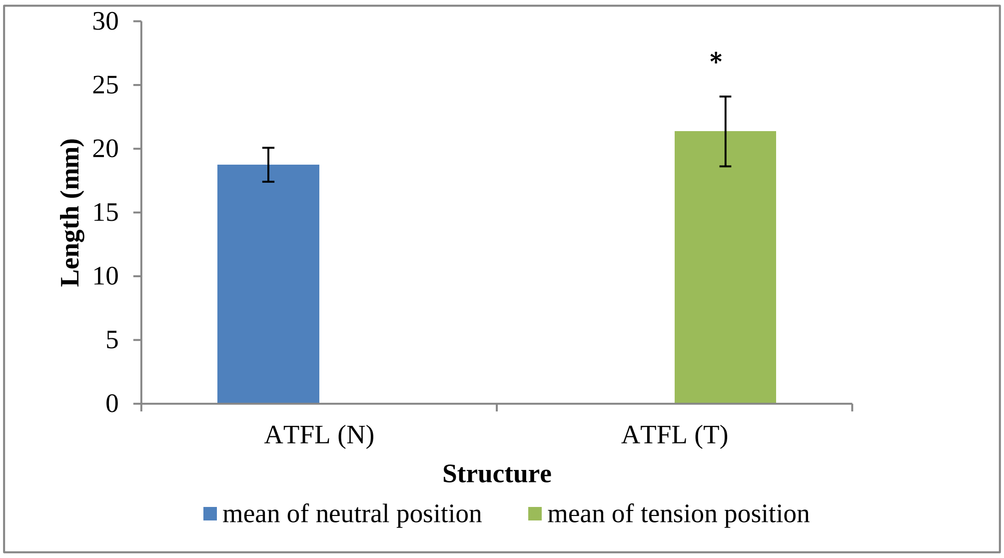


Figure 3-30: The mean and SD of the length (mm) of ATFL in neutral and tension positions. (N) Neutral, (T) Tension
** ATFL is statistically significant longer in tension position compared to neutral position*

The thickness of the measured structures is represented in Figure 3.31. Differences between neutral and tension positions were not statistically significant for the thickness of the selected ligaments, tendons, and muscles.

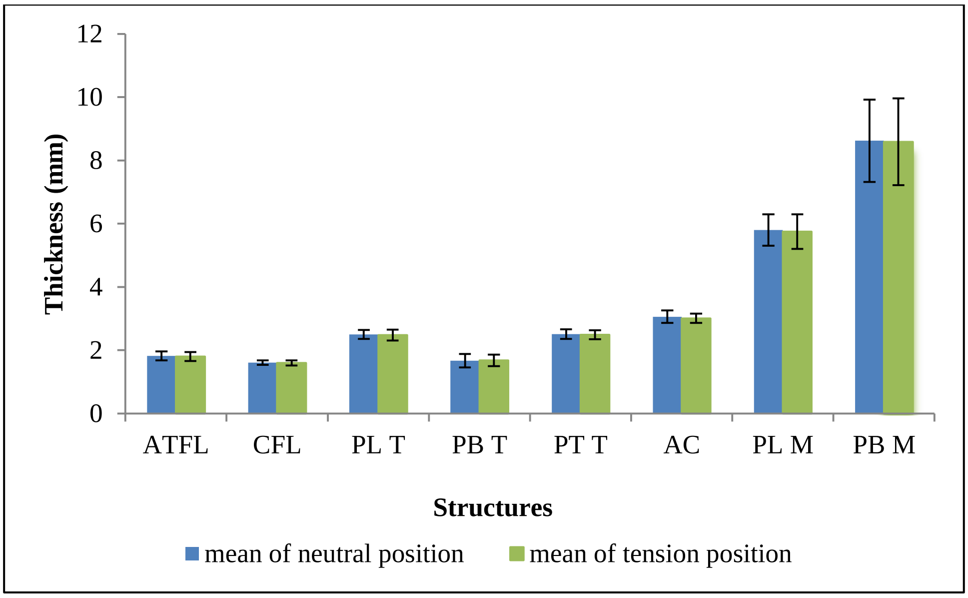


Figure 3-31: The mean and SD of the thickness (mm) of selected ankle structures in neutral and tension positions

The CSA of the measured structures is represented in Figure 3.32. Differences between neutral and tension positions were not statistically significant for the CSA of the selected tendons, and muscles.

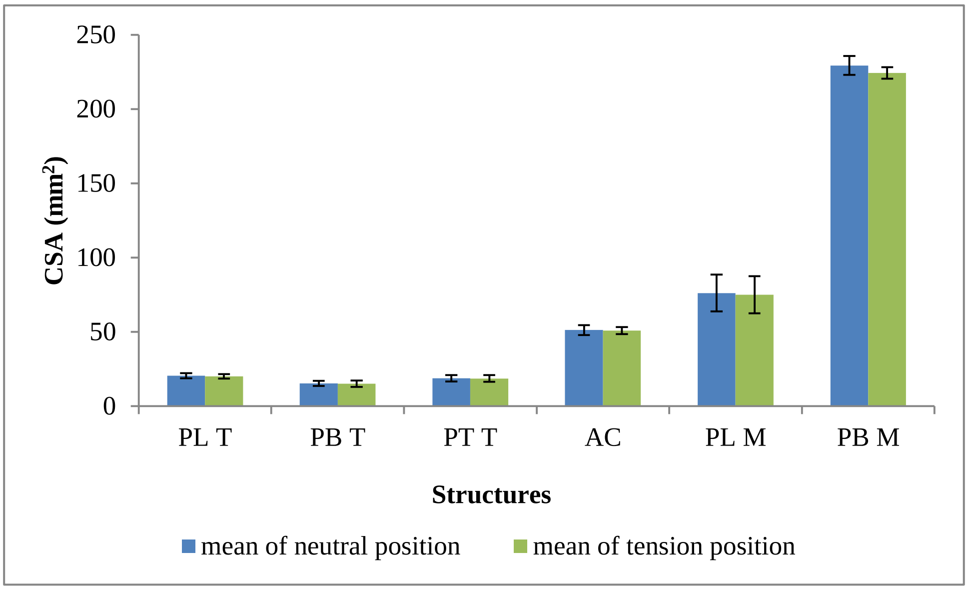


Figure 3-32: The mean and SD of the CSA (mm^2) of selected ankle structures in neutral and tension positions

3.8.4 Comparison between right and left limbs among healthy participants

The descriptive values for selected ankle structures for right and left limbs are presented in detail in Appendix 14.

The length of ATFL in two positions (neutral and tension) and a comparison of the right (RT) and left (LT) limbs are represented in Figure 3.33. Differences between RT and LT limbs were not statistically significant for the length of ATFL (Figure 3.33).

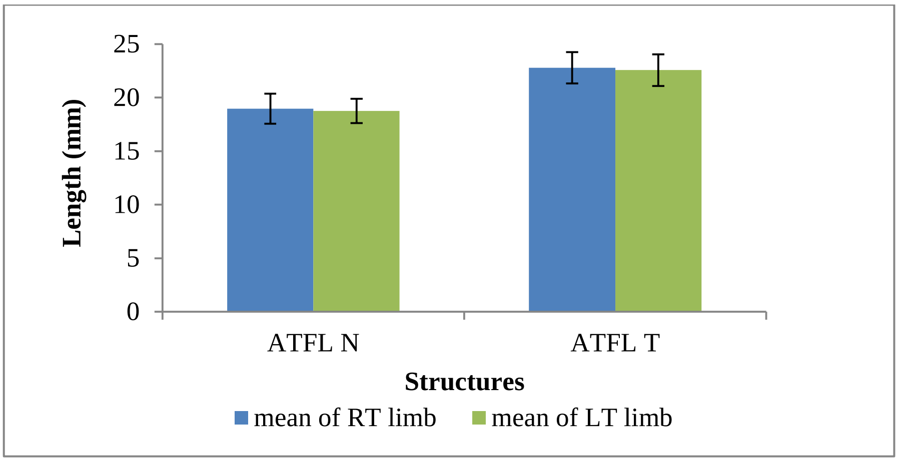


Figure 3-33: The mean and SD of the length (mm) of ATFL in two positions in RT and LT limbs

The thickness of the measured structures and comparison of the RT and LT limbs are represented in Figure 3.34. Differences between RT and LT limbs were not statistically significant for the thickness of the selected ligaments, tendons and muscles (Figure 3.34).

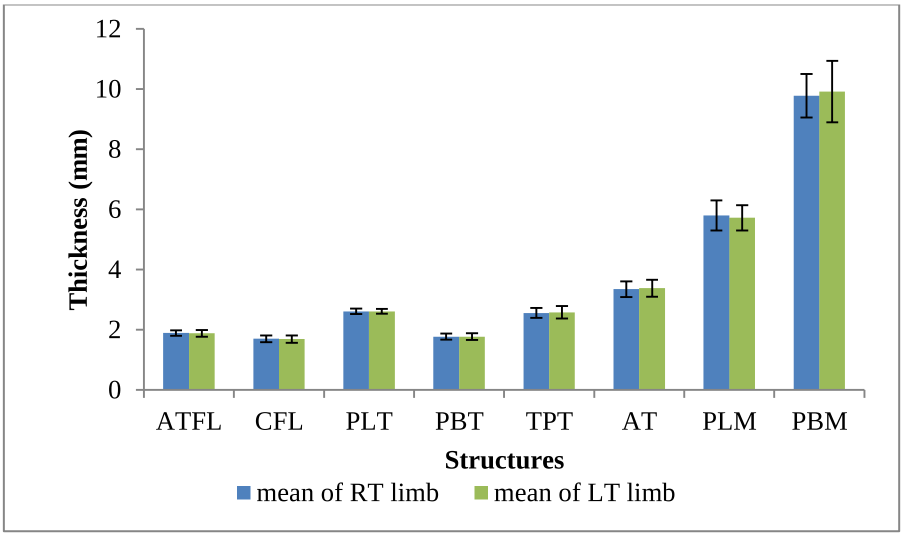


Figure 3-34: The mean and SD of the thickness (mm) of selected ankle structures in RT and LT limbs

The CSA of the measured structures is represented in Figure 3.35. Differences between RT and LT limbs were not statistically significant or meaningful for the CSA of the selected tendons and muscles (Figure 3.35).

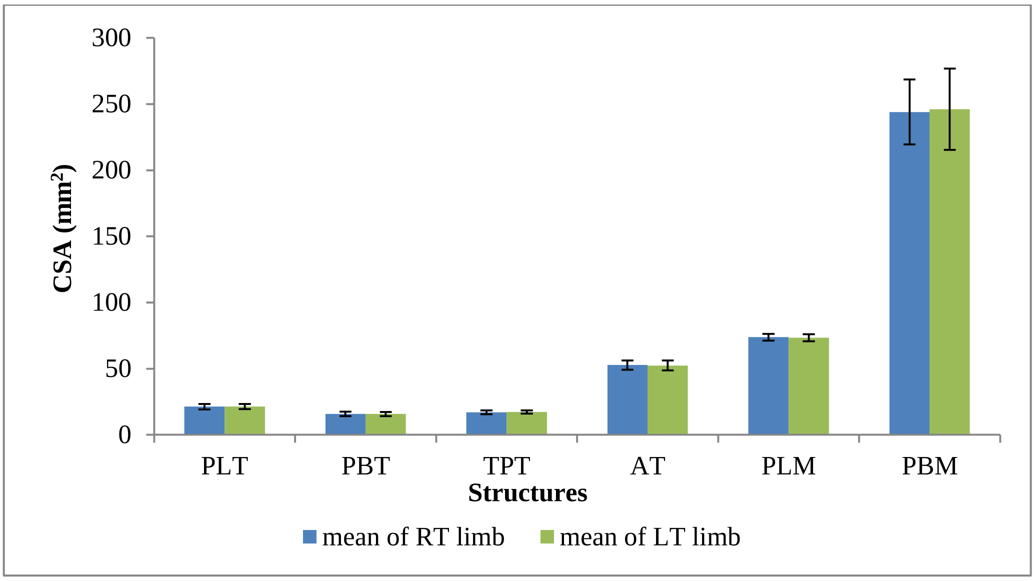


Figure 3-35: The mean and SD of CSA (mm^2) of selected ankle structures in RT and LT limbs

3.8.5 Comparison between male and female healthy participants

The descriptive values of selected ankle structures compared between male and female healthy participants are presented in detail in the Appendix 15.

The length of ATFL in two positions (neutral (N) and tension (T)) and a comparison the female and male participants are represented in Figure 3.36. Differences between male and female participants were not statistically significant for the length of ATFL (Figure 3.36).

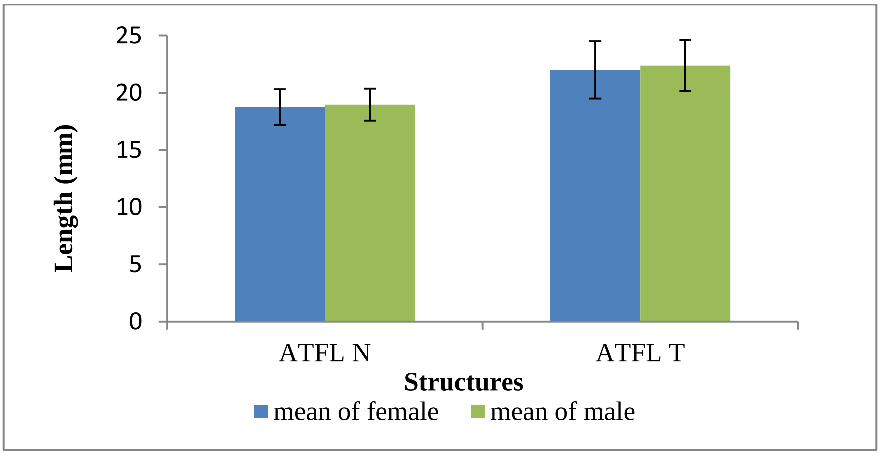


Figure 3-36: The mean and SD of the length (mm) of ATFL in two positions in female and male healthy participants

The thickness of the measured structures and a comparison of male and female participants are represented in Figure 3.37. Differences between male and female participants were not statistically significant or meaningful for the thickness of the selected ligaments, tendons and muscles (Figure 3.37).

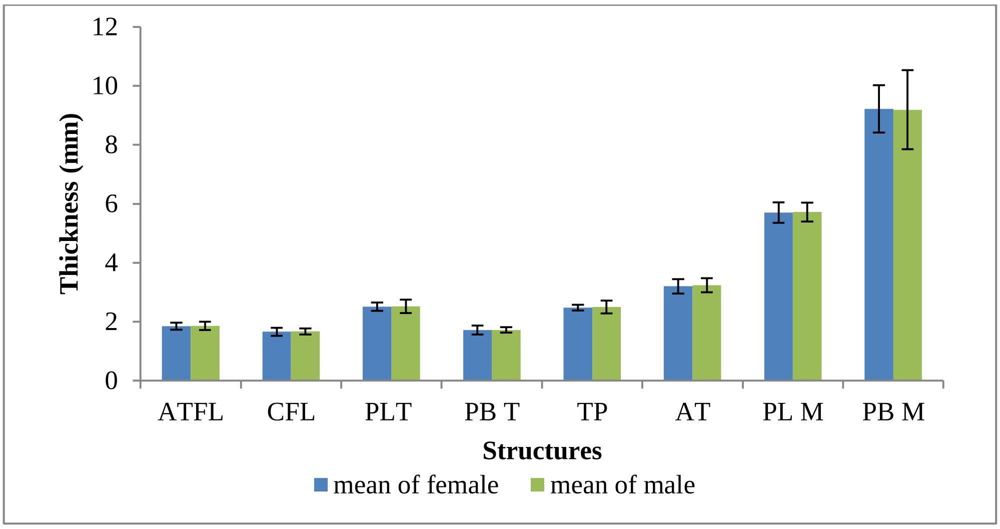


Figure 3-37: The mean and SD of the thickness (mm) of selected ankle structures in female and male healthy participants

The CSA of the measured structures is represented in Figure 3.38. Differences between male and female participants were not statistically significant for the CSA of the selected tendons and muscles (Figure 3.38).

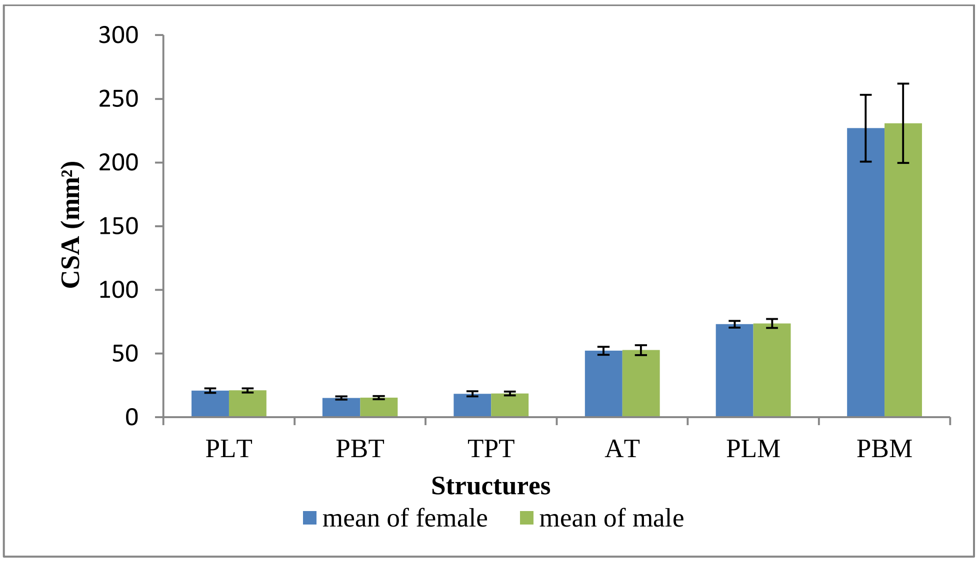


Figure 3-38: The mean and SD of CSA (mm²) of selected ankle structures in female and male healthy participants

3.8.6 Comparison between normal weight and overweight participants

The descriptive values of selected ankle structures for normal weight and overweight participants are presented in detail in the Appendix 16.

The length of ATFL in two positions (neutral and tension) and a comparison of the normal weight and overweight participants are represented in Figure 3.39. Differences between normal weight and overweight participants were not statistically significant or meaningful for the length of ATFL (Figure 3.39).

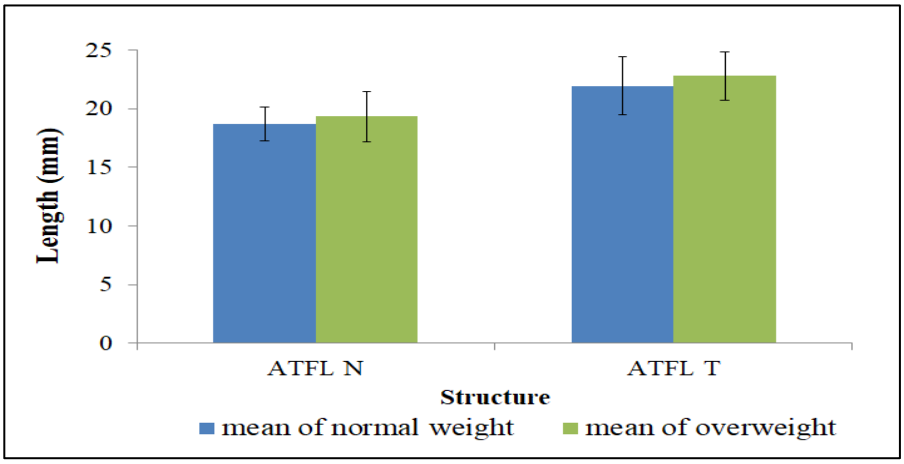


Figure 3-39: The mean and SD of the length (mm) of ATFL in normal weight and overweight groups

The thickness of the measured structures and a comparison of the normal weight and overweight participants are represented in Figure 3.40. The thickness of the selected tendons and muscles was greater in overweight participants compared to normal weight participants. Whilst the p-value showed no statistically significant difference in the thickness of ATFL and CFL between the normal weight and overweight participants (0.06 and 0.20 respectively), the effect sizes showed a small to moderate effect (0.61 and 0.47 respectively).

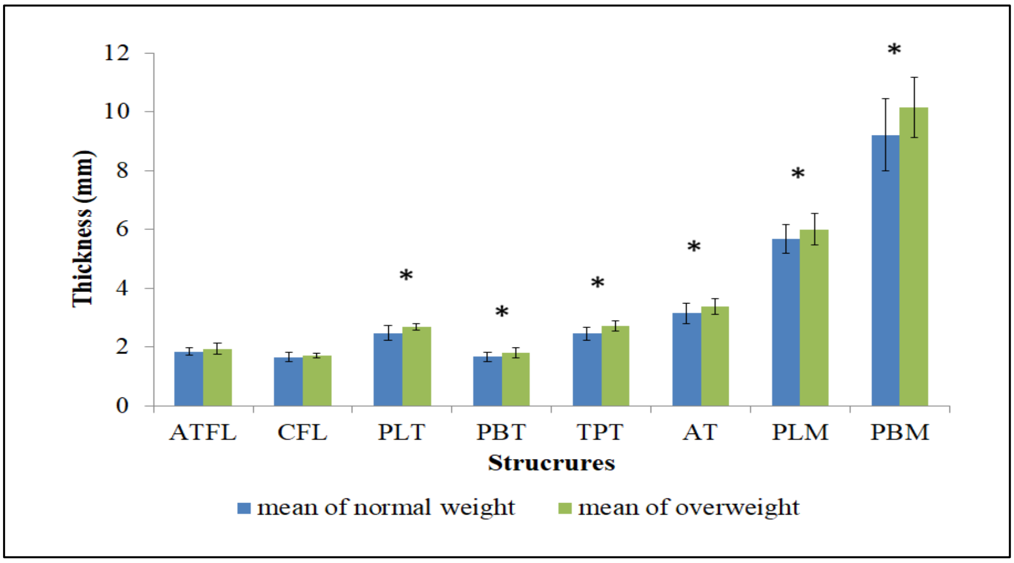


Figure 3-40: Thickness (mm) of the selected ankle structures in normal weight and overweight participant
** Thickness was statistically significant greater in overweight participants compared to normal weight participants*

The CSA of the measured structures is represented in Figure 3.41. Differences between normal weight and overweight participants were statistically large significant for the CSA of all of the selected tendons and muscles.

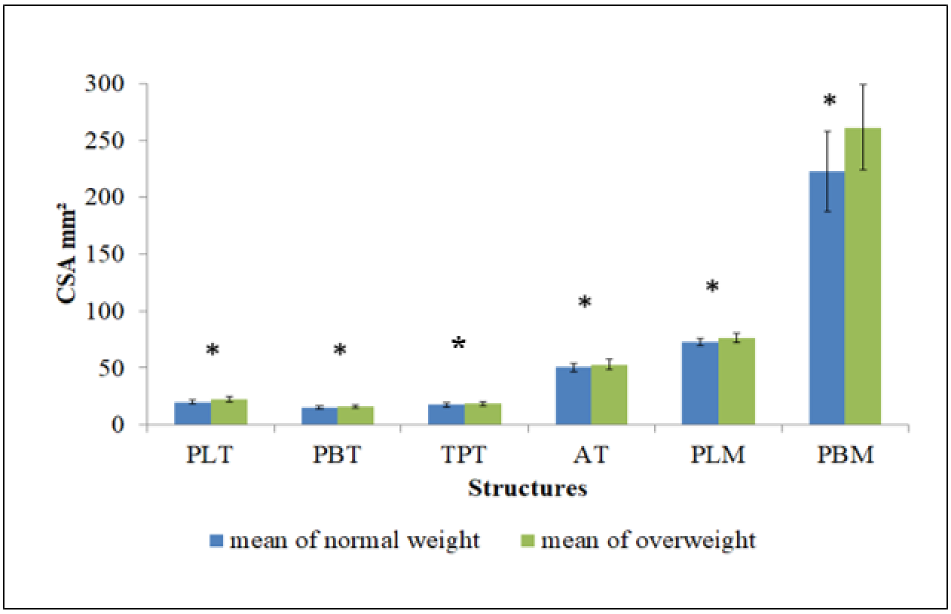


Figure 3-41: The mean and SD of CSA (mm²) of the selected ankle structures in normal weight and overweight participants
** CSA was statistically significant greater in overweight participants compared to normal weight participants*

3.9 Discussion

3.9.1 Comparison of the length and thickness of the ATFL between healthy, coper and CAI groups

The main finding of this study is that participants with a history of lateral ankle sprain had a significantly greater change in the length of their ATFL when the ankle was placed in tension position, and change in the thickness of the ATFL and CFL compared to healthy participants. Even though CAI and coper participants showed increased laxity and thickness of the ATFL, coper participants did not report ankle instability or score low on CAIT.

The ATFL was significantly more elongated at tension position in CAI participants followed by coper and then healthy participants (23.61 ± 1.79 mm, 23.48 ± 0.82 mm, and 22.22 ± 1.27 mm respectively). While no statistically difference was found in neutral position (18.82 ± 2.1 mm, 18.76 ± 0.99 mm, and 19.15 ± 1.52 mm). Hypothetically, lengthening the ATFL leads to reduced constraint on the talus relative to the fibula and tibia, allowing it to translate anteriorly or rotate medially relative the fibula (Croy et al., 2012). In other words, stretched or elongated ligaments over a particular point for long period of time become unable to retain to their original shape. As it happened in our coper and CAI participants, with the ATFL of CAI participants become more lax and lose their ability to support the joint properly which could lead to ankle joint instability which demonstrated in decrease reach distance of the SEBT as shown later in chapter five.

Even though the study demonstrated that ATFL was significantly longer in coper and CAI compared to healthy group, no significant difference was observed between the coper and CAI participants. This finding supports Croy et al. (2012) and Liu et al. (2015) who also found no statistically significant differences in the length of ATFL between coper and CAI groups. Given one of our criteria for CAI group was a feeling of “giving away”, this infers that changes in the ATFL are not an obvious explanation for these experiences. In addition, the syndrome of CAI is probably precipitated by a traumatic injury, getting worse by the dysfunction of sensorimotor and several episodes of ankle sprain reoccurrence. These could be aggravated by potential undiagnosed cartilage injuries which could be related to osteoarthritis and poor outcomes (Choi, Lee, & Han, 2008; Valderrabano, Hintermann, Horisberger, & Fung, 2006).

Baezegari and colleagues (2017) reported that the ligament is considered to be thick when the width is increased more than 2.4 mm or more than 20% of normal lateral ligament. Several previous studies have compared the thickness of the ATFL in healthy and injured ankles. The present study measured the thickness of healthy ATFL to be 1.90 ± 0.16 mm which increased to 2.45 ± 0.38 mm in coper and to 2.93 ± 0.31 mm in CAI. The thickness of ATFL was found to have increased by approximately 54.21% compared in CAI participants to healthy participants and increased by almost 29% compared in coper participants to healthy participants. The thick ligament could be occur due to the scar because of the healing of the ligament (Cai et al., 2017).

Hua et al. (2008) also reported an increased thickness of the ATFL in acute ankle sprains, from 1.46 ± 0.21 mm in healthy ankles to 2.71 ± 0.49 mm in injured ankles. An ultrasound study measured the thickness of the ATFL in healthy and chronic ankle instability groups (Liu et al., 2015). The authors reported that ATFL was thicker in people with CAI (2.28 ± 0.53 mm) compared with the healthy group (1.97 ± 0.42 mm). Using MRI rather than ultrasound, Kijowski and Tuite (2015) deduced that a chronic ankle sprain leads to a thickening of the ligament without surrounding haemorrhage and edema.

Contrary to the results of Dimmick et al. (2009), whose MRI-based data study indicated there was no statistically significant difference in the thickness of the ATFL between normal and injured participants. In men, the mean thicknesses of healthy and previously injured ATFLs were 2.44 ± 0.49 mm and 2.26 ± 0.53 mm respectively, and 2.16 ± 0.47 mm and 2.18 ± 0.61 mm respectively for females. It is possible that these results were due to the very small sample size used in the study (male: ten healthy and nine injured, female: nine healthy and five injured). Moreover, there was no information regarding the type of ankle injury, whether it was acute or chronic, or how long it had been since the participants were injured.

Whilst not statistically significant, the ATFL in the present study was almost 20% thicker in the CAI group compared to copers. This contrasts with Liu et al. who found no such difference, although they only reported a 15% greater thickness in CAI compared to healthy participants, and the equivalent figure in this work is 54.21%. The critical difference between Liu et al. and this work is that in Liu's CAI participants were selected based on CAIT score regardless the number of previous ankle sprains and the sensation of "giving away". This is contrary to recent definitions of CAI (Gribble et al., 2014). In contrast we did not differentiate participants exclusively by CAIT scores, but used the number of prior sprains and the sensation of “giving away” to differentiate CAI and copers.

In order to decrease 70% of the reoccurrence of ankle sprain, it is important to fully understand the reason that prevents coper people from having another sprain (Liu et al., 2015). The finding of our study showed that the morphologies of the coper are different from healthy ankles. This is contrary to previous studies which have suggested that morphologies of the coper are similar to healthy ankles. They concluded that structure of the ligament is not the only caused for ankle instability.

3.9.2 Comparison of the thickness of CFL between healthy, coper and CAI groups

The CFL is often involved in ankle ligament sprains and chronic lateral instability (Kitsoulis et al., 2011). In other words, the CFL affected in 50 - 75 % of cases of ankle sprain (Apoorva et al., 2014). Thus, a good knowledge and understanding of ankle anatomy is essential for diagnosis injuries. In the current study, the mean thickness of CFL for healthy participants was 1.68 ± 0.13 mm, which matches results from earlier cadaveric studies by Apoorva et al. (2014) and Kitsoulis et al. (2011) (1.64 ± 0.43 mm and 1.60 mm respectively). The result of our study showed increased CFL thickness by 8.3% in CAI participants compared to healthy participants. This is in line with Hua et al. (2008) who used MRI and CT to report thickness increased in acute ankle sprain. Whilst not statistically significant the thickness of CFL had a large effect size when comparing copers to healthy (p =0.87, d =1.03) and copers to CAI (p = 0.08, d = 0.90). To the best of our knowledge this is the first study to measure the CFL in healthy, coper and CAI groups by using musculoskeletal ultrasound.

3.9.3 Comparison of the thickness and CSA of selected ankle structures between healthy, coper and CAI groups

It has been reported that inversion ankle sprain may not only damage the ATFL and CFL, but it may also disturb the peroneal tendons (Park et al., 2010). However, the data in this present study does not support this, with no statistically significant change observed in the thickness and CSA of peroneal tendons between healthy, coper and CAI participants.

The thicknesses of healthy peroneal longus and brevis muscles are in line with those of previous studies. Angin et al. (2014) reported the average thickness of peroneal muscles as 13.0 ± 1.8 mm and Crofts et al. (2014) measured the thickness of peroneal muscles as 13.5 ± 1.9 mm. However, these two studies measured the full thickness of peroneal muscles without separating PLM from PBM. Furthermore, the results of the present study did not reveal any significant change in the thicknesses of peroneal muscles between the three groups. This is the first data to demonstrate the thickness of peroneal muscles separately in healthy, coper and CAI groups.

In addition to measuring the thicknesses of the peroneal muscles, the present study measured the CSA of the peroneal muscles; PLM values in healthy ankles (73.9 ± 4.2 mm^2), in coper (74.0 ± 4.1 mm^2), and in CAI (74.5 ± 5.2 mm^2). However, Lobo et al. (2016) reported that the CSA of PLM in healthy ankles was 50 ± 20 mm^2 and in LAS was 40 ± 20 mm^2. On the other hand, CSA of PBM in the current study was 234.7 ± 31.7 mm^2 for healthy, 246.2 ± 28.1 mm^2 for coper, and 256.4 ± 31.5 mm^2 for CAI ankles. A previous study demonstrated that CSA of PBM in healthy ankles was 350 ± 70 mm^2 and in LAS was 320 ± 70 mm^2 (Lobo et al., 2016). Furthermore, Angin et al. (2014) and Crofts et al. (2014) reported the CSA of peroneal muscles for healthy ankles to be 382 ± 63.0 mm^2 and 409 ± 125.0 mm^2 respectively. However, they do not separate the PLM from PBM during the measurement. The findings of the current study demonstrated that the CSA of peroneal muscles in coper and CAI groups does not differ from that of healthy group. However, this finding does not support the previous research, which demonstrated that the CSA of the peroneus longus muscle is reduced in LAS participants compared to healthy ones. The age of control and injured participants in Lobo's study were statistically significantly different (p <0.05) and Kim, Ko, Lee, Ha, & Lee, (2015) and Fujiwara et al. (2010) found that the thickness and CSA of lower extremity muscles changes with age. The reasons why PLM are the only muscles to be changed with LAS in Lobo's study were unclear. Moreover, the differences in the measurement of peroneal muscles between the studies could be due to the differences in inclusion criteria for the groups, differences in the techniques or the traces that are used to outline the boundary of muscles.

Since unrecognised acute Achilles tendon has been reported in severe ankle sprain (Lam & Lui, 2015), and it has been reported that 12.2% of ankle sprain cases involved Achilles tendinitis (Fallat et al., 1998), it was important to include Achilles tendon in this study to have a baseline knowledge for accurate diagnosis. The results from the present study demonstrates that the thickness and CSA of AT in healthy was 4.01 ± 0.61 mm and 51.0 ± 4.5 mm^2, in coper was 4.03 ± 0.53 mm and 51.40 ± 2.90 mm^2, and in CAI was 4.01 ± 0.62 mm and 51.90 ± 3.80 mm^2 respectively which reveal no statistically significant differences being found between the three groups. Daftary & Adler (2009) stated that the thickness of a normal AT should not exceed 6 mm, based on their ultrasound study. Hodgson et al. (2012) evaluated the thickness of AT in patients with spondyloarthritis and a healthy control group using MRI and ultrasound imaging. They reported that the close to the data obtained from previous studies. Ying et al. (2003) found that the CSA of AT at the level of the medial malleolus was 56.91 ± 7.58 mm², and Yildiz and Turksoy (2007) reported the CSA as 60.78 ± 13.09 mm² (based on 80 ankles). A possible explanation of the smaller value in the present study may be due to the differences in calliper settings. The present study does not include the paratenon (superficial areolar connective tissues upon which the AT rests) (Shapiro, 2015) of the tendon during the measurements.

Results of this study showed that there were no statistically significant differences in the thickness and CSA of TPT between the three groups. The present study followed Neville et al's (2010) protocol, according to which the thickness was measured at approximately 2 cm proximal to the tip of the medial malleolus, and finding the thickness of TPT to be 2.9 mm, while in this study it was measured as 2.50 ± 0.17 mm. However, Perry et al. (2002) measured the thickness of TPT using longitudinal ultrasound images at around 1 cm distal to the end of the medial malleolus and found the thickness to be 3.0 ± 0.7 mm. The results of these studies are consistent despite that the authors taking measurements from different locations.

The researcher used CSA and the thickness as surrogates of the force passing through the structures and their functional role. Increased thickness and CSA could reflect increased mechanical loading on tendons due to increases in muscle strength or use (Taş et al., 2017). In the face of evidence for no differences in muscle or tendon structures, the efficiency of motor control strategies during inversion incidents is perhaps a more likely explanation for differences between copers and CAI.

Knowing the CAI is a complicated issue, it is essential to acquire a comprehensive understanding of all the possibility changes that occurred to an individual to realise the differences between who experience several ankle sprains and those who are classify as ankle copers (Liu et al., 2015).

3.9.4 Comparison of selected ankle structures between neutral and tension position

It has been stated that an increase in the ATFL length beyond 20%, equivalent to 4 mm, is enough to cause ligament failure and joint instability (Croy et al., 2012; Jeys, 2004). No previous studies involving healthy ankles have observed 20% ATFL lengthening.

However, in this current study, the length of the ATFL in CAI participants increased by 4.79 mm between the neutral (18.82 mm) and the tension (23.61 mm) positions, a change of approximately 25.45%. This is in line with Croy et al. (2012) who used stress ultrasonography during inversion and anterior drawer tests. They found that the length of the ATFL in CAI participants increased by 4.7 mm from the neutral (18.8 mm) to the inversion positions (23.5 mm), a change of 25%. However, the length in healthy participants did not exceed 15% from neutral (19.15 mm) to tension (22.22 mm) positions. This illustrates the importance of studying ligament competency under loaded conditions and not simply passive ligament morphology. Increases were circa 16% in our healthy group, 25.2% in the coper group, and 25.45 % in the CAI group.

These findings compare favourably with the finding of de Asla et al. (2009), who measured the change in the ATFL healthy male participants when the foot was moved from a neutral position (16.3 ± 3.00 mm) to a maximal plantarflexion (20.8 ± 2.7 mm) using a combined dual-orthogonal fluoroscopic and MRI techniques. Khawaji and Soames (2015) also measured the changes in ATFL length in their sample of 50 cadaveric feet. They reported that the length of the ATFL changed from 20.17 ± 3.4 mm to 21.06 ± 06 mm during plantar flexion from a neutral position, in a study using electronic digital Vernier callipers.

When the foot is positioned in the plantar flexion position, the alignment of the ATFL fibres adapts to this position and most likely plays the role of the main collateral ligament in constraining further movement. Because most lateral ankle sprains happen during plantar flexion, the ATFL would therefore be the initial lateral collateral ligament to be affected and damaged (de Asla et al., 2009). This concept has been corroborated by several in-vitro studies which have investigated strain in the ATFL in response to loading in different foot positions. Renstrom et al. (1988) found that the strain of ATFL increased by 3.3% when the ankle joint moved from 10° of dorsiflexion to 40° of plantarflexion. Stephenson, Charlton & Thordarson (2012) found that strain on the ATFL increased as the ankle moves from 20° of dorsiflexion to 30° of plantar flexion. The highest rate of increase occurred between the neutral position and 20° of plantar flexion (Colville et al., 1990). Colville et al. (1990) evaluated the strain in lateral ankle ligaments using implanted strain gauges, by moving the foot through inversion, eversion, and internal and external rotation whilst flexing, along with on-extension arc of motion in ten cadaveric ankles. They found that the greatest rate of increased ATFL strain occurred when the foot changed position from neutral to 20° of plantar flexion. Moreover, Ozeki et al. (2002) studied strain in the central fibres of the ATFL in 12 cadaveric ankles in plantar flexed and dorsal flexed positions. Strain was found to increase as the ankle was plantar flexed, inverted and internally rotated. This helps to explain why the ATFL is the ligament affected in all cases of inversion ankle sprains, since it is the most important for movement restraint and yet also the weakest (Attarian, McCrackin, Devito, McElhaney, & Garrett, 1985; Jeys, 2004). These studies proposed that the ATFL would tighten in plantar flexion. Thus, excessive and explosive plantar flexion when the ankle joint is inverted could produce stress and may rupture the ATFL (Fong et al., 2012b).

The viscoelastic properties of the ATFL along with the crimped arrangement of collagen fibres, influence the mechanical behaviour of the ATFL under load (Hauser et al., 2013). The relationship between the mechanical properties of the ligaments and changes in the crimp pattern is expressed in terms of their stress-strain relationship. The four regions of the stress-strain curve are illustrated in Figure 3.42.

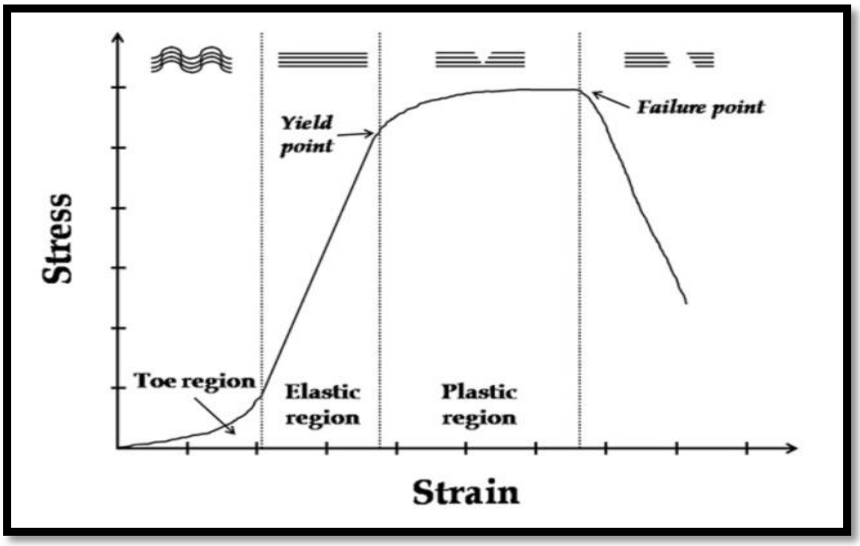


Figure 3-42: Four regions of the stress-strain curve (Korhonen & Saarakkala, 2011)

When the foot is in a neutral position, the ATFL is largely unstressed and the collagen fibres bind with elastic and reticular fibres to create the sinusoidal pattern referred to as the "crimp" pattern (Raut & Mate, 2016). This pattern is demonstrated by the first region of the stress-strain curve, or the toe region. When force is initially applied to the tissue it is transferred to collagen fibrils, causing the fibrils to laterally shrink, water to be released, and the crimp of the ligament to be progressively lost as the ligament is elongated, until all fibres are almost straight. This second region of stress-strain curve is called the elastic (linear) region (Raut & Mate, 2016). As the ligament is exposed to more strain, the microstructure of the ligament is disrupted and partially tears (third, or plastic region). Further elongation causes a complete rupture of the ligament (failure region) (Raut & Mate, 2016).

A computational biomechanics study has been done to study the effect of different combination of joint movement on the strain of different ankle ligaments (Fong et al., 2011). The authors found that the highest strain was seen in the ATFL with combination of plantar flexion, inversion, and internal rotation (Fong et al., 2011) which could be the most probably the mechanism of the ankle sprain injury for our participants since ATFL was the most affected ligament compared to CFL.

Whilst this study demonstrated no statistically significant differences in the thickness and CSA of the provided ankle structures between the neutral and tension positions, it is important for the sonographer to know the appearance of each structure in different ankle joint positions in order to provide an adequate patient position which plays a significant role in providing an appropriate ultrasound image. In addition, knowing that there was no difference between the neutral and tension positions. Neutral position was considered to be the optimal position for ultrasound imaging of the selected ankle structures (PBT, PLT, TPT, AT, PBM, and PLM) without placing the participant's feet into tension position, making the scan easier for both participants and sonographer.

3.9.5 Comparison between right and left limbs of healthy participants

The present study evaluates the length, thickness and CSA of selected ankle structures in 20 healthy ankles to find the degree of symmetry between the right and left limbs. The present study found that the length of the ATFL and thickness of the ATFL and CFL were the same for the right and left ankles. This finding supports Khawaji and Soames (2015), who showed that the length of the ATFL did not differ between left and right ankle in males and females, and Liu et al. (2015), who also found no differences between right and left in healthy ankles. In addition, the mean CFL thickness was found to be similar between right and left healthy ankles (Apoorva et al., 2014).

No significant variation in the thickness and CSA of the tendons and muscles was found. This result is in line with those of previous studies. Bjordal et al. (2003) concluded that the differences in Achilles tendon thickness between the two sides were very small and a difference of only 0.8 mm could be established for healthy tendons. Andrade et al. (2006) reported that the thickness of Achilles tendons between right and left in 100 healthy ankles was similar. Furthermore, Ying et al. (2003) found that the thickness of Achilles tendons in the dominant and non-dominant ankles was similar in both the frequent exercise group (> 6 hours every week) and the infrequent exercise group (< 1 hour every week). It has been reported previously that there was a significant difference in the thickness of leg muscles between dominant and non-dominant legs of junior soccer players because of the preferential use of one limb over another (Kearns, Isokawa, & Abe, 2001). This is consistent with previous research on Gaelic footballers which found a similar dominance effect in the anterior tibial muscles with the dominant side being 7.3% thicker. These differences were not seen in non-football players and thus the difference observed in footballers could be related to sport-specific performance (McCreesh & Sinead, 2011). Sonographers may take this difference into consideration when scanning sports players to evaluate the dimensions of their muscles.

It was important for the researcher to compare the length, thickness and CSA of selected ankle structures between the right and left limbs to have a complete characterisation of healthy ankles and to know if it is necessary for the researcher to scan either healthy limbs or just one of them for comparison with injured limb.

3.9.6 Comparison between female and male healthy participants

To gain greater insight into sex-based differences in selected ligaments, tendons and muscles, the researcher assessed the length, thickness, and CSA of selected ankle structures from healthy male and female using ultrasound scanning. This ultrasonographic study found that although the ultrasound measurements of the thickness, CSA and the length selected ankle structures (ATFL, CFL, PBT, PLT, TPT, AT, PBM, and PLM) were greater in males with the same BMI, this was not statistically significant. Sarver et al. (2017) recently evaluated the gender differences in tendon structure and function in an in-vitro study. Authors reported that there was a great level of similarity in the morphology and behaviour of tendon between male and female mice. They concluded that, even though there was some variance in the size, cellular composition, transcriptome (i.e. the total set of Ribonucleic acid (RNA) species in a tissue), and proteome (i.e. the total set of proteins in a given tissue) (Passos, 2015), the gender variances in the structure and function of tendon were small (Sarver et al., 2017).

In contrast, Khawaji and Soames (2015) reported significant differences in the length of the ATFL between males and females, and Chow et al. (2000) found that the average muscle thickness was greater in males compared to females. The number and size of muscle fibres play an important role in muscle characterisation (Cheuvront, Moffatt, & DeRuisseau, 2016). While some studies have concluded that males have a greater number of muscle fibres

(Meth, 2001), others have shown males to have the same number as females (Cheuvront et al., 2016). Moreover, authors demonstrated that increased the thickness of the muscles is a result of increase the diameter of a muscle fibre with constant fibre number and length, and muscle length (Maxwell, Faulkner, & Hyatt, 1974). However, Kubo et al. (2003) found that fascicle length of gastrocnemius muscles was similar between male and female. While Chow et al. (2000) demonstrated that female had longer fascicle length of the triceps surae muscles than male. These contradictory results may be due to variations in the methodology and sampling used, and it is difficult to determine if the number of muscle fibres is actually different between genders. Another possible explanation is the changing sizes of muscle fibres. Muscle fibres have different diameters, from 10 to 100 μ (Mair & Tome, 2013) with Cheuvront et al. (2016) and Meth (2001) having deduced that the individual muscle fibres in females are smaller than those in males.

3.9.7 Comparison between normal weight and overweight healthy participants

The present study was also designed to determine the effect of BMI on the length, thickness and CSA of selected ankle structures. BMI is the metric that is now utilised to define adult anthropometric weight/height characteristics and to categorise them into different groups (Nuttall, 2015). The results of this study highlight a significant aspect for consideration in the measurement of selected ankle structures. The thicknesses and CSA of the selected ankle structures (PLT, PBT, AT, TPT, PLM, and PBM) were found to be statistically significant thicker among overweight participants compared to those with normal BMI with p-value less than 0.05. Whilst the p-value showed no significant differences in the thickness of ankle’s ligaments, overweight participants had thicker ligaments with small to medium effect size (Cohen’s $d = 0.47 - 0.61$ for CFL and ATFL respectively). The results of the present study agree with Abate (2014) and Klein et al. (2013), who deduced that a higher BMI can lead to increased AT thickness. The most significant reason behind increased thickness of the tendon could be due to increased mechanical loadings on the tendon because of increased body weight and the increased strength of the muscle (Taş et al., 2017). Mechanical loading is necessary for tendon homeostasis to continue. If the mechanical loading continues increasing, anabolism becomes dominant over catabolism, and the thickness and CSA of the tendon increase through the generation of new extracellular matrix and collagen fibres (Abate et al., 2009; Kubo et al. 2003). Taş et al. (2017) mentioned that the thickness of the tendon increases as a compensatory mechanism to reduce increased mechanical loadings on the tendon and avoid deformation of the tendon due to increased body mass and/or muscle strength.

A number of studies have tested the association between BMI and the size of some ankle structures (Abate, 2014; Klein et al., 2013). Mirza (2016) proposed that BMI is a factor that engaged in altering the physical form of Achilles tendon by changing its thickness. The author concluded from his ultrasound study that the thickness of the AT is BMI-dependent, as overweight participants have a thinner Achilles tendon. In other words, thickness of Achilles tendon decreases as the BMI increases. This, however, contradicts the findings of the current study, where Achilles thickness was found to be greater in those who were overweight. The classification of overweight participants differs between the two studies, with Mirza's overweight category overlapping with the obese in the current study. In addition, Mirza included a very small sample size (5 participants in each group).

3.10 Limitation

The researcher acknowledges some limitations to this study. Ultrasound is considered to be an operator-dependent. It needs a training course to master its scanning techniques. Based on the previous studies, agreement between senior sonographer with 10 years experiences and the beginner sonographer improved within two months (Backhaus et al., 2010). Gun et al. (2013) reported that after a six hour training program to a strict protocol, emergency physicians could utilise bedside ultrasonography to assess patients with suspected ATFL injury. Therefore, they suggested that a relatively short period of training has the ability to allow sonographers to provide adequate US imaging (Backhaus et al., 2010). In addition, the researcher did not classify if injured participants had combined ligament injures to ATFL and CFL, and it could be that these kind of injuries have impact on the results. Despite the limitation, the present study provides novel insight into the structural features of selected ankle structures in healthy, coper and CAI groups.

3.11 Conclusion

Foot and ankle injuries may affect the body function. Therefore, the early diagnosis and particularly designed treatment will improve the prognosis. Ultrasound scanning has been an imaging modality to study muscular tissue for many years. This study characterised and compared selected ankle structures by using ultrasound in participants with and without a history of previous ankle sprain. The findings of study indicate that the ultrasonography is a reliable and effective method that can be utilised to detect participants' ankle ligamentous injury.

Among the selected ankle structures, the length of the ATFL and the thickness of the ATFL and CFL were the only ones which showed statistically longer and thicker in injured groups (coper and CAI) compared to healthy participants. ATFL was longer and thicker in both coper and CAI participants and thicker but not longer in CAI compared to coper participants. For the healthy group, no statistically significant differences were found in the length, thickness, and CSA of the selected ankle structures between the right and left limb, nor females and males. However, the thickness and CSA of most of the selected ankle tendon and muscles was larger in overweight compared to normal weight healthy participants.

Knowledge the characterisation of normal and injured ligaments, tendons, and muscles is important for diagnosing the ankle sprains. Moreover, it is important for planning rehabilitation programmes for patients with ankle sprains, as changing in ligament size has been reported in injured participants. Structural changing of the ankle ligaments opens new horizons to investigate if the thickness of the ankle ligaments is related to functional deficits in injured ankles which lead the researcher to conduct a functional study (chapter five). On the basis that no differences were found in the selected muscles and tendons structures we measured, motor control strategies might be a more likely explanation for differences between coper and CAI cases.

4 Chapter four: Quantitative evaluation of ultrasound images to compare healthy and injured anterior talofibular ligaments

4.1 Chapter overview

A digital ultrasound image is a two-dimensional numerical matrix (Diaz et al., 2015) that reflects signal echoes that have experienced different attenuation (less or high) after passing through various tissue. Digital images are created from a large number of elements named pixels and a pixel has a numerical value and is the fundamental quantify the ultrasound signal (Diaz et al., 2015). Each pixel is represented by grey-level (brightness) intensity, which helps radiologists and sonographers to interpret the structure together with the related anatomical and clinical information (Diaz et al., 2015).

According to Tamborrini (2014), musculoskeletal structures have various echogenicities and different reflection ratios which define their ultrasound appearance. Changes in echogenicity of an injured ligament and tendon are one of the most significant criteria in the use of musculoskeletal ultrasound images (Agut et al., 2009). What we know about the normal sonographic appearance of tendons and ligaments is largely based on subjective evaluations of their echogenicity. The echogenicity of an injured ATFL is 'darker' than the healthy equivalent, but the degree of difference in echogenicity is unknown. Work in this chapter sought to quantify echogenicity in the ATFL, focusing on this structure because chapter three revealed it to be the most structure differing between healthy, coper, and CAI groups. The assumption made in this study was that quantitative analysis of the echogenicity may offer further insights into the nature and scale of changes in the ligaments post-injury.

This study, which is the first of its kind to deal with the ATFL ultrasound images, will benefit and use the information provided by healthcare providers to distinguish of the ATFL between healthy, coper and CAI participants. The quantitative analysis was performed by ImageJ as traditional method in the literature.

4.2 Aims, objectives, and hypothesis of the study

Since the length and the thickness of the ATFL were the only structures different in the injured groups (coper and CAI) compared to healthy participants from the previous study, this study aimed to provide a quantitative analysis of echogenicity of the ATFL. Providing a numerical and objective estimate of echogenicity could enhance our understanding of the structural changes that might have occurred in previously sprained ankles. To achieve this aim, the following objectives were established:

- Evaluate the echo intensity value of the ATFL in healthy, coper and CAI participants by using computer-aided greyscale analysis.
- Evaluate the differences in ATFL echo intensity between the three groups.

Hypothesis: There will be statistically significant differences in the echo intensity value of the ATFL between healthy, coper, and CAI participants.

4.3 Methods

4.3.1 Image dataset

Ultrasound images of the ATFL were obtained using a linear array transducer (12.7 mm x 47.1 mm) with 5-13 MHz of a portable Venue 40 musculoskeletal ultrasound system (GE Healthcare, UK). Data were collected from 60 participants (20 healthy, 20 coper, and 20 CAI) with mean age 28.13 ± 7.33 years and mean BMI 24.47 ± 2.51 kg/m^2. The size of the ultrasound images was 600 x 655 pixels with 256 grey-levels. Ultrasound machine settings (gain and probe frequency) were constant to ensure that the value of greyscale pixel was the same (described in 3.5.8.1). The ultrasound protocol used to scan the ATFL was described previously in section 3.5.8.1.1.

4.3.2 Quantification echogenicity of ATFL

Echo intensity can be measured by taking the average of the greyscale value of every pixel in a particular “region of interest” (ROI) on an ultrasound image (Varanoske et al., 2016). Differences in shades of grey are used to discriminate different kinds of tissue, analyse relationships between the structures, and may reflect physiological function (Balakrishnan, 2014). The mean echo intensity of the ATFL was determined using a computer-aided greyscale analysis of the echo signals in regions of interest.

Longitudinal images of the ATFL were taken as per the previous study (chapter three) in JPEG (Joint Photographic Experts Group) format. However, the images were displayed on a computer monitor which is 5 mega-pixel DOME E5 (NDSsi, Santa Rosa, CA, USA; 2048 by 2560 pixels) calibrated to the DICOM Grey Scale Display Function Standard. All ultrasound images were analysed offline through the standard histogram function in image analysis software (ImageJ, National Institutes of Health, Bethesda, Maryland, USA) to provide quantitative data for echo intensity. The research was blind to the group classification of the participants during image analysis. In each image, the researcher selected three ROIs manually as a circle in the ATFL. The area of each ROI was between 0.045 and 0.05 cm^2. Three ROIs were selected for each ultrasound image to determine three mean grey-level values, The first ROI was midway (50%) between the talus and lateral malleolus and the remaining two were 25% away from the first ROI in both directions. However, defining the boundary of the ligament (talus and lateral malleolus) could be subjective. Therefore, the total area of the three ROIs covered most of the ligament. The average of the three was taken for further analysis. An example of the ROIs is in Figure 4.1. For AT, three ROIs were also selected as a circle (Figure 4.2).

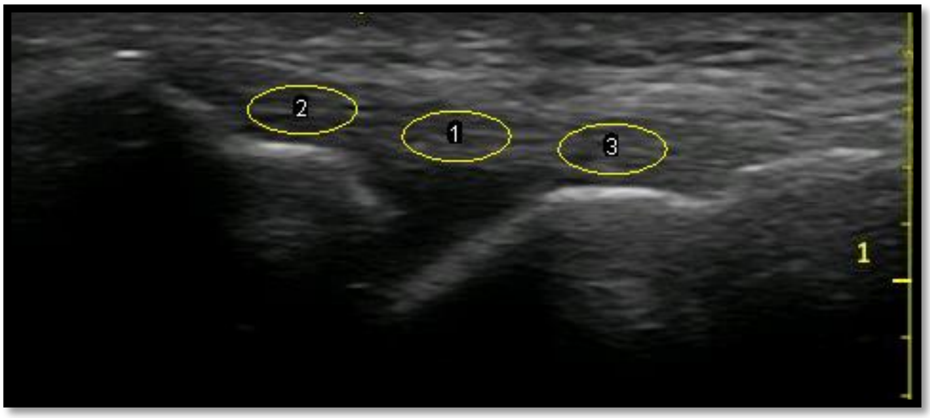


Figure 4-1: Longitudinal US image of ATFL. Three regions of interest on healthy ATFL.

Figure 4-2: Longitudinal US image of AT. Three regions of interest on healthy AT.

Echo intensity in each ROI was determined by computer-aided greyscale analysis using the standard histogram function in ImageJ and measured in greyscale levels. Histograms presented the distribution of the grey-level within each ROI, example is demonstrated in Figure 4.3. The value of the mean grey-level was measured numerically based on the 256 degrees of greyscale labelled by the software, from 0 (black) to 255 (white), for each pixel within the ROI. The mean grey-level of the three ROIs values was calculated as the final grey scale value for each image.

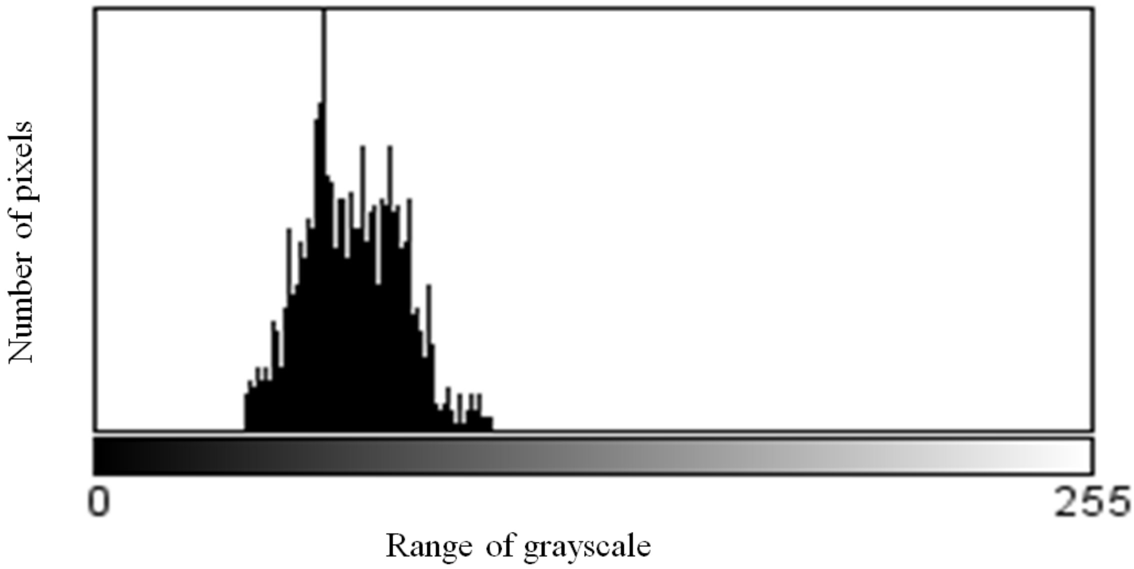


Figure 4-3: Histogram analysis shows the distribution of the intensity of ATFL tissue. The vertical axis demonstrates the amount of pixels; the horizontal axis demonstrates the range of greyscale. The mean echo intensity of this healthy ligament is 65.28 ± 12.67

4.4 Statistical Analysis

The mean grey-level values were expressed as mean ± standard deviation (SD). The Shapiro-Wilk test was applied to test the normal distribution of the data. Significant differences in echo intensity between the three groups were investigated using one-way ANOVA (SPSS Inc., version 24, Chicago, IL, USA). In case of significant effect ($p < 0.05$), a Bonferroni post hoc comparison was applied between the groups.

4.5 Results

All data were normally distributed. There was a significant difference in ATFL echo intensity between the three groups ($p < 0.001$). Mean grey-level intensity for healthy, coper and CAI participants was 52.57 ± 5.68, 39.07 ± 4.14, and 31.71 ± 5.66 respectively (Figure 4.4). This indicated that healthy participants had the highest intensity and CAI participants had the lowest intensity. When compared to AT, the normal echo intensity of the ATFL is lower than AT by 5% (52.57 ± 5.68 vs. 55.10 ± 4.58) even though there was no statiscal significant difference (p=0.71, d=0.75). ATFL in coper and in CAI were significantly different compared to healthy AT (p<0.01, d=3.67, p<0.01, d=4.54 respectively).

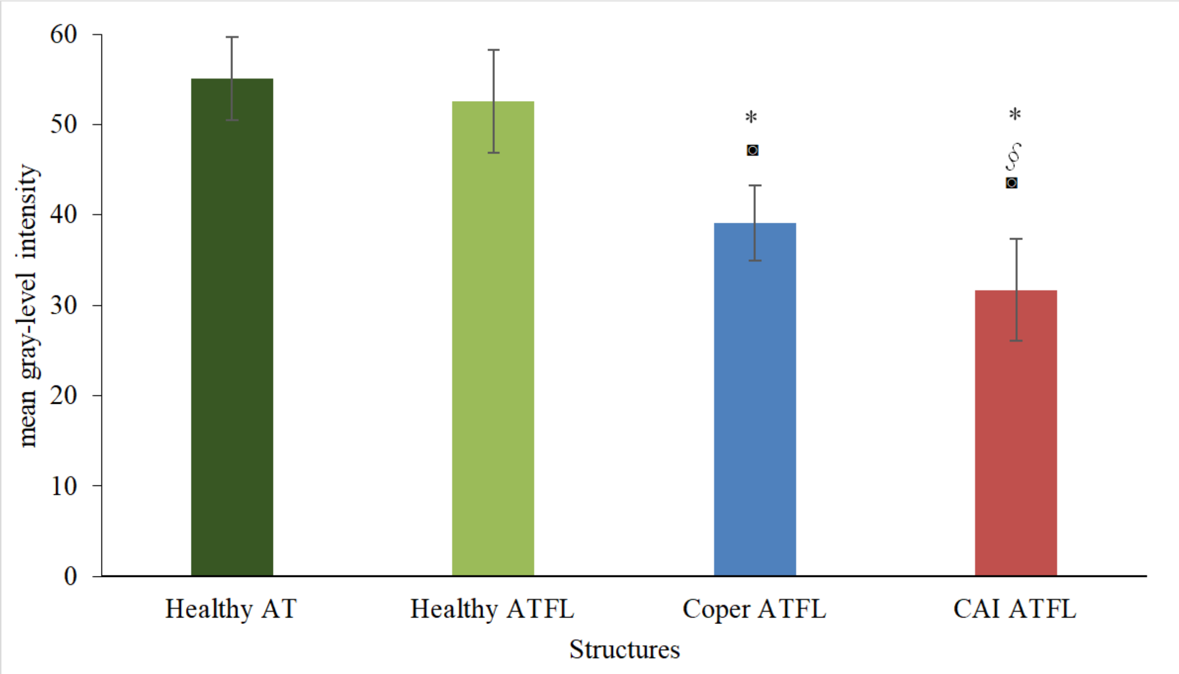


Figure 4-4: mean grey–level intensity of healthy AT, healthy ATFL, coper ATFL and ATFL in CAI participants.
** A significant difference was found in ATFL between healthy and coper participants (p<0.001, d=2.72), between healthy and CAI participants (p<0.001, d= 3.68)*
§ A significant difference was found in ATFL between coper and CAI participants (p<0.001, d=1.48).
◙ A significant difference was found between AT healthy and ATFL coper participants (p<0.01, d=3.67), between AT healthy and ATFL in CAI participants (p<0.01, d=4.54)

The ATFL intensity of coper participants was 25.69 % lower than the intensity of healthy ATFL, while the ATFL intensity of CAI participants was almost 40 % lower than the intensity of healthy ATFL. Interestingly, CAI participants had 18.85 % lower echo intensity than copers.

4.6 Discussion

Beside differences in the length and thickness of the ATFL demonstrated in chapter three, the composition of ligament tissue may also affect the function of the ligament and differentiate injured and uninjured ligaments. In this chapter echo intensity has been proven to differ between the three groups investigated. Previous research had reported qualitatively that injured ligaments are less echogenic compared to healthy ligaments (Ahmed and Nazarian, 2010; Oae et al., 2010), but to what extent was unknown. This is the first study reporting quantitative information about altered ATFL echogenicity between healthy, coper and CAI participants.

Several authors have described the normal echogenicity of tendon and ligament as hyperechoic, with ligament being less echogenic than tendon (Hodgson et al., 2012; Pinzon & Moore, 2009). Moreover, the echogenicity of injured ankle ligaments has been evaluated subjectively and described as hypoechoic (Langer, 2011; McNally, 2014; Oae et al., 2010). So, by subjective evaluation, both healthy and injured ligaments are hypoechoic compared to tendon and there is a lack of evidence that quantitatively compared healthy and injured ankles. Therefore, healthy ATFL was compared to healthy AT to provide the baseline quantitative value for healthy ATFL. The result of the study demonstrated that healthy ATFL was lower than healthy AT by 5%. The result of present supports Hodgson et al. (2012) who found that echogenicity of ligaments is less than tendon in healthy ankles which may be explained by the fact that the healthy ligament contains more water than tendon (Hodgson et al., 2012). Injured ATFL was lower by 29% compared to healthy AT, and ATFL in CAI was lower by 43%. This value could help sonographers to determine the echogenicity of the ligaments and differentiate between coper and CAI ligament.

The echo intensity of the injured ATFL in coper and CAI participants was lower than that of the normal ATFL and this concurs with previous qualitative evaluation. Oae and colleagues (2010) evaluated the efficacy of ultrasonography in detection the ATFL injury in 19 acute injures and 15 cases of chronic ankle instability. They stated that hypoechoic lesions are one of the diagnostic criteria of injured ligaments. In 2011, Langer suggested that there was an anechoic band along the upper border of injured ATFL, or a decrease in echogenicity along the whole ligament. McNally (2014) also stated that disrupted ligament appears as a hypoechogenic region below the fibula. Fessell and Jacobson (2009) and Ahmed and Nazarian (2010) both reported that acute and mild ankle sprains can be identified by loss of the normally echotexture (i.e. be hypoechoic). Therefore, prior findings appear consistent with ours, that an injured ligament is darker than a healthy ligament.

However, some authors described the echogenicity as anechoic, while others mentioned it as hypoechoic. Thus, the degree of darkness is not well defined, reflecting the limitations of subjective evaluation. One possible explanation for the anechoic or hypoechoic appearance is the injury being in an acute phase and the series of cellular events that occur post injury, across three sequential phases described in Table 4.2 (Buschmann & Burgisser, 2017).

Table 4.1: Overview and duration of the three phases in ligaments healing (Buschmann & Burgisser, 2017)

Phase		Process	Time	Repair tissue
1		Initial phase with immigration of erythrocytes and inflammatory cells	< day 1 Within in minutes of injury and continues over the 48-72 hours.	Cellular phase
2		Remodelling phase	Day 2 - 5.9 weeks	
3 Modelling phase	a	Consolidation stage	6 - 9.9 weeks	Fibrous phase
	b	Maturation stage	10 weeks - 1 year	Scar- like tissue

The initial phase, known as the acute inflammatory phase starts within minutes of an injury and lasts up to 72 hours. In this phase, the blood begins to collect where the injury occurred and the process of clot formation is initiated, with increased vascularity in the area (Buschmann & Burgisser, 2017). Therefore, the injured area will appear anechoic due to the vascularity. For the next six weeks, as the second regenerative or proliferative phase commences, tissue starts to remodel, and type III collagen becomes more prevalent. In addition, the water and glycosaminoglycans' content of healing tissue is greater than healthy tissue (Buschmann & Burgisser, 2017), with assumed changes in echogenicity too. It seems possible that an anechoic to hypoechoic appearance of injured ligaments could occur during these two phases due to increase in the vascularity initially and water content latterly. However, previous studies only described participants "post injury" without specifying the time after injury or the stage of healing process.

In the current study, participants had the last prior lateral ankle sprain at least 12 months ago for copers and 3 months ago for CAI participants. By this stage, the ligament is in the modelling phase of the healing process. The reduction of cellular density would have occurred and the cellular tissue has altered to more fibrous tissue (Buschmann & Burgisser, 2017). Furthermore, scar-like tissue is generally the most common tissue found around 10 weeks post injury (Buschmann & Burgisser, 2017). Evidence suggests that remodelled ligament is morphologically inferior to normal ligament and the ligament fibres are not packed as densely (Elliot & Giesen, 2013; Hauser & Dolan, 2011). Several identifiable variations have been found between the remodelled and normal ligament matrix such as changing the types of collagen, decreased collagen crosslinks, increased vascularity, abnormal innervation, having some inflammatory cell pockets that contribute to ligament's weakness (Hauser et al., 2013). This leads to formation of fibro-vascular scar tissue that never resembles healthy ligament tissue (Buschmann & Burgisser, 2017). The fibro-vascular scar tissue contains more vascular tissue than the normal ligament tissue and so, tends to show up as darker than normal ligament in ultrasound images.

In addition, normal tissue of healthy ligament is mainly composed of type I collagen which is responsible for the strength and stiffness of the tissue (Hsu, Liang, & Woo, 2010). Following injury, fibroblasts mainly synthesize type III collagen that is responsible for ligament repair. The variation in matrix structure persist such as collagen disorganised and the vascularity may play a role in decreasing the echogenicity that found in coper and CAI participants compared to healthy participants. The abnormal collagen cross-linking of the remodelled ligament leads to reduced tissue strength and stiffness for up to several years after the first sprain (Hauser & Dolan, 2011). This, may result in abnormal biomechanical, biochemical and ultrastructural properties which could lead to further injury (Hauser & Dolan, 2011; Hildebrand & Frank, 1998).

Even though it has been reported that coper and healthy ankles were more functionally similar (Liu et al., 2015), copers were structurally different. This finding agree with the ultrasound findings that copers demonstrated thicker ATFL than healthy participants. Since the echo intensity of the coper ligaments was lower value compared to healthy values, it seems their ligaments are likely different from healthy ligaments and the thick ligament that observed in the ultrasound could be occur due to the scar because of the healing of the ligament.

Changes in tissue components in copers was also differ from CAI participants. This could be due to the difference in the grade of ankle sprain or the interventions used after the ligament injury and any beneficial effect on the healing process. Understanding the differences in tissue component between coper and CAI participants might help guide more personalised rehabilitation programs that could protect copers from developing ankle instability and/or prevent further sprain for CAI participants.

It has been stated that understanding the structure and function of injured ligaments becomes more complicated because of the inconstantly and unpredictable nature of ligament healing (Hauser & Dolan, 2011). This could be due to the structural and physiological alteration of the ligament after being sprained, in addition to the complex and dynamic cellular processes which occur during healing. These process generate changes in the biomechanics and biology of the sprained ligament, leading to inappropriate tissue formation, which is inferior to the tissue it seeks to replicate (Hauser & Dolan, 2011). The remodelled ligament tissue in coper and CAI participants is characterised by different degrees of fibro-vascular scare tissue with lower tissue quality and changes in morphological and biomechanical properties. This may cause functional disability of the influenced joint and predispose other structures around the joint to further injury. Whether structural changes in the ATFL are linked to functional performance of the ankle is the subject of the next chapter.

4.7 Limitation

A possible limitation of this study is that the value of echo intensity is sensitive to the ultrasound machine settings, therefore limiting the generalisability of the current findings to other machines and the normal values for greyscale analysis should be defined for each ultrasound system and machine. Further researches are important to standardise the setting of ultrasound for various machine. Moreover, ultrasound images do not have the ability to precisely identify the type of the intramuscular tissue such as fat or connective tissue which might impact the value of the echo intensity.

4.8 Conclusion

Musculoskeletal ultrasound was able to provide quantitative of echogenicity using computer-aided analysis image analysis software. The injured ATFL has lower echo intensity than healthy ATFL, with CAI having the lowest echo intensity, followed by copers. Histograms image analysis can decrease subjectivity in injury evaluation and perhaps help to improve diagnosis of ATFL injuries leading to more targeted treatments or injury classification.

5 Chapter five: SEBT and 3D kinematics as measure balance performance in injured ankles compare to healthy controls

5.1 Chapter overview

Since lateral ankle sprain has a high rate of reoccurrence and there is evidence of structural change in the ankle (detailed in the previous chapter), it is important to understand the effect that structural changes might have on function of the ankle joint. Ligamentous injury can lead to proprioceptive deficit which can increase body sway and disturbed balance in such cases (Mettler et al., 2015).

Gribble et al. (2016b) stated that there could be pairs of functional and sensorimotor deficits that persist in the months after ankle sprain with consequences for important movement tasks. Individuals with a history of ankle sprains and chronic ankle instability have deficit in their postural control (Olmsted et al., 2002, Shaffer et al, 2013; Mettler et al., 2015). Most studies have measured this deficiency during a static test such as quiet standing (McKeon & Hertel, 2008a; Trojian & McKeage, 2006; Wikstrom et al., 2010). However, using a static balance test may not be sensitive enough to identify the deficits in motor control associated with impaired functional activity and sporting performance (Olmsted et al., 2002). For example, the task of keeping the body still during quiet standing may not make sufficient demands on the postural control system compared to a dynamic task during which load at the ankle and ankle movement are changing. Thus, it is important to adopt a method which is simple, valid, sensitive, reliable and, if possible, cost effective for dynamic movement assessment of ankle function (Gribble et al., 2012).

The SEBT is a dynamic balance test which has used in research and clinical settings (Gribble et al., 2012). Researchers have proposed that, with proper instruction, good practice by the participants and normalisation of the reach distances, the SEBT can give an objective measure and distinguish deficits and progress in dynamic balance associated with lower limb injury (Gribble et al., 2012).

During the performance of the SEBT, it was important to measure how the task of maintaining balance is actually achieved. One way to do this is to study the motion of the foot and ankle during the SEBT using 3D motion-analysis systems. These systems have been commonly used in many previous studies to measure the kinetics and kinematics of the lower limb (Fong et al., 2014; Ford, Myer, & Hewett, 2003; Hoch et al. 2016; Jones, Herrington, Munro, & Graham-smith, 2014; Pionnier et al., 2016; Sigward & Powers, 2006). This enables researchers to measure motion in all body planes during performance of dynamic tests. The skeletal system is reconstructed by placing reflective markers on particular anatomical landmarks and the kinematic data is recorded and measured during dynamic tests. Therefore, by combining a measure of dynamic balance and an explanation of how the balance is achieved we can learn the functional consequences of the lateral ankle sprain on the ankle joint.

This chapter describes the method used to evaluate balance in people with and without a history of ankle sprains. Maximum reach distances for the SEBT were measured; 3D motion of the ankle of the leg being tested was recorded for eight reach distances utilising a motion capture system. Discrete values were defined for the ankle joint in the sagittal, frontal, and transverse planes at the point of maximum reach distance and were compared between healthy, coper and CAI participants. Correlation tests were used to investigate any relationship between changes in the ligament (as shown in chapter 3) and changes in reach distance. An overview of this chapter is demonstrated in Figure 5.1.

5.2 Aim, objectives, and hypothesis of the study

The aim of this study is to investigate the dynamic balance in people with and without a history of ankle sprain. To achieve the aims of the study, the following objectives were established:

- Evaluate the dynamic balance in healthy, coper and CAI participants and to determine which directions are most affected by ankle sprains during the SEBT.

- Investigate reach strategy differences in the sagittal, frontal and transverse planes of ankle motion between healthy, coper and CAI participants.

- Investigate a potential correlation between the differences in the ligament thickness and the most affected direction of reach distance of the SEBT.

Hypothesis: There will be statistically significant differences in the dynamic balance between healthy, coper, and CAI participants.

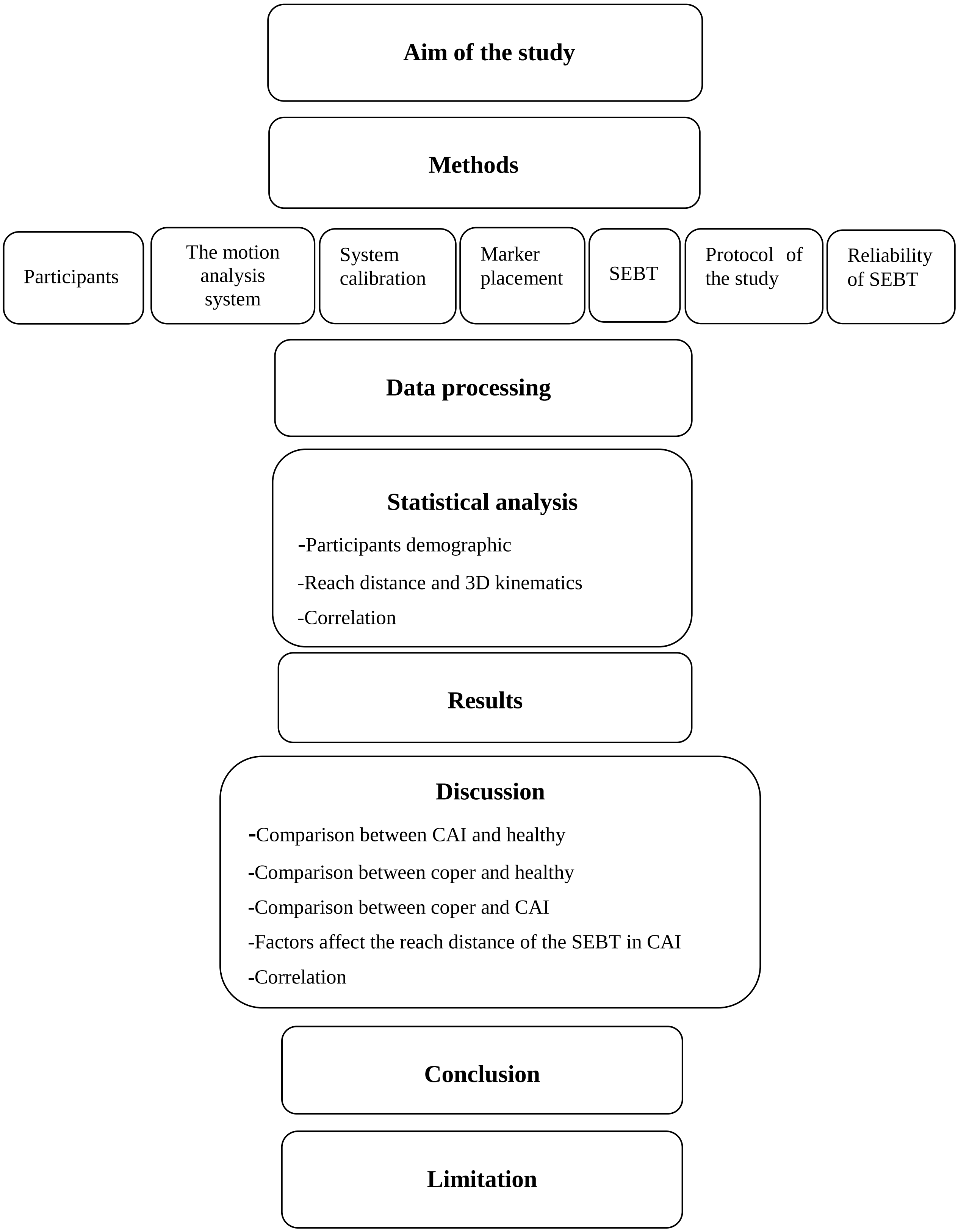


Figure 5-1: Flowchart demonstrating the structural of this study

5.3 Method

5.3.1 Participants

A total of 68 participants (33 male, 35 female) took part in the study. Participants were classified into healthy, coper and CAI groups. The demographic data is presented in Table 5.1. Participants were recruited from the University of Salford through advertisements and flyers posted in the university. Before starting the test, all participants signed a written consent form which was approved by the ethical committee of the University of Salford. The inclusion and exclusion criteria were identical to those in chapter three. The study was conducted in the gait laboratory at the University of Salford. Before the study, the researcher prepared the laboratory by checking the connectivity of each camera, performing calibration of the system, and preparing the markers for each participant.

Table 5.1: Demographic data of healthy, coper and CAI participants

	Healthy Mean ± SD	**Coper Mean ± SD**	**CAI Mean ± SD**	**P-value, effect size**
Number of participants	28	18	22	
Sex male/female	10/18	10/8	13/9	
Years (y)	28.07 ± 6.32	28.53 ± 6.83	28.02 ± 7.71	0.95, 0.04
Weight (Kg)	68.46 ± 12.43	70.07 ± 15.41	69.73 ± 12.56	0.54, 0.14
Height (m)	1.68 ± 0.10	1.71 ± 0.09	1.69 ± 0.10	0.59, 0.10
BMI (kg/m²)	24.30 ± 3.19	24.00 ± 4.71	24.40 ± 3.64	0.85, 0.07
CAIT score	29.50 ± 0.70	27.53 ± 1.40[a]	13.62 ± 3.74[a]	<0.01, 3.04
Time since last injury (months)	0.0 ± 0.0	18.12 ± 03.37[a,b]	08.13 ± 2.83[a]	<0.01, 3.21

Abbreviations: CAIT, Cumberland Ankle Instability Tool; BMI, Body Mass Index.
*Values are mean ± SD.
[a] Indicates statistical differences between CAI and coper, and between CAI and healthy participants.
[b] Indicates statistical differences between coper and healthy.

5.3.2 The motion analysis system

Motion analysis includes particular techniques and procedures to systematically analyse movement. Modern gait laboratories utilise motion analysis systems to describe dynamic alteration in 3D coordinates of particular body landmarks from the images taken by each camera. The 3D foot and ankle kinematic data (sample frequency 100 Hz) for this study were recorded using twelve Qualisys infrared cameras (ProReflex, Qualisys Inc., Gothenburg, Sweden) that are permanently wall mounted to permit faster set up and calibration. Four 400mm x 600mm force platforms (AMTI: Advanced Mechanical Technology Incorporation, Watertown, USA, model BP4006001500) were embedded in the middle of the gait laboratory to collect kinetic data at a frequency of 1000 Hz. In this study, an in-ground force plate measured the kinetics of the ankle motions for each participant's foot.

The orientation of the force platforms and the positions of the motion capture cameras are demonstrated in Figure 5.2. Qualisys proprietary software (Qualisys Track Manager (QTM)) was utilised in order to connect the cameras. There are three steps in collecting the kinematic data: calibration, data collection and 3D reconstruction of retroreflective markers.

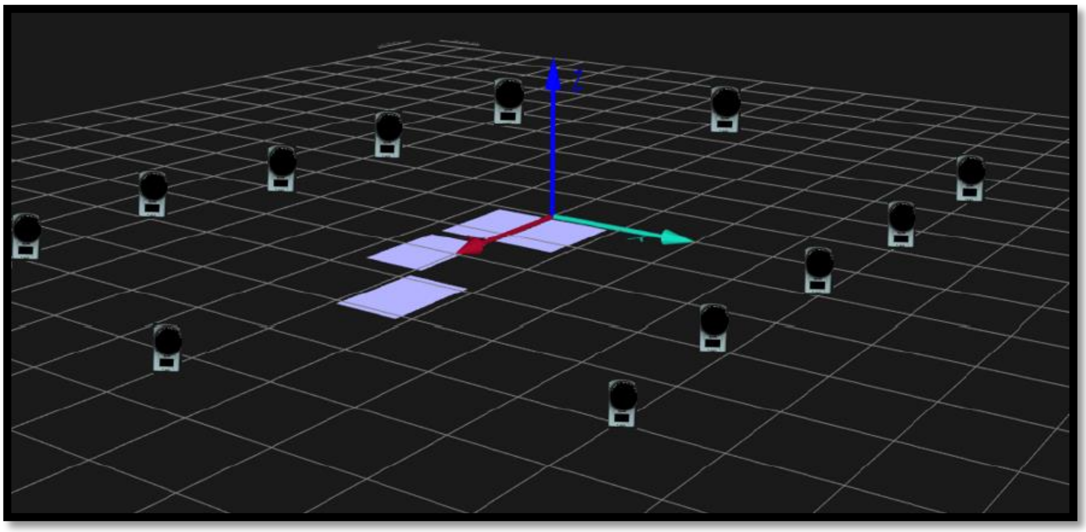


Figure 5-2: Set up the gait laboratory with orientation of cameras and position of force platforms

5.3.3 System calibration

The aim of the calibration is to determine the accurate location of the markers in the 3D coordinate system utilising a direct-linear transformation (DLT) technique. The algorithm for this technique builds a linear relationship between the 2D image and the real life 3D coordinates of the markers (Grimshaw, Lees, Burden, & Fowler, 2006); each Qualisys camera is synchronised and records a 2D (X and Y) image which is then rebuilt and presented as a real life set of 3D (X, Y and Z) coordinates. It is important to ensure that the volume of data is captured during calibration (Grimshaw et al., 2006). The location of the markers in 3D space can only be determined based on the accuracy with which the system is calibrated (Payton & Bartlett, 2008).

A rigid L-shaped calibration frame with four retro-reflective markers was used in the static calibration of the motion-capture system (Figure 5.3A). This was placed on the floor at the medial corner of the force plate to determine the origin of the global coordinate system (0, 0, and 0 for X, Y, and Z). The X-axis was equated to the anterior/posterior (forward/backward) direction, the Y-axis to the medial/lateral (left/right) direction, and the Z-axis to the proximal/distal (upward/downward) direction (Figure 5.4). A T-shaped handheld wand (Figure 5.3B), with two reflective markers, one attached to each end 749.3 mm apart, was utilise to calibrate the volume which would be used through the dynamic balance trials. A capture time of 70 seconds was set for calibration to ensure that both of the lower limbs were seen completely during the dynamic balance trials by at least two cameras. The wand was moved manually throughout the area as many directions as possible where kinematic data were collected. Therefore, a calibration volume was built to track the positions of the lower limb markers (Figure 5.5). The most significant factor for successful calibration is low error accuracy, which describes the relationship between the actual position of the reflective markers and their position on the system. The results demonstrated a numerical value as an average for each camera and displayed the standard deviation, expressed in mm, of the wand length. A successful calibration is essential for high quality data collection. The result of the calibration was calculated by the Qualisys system; the lower the residual error (the mean error from all cameras), the higher the accuracy.

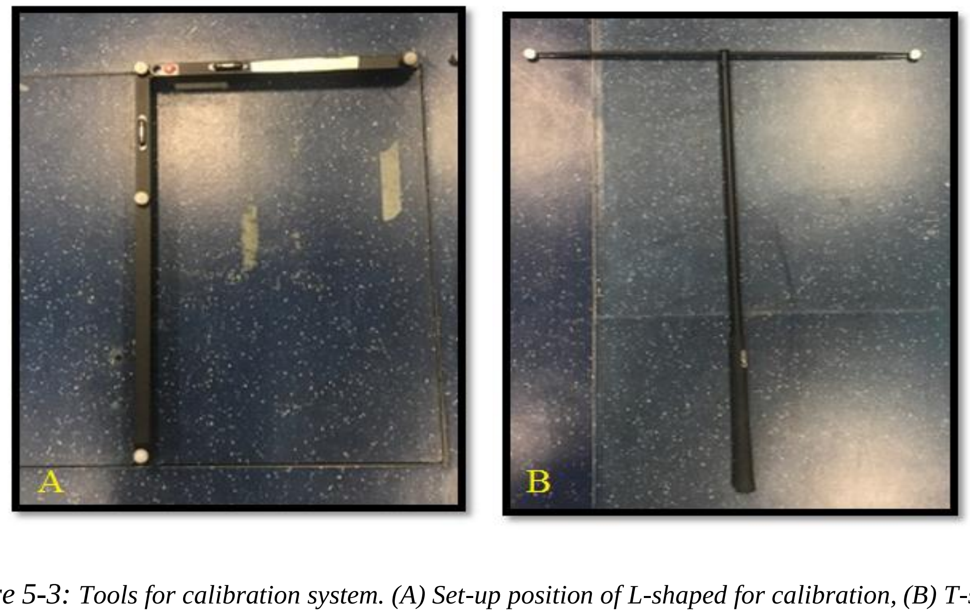


Figure 5-3: Tools for calibration system. (A) Set-up position of L-shaped for calibration, (B) T-shaped handheld wand

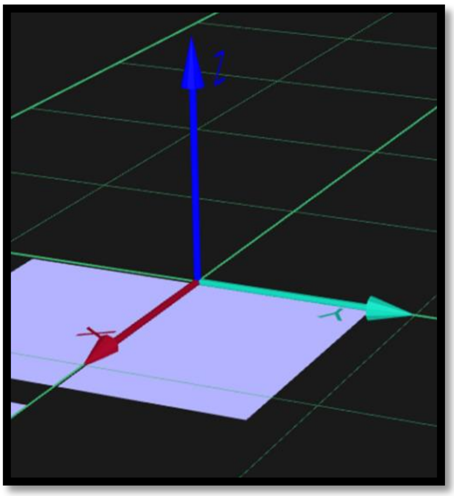


Figure 5-4: Illustration of the orientation of force platform

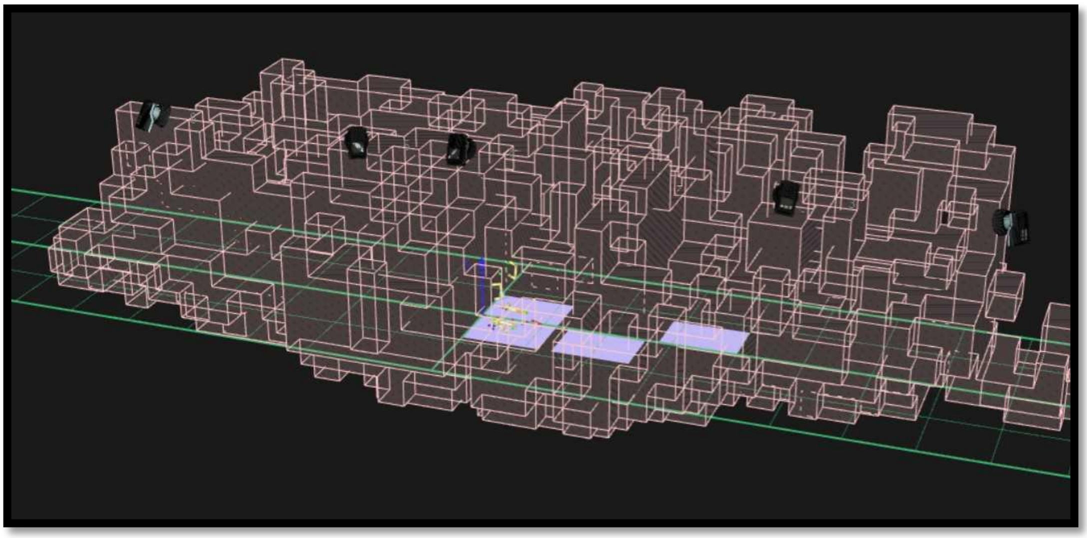


Figure 5-5: All the irregular cube- like shaped represent all spaces that has been calibrated

For a successful calibration, the manufacturer recommends that the value of the residual error should be less than 1 mm (Grimshaw, 2006). The result of the average residuals in this study was accepted as the values for all 12 cameras were < 0.8 mm, which mean the locations of the markers in space were < 0.8 mm from their true positions.

To develop 3D coordinates, at least two cameras have to create a 2D coordinate set for each marker. This procedure occurs for every time frame, following which the marker trajectory is determined. These trajectories of the markers can therefore be applied for kinematic analysis.

5.3.4 Marker placement

Lightweight spherical retro-reflective markers (14 mm and 9.5 mm in diameter) (Figures 5.6A-B) were used to determine and track the anatomical segments. Karlsson and Tranberg (1999) reported that the weight of the markers can create movement artefacts. Thus, very lightweight markers were utilised in this study. A total of 30 anatomical markers were used for each participant in order to determine anatomical reference frames of the shank, foot, the centres of ankle and subtalar joint rotation. These markers were attached directly to the skin by utilising hypoallergenic double-sided adhesive tape that was attached to a flat-based marker. The ankle markers were attached to the medial and lateral malleolus; the foot markers were placed on first, second and fifth metatarsal heads, and posterior heel, the cluster (wand) markers were placed on calcaneal tubercle to tracked the calcaneus relative to the shank which provide rearfoot eversion/inversion, while knee markers were placed on the medial and lateral femoral condyle (Figure 5.8). Another four markers, known as cluster markers, were attached to the four corners of the rigid polypropylene pads (Figure 5.7) that were used for the shank segment. The shank segment was identified as a rigid structure between the medial and lateral epicondyle of the knee joint proximally and the medial and lateral malleolus of the ankle joint distally. The cluster pad was placed on the anterior lateral of the shank and was fastened with an elastic strap to define the segment through the dynamic balance movement. These clusters were used to decrease soft tissue artefacts through movement (Cappozzo, Catani, Della Croce, & Leardini, 1995). Moreover, authors have pointed out that the use of rigid clusters gives the optimal arrangement compared to individual skin markers (Manal, McClay, Stanhope, Richard, and Galinat, 2000). Figure 5.8 illustrates the markers on a participant's lower limb.

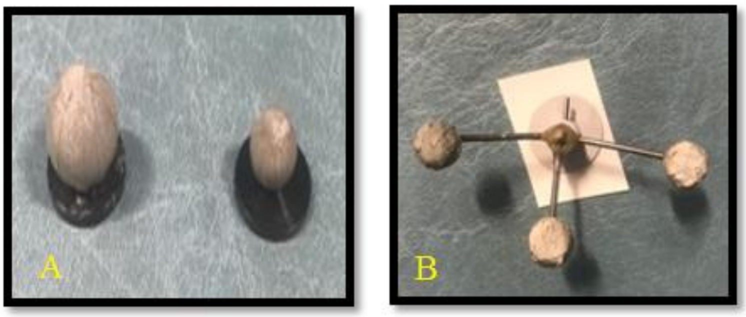


Figure 5-6: Types of markers. A: spherical retro-reflective marker, B: wand markers

Figure 5-7: cluster markers

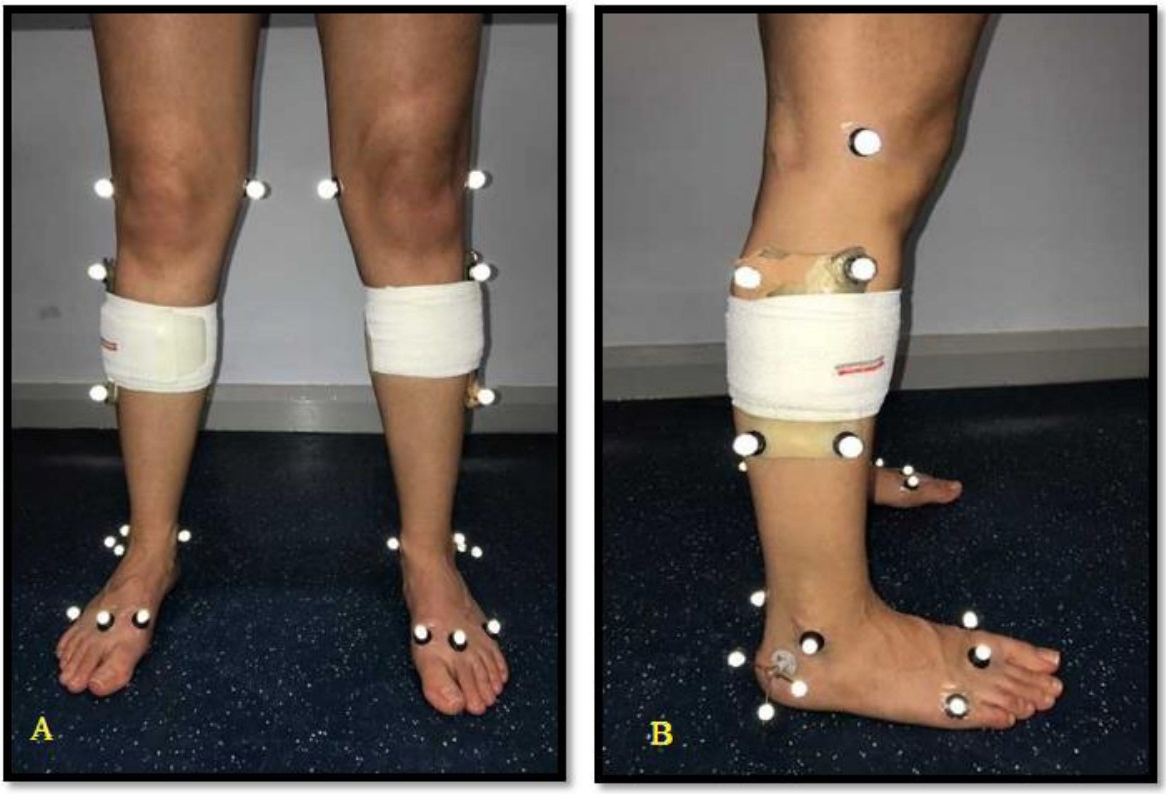


Figure 5-8: Location of markers and clusters on participant's lower limb

5.3.5 SEBT

The eight lines of the SEBT were arranged over a force plate, at 45° to one another and are named based on the direction of excursion related to the stance (test) limb: anterior (Ant), anterolateral (AntLat), lateral (Lat), posterolateral (PostLat), posterior (Post), posteromedial (PostMed), medial (Med), and anteromedial (AntMed) (Figure 5.9).

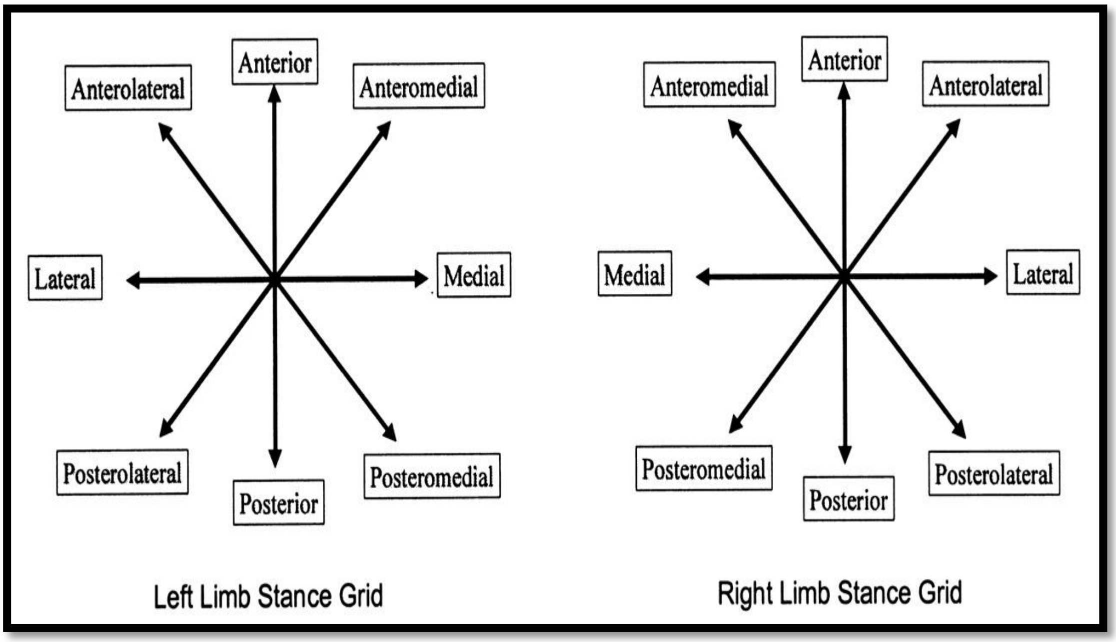


Figure 5-9: Eight directions of the SEBT. The directions are labelled based on the reach direction in reference to the stance limb (Mahajan, 2017).

5.3.6 Protocol of the study

The study and the procedure for the test were explained to each participant clearly on arrival, and the consent form was completed and signed. Once the participants completed the ultrasound exam (described in detailed in chapter three), they had a rest for two-five minutes. Then, the researcher began to place the retro-reflective markers on the lower limbs to start the balance test.

The study began with the researcher asking the participants to complete a static trial which include participants standing barefoot on the force plates. Each participant stood with all of the markers attached to their lower limb, as described in the previous section (5.3.4), in an upright position with their knee extended completely, their feet pointing forward towards in anterior direction of the SEBT, and with both legs symmetrically positioned. The participants were asked to remain in a static position for 20 seconds with their arms held away from the markers to ensure that they did not cover them. This trial was conducted to align the participant with the laboratory coordinate system and to record this as a reference (zero degrees) position for further kinematic analysis (Fullam et al., 2014). The researcher verified the placement of the markers and ensured that participants' movements were exactly mimicked by the computer QTM software before starting the test.

Following the static trial, dynamic trials were measured during the SEBT. The purpose of the SEBT is to reach with one limb as far as possible along each of the eight lines designed by tape measures taped to the laboratory floor (Figure 5.10), whilst preserving stability of the contralateral limb (Khuman et al., 2014). Both limbs were tested for the comparison between injured and uninjured limb. The protocol for performing the SEBT was taken from published practice (Doherty et al. 2015b; Gribble et al., 2012; Hoch et al., 2016; Khuman et al., 2014). The instructions and the procedures for the SEBT were given verbally to the participants by the researcher.

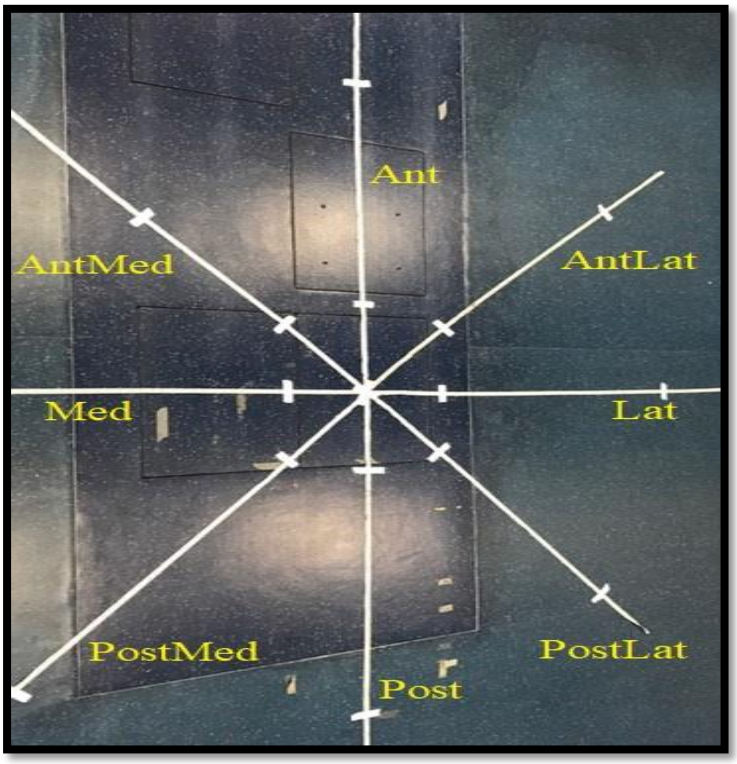


Figure 5-10: The eight directions of the SEBT with right limb stance

Participants started the test by placing the stance (test) limb on the middle of the SEBT grid, while the untested limb was placed outside the force plate. They stood with their hands on their hips for three seconds. They were then asked to reach as far as they could with the non-weight bearing (untested) limb, making a light touch on the furthest point on the measuring tape with the most distal part of the big toe, while maintaining appropriate control of the standing limb. Participants leant backward during the performance this movement in the AntLat and AntMed directions, and abducted the untested leg to the side of the body for Lat reach distance (Lockie, Schultz, & Callaghan, 2016). For the PostLat and PostMed reach distances, the participants leant forwards, adducting the leg to a position behind the body, before extending the leg medially (Lockie et al., 2016). Examples of these excursions are demonstrated in Figure 5.11.

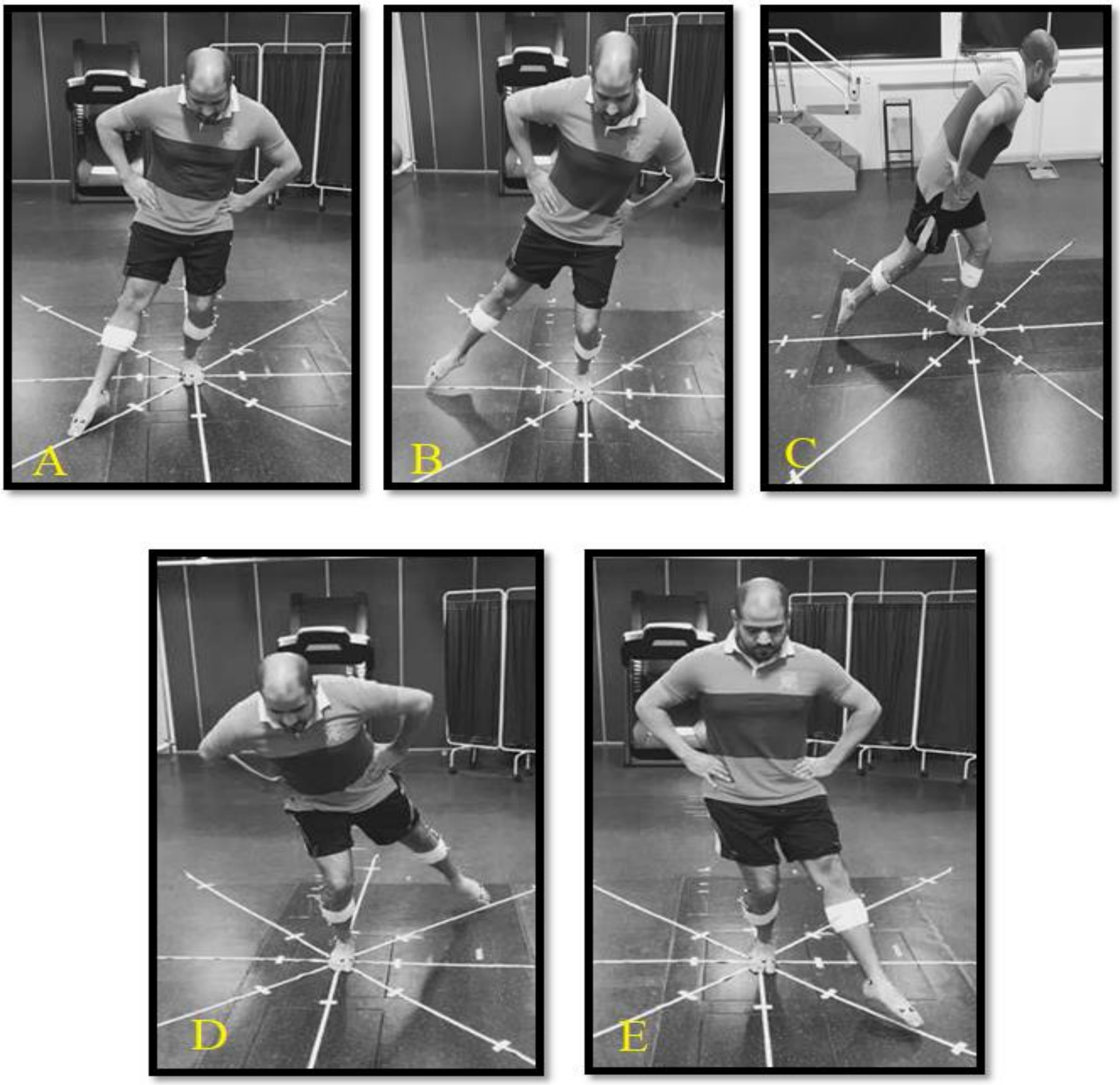


Figure 5-11: Demonstration of SEBT directions with testing the left leg, (A)Anterolateral, (B)Lateral, (C)Posterior, (D)Posteromedial, and (E)Anteromedial

Reach distance was measured by the researcher in centimetres using a one-meter measuring tape extended from the middle of this grid in the relevant direction over the SEBT (Appendix 17). Using the same technique, the researcher also took measurements for the other seven directions. The participants were then asked to return the reaching limb to the starting point and to stay still for three seconds. All trials were completed in sequential order in either the counter clockwise (right limb) or clockwise (left limb) directions (Khuman et al., 2014).

Following Pionnier's (2016) techniques, the participants performed four practice trials to decrease the learning effect and to become familiar with the SEBT grids, with a 30 second break between each trial, then the average for the three trials in each direction were recorded for analysis.

The motion of the foot and ankle structures in this study was quantified by applying a technique known as Calibrated Anatomical System Technique (CAST) which was developed by Cappozzo et al. (1995). The CAST was utilised to determine the six degrees of freedom movement for each segment during the performance of dynamic tasks, involving three rotations (sagittal, frontal and transverse) and three translations (anterior/posterior, medial/lateral, and distraction/compression) (Cappozzo et al., 1995). This system develops an anatomically related reference frame for the lower limb segments through the reflective markers being placed on the external anatomical landmarks to give an external indication of the orientations of the internal bones.

The trials had to be repeated in some cases of error. Errors involved: (1) a marker falling off; (2) the stance leg losing normal contact or the heel lifting the force plate; (3) the big toe not touching the tape or making a heavy or prolonged touch; (4) the hands changing position or moving from the hips; (5) the participant losing balance or being unable to return to the starting point in a controlled way (Doherty et al., 2015b).

As before any study, a reliability study was required to confirm that the researcher could collect reliable data and to prove that the data could be repeated for the eight reach distances of the SEBT. This will be discussed in the next section.

5.3.7 Reliability of the SEBT

Higher reliability of the SEBT has been previously reported on healthy recreational athletes (Munro & Herrington, 2010). A reliability study of the SEBT was undertaken before the main study conducted for both healthy and injured participants. This involved ten participants who understood the aim and voluntarily consented to take part. The ten participants (5 healthy, 5 injured), had a mean age of 27.20 ± 5.86 years, mean height of 1.67 ± 0.11 m, mean weight of 68.17± 13.19 kg, and mean BMI of 24.33± 3.28 kg/m^2. The participants followed the protocol of the study [as described in the previous section (5.3.6)], each performing the test twice, one week apart, with the same researcher in order to test the intra-tester reliability.

The two-way mixed model with absolute agreement types of intraclass correlation coefficient (ICC) and limits of agreement (LoA) were used to evaluate the reliability. The data collected were analysed using SPSS. The descriptive characteristics of the measured reach distances of the SEBT and their corresponding ICC and LoA values for healthy and injured participants are listed in Tables 5.2-3. There was a good to excellent reliability for the eight reach directions of the SEBT for the healthy participants, with ICC ranging from 0.87 to 0.99 and a limit of agreement between 2.20 and 14.5 % (Table 5.2). The reliability was also excellent for the injured limb of injured participants, with ICC ranging from 0.91 to 0.99 and limit of agreement from 3.68 to 14.6 % (Table 5.3).

The reliability of uninjured limbs for healthy and injured participants are presented in Appendix 18. Examples of the Bland-Altman plot are demonstrated in Figures 5.12 and 5.13. More plots for several directions for both healthy and injured participants are provided in Appendix 19.

Table 5.2: Reliability and limit of agreement results for eight reach distances of injured limb in healthy participants

	Day 1 mean (SD)	Day 2 mean (SD)	ICC (95% Confidence Interval)	95% LoA		LoA
				lower	upper	
Ant	86.78 (6.19)	86.05 (3.64)	0.92 (0.70-0.99)	-5.43	7.07	14.50
AntLat	91.24 (2.06)	91.80 (1.30)	0.87 (0.62-0.95)	-2.79	1.67	4.87
Lat	92.01 (3.47)	91.80 (3.56)	0.99 (0.91-0.99)	-1.34	1.76	3.37
PostLat	97.02 (3.73)	96.40 (4.16)	0.93 (0.50-0.99)	-3.46	4.70	8.44
Post	98.57 (3.89)	98.20 (3.42)	0.95 (0.60-0.99)	-2.88	3.62	6.60
PostMed	91.26 (6.64)	90.85 (6.43)	0.99 (0.97-1.00)	-0.59	1.41	2.20
Med	84.71 (2.22)	84.72 (2.57)	0.89 (0.30-0.98)	-3.19	3.27	7.62
AntMed	85.92 (6.25)	84.20 (8.29)	0.95 (0.63-0.99)	-4.24	7.68	14.00

Table 5.3: Reliability and limit of agreement results for eight reach distances of injured limb in injured participants

	DAY 1 mean (SD)	Day 2 mean (SD)	ICC (95% Confidence Interval)	95% LoA		LoA
				lower	upper	
Ant	74.58 (3.75)	74.38 (2.82)	0.97 (0.70-0.99)	-2.29	2.69	6.68
AntLat	79.61 (4.55)	79.39 (5.12)	0.91 (0.60-0.99)	-5.58	6.02	14.60
Lat	80.90 (5.62)	80.83 (6.26)	0.99 (0.94-0.99)	-1.92	2.06	4.90
PostLat	81.64 (6.49)	81.60 (5.43)	0.98 (0.83-0.99)	-3.29	3.37	8.16

Post	81.80 (6.85)	81.90 (6.43)	0.98 (0.91-0.99)	-2.16	3.40	6.79
PostMed	82.45(5.18)	81.56 (5.21)	0.98 (0.71-0.99)	-0.59	2.43	3.68
Med	69.08 (7.43)	68.39 (6.77)	0.98 (0.85-0.99)	-3.30	4.68	11.61
AntMed	68.82 (4.79)	68.74 (5.29)	0.96 (0.68-0.99)	-3.74	3.90	11.11

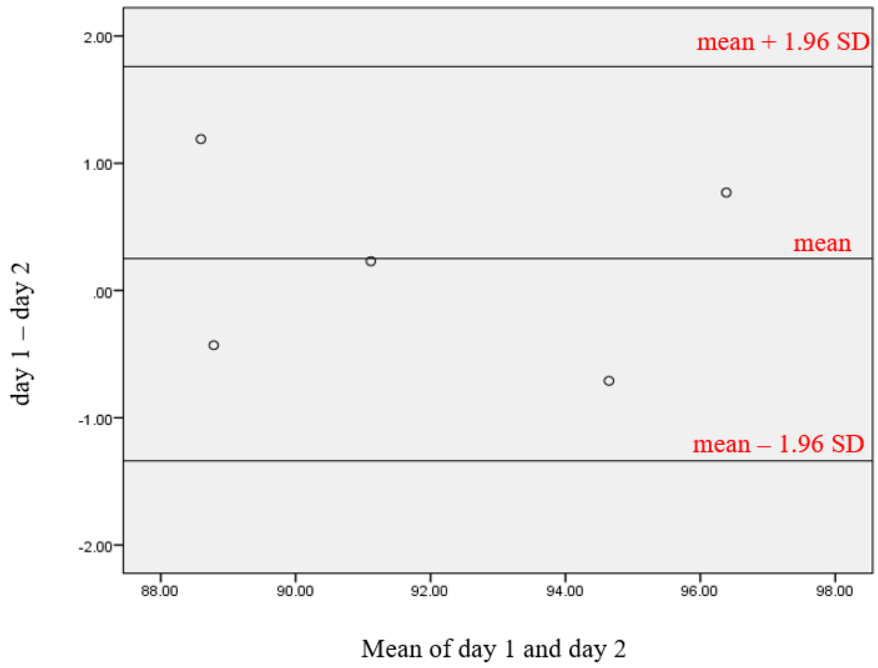


Figure 5-12: Bland and Altman plot for lateral direction of injured limb of healthy participant with representation of limit of agreements

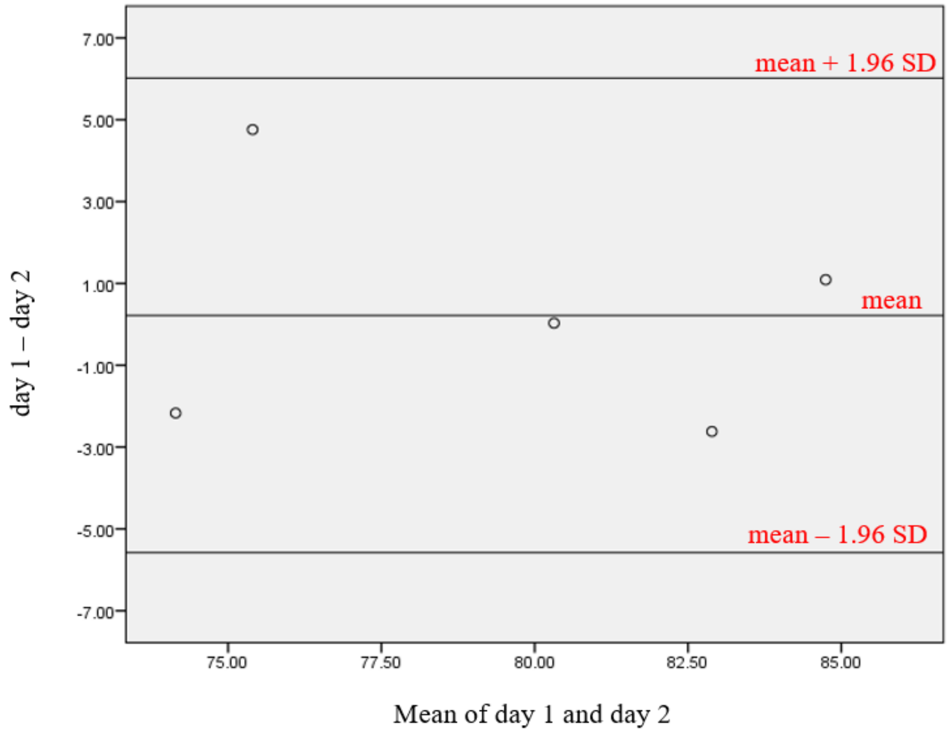


Figure 5-13: Bland and Altman plot for anterolateral direction of injured limb of injured participant with representation of limit of agreements

5.4 Data processing

Because leg length is a significant factor in determining reach distance (Gribble & Hertel, 2003), each reach distance was normalised to the participant's leg length. Normalisation of the reach distance was achieved by dividing the reach distance by limb length and multiplying it by 100 (reach distance/limb length X 100 = % of limb length) (Khuman et al., 2014). Therefore, the normalised value is expressed as a percentage of each distance relative to the participant's leg length (Pionnier et al., 2016); this percentage permits a more precise comparison of the SEBT performance among participants (Gribble & Hertel, 2003).

The segments and planes were based on the recommendation of International Society of Biomechanics. For the left and right foot (calcaneus, talus, cuboid, navicular, lateral, medial, intermediate cuneiform, and metstarsal):

O_f - The origin is located at the calcaneus landmark.

y_f - The calcaneus and the first and fifth metatarsal heads define a plane that is quasi-transverse. Orthogonal to this plane is quasi-sagittal plane which is defined by the calcaneus landmark and the second metatarsal head. The intersection of these two planes is the y axis and its positive direction is proximal.

z_f – The axis lies in the quasi-transverse plane and its positive direction is from left to right.

x_f - The axis is orthogonal to the yz plane with its positive direction is dorsal.

Cappozzo CAST model was also applied, which used clusters on the shank to track movement. Then angles were calculated as flexion/ rotation – angle between shank and foot at ankle axis in sagittal and transverse plane. The eversion/ inversion, were based on angle between shank and calcaneus (calcaneus segments based on calcaneus markers/ foot position) in the frontal plane.

The QTM software was used to label the 3D trajectory markers (Figures 5.14A-B). The gaps in the data were completed to the maximum through applying a polynomial cubic spline interpolation in QTM, which has the ability to refine the digitising errors of the markers that could occur randomly during movement. The data were exported to C3D (Coordinate 3 dimensional) files in order to be imported into Visual 3D motion software (Version 6, C-Motion, Inc., Rockville, MD) to calculate the ankle joint kinematic data. This software is commonly used in studies calculating the kinematics of the lower extremity joints (Hsu et al., 2014; Liu et al., 2012; Telfer, Abbott, Steultjens, & Woodburn, 2013;).

Bi-directional Butterworth low pass filters were applied with cut-off frequencies of 6 Hz to smooth the kinematic data. This kind of filter permits low frequency data to pass but not high frequency data, thus decreasing noise related to skin movement artefacts (Richards, 2008).

Foot and shank segment models (Figure 5.14C) were built to calculate the ankle joint kinematic data. Kinematic data of the ankle (dorsi-plantar flexion, eversion-inversion, internal-external rotation) were measured at the maximum reach distance in each direction of the SEBT (Figure 5.15). The moment of maximum reach distance was defined as the maximum difference between left and right foot segment centres. The averages for the three trials for each kinematic variable were used for analysis. All of the data were exported as a text file to Microsoft Excel to facilitate the analysis and presentation. For all kinematic variables, positive values indicated dorsiflexion in the sagittal plane, inversion in the frontal plane, and internal rotation in transverse plane. Negative values indicated plantar flexion in the sagittal plane, eversion in the frontal plane, and external rotation in the transverse plane.

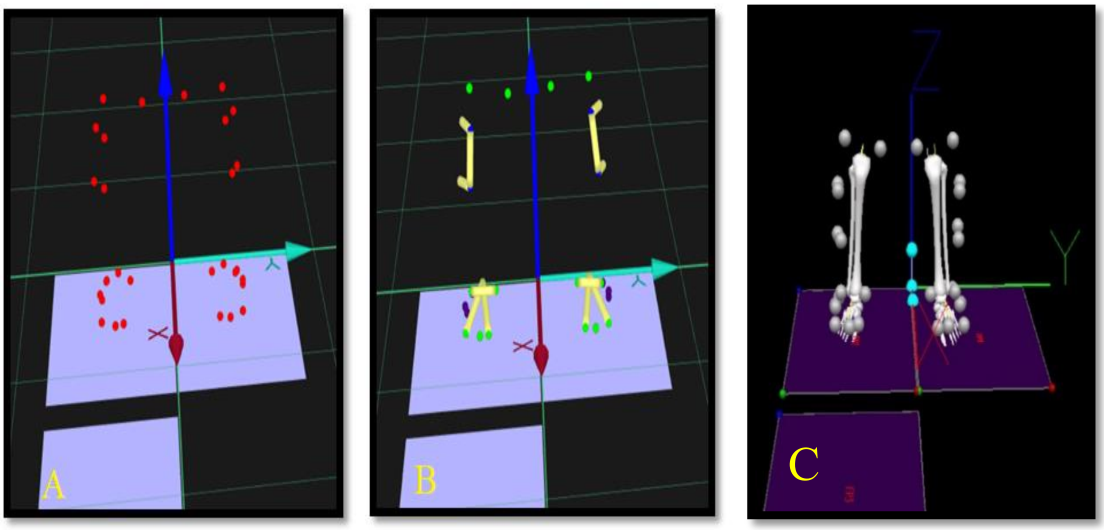


Figure 5-14: (A)QTM ™ static model and, (B) Labelling (C) Modelling

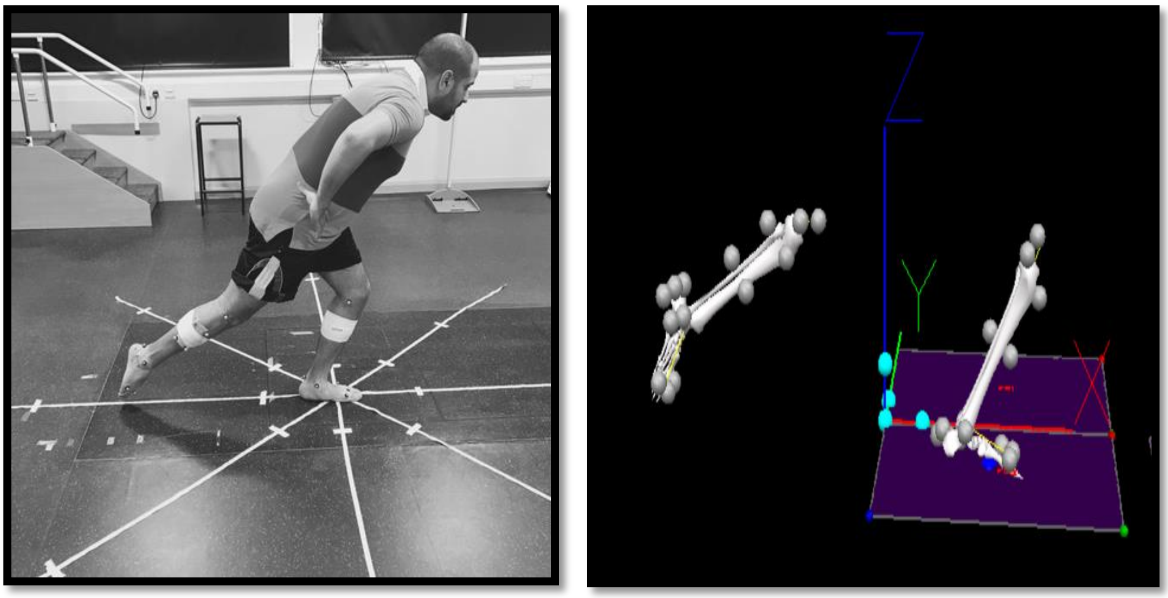


Figure 5-15: Image demonstrated the appearance of the posterior direction in visual 3D

5.5 Statistical analysis

A Shapiro-Wilk test was performed and demonstrated that the data was normally distributed. This proves the appropriateness of parametric analysis. Several statistical methods were used to answer the research questions and the analyses were conducting using SPSS version 23 (SPSS Inc., USA), these methods are outlined below.

5.5.1 Participants demographic and questionnaires

The demographic variables (sex, age, weight, height, BMI, CAIT score) were compared between healthy, coper and CAI participants using a one-way ANOVA. The independent variable was group (healthy vs coper vs CAI). The significance level for the analysis was set a priori at $p < 0.05$. If a significant difference was observed, the Bonferroni post-hoc analysis was applied.

5.5.2 Reach distances and 3D kinematics

One-way ANOVA was applied for each reach direction of the SEBT to evaluate whether there was a difference in reach distance and ankle motion between the groups (healthy, coper, and CAI). If significant, a post-hoc (Bonferroni) pairwise comparison was applied to determine the significant main pairwise effects. The independent variable was group (healthy vs coper vs CAI), while the dependent variable consisted of the eight reach distances and the ankle motions in the three planes. Moreover, paired t-tests were used to compare left to right differences in each group. The significance level for the analysis was set a priori at $p \leq 0.05$. Cohen's *d* effect sizes were calculated with $d = 0.20$ to 0.49 to be considered a 'small' effect size, 0.50 to 0.79 representing a 'medium' and > 0.80 representing a 'large' effect size (McGough & Faraone, 2009).

5.5.3 Correlation between the thickness of lateral ligaments and anterolateral direction of the SEBT

Pearson product-moment correlation coefficients were utilised to evaluate correlation (*r*) and to characterise any relationship between changes in the thickness of ankle ligaments (ATFL and CFL) in the three groups and the anterolateral direction of the SEBT for the three groups. These relationships were described as weak (≤0.39), moderate (0.40–0.69) or strong (≥0.70) (Lomax, 1998).

5.6 Results

5.6.1 Participants demographic and questionnaires

The demographic data for the three groups were homogenous at the baseline with p-value >0.05, except for CAIT which showed significant differences between the groups with p-value <0.01. Bonferroni post-hoc analysis demonstrated significant differences between the healthy and CAI participants, between the healthy and coper participants, and between the coper and CAI participants ($p<0.01$, $p=0.02$ and $p<0.01$ respectively).

5.6.2 SEBT reach distances

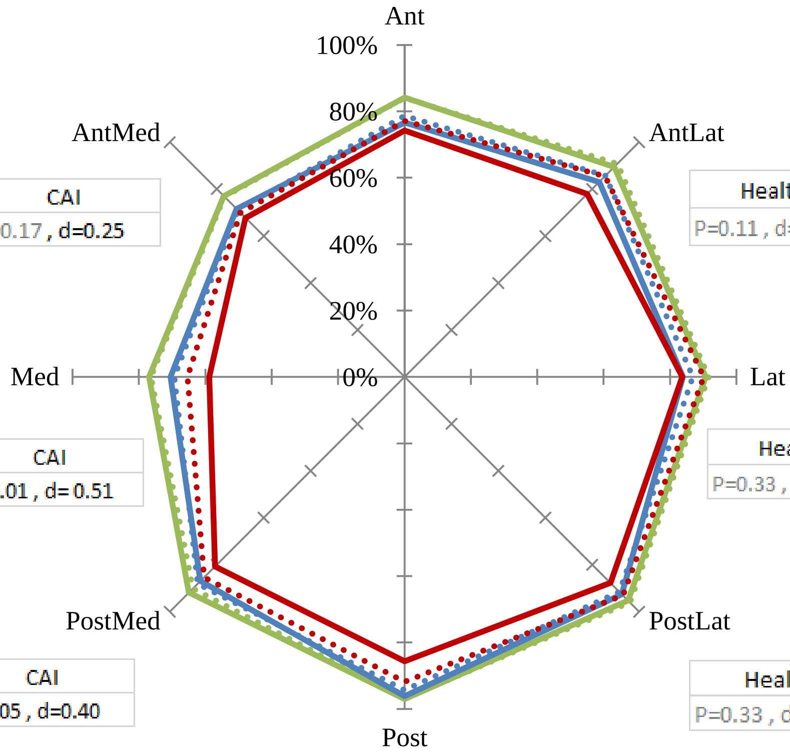
The within-group comparisons of injured and uninjured limbs of CAI participants demonstrated significant differences in normalised reach distances in all eight directions of the SEBT ($p<0.05$) except AntMed and Ant directions which demonstrated small to medium effect size (Figure 5.16). There were no statistically significant differences in coper participants between their injured and uninjured limbs in all eight directions with small effect size in the Ant, AntLat and Lat directions of the SEBT (Figure 5.16). For healthy participants, there were no statistically significant differences between both limbs. Therefore, the side-matched ankle of healthy participants with injured ankle of coper and CAI was selected for further analysis.

There were statistically significant differences in reach distances in all eight directions between the three groups ($p<0.05$), with CAI participants achieving the shortest distances (Figure 5.17). The Ant and AntLat reach distances were around 10% shorter in the CAI group (74.2%, 77.9% respectively) compared to healthy participants (84.1%, 89.4% respectively) (Figure 5.17).

There were also statistically significant differences in reach distances between healthy and coper participants in the Ant, AntLat and Lat directions (Figure 5.17). Whilst there were no significant differences in the PostLat, PostMed, Med and AntMed directions with only small to moderate differences observed (Figure 5.17). However, the Post direction did not show any significant differences between healthy and coper participants (Figure 5.17).

When comparing coper and CAI participants, there were statistically significant differences in the AntLat, Post and Med reach distances (Figure 5.17). Whilst there were no significant differences in the Ant, PostLat, PostMed and AntMed directions with small to moderate differences observed (Figure 5.17). However, the Lat direction did not show any significant difference between coper and CAI participants (Figure 5.17).

Healthy	Coper	CAI
P=0.96 , d=0.00	P=0.14, d=0.34	P=0.06 , d=0.50



AntMed:

Healthy	Coper	CAI
P=0.82 , d=0.03	P=0.67 , d=0.14	P=0.17 , d=0.25

AntLat:

Healthy	Coper	CAI
P=0.11 , d=0.17	P=0.12, d=0.41	P<0.01 , d=0.97

Med:

Healthy	Coper	CAI
P=0.76 , d=0.06	P=0.65 , d=0.09	P<0.01 , d= 0.51

Lat:

Healthy	Coper	CAI
P=0.33 , d=0.17	P=0.22 , d=0.39	P<0.01 , d=0.93

PostMed:

Healthy	Coper	CAI
P=0.30 , d=0.10	P=0.72 , d=0.10	P=0.05 , d=0.40

PostLat:

Healthy	Coper	CAI
P=0.33 , d=0.17	P=0.61 , d=0.11	P=0.02 , d=0.60

Post:

Healthy	Coper	CAI
P=0.87 , d=0.01	P=0.17 , d=0.17	P=0.05 , d=0.50

Healthy injured imb · Healthy uninjured limb · Coper injured limb · Coper uninjured limb · CAI injured limb · CAI uninjured limb

Figure 5-16: Within groups comparison of normalised reach distance between injured and uninjured limbs of healthy, coper and CAI participants

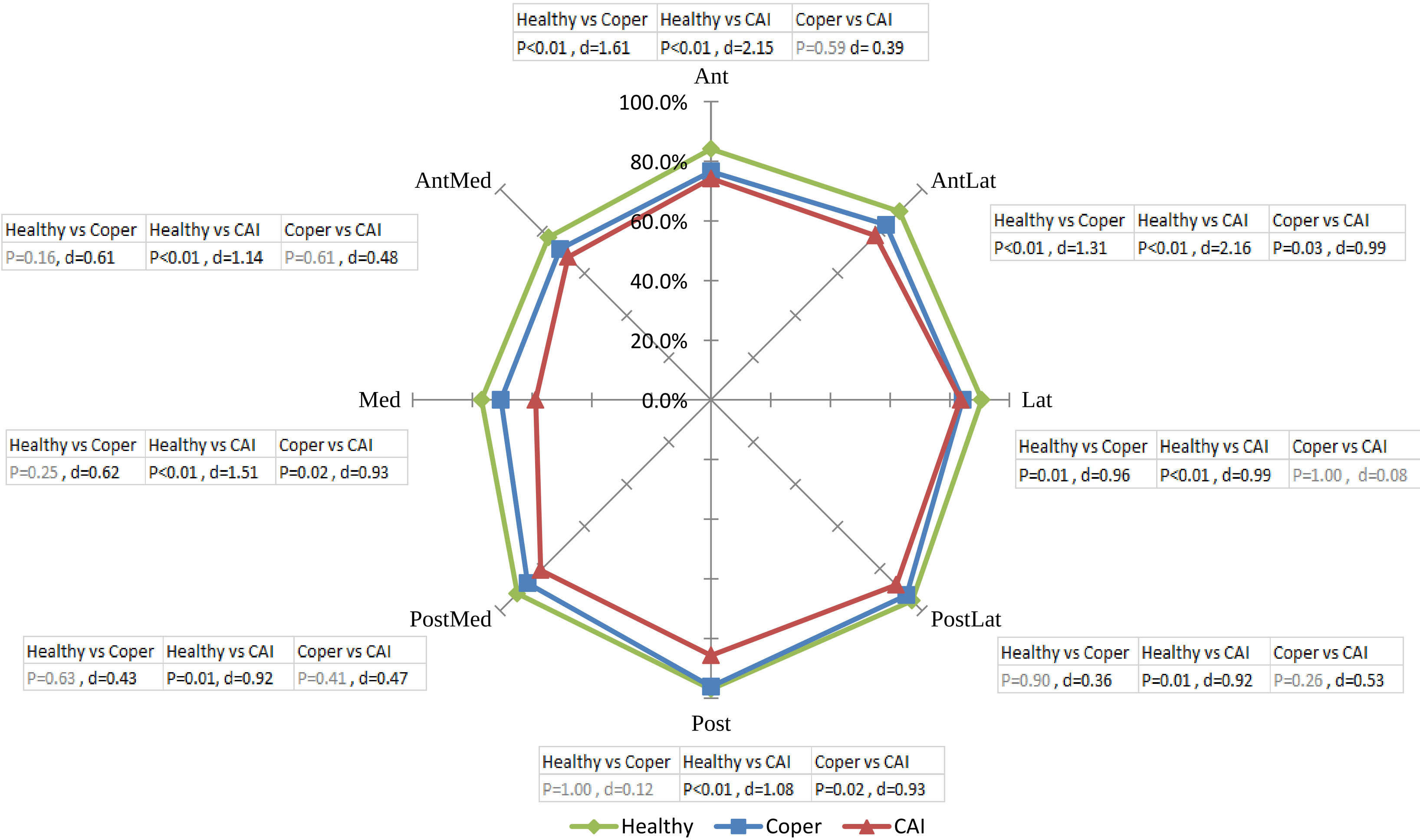


Figure 5-17: Normalised reach distance of the SEBT of the injured limb of healthy, coper, and CAI participants

5.6.3 3D kinematics

The sagittal plane kinematics reflected a dorsiflexion movement in all eight directions of the SEBT for all groups. In the frontal plane, there was eversion for all three groups in all eight directions, except in the AntMed direction for healthy participants, for which there was inversion. Regarding the transverse plane, there was internal rotation in all eight directions for healthy participants. The coper and CAI participants also demonstrated internal rotation for all eight directions, except in the AntMed direction, for which there was external rotation.

Figure 5.18 represents the differences in the kinematics of the ankle joint between the three groups (healthy, coper and CAI) in each plane (sagittal, transverse and frontal). The largest difference was seen in sagittal plane when compared to the frontal and transverse planes. The differences in the sagittal plane were largest between healthy and CAI participants with the AntLat direction being the most affected ($p<0.01$) (Figure 5.18). On the other hand, the frontal plane was the smallest differences among the three planes (Figure 5.18). The descriptive values of the differences between the three groups in the three planes are given in Appendices 20-22.

In the sagittal plane, CAI participants demonstrated a statistically significant decrease in ankle dorsiflexion compared to healthy participants in all eight directions ($p<0.05$), except in the Post and Med directions. Whilst, the effect size showed large effects for all directions (Figure 5.18). Moreover, statistically significant decreases in ankle dorsiflexion were demonstrated in the CAI participants compared to coper participants in the AntLat and AntMed directions. Whilst, there was a moderate to large effect size in all eight directions (Figure 5.18). Interestingly, there were no significant differences in ankle dorsiflexion between healthy and coper participants in all directions; whilst, a small effect size was demonstrated in the Ant, AntLat, Lat and PostMed (Figure 5.18). Figure 5.18 revealed that the largest differences in ankle dorsiflexion were seen between healthy and CAI participants, followed by coper and CAI participants, with no differences seen between healthy and coper participants.

In the transverse plane, there was a statistically significant decrease in ankle internal rotation between healthy and CAI participants in the Lat, Post and Med directions with a moderate to large effect size was demonstrated in all eight directions (Figure 5.18). There were no statistically significant differences in ankle internal rotation between coper and CAI participants in all directions with a small to moderate effect in all directions, except in the

Ant and AntMed directions (Figure 5.18). There were no significant differences between healthy and coper participants with only small to moderate differences observed in all eight directions (Figure 5.18).

In the frontal plane, there were no statistically significant differences in ankle eversion between healthy vs CAI, healthy vs coper, and coper vs CAI participants in all eight directions ($p>0.05$) (Figure 5.18). Whilst, the effect size showed a small to moderate effect between healthy and CAI participants in all directions except in the Ant and PostMed directions (Figure 5.18). Furthermore, a small to moderate effect between coper and CAI participants was found in all directions except the Ant, AntLat, and PostLat directions (Figure 5.18). There was also a small to moderate effect size between healthy and coper participants in all directions, except the Med and AntMed directions (Figure 5.18).

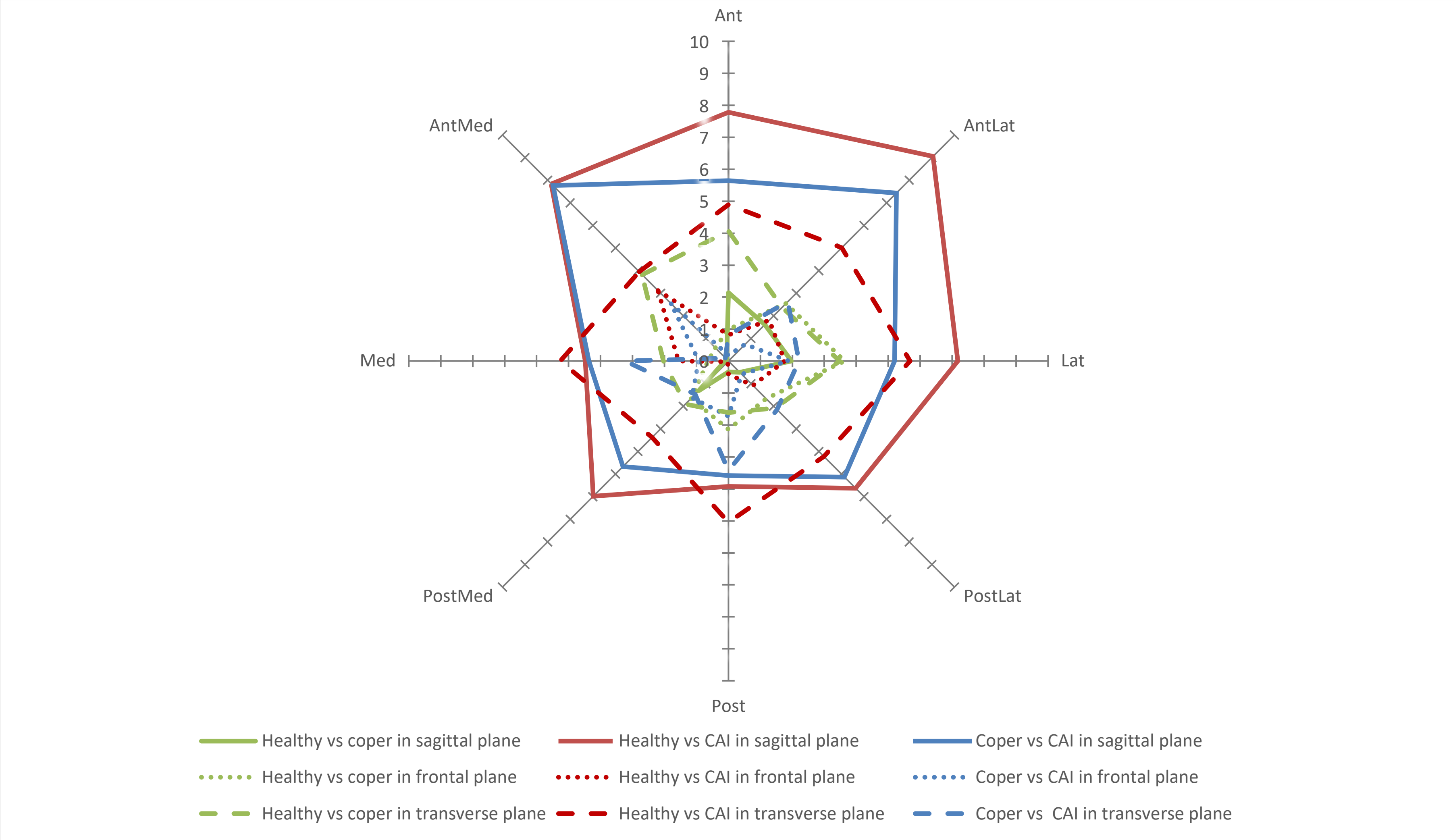


Figure 5-18: The differences of kinematics of ankle joint between the three groups (healthy, coper and CAI) in each plane (sagittal, transverse and frontal)

The within-group comparisons of injured and uninjured limbs for CAI participants demonstrated a large significant difference in kinematics of the ankle joint in the sagittal plane for all eight directions of the SEBT ($p<0.01$), except for the Med and AntMed directions ($p=0.06$); whilst, the effect size showed a moderate effect in these directions (Figure 5.19). Comparing the injured and uninjured limbs of coper participants showed no statistically significant differences in the kinematics of the ankle joint in the sagittal plane ($p>0.05$), except in the AntMed direction ($p=0.04$) (Figure 5.19); whilst, the effect size showed a small effect in all directions except the PostLat, Post and Med. For healthy participants, there were no statistically significant differences between injured and uninjured limbs in all directions ($p>0.05$) (Figure 5.19).

Even though injured limb of CAI participants demonstrated a decrease eversion compared to uninjured limb, it was only statistically significant different in the Lat and PostLat directions of the SEBT, with large effect sizes (Figure 5.20). Whilst, the effect size showed a small to moderate effect in all eight directions except, the AntMed direction. There were no statistically significant differences between injured and uninjured limbs in coper and healthy participants in the transverse plane (Figure 5.20). Whilst, a small effect size was demonstrated in the Ant and Lat directions between injured and uninjured limbs among coper participants (Figure 5.20).

Interestingly, there were no statistically significant differences in the kinematics of the ankle joint between injured and uninjured limbs of the three groups in the frontal plane (Figure 5.21). Whilst, there was a small effect size in the AntLat, Lat, Med and AntMed directions for injured and injured limbs of coper and CAI participants (Figure 5.21). Moreover, a small effect size was demonstrated in the PostLat and Post directions between injured and injured limbs of healthy participants (Figure 5.21).

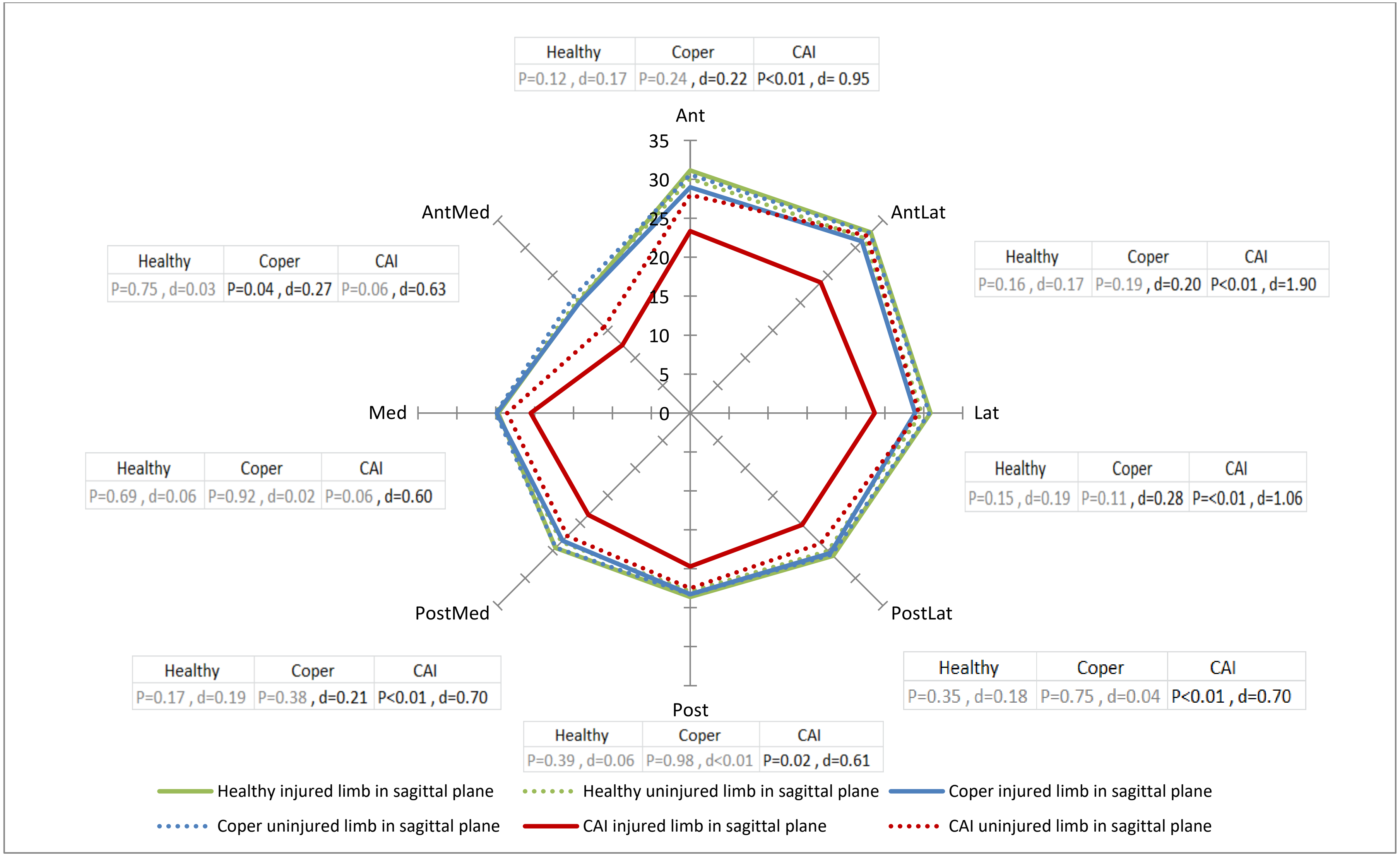


Figure 5-19: Kinematics of ankle joint for injured and uninjured limbs in healthy, coper, and CAI participants in sagittal plane

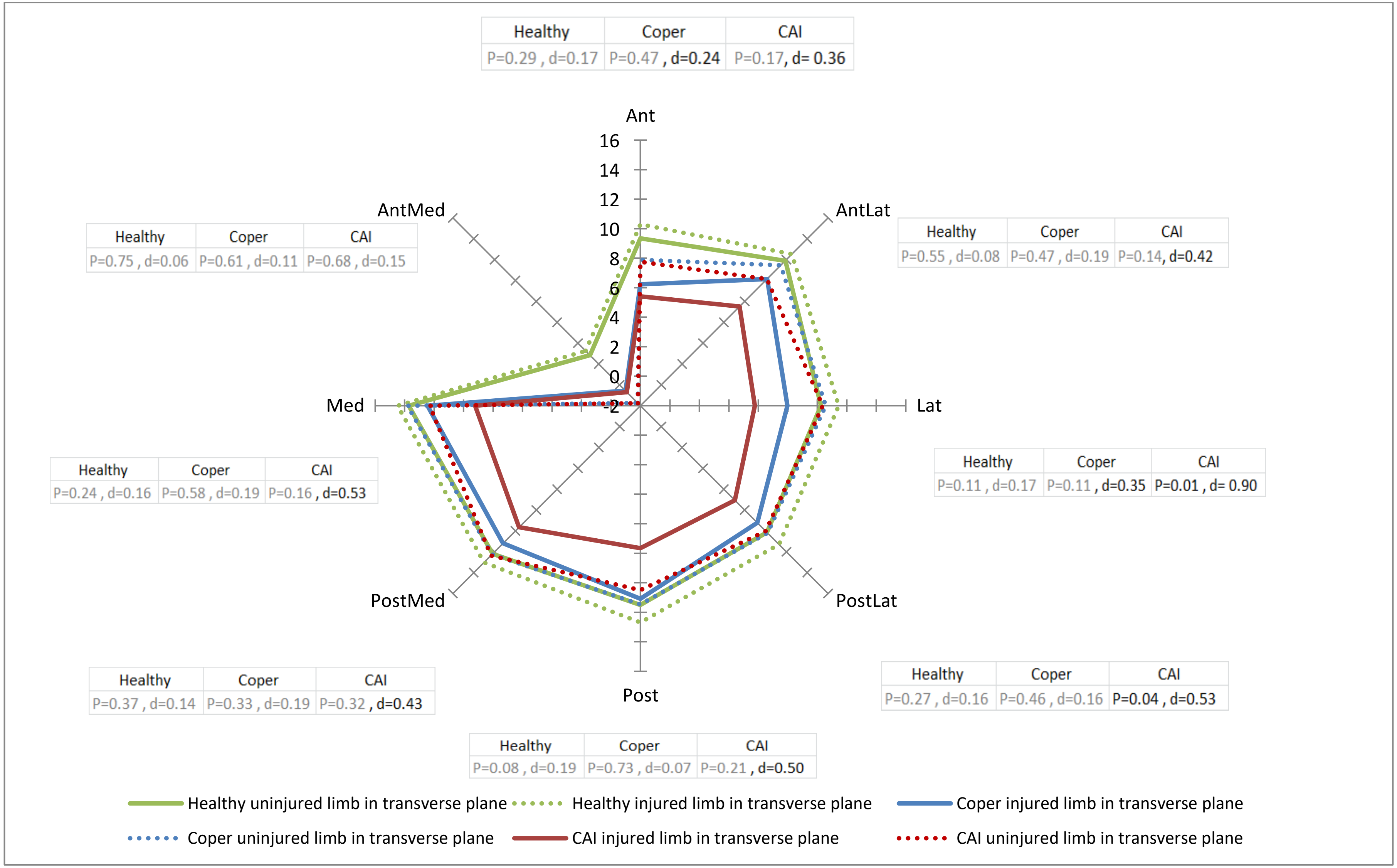


Figure 5-20: Kinematics of ankle joint for injured and uninjured limbs in healthy, coper, and CAI participants in transverse plane

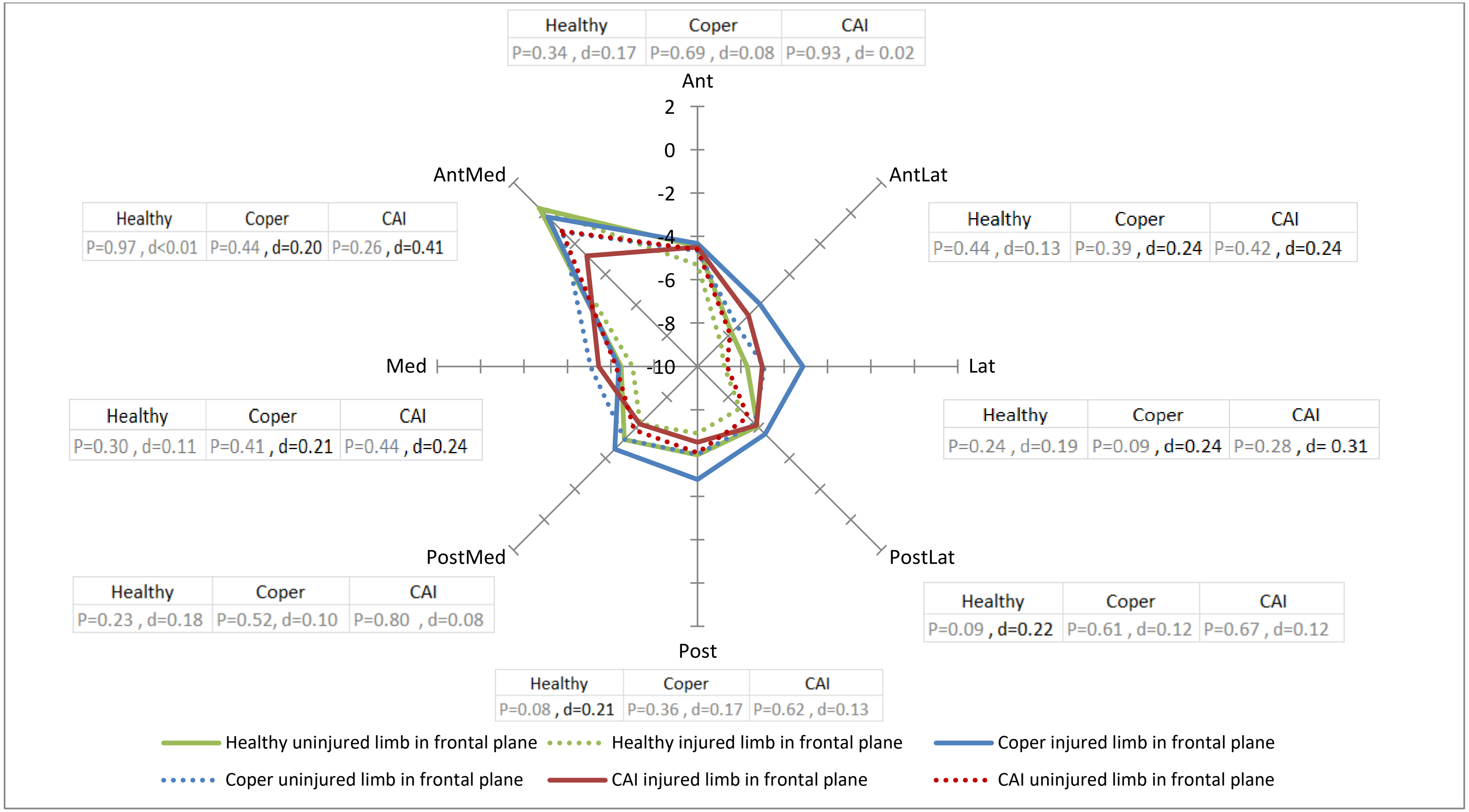


Figure 5-21: Kinematics of ankle joint for injured and uninjured limbs in healthy, coper, and CAI participants in frontal plane

5.6.4 Correlation between the thickness of lateral ligaments and the most affected direction of the SEBT

Since the previous results have demonstrated that reach distance in the AntLat direction of the SEBT is the most affected, a correlation was calculated for this direction. Figures 5.22 and 5.23 showed that the thickness of the ATFL and CFL had a significant moderate correlation with the AntLat direction of the SEBT (r = -0.53, p<0.001and r = -0.40, p<0.001 respectively).

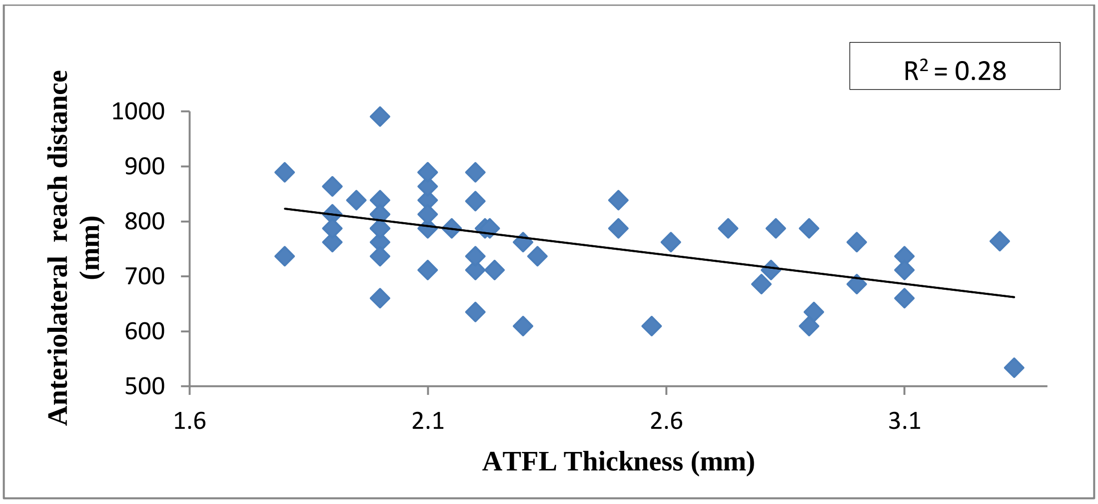


Figure 5-22: Pearson correlation between anterolateral reach distance on the SEBT and thickness of ATFL of the involved limb for healthy, coper, and CAI groups. The trend line symbolises the overall correlation for the three groups

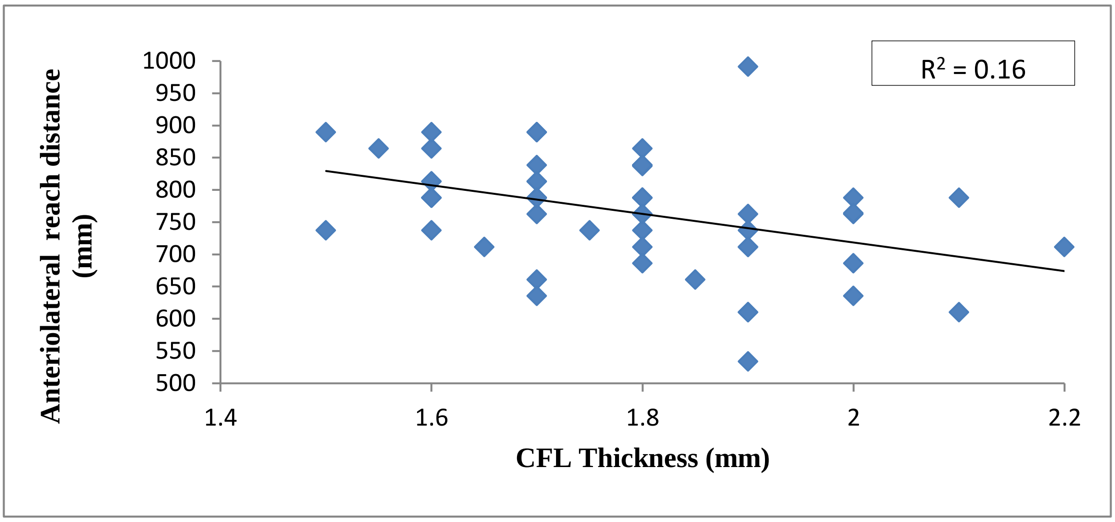


Figure 5-23: Pearson correlation between anterolateral reach distance on the SEBT and thickness of CFL of the involved limb for healthy, coper, and CAI groups. The trend line symbolises the overall correlation for the three groups

5.7 Discussion

5.7.1 Comparison of the reach distance of the SEBT between CAI compared to healthy participants

Capturing the deficits that participants report related with CAI in measurable laboratory test is important for the development of objective outcomes for diagnosis, prognosis, and rehabilitation. The most significant finding was that participants with chronic ankle instability demonstrated poorer dynamic balance than healthy participants in all eight directions of the SEBT. As a result from the ultrasound study (chapter three), the length, thickness of ATFL and the thickness of CFL were greater in CAI participants compared to healthy participants. This demonstrated both mechanical instability and structural adaptations occur in participants with CAI who exhibited in shorter reach distances.

The anterior directions of the SEBT (anterior, anterolateral, and anteromedial) demonstrated great differences than the posterior directions (posterior, posterolateral, and posteromedial) in CAI compared to healthy participants with the anterolateral direction was the most significantly difference, followed by the anterior direction. This result agrees with those seen by Ahn, Kim, and Kim (2011) and Doherty et al. (2016) who found the anterior directions were shorter than posterior directions in CAI participants compared to healthy participants. Olmsted et al. (2002) and Pionnier et al. (2016) also found that CAI participants had significantly shorter reach distances than healthy participants when standing on the injured limb. However, both studies averaged the scores for all eight reach distances; their analyses did not treat each direction independently. It could be that they averaged the scores for all eight directions as they aimed to test the efficiency of the SEBT in detecting dynamic balance deficits in participants with CAI. Several studies used three directions (YBT) instead of the eight directions of the SEBT (Doherty et al., 2016; Plante & Wilkstrom, 2013). Payne et al. (2016) compared distances during the YBT (anterior, posteromedial, and posterolateral) among soccer players with and without CAI. The result demonstrated that the anterior direction was the only difference between these groups.

In ankle joint motion, dorsal flexion and plantar flexion were defined as rotation around the medial-lateral axis, inversion and eversion were defined as around a rotation around the anterior-posterior axis, and external and internal rotation were defined as rotation around the proximal-distal axis (Fong, Chung, Chan & Chan, 2012).

Gabriner and colleagues (2015) studied the contributions of the ankle dorsiflexion range of motion, strength, static postural control and plantar cutaneous sensation to the performance of the YBT in chronically unstable ankles. They found that ankle dorsiflexion range of motion and plantar cutaneous sensation contribute to 16% of the difference related to anterior direction, and eversion strength and time-to-boundary mean minima (TTBMM) in the medial-lateral direction contribute to 28% of the difference related to posteromedial. Their results suggested different physical demands required to perform the anterior and posterior directions of the SEBT which could explained the differences found in the present study. The anterior direction might be more affected by sensory deficits and mechanical restrictions at the ankle complex, while the posterior directions depend more on the postural control and strength (Gabriner et al., 2015). This result emphasised Terada et al.'s (2014) finding that mechanical restriction (smaller dorsiflexion ROM) at the ankle affect only the anterior direction of the YBT in participants with CAI.

Another possible explanation of the anterior directions being most affected than posterior directions could be due to different of muscle activity in each direction. Feger and colleagues (2014) studied the activation of the lower limb muscles during YBT for participants with and without CAI. They found that CAI participants showed less activity of the ankle, knee and hip musculature during YBT compared to healthy participants which decrease the angles of hip and knee flexion. Thus, the ankle joint instability is influenced by the impairment of neuromuscular adaptation of the knee joint caused by decrease the torque of the ankle plantar flexor and knee flexor and extensors (Gribble & Robinson, 2009).

Most of the ankle sprains create injury to the lateral ligaments (also reported in our ultrasound study). Ahn et al. (2011) found that functionally unstable ankles had weaker plantar flexors compared to healthy group. The electromyography (EMG) activities of peroneal longus, tibialis anterior, and biceps femoris were also significantly lower compared to healthy participants in the anterior, anterolateral, and anteromedial directions of the eight directions of the SEBT. Although, peroneus longus muscles, play a significant role in controlling ankle inversion, there were no significant differences in the thickness and the CSA in CAI participants compared to healthy and copers (as demonstrated previously in chapter 3), suggesting that the peroneals may have similar structure, yet, functionally act differently when controlling movement. Ahn et al. (2011) reported tibialis posterior and soleus activation was lower in the posterior, posteromedial and posterolateral while vastus lateralis activation was lower in the lateral direction. The EMG differences between different reach distances could assist clinicians to decide which reach directions to apply as outcome measures in patients with particular deficiency in the muscle strength. The tibialis posterior must be strengthened rather than strengthened peroneus longus. Thus, strengthened invertor as well as evertor must be desired for preventing the ankle instability. The results suggest that preventing the reoccurrence of ankle sprain can be occur by strengthened muscles contributing to the ankle stabilisation specially in the anterior directions and activation feed-forward motor control by the analysis of muscle activities involved based on the SEBT in CAI.

In contrast to the current and previous research, De La Motte et al. (2015) studied the medial reach distances (anteromedial, medial, and posteromedial) between healthy and CAI participants and found no statistically significant differences in these three directions (p=0.66, p=0.35 and p=0.45 respectively). These results are similar to those reported by Sefton et al. (2009), who also found no differences in these three reach distances in CAI participants compared to healthy participants. Furthermore, Razeghi et al. (2016) studied the ability of the YBT (anterior, posteromedial and posterolateral) to differentiate between women with and without CAI. They found no statistically significant differences in the three directions (p>0.05) between the two groups. These studies emphasised the need to perform eight directions of the SEBT instead of only three directions when testing participants with previous history of ankle sprain. Since some of the SEBT directions could not be sensitive enough to detect the deficiency and based on our result AntLat direction was the most significant different between the three groups and it was not test in these previous studies.

Stiffness of the lateral ligaments and weakness of the muscles that are in charge for eversion and pronation of the ankle complex contributes to functional instability in the lateral ankle sprain. Changes in the structures can possibly reduce the ankle dorsiflexion which restrict the movement of the ankle joint and decrease the reach distance in participants with CAI. The movement of the ankle joints during the SEBT was measured by using the motion analysis system. Biggest differences between the groups were seen in the sagittal plane, with the differences between CAI and healthy participants being the most affected, particularly in the anterior directions (anterior, anterolateral and anteromedial). In other words, ankle dorsiflexion was most significantly decreased in CAI participants, this outcome supports the evidence that limitation in ROM is contributing factor in individuals with CAI (Collins, Teys, & Vicenzino, 2004; Morrison & Kaminski, 2007). Moreover, the smallest difference in the sagittal plane (dorsiflexion) occurred in the posterior direction, which could also reflect the fact that the CAI participants was able to achieve longer reach in the posterior direction of the SEBT than in the anterior direction, as discussed previously. Using the kinematic in this study helps to explain the shorter reach distance demonstrated in CAI.

Many studies support the findings of this study and have observed a restriction in ROM after an ankle sprain and commonly in dorsiflexion (Collins et al., 2004; Hubbard & Hertel, 2006). It has been reported that stiffness of the gastrocnemius and soleus muscles can create restrictions in dorsiflexion (Denegar & Miller, 2002). This plays a role in abnormal movement during physical activity, which could affect the tissue around the ankle in generating a compensatory movement in order to maintain function (Almansour, 2015). Therefore, this will predispose the individual to a high risk of sustaining another sprain (Hubbard & Hertel, 2006).

Doherty et al. (2015b) evaluated the 3D kinematics of the joints of the lower extremity during the performance of the YBT in participants within two weeks of having a first time lateral ankle sprain and in a control group. Sagittal plane kinematic profiles demonstrated decreased flexion displacements at the hip, knee and ankle during reach performance. In addition, the frontal plane of the ankle joint showed a decreased eversion displacement in the posteromedial direction only which contradict our findings in which there was no difference in the frontal plane between the groups.

In a follow-up study, Doherty et al. (2015a) conducted a study on participants within six months after their initial acute sprain to measure the sagittal plane kinematics of the lower limb at the point of maximum reach distance in the YBT by using 2D video analysis. The injured limb of the lateral ankle sprain participants presented with decreased hip and knee flexion, and ankle dorsiflexion in all three directions compared to healthy participants. Doherty et al. (2016) repeated the study in CAI and coper participants within one year after a first time lateral ankle sprain. They found that CAI participants showed decreased hip and knee flexion in posteromedial direction compared to coper and healthy participants. While CAI participants demonstrated decreased knee flexion and ankle dorsiflexion displacement only in the posterolateral direction compared to coper and healthy participants.

In recent research, Pionnier et al. (2016) found that, in addition to decreased medial rotation of the hip and knee, ankle pronation/supination and abduction/adduction ROM were reduced in CAI participants compared to healthy participants ($p<0.01$ and $p=0.04$ respectively). However, there was no statistically significant difference in ankle flexion/extension between CAI and healthy participants (p=0.45). The results from Pionnier et al.'s (2016) study revealed that the ROM in CAI participants decreased, especially in the transverse and frontal planes, reducing medio-lateral restraints at the ankle joint, and therefore limiting stresses on the lateral ligaments (Pionnier et al., 2016). A possible explanation for the differences in ankle dorsiflexion ROM in the reach directions between the present study and the previous studies could be the differences in inclusion criteria used for CAI participants. For example, in Pionnier et al.'s (2016) study, CAI participants should have had at least one sprain and at least three episodes of "giving-way". In addition, different directions of the SEBT used in different studies.

The present study also demonstrated significant differences in transverse plane between CAI and healthy participants. In addition, it seems that CAI and coper participants adopt different strategies to healthy participants in the AntMed direction. Their ankles demonstrated an external rotation, while healthy participants performed an internal rotation motion. Interestingly, it seems that all three groups (healthy, coper and CAI) rely least on the frontal plane during the performance of the test. This finding reflected De la Motte et al.'s (2015) previous finding that CAI participants may use various movement strategies that could be reliant more on motion in the transverse or frontal planes to achieve the movement goal. A possible explanation for those with CAI adopting transverse strategies is the limitation in ankle dorsiflexion range of motion which is frequently recognised in these people (Hoch et al., 2012).

Cumulatively, the alteration and deficits in proximal muscles and controlling of the joint as well as stereotypical movement patterns demonstrate global deficits in sensorimotor function. This study demonstrate the need of better and longer protective of ankle joint following an acute sprain to assist restore mechanical stability. Increased the laxity of the ligament can produce further mechanical adaptation, impairment in controlling the sensorimotor, ankle sprain reoccurrence, and reduction in global function as maladaptive compensation of the change in joint laxity and/or sensorimotor control.

5.7.2 Comparison of the reach distance of the SEBT between coper and healthy participants

Few researchers have conducted studies that include copers in addition to CAI and healthy participants (Doherty et al., 2016; Plante & Wilkstrom, 2013). Even though coper participants did not develop CAI, the present study showed that they are quit differ from healthy participants. They reported a decrease normalised reach distance in the anterior, anterolateral, and lateral directions of the SEBT compared to healthy participants ($p<0.05$). Whilst, there was a small to moderate ($d=0.36 – 0.62$) difference in the remaining directions except the posterior direction. This difference between coper and healthy participants is consist with structural change in the thickness of ATFL that found in the ultrasound study. These results shad the light that copers could go to the same way as CAI, however, it is not sever enough to develop CAI. In contrast to the present study, Plante and Wilkstrom (2013) and Doherty et al. (2016) did not find differences in the YBT between coper and control participants. However, they only tested three directions which may not be sensitive enough to detect the deficiency.

Even though there was no statistically significant difference in the kinematics data between the coper and healthy participants, lateral ligament structure and reaching distance of the coper demonstrated significant different. It seems that copers are a high risk to develop CAI as their structural and functional of the ankle joint are not perfectly well as healthy participants. Therefore, it is suggested that a rehabilitation plan can be drawn which include selective muscle strengthening for coper participants to avoid further disability and develop CAI.

5.7.3 Comparison of the reach distance of the SEBT between coper and CAI participants

When comparing coper and CAI participants, normalised reach distances were significantly decreased in the anterolateral, posterior and medial directions with a small to moderate ($d=0.39 – 0.53$) effect size in the remaining directions except the lateral direction. The differences in balance between coper and CAI participants in the present study are consistent with previous studies (Wikstrom, Fournier, & McKeon, 2010a; Wikstrom et al., 2010b) in which deficits in static and dynamic balance were found. Therefore, it seems that impaired balance is a significant consequence of CAI that most probably has significant negative consequences for functional performance (Plante & Wikstrom, 2013) as demonstrated by the fact that balance deficit is related to increase the risk of ankle injury (Wang et al., 2006). Furthermore, differences between coper and CAI participants in some directions suggest that this result could represent a side of the coping mechanism that is absent in CAI participants.

Changing kinematic patterns have been reported previously in people with ankle instability during the performance of more dynamic jumping and landing tasks (Brown, Padua, Marshall, & Guskiewicz, 2011; Caulfield & Garrett, 2002). Caulfield and Garrett (2002) proposed that these changes may point to a learned adoptive strategy as a consequence of previous injury and could be reflected in other tests in which CAI dysfunction is presented. These learned strategies may assist in elucidating the finding of this present study in each of the eight directions between the groups. SEBT is a close chain exercise which permits participants to achieve the reach distances in a self-selected manner at a self-selected pace (De La Motte et al., 2015). Therefore, it could be that the coper and CAI participants in the current study have utilised a learned adaptive compensatory motor-control strategy, representing a change in the sagittal plane kinematics of the ankle during the performance of the SEBT test.

Even though the present study showed a significant difference of ankle dorsiflexion at the anterior directions only, Plante and Wilkstrom (2013) and Doherty et al. (2016) found a difference in posterolateral reach distances between coper and CAI participants. However, coper participants in Doherty et al.'s (2016) study had their sprain for exactly one year and achieved better reach distances than those in the previous studies already mentioned. For instance, the mean reach distance for coper participants in the PostLat direction was (99.80 ± 8.70 %) which is significantly higher than the finding in the present study and in Plante and Wilkstrom's (2013) study (92.6 ± 8.40 %, 83.0 ± 8.0 % respectively). It could be that the participants in Doherty et al.'s (2016) study represent a population who are at an early stage of the disease process; it is possible that the pattern of ankle instability becomes worse with time (Doherty et al., 2016). Meanwhile, participants with years of ankle instability are specifically prone to time-dependent deteriorations in functional capability, as might have been the situation in the present and previous studies (Hertel et al., 2006; Hoch et al., 2012; Plante & Wikstrom, 2013); it is improbable that a precise one-year cut-off occurs whereby the degenerative ankle instability process reach relative stasis (Doherty et al., 2016).

Moreover, Jaber et al. (2018) found a higher activation of the anterior tibialis and gluteus maximus in coper and healthy participants might be explained as a strategy utilised by these groups to maintain the stability in case of more challenge task such as anterior and posterolateral direction. Therefore, the poorer achievement demonstrated in CAI participants during these directions may be attributed to the lack of such strategy. Moreover, coper and healthy participants utilised both ankle and hip muscles during the posterolateral direction while CAI participants depends more on the ankle muscles to complete the task. Furthermore, Jaber et al. (2018) found that CAI participants demonstrated a significantly delayed in only gluteal muscles activation onset time but not in the ankle muscles. This dependence on ankle strategy to maintain stability could be considered a suboptimal muscle recruitment pattern and it might clarify the higher injury risk in people with CAI.

Even though the YBT has commonly been used in studies, Coughlan and colleagues (2012) argued that various strategies for postural control could be utilised to perform this test. Therefore, it is difficult to directly compare the current finding with previous findings from using the YBT or from using only the medial directions of the SEBT. This is because it is also demonstrated in this study that each direction could represent a different movement strategy for each group. For instance, during reach movement in the anterior direction in the transverse plane, the greatest difference was between healthy and coper participants while the smallest difference was between coper and CAI participants. However, in the posterior direction, the opposite occurred, with the biggest difference being between the coper and CAI participants and the smallest between coper and healthy participants. This means that coper participants were able to adapt a strategy similar to healthy participants in the posterior direction but not in the anterior direction, which is similar to CAI. This finding could also explain why the coper participants were able to achieve better reach distances in the posterior than the anterior direction. Coper participants in this study demonstrated no significant differences at the ankle joint compared to healthy and CAI participants in transverse and frontal planes, this explained that coper participants could rely more on proximal joints and muscles which need a further investigation.

Therefore, it seems important to investigate the three kinematic planes along with the eight directions of the SEBT in order to complete understanding functional consequences of lateral ankle sprain. All of these findings can point to an adaptation in the central nervous system, which sets up a protective motor command relying on previous experiences of lateral ankle sprain or ‘‘giving-way’’ of the ankle. This motor adaptation could decrease the risk of ankle instability which can be induced by whole body-wide movements or accelerations (Pionnier et al., 2016).

5.7.4 Other factor could affect the reach distance of the SEBT in CAI participants

Interestingly, it has been reported that participant apprehensiveness could be the most crucial factor in inhibiting performance (Olmsted et al., 2002). It could be that after sustaining their injury, many participants are hesitant in attempting to accomplish the SEBT which asks them to challenge their limits of stability. Olmsted et al. (2002) reported that many participants with CAI in their study experienced a feeling of apprehension while reaching in a specific direction of the SEBT and trying to keep their injured limb stable. However, in a quiet standing balance test apprehension does not play a significant role, because participants are rarely required to challenge their limits of stability (Olmsted et al., 2002).

5.7.5 Differences of reach distance of the SEBT between anterior and posterior directions in healthy participants

The posterolateral and posteromedial directions were significantly longer than the anteromedial and anterolateral directions. A possible explanation for this could be that muscle activation differs in each direction. Earl and Hertel (2001) demonstrated that muscle activation (measured using EMG), was significantly different during movement in the eight different reach directions of the SEBT for healthy participants. Reach distance is therefore sensitive to direction. They reported that during the performance of the three anterior reaches of the SEBT, the quadriceps were the most active muscles in the lower limbs, with the vastus medialis being the most active in the anterior reach direction compared to all other directions. In addition, the medial hamstring showed greater activity in the anterolateral direction than in the other anterior directions (Earl & Hertel, 2001). During the performance of posterior reach distance, the anterior tibialis was the most active muscle. The activity of the biceps femoris was greater in the posterior, posterolateral and lateral directions compared to the anterior and anteromedial directions (Earl & Hertel, 2001). Based on these findings, it seems that the activity of the lower extremity muscles is reach dependent.

5.7.6 The inconsistency of the results between this study and previous studies

This inconsistency in the results for reach distances and kinematic data in the SEBT between CAI and healthy participants, coper and healthy participants, and coper and CAI participants between the previous studies may be due to numerous differences in inclusion criteria, statistics and methodology, such as variations in the number of practice trials. Some studies asked the participants to perform six practice trials (De La Motte et al., 2015) while other required three or four trials. Moreover, hand placement was not consistent among the previous studies. In the present study, along with several previous studies, (De La Motte et al., 2015; Doherty et al., 2016) the participants positioned their arms on the iliac crest of the waist during each trial to avoid supporting their balance with their hands. On the other hand, in Earl and Hertel's (2001) study, the participants were allowed to move their arms freely. This free arm movement made the task of maintaining balance less challenging by increasing the counterweight moment while performing the reach distance and therefore changing the function of the muscles (Anderson, 2016). In addition, the inclusion criteria could also explain the differences in findings. As it generate heterogeneous sample of participants which limits our ability to compare the results across different studies. Even though all studies need an ankle sprain for inclusion, the severity, the number of the sprain, and the time since last injury could be different. It is assumed that participants with many sprains or regular episodes of ankle giving way had increased ankle disability and decrease function compared to participants with only a feeling of giving away. In addition, it is important to recognise the time since last injury because an early stage after most recent ankle sprain could affect the results of the study as full recovery may have not taken place and the deficits occur could be due to the consequences of acute phase (Delahunt et al., 2010).

5.7.7 Correlation between the thickness of lateral ligaments and the most affected direction of the SEBT

In addition to any changes in the associated muscles and tendons of the ankle joint, which may simply reduce active ROM, potentially due to disuse post injury, the findings indicate that participants with thicker ligaments achieved shorter distances in the SEBT. One possible explanation for this is that thicker ligaments are stiffer and restrain joint motion, therefore prevent longer reach distances as kinematic data demonstrated decrease in the dorsiflexion ROM of coper and CAI participants compared to healthy participants.

It is assumed that changing morphology of the ligament influences the stability of the ankle and thereafter the ability to control balance during reaching movements that involve joint motion placing the thicker ligaments under strain. In addition, the skeletal muscle and tendons will also play a role during the dynamic tasks. If however thicker ligament infers more compliant structure, perhaps due to greater water content, or disorganised collagen fibres post healing, then deficiency in maintaining balance occur and participants with injured ankles are not able to achieve long reach distance as healthy ankles.

5.8 Limitation

Even though the study evaluates the three planes of movement for the eight directions of the SEBT, it has only evaluated the ankle joint. It should be acknowledged that the hip and knee joints are involved in the performance of the SEBT. As such, future study should evaluate the kinematics of the whole lower extremity of healthy, coper and CAI participants and to include coordination training for the whole lower limb into rehabilitation following lateral ankle sprain. Moreover, the researcher did not measure the EMG of the lower extremity muscles which related to the performance of SEBT and could provide additional insight to reach distance behaviour during the SEBT. Even though the demographic data (age, height, weight, and BMI) was matched between the groups, the number of male and female participants in each group was differ and future researches should assess a single gender to reduce the possibility of the effect of gender in the test performance.

5.9 Conclusion

Differences in dynamic balance exist between CAI and healthy participants in all eight directions of the SEBT, coper and healthy participants in the anterior, anterolateral, and lateral directions, and CAI and coper participants in the anterolateral, medial, and posterior directions.

The differences in reach strategies were mainly in the sagittal plane which greater decrease dorsiflexion motion/angle in the CAI participants compared to coper and healthy participants, and during all directions except medial and anteromedial directions of SEBT. This study also provides an insight into the negative relationship between the thickness of ankle ligaments (ATFL and CFL) and the anterolateral reach distance in the SEBT.

6 Chapter six: Overall summary, conclusion, limitation and recommendations for future work

6.1 Chapter overview

This final chapter starts with an overall summary of the thesis. A brief discussion of the lack of detailed sonographic studies and quantitative analysis of the echogenicity and literature on ankle sprain are included as a reminder of the rationale for the thesis. The novelty of this work is also highlighted, limitations, clinical relevance and recommendations for future work presented.

6.2 Overall summary of the thesis

The ankle is considered one of the most traumatised body sites and accounts for 3-5% of all admissions to hospital emergency rooms in the UK. Sprains account for approximately 85% of all ankle injuries with the lateral ankle ligaments the most frequently injured site. Physical examination of the anterior drawer and talar tilt tests assist in defining the extent of injury, especially in most severe injuries. However, these tests are subjective and appear to be unreliable. Imaging of the foot and ankle area help in diagnosis, therapeutic decision-making, and evaluation of functional results. Foot and ankle structures have been scanned using plain radiographs but these are not helpful in ligamentous sprains and poor utility of x-rays led to the development of the "Ottawa Ankle Rules", as a means to help management of acute ankle injuries. On the other hand, MRI is considered to be the gold standard for diagnosing the ankle ligaments after ankle sprain, but access can be limited. Using musculoskeletal ultrasound to detect ankle abnormalities has revealed positive findings in relation to make sprains and more screening of the precise soft tissue injury may help inform the design of individual intervention programmes.

The review of the literature (chapter two) identified a knowledge gap regarding our understanding of ankle structures in people with previous ankle sprain, specifically between those who develop further ankle sprains after the first, and individuals who do not report any complications after the first sprain. This is important because it may improve our understanding of whether structural features of the injury explain why some individuals experience ongoing ankle instability while others do not. However, it is also important to ask how changes in any structural features may affect the functional performance of the ankle.

The first aim of this PhD thesis was to characterise and compare selected ankle structures in people with and without a history of ankle sprain. The second study was aimed to provide a quantitative analysis of echogenicity of the anterior talofibular ligament in healthy, coper and CAI participants using computer-aided greyscale analysis software. The last study was aimed to investigate the dynamic balance in people with and without a history of ankle sprain. To achieve these aims, the thesis was conducted in three studies.

The study presented in chapter three was designed to investigate structural changes in selected ankle structures in people with and without previous ankle sprain. An ultrasound protocol was developed to assess the length, thickness and cross sectional area of ATFL, CFL, peroneal tendons and muscles, TPT and AT. ATFL was found to be longer and thicker in both coper and CAI participants compared to healthy and thicker but not longer in CAI compared to copers. An interesting finding among healthy participants was that overweight participants demonstrated thicker and larger CSA of most of the selected ankle tendon and muscles compared to normal weight participants that is likely due to the additional strain placed on these structures.

In addition to the thickness and the length of the ATFL, it was important to characterise the ligaments in term of echogenicity to have a great understanding of the structural changes. Injured ligaments were defined in the literature as "hypoechoic" which is considered to be a qualitative definition, and it does not give any absolute quantitative information related to the degree of echogenicity. Therefore, there was a need to quantify the echo intensity in order to objectively characterise and compare the ATFL in healthy, coper and CAI participants. Since ultrasound image included different value of echo intensity, computer-aided greyscale analysis software was used in chapter four in order to provide numerical data of the ATFL intensity which overcome the limitations of qualitative and subjective definitions of the echogenicity. The intensity of the ATFL was lowest in CAI following by coper then healthy participants. Ultrasound evaluation of selected ankle structures could improve the ability of the clinician to determine the current situation of the ligaments injury and produce more knowledgeable treatments and rehabilitation decisions regarding each individual.

While the previous studies (chapter three and four) characterise structural changes, they do not provide any insight into the functional consequences of any changes. Functional deficits were investigated using the SEBT whilst limb kinematic and kinetics were monitored, and compared between healthy and injured ankles. Results demonstrated poorer performance of the balance test, in all eight directions, in CAI participants compared to coper and healthy participants, poorer performance of the balance test in anterior, anterolateral and lateral directions in copers compared to healthy participants. In addition, significant differences were found in ankle kinematics between the three groups. The findings demonstrate decreased ROM mainly in the sagittal plane in CAI participants compared to coper and healthy participants.

Since the thickness of ATFL and CFL were the most structurally significant different among the three groups and the anterolateral reach distance of the SEBT was the most functionally significantly different, a correlational analysis was conducted to evaluate the association between the thickness of the ligaments (ATFL and CFL) and the anterolateral reach distance of SEBT. The study showed that the thickness of ATFL and CFL had a significant negative moderate correlation with anterolateral direction of SEBT ($r = -0.53$, $p<0.001$ and $r = -0.40$, $p<0.001$ respectively), as the thickness increased, the reach distance decreased.

6.3 Thesis novelty

This thesis has added to the literature on ankle sprain, highlighting the importance of accounting for the differences in length and thickness of lateral ankle ligaments post injury. Characterise selected ankle structures in people with and without a history of ankle sprain has been quantified for the first time. Throughout this work differences between healthy, coper and CAI participants have been identified. CAI participants demonstrated longer and thicker ATFL and thicker CFL compared to coper and healthy participants. The thickness of ATFL was also thicker in coper compared to healthy participants. CAI participants had the lowest ATFL echogenicity and coper participants had lower than healthy. Regarding functional consequences of ankle sprain, CAI participants had the shortest distance in the SEBT and copers performed shorter distances than healthy participants. Interestingly, coper participants fell somewhere on a spectrum between healthy and CAI participants

Over the last 10 years, the literature has contained about 47 studies of ankle structures post sprain, and 45 studies of ankle function post ankle sprain (Appendix 23). However, this is the first study that combined both structural changes and functional consequences of lateral ankle sprain and investigate any relationship between them to provide an overall understanding of how these two factors are related. It was hypothesised that changing morphology of the lateral ligament structures affects the stability of the ankle and thereafter the ability to control balance during reaching movements that place the lateral ligaments under strain.

Additional novel contributions include being the first study to characterise and compare selected ankle structures in healthy, coper and CAI groups using ultrasound, the first study to provide quantitative evaluation of the echogenicity of the ATFL and compared the echogenicity between healthy, coper and CAI participants using computer-aided greyscale analysis. It is also first to compare the reach distance and 3D kinematics of healthy, coper and CAI participants during the performance of eight directions of the SEBT. This provides an explanation of how structural changes might affect functional performance. Differences between groups point to a learned adoptive strategy as a consequence of ligament injury and impaired joint stability. Specifically that the coper and CAI participants utilise an adaptive compensatory motor-control strategy, reflected in a change in the ankle kinematics during reach and balance tasks (such as the SEBT).

6.4 Clinical relevance

Ultrasound which is a non-invasive imaging modality was able to identify differences in lateral ankle ligament structures in people with a previous history of ankle sprain. The protocol provided in this thesis could help presumably fully trained sonographers to apply the appropriate patient and transducer positions when scanning the selected ankle structures to provide accurate diagnosis and characterisation. The accurate diagnosis of chronic lateral ankle ligament injury could be important for deciding appropriate clinical pathways and guiding choices of surgical intervention. In addition, the findings of comparisons between different healthy groups can be used as a baseline for the normal ultrasound measurements and provide an initial understanding for the importance of foot and ankle position during ultrasound scanning. Evaluating echo intensity by the quantitative analysis of greyscale could be suggested as valuable tool to compare the degree of the darkness in injured ligaments especially between coper and CAI in order to characterise the tissue and understand the status of the injured ligaments.

Utilising optoelectronic cameras and a force platform, along with their related variables has demonstrated that copers and individuals with CAI have developed a protective strategy which restricts ankle movements and acceleration of the body during the reaching involved in the SEBT. This may indicate a balance deficit in copers and individuals with CAI that could be addressed through retraining. The findings of the current study could assist clinicians to improve their understanding of the motor strategies utilised by coper and CAI people throughout the performance of dynamic balance and could guide the process of rehabilitation.

Since the study demonstrated that CAI participants showed altered movement strategies especially lower extremity kinematics across different directions of the SEBT compared to coper and/or healthy participants. It is significant to recognise that both copers and CAI participants who have a history of ankle sprain exhibited restriction dorsiflexion range of motion during the performance of the SEBT. Since it has been reported that restricted ankle dorsiflexion range of motion makes ankle sprains more likely to reoccur, healthcare practitioners must focus on retrieving full dorsiflexion range of motion utilising open chain posterior talar glide, talocrural joint mobilization with movement (e.g., weight-bearing lunge), and/or calf stretching after ankle sprain injury to decrease the reoccurrence of ankle sprain (Son et al., 2017).

It also provides quantitative data that can be used as a reference for healthy, coper and CAI. By understanding the strength, neuromuscular control, and range of motion that contribute to the performance of the SEBT, clinicians may be able to detect specific limitation of their patients and provide classification schema for clinical predictive tools. In addition, because the lower limb is linked as kinematic and kinetic chains, healthcare practitioners must know that a single ankle joint issue would change the whole biomechanics of lower limb during functional movement (Son et al., 2017). Therefore, healthcare practitioners must considered a multi-joint rehabilitation program for these coper and CAI participants.

6.5 Limitations

This thesis has some limitation as the researcher did not differentiate the CAI participants into functional and mechanical instability. It could be that those with functional ankle instability have different characteristics compared to those with mechanical instability. Hiller et al. (2011) suggested that participants with functional and mechanical instability may perform differently than those with only functional instability. Thus, understanding functional and mechanical ankle instability separately with additional exploration of the sensorimotor and mechanical characteristics related to CAI is needed to further improve our understanding of ankle sprain injury.

The numbers of ankle sprains in injured participants were recorded based on the participant's recall (self-reported), we attempted to minimise the impact of this limitation by limiting the participant's recall to the 24 months prior to the test. In addition, this study compared participants who varied degree of their ankle injury history and reports of instability. Furthermore, the number of participants involved in this study was relatively small given the number of factors affecting ligament presentation. This may have led to a lower statistical power to the comparisons between groups, the number of participants in each groups was also unequal. Injury specific factors, such as severity of initial injury, specific ligaments injured, generalized joint laxity, type and timing of rehabilitation, or sensorimotor measures, limits the ability of the researcher to justify the differences between these groups. Further researcher included these factors will increase the understanding of changing the ankle joint after injury.

6.6 Recommendations for future work

One of the outcomes of addressing research questions is the development of new research ideas but also reflections of how future research might be improved. The outcome of this thesis could inform several future studies. Future research should use electronic medical records to identify the full history of the ankle sprains. This would help validate the self-reported injury history but also provide details of healing periods, frequency of repeated sprains and rehabilitation used.

Chronic ankle instability might happen due to functional or mechanical instability. There is therefore an opportunity to further research sensiomotor function during balance and other tasks that challenge ankle control, such as landing. This could include proprioception and joint movement detection and profiles differences in these characteristics between different individuals with different injury histories.

Future work should define standard criteria to describe the CAI and follow the National Centre for Biotechnology Information which reported that CAI is a condition characterised by a feeling of "giving way", six months or more following the initial ankle sprain so comparisons can be made between studies.

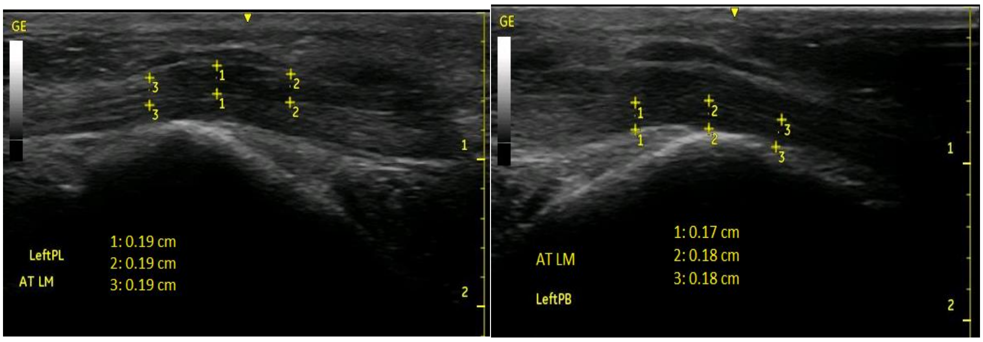
This study is cross sectional in nature and prospective studies would increase our understanding of changes in ligament, tendon and muscle structures as a result of single or multiple lateral ankle sprains. It would also allow predictive factors to be identified by comparing foot and ankle features of those who do and so not subsequent experience sprains.

Sonoelastography is improving constantly as diagnostic tool in musculoskeletal medicine, and applying this tool to the post-traumatic tissue could assist clinicians to customise treatment plane for each injured ankles.

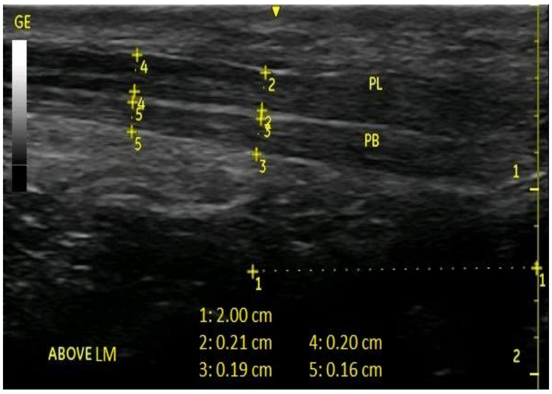
Further research on healthy, coper and CAI groups could specifically involve athletes with different types of sports. This would allow greater homogeneity of the sample groups because training and physical fitness levels would be more similar.

Appendix 1- Measuring peroneal tendons at three different locations

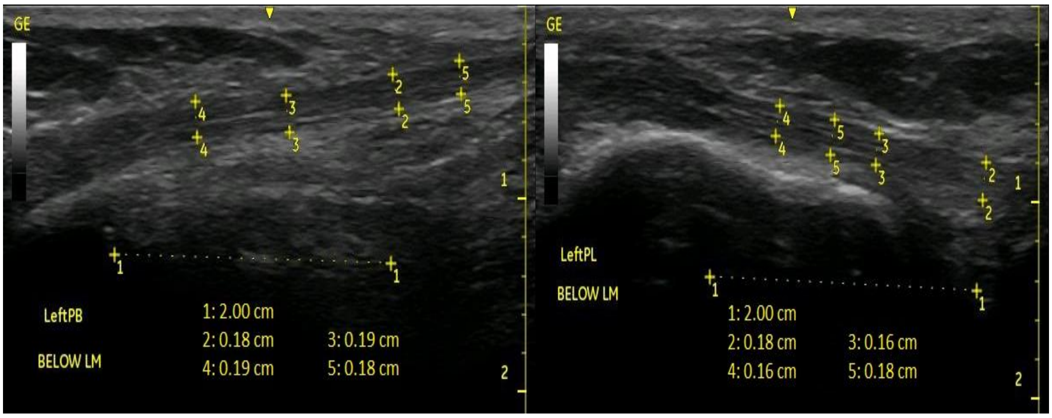
Measurement of peroneal tendons at the level of LM: lateral malleolus, PL: peroneal tendon. PB: peroneal brevis.



Measurement of peroneal tendons above LM: lateral malleolus, PL: peroneal tendon. PB: peroneal brevis.



Measurement of peroneal tendons below LM: lateral malleolus, PL: peroneal tendon. PB: peroneal brevis.



Appendix 2- Ethical approval letter for reliability study

University of Salford MANCHESTER

Research, Innovation and Academic Engagement Ethical Approval Panel

Research Centres Support Team
G0.3 Joule House
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M5 4WT

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12 December 2016

Dear Rawan,

RE: ETHICS APPLICATION–HSR1617-19–'Reliability of Ultrasound for measurement of selected foot and ankle structures.'

Based on the information you provided I am pleased to inform you that application HSR1617-19 has been approved.

If there are any changes to the project and/or its methodology, then please inform the Panel as soon as possible by contacting Health-ResearchEthics@salford.ac.uk

Yours sincerely,

Sue McAndrew
Chair of the Research Ethics Panel

Appendix 3- Consent form for reliability study

Title of study: Characteristics of selected ankle structures in chronic ankle instability and healthy individuals by ultrasound and centre of pressure analysis
Name of Researcher:

Please complete and sign this form **after** you have read and understood the study information sheet. Read the statements below and yes or no, as applicable in the box on the right hand side.

1.	I confirm that I have read and understand the study information sheet Version 1, dated 12/02/2017, for the above study. I have had opportunity to consider the information and ask questions which have been answered satisfactorily.	Yes/No
2.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my rights being affected.	Yes/No
3.	If I do decide to withdraw I understand that the information I have given, up to the point of withdrawal, may be used in the research. I can withdraw from the research at any time without giving any reason	Yes/No
4.	I agree to participate by having my ankle and foot scanned with ultrasound	Yes/No
5.	I understand that my anonymised data will be used in the (researcher's thesis/ research report) other academic publications and conferences presentations.	Yes/No
6.	I agree to take part in the study	Yes/No

Name of participant	**Date**	**Signature**
Name of person taking consent	**Date**	**Signature**

Appendix 4- Data collection sheet

Structural characteristics and functional consequences of lateral ankle sprains

Today's Date	**Name**	**Number**
Leg Dominance **Right** **Left**	**Date of Birth**	**Age** **gender**

Height	Weight	BMI

Have you ever had an Ankle Injury?	Yes	No
If so which foot	Right Left	Both
How long ago?	Right Ankle 3-6 months 7-10 months 11-14 months 15-18 months 19-22 months 23-24 months	Left Ankle 3-6 months 7-10 months 11-14 months 15-18 months 19-22 months 23-24 months
Does the ankle injury re-occur	Yes	No
Have you ever feel a "giving away"	Yes	No
If so which foot	Right Left	Both
How Many?	Right Ankle	Left Ankle

Did you receive treatment for your Ankle Injury?	Yes	No
	Right Left	Both
If so what treatment did you receive?	**Right Ankle** Self A/E Physiotherapy Rehab	**Left Ankle** Self A/E Physiotherapy Rehab
Did you take any medication for your ankle injury	Yes	No
If so what did you take?		

How long did it take to return to normal activity/ playing?	**Right Ankle** <2 months 2-4 months 4-6 months 6-9 months 9-12 months 12-18 months 18-24 months 24-36 months	**Left Ankle** <2 months 2-4 months 4-6 months 6-9 months 9-12 months 12-18 months 18-24 months 24-36 months

Appendix 5- The CAIT Questionnaire

Please tick the ONE statement in EACH question that BEST describes your ankles.

	LEFT	RIGHT	Score
1. I have pain in my ankle			
Never	☐	☐	5
During sport	☐	☐	4
Running on uneven surfaces	☐	☐	3
Running on level surfaces	☐	☐	2
Walking on uneven surfaces	☐	☐	1
Walking on level surfaces	☐	☐	0
2. My ankle feels UNSTABLE			
Never	☐	☐	4
Sometimes during sport (not every time)	☐	☐	3
Frequently during sport (every time)	☐	☐	2
Sometimes during daily activity	☐	☐	1
Frequently during daily activity	☐	☐	0
3. When I make SHARP turns, my ankle feels UNSTABLE			
Never	☐	☐	3
Sometimes when running	☐	☐	2
Often when running	☐	☐	1
When walking	☐	☐	0
4. When going down the stairs, my ankle feels UNSTABLE			
Never	☐	☐	3
If I go fast	☐	☐	2
Occasionally	☐	☐	1
Always	☐	☐	0
5. My ankle feels UNSTABLE when standing on ONE leg			
Never	☐	☐	2
On the ball of my foot	☐	☐	1
With my foot flat	☐	☐	0
6. My ankle feels UNSTABLE when			
Never	☐	☐	3
I hop from side to side	☐	☐	2
I hop on the spot	☐	☐	1
When I jump	☐	☐	0
7. My ankle feels UNSTABLE when			
Never	☐	☐	4
I run on uneven surfaces	☐	☐	3
I jog on uneven surfaces	☐	☐	2
I walk on uneven surfaces	☐	☐	1
I walk on a flat surface	☐	☐	0
8. TYPICALLY, when I start to roll over (or "twist") on my ankle, I can stop it			
Immediately	☐	☐	3
Often	☐	☐	2
Sometimes	☐	☐	1
Never	☐	☐	0
I have never rolled over on my ankle	☐	☐	3
9. After a TYPICAL incident of my ankle rolling over, my ankle returns to "normal"			
Almost immediately	☐	☐	3
Less than one day	☐	☐	2
1–2 days	☐	☐	1
More than 2 days	☐	☐	0
I have never rolled over on my ankle	☐	☐	3

Appendix 6- Ethical approval letter for main study

University of Salford MANCHESTER

Research, Innovation and Academic Engagement Ethical Approval Panel

Research Centres Support Team
G0.3 Joule House
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M5 4WT

T +44(0)161 295 2280

www.salford.ac.uk/

27 March 2017

Dear Rawan,

RE: ETHICS APPLICATION–HSR1617-106–'Characteristics of ankle structures and balance in people with and without a history of ankle sprains.'

Based on the information you provided I am pleased to inform you that application HSR1617-106 has been approved.

If there are any changes to the project and/or its methodology, then please inform the Panel as soon as possible by contacting Health-ResearchEthics@salford.ac.uk

Yours sincerely,

Sue McAndrew
Chair of the Research Ethics Panel

Appendix 7- Participants information sheet for main study

Title of study: Structural characteristics and functional consequences of lateral ankle sprains

Name of Researcher:

Invitation paragraph

You are being invited to take part in a research study to help us better understand how we can characterise the ligaments and tendons around the ankle based on ultrasound images and balance test. We would like to do this in people with and without ankle sprains, too see the differences of the structure and functional status between them.

Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve. This document provides information about the purpose, any risks, and possible benefits of participating. Please take time to read the following information carefully. If you have any questions then feel free to contact the researcher whose details are given at the end of the document.

What is the purpose of the study?

Sprained ankles are common, painful and can leave people with long term instability, whereby their ankles frequently "give way". We want to improve this situation by better understanding whether (and how) factors such as age, gender, activity levels, and prior ankle sprain affect ankle ligaments. We want to see whether changes in the ankle ligament are associated with changes in balance.

The study will involve 100 participants. Participating in this study is voluntary and you may withdraw at any time without any consequences.

Why have I been invited to take part?

You are being invited to take part in a research study to help us better understand how we can characterise the ligaments and tendons around the ankle. We would like to do this in people with and without ankle sprains and investigate whether it affects people's ability to maintain their balance.

Do I have to take part?

Participating in this study is voluntary.

What will happen to me if I take part?

If you agree to take part, you will be required to have an ultrasound scan of your feet. Ultrasound is a safe method used to image structures inside the body and is used most commonly to image babies during pregnancy. It sends sound waves inside the body and records the sound waves that bounce back off different structures such as bones and muscles. These 'sound reflections" (like echoes) can be used to create images of the internal structures. Unlike x-ray, ultrasound does not expose you to any radiation. In addition to the ultrasound, you will be perform a balance test during which you stand on one leg and point your feet in different directions. We will measure your balance as you do this.

The study will involve:

1. Taking your written consent (2 minutes).
2. Researcher will collect information on your foot health, any physical activity you undertake, and any history in injuries (3 minutes).
3. Measuring the length of the limb (2 minutes)
4. Ultrasound scan (15 minutes). This will involve you lying down. You will have to remove your shoes and socks. The equipment and process is similar to that used to scan pregnant women. Gel will be applied to the skin surface and a small handheld device will be moved over the skin to image the ankle. Your foot will be held in a simple plastic brace to help keep it steady.
5. Balance test (20 minutes). You will be asked to stand on a plate that measure the forces under your feet and balance on one leg. You will be asked to place the foot which is off the ground in a specific direction (e.g. forwards, or to the side). We will measure how far your feet can reach as you maintain your balance and also measure your balance using the plate. You can put your hands on your hips to help maintain your balance. You will be asked to repeat this in 8 different directions but have a short rest in between. There will be chance to practice this too.

Expenses and payments?

You will be provided with a £10 voucher to recognise your participation.

What are the possible disadvantages and risks of taking part?

There are very few risks associated with this study. All the measurements will be undertaken by a trained researcher and involve equipment and procedures used routinely by health professsionals. Although the ultrasound transducer gel used in the study is hypoallergenic and non-hazardous, there is a small risk of an allergic skin reaction. A patch test will therefore be performed prior to make sure there is no reaction to the gel.

What are the possible benefits of taking part?

Your participation in this study will help improve our understanding of ankle structures and function and how age, gender, sport participation and the number of previous ankle injuries might affect these. This will help us to do further research that aims to help people suffering from ankle sprains and the long term effects of sprains.

What if there is a problem?

It is unlikely that anything will go wrong. The University has insurance to cover any issues that may arise whilst you are taking part in these tests. However, if you decide to take legal action, you may have to pay for this. If you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, you can approach the University of Salford; please contact *Anish Kurien*, *Research*

Will my taking part in the study be kept confidential?

All information that is collected about you will be kept strictly confidential. Any information related to your participation that leaves the University of Salford will not have your name or any other have identifying features on it (e.g. ultrasound images).

What will happen if I don't carry on with the study?

You can withdraw from this study at any time without loss of any non-study related benefits to which you would have been entitled before participating in the study. If you want to withdraw you may do so by notifying the study representative listed in the "Contact Information" section below. Moreover, all your personal details will be kept confidential and not revealed to people outside the research team. However, the researcher will have to share the data in appropriate manner.

What will happen to the results of the research study?

A summary of the research findings will be available to everyone who participates. Significant findings may be published in clinical and sports journals and form chapters in the PhD thesis of the researcher.

Who is organising or sponsoring the research?

University of Salford

Further information and contact details:

If you require more information about the study, want to participate, or if you are already participating and want to withdraw, please contact;

xxxxxxxx

Email: xxxxxxx

Phone: xxxxxxx

Address: School of Health Science

Appendix 8- Consent form of main study

Title of study: Characteristics of selected ankle structures in chronic ankle instability and healthy individuals by ultrasound and star excursion test

Name of Researcher:

Please complete and sign this form **after** you have read and understood the study information sheet. Read the statements below and yes or no, as applicable in the box on the right hand side.

1.	I confirm that I have read and understand the study information sheet Version 4, dated 06/10/2017, for the above study. I have had opportunity to consider the information and ask questions which have been answered satisfactorily.	Yes/No
2.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my rights being affected.	Yes/No
3.	If I do decide to withdraw I understand that the information I have given, up to the point of withdrawal, may be used in the research. I can withdraw from the research at any time without giving any reason	Yes/No
4.	I agree to participate by having my ankle and foot scanned with ultrasound, and doing the balance test	Yes/No
5.	I understand that my anonymised data will be used in the (researcher's thesis/ research report) other academic publications and conferences presentations.	Yes/No
6.	I understand that the information about me and the data generated from the study is anonymized and data will be kept on a UoS database and may be used in future studies.	Yes/No
7.	I agree to respect other people's confidentiality and privacy as well and therefore, will not contact or approach any other patients/athletes on the premises as to do so may lead to legal action being sought.	Yes/No
8.	I understand that I will not be allowed to take pictures of any other patients/athletes on the premises, and it is better if the phone is put away in your bag.	Yes/No
9.	I agree to take part in the study	Yes/No

Name of participant **Date** **Signature**

Name of person taking consent **Date** **Signature**

Appendix 9- Risk Assessment Summary of Student Projects

1. What is the title of the project?

Characteristics of selected ankle structures in chronic ankle instability and healthy individuals by ultrasound and star excursion test

2. Is the project purely literature based? NO

3. Identifying the Risks

Hazards	**Risks**	**If yes, consider what precautions will be taken to minimise risk and discuss with your Supervisor**
Use of ionising or non-ionising radiation	Exposure to radiation **NO**	
Use of hazardous substances	Exposure to harmful substances **YES**	Although the ultrasound transducer gel used in the study is hypoallergenic and non-hazardous, there is still a risk of an allergic reaction occurring with the participants. Therefore a patch test will be performed prior to testing to make sure the participants have no reaction to the gel.
Use of face-to-face interviews Interviewees could be upset by interview and become aggressive or violent toward researcher	Interviewing; Own classmates=Low risk **NO** Other University students=Medium risk **NO** Non-University personnel=High risk **NO**	
Use of face-to-face interviews Participants or interviewees could become upset by interview and suffer psychological effects	**NO**	

Sensitive data	Exposure to data or information which may cause upset or distress to Researcher **NO**	
Physical activity	Exposure to levels of excerption unsuitable for an individual's level of fitness. **NO**	
Equipment	Exposure to faulty unfamiliar equipment. **YES**	All equipment has been checked and verified by the manufacture before delivery. Throughout testing sessions all equipment will be regularly checked and maintained.
Sensitive issues i.e. Gender / Cultural e.g. when observing or dealing with undressed members of the opposite sex	Exposure to vulnerable situations/ sensitive issues that may cause distress to interviewer or interviewee **NO**	
Manual Handling Activities	Exposure to an activity that could result in injury NO	

If you have answered yes to any of the hazards in question 3, please list the proposed precautions below:

Recruitment of experienced participants. Completion of Health and medical questionnaire before testing takes place. Appropriate maintenance of equipment. Performing a patch test prior to testing to see if the participant is allergic to the ultrasound transducer gel.

Appendix 10- Flyer/poster of the main study

University of Salford MANCHESTER
Participants needed for ankle research!!
We are looking for people that:
▪ Have had an **ankle sprain** in the last 2 years or
▪ Have never had an ankle sprain and are otherwise **healthy**
We require volunteers to help us characterize healthy and previously injured ankle ligaments and tendons by using ultrasound and balance test.
If you think you might like to take part, we would like to hear from you or if you have any questions please contact PhD researcher: Rawan Abdeen on either email : r.abdeen@edu.salford.ac.uk or text FOOT to 83123
r.abdeen@edu.salford.ac.uk
FOOT to 83123
r.abdeen@edu.salford.ac.uk
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r.abdeen@edu.salford.ac.uk
FOOT to 83123

Appendix 11- General Practice Physical Activity Questionnaire

General Practice Physical Activity Questionnaire

Date……………………….

Name……………………..

1. Please tell us the type and amount of physical activity involved in your work.

		Please mark one box only
a	I am not in employment (e.g. retired, retired for health reasons, unemployed, full-time carer etc.)	
b	I spend most of my time at work sitting (such as in an office)	
c	I spend most of my time at work standing or walking. However, my work does not require much intense physical effort (e.g. shop assistant, hairdresser, security guard, childminder, etc.)	
d	My work involves definite physical effort including handling of heavy objects and use of tools (e.g. plumber, electrician, carpenter, cleaner, hospital nurse, gardener, postal delivery workers etc.)	
e	My work involves vigorous physical activity including handling of very heavy objects (e.g. scaffolder, construction worker, refuse collector, etc.)	

2. During the *last week*, how many hours did you spend on each of the following activities? *Please answer whether you are in employment or not*

Please mark one box only on each row

		None	Some but less than 1 hour	1 hour but less than 3 hours	3 hours or more
a	Physical exercise such as swimming, jogging, aerobics, football, tennis, gym workout etc.				
b	Cycling, including cycling to work and during leisure time				
c	Walking, including walking to work, shopping, for pleasure etc.				
d	Housework/Childcare				
e	Gardening/DIY				

3. How would you describe your usual walking pace? Please mark one box only.

Slow pace (i.e. less than 3 mph)		Steady average pace	
Brisk pace		Fast pace (i.e. over 4mph)	

Appendix 12- Ultrasound measurements of selected ankle structures between healthy, coper and CAI

- Thickness of selected ankle structures among healthy, coper and CAI

Structures	Healthy	Coper	CAI
ATFL L	22.22 ± 1.47	23.48 ± 0.82 [a]	23.61 ± 1.10 [b]
ATFL T	1.90 ± 0.16	2.45 ± 0.38 [a,c]	2.93 ± 0.31 [b]
CFL	1.68 ± 0.13	1.72 ± 0.10	1.82 ± 0.12 [b]
PLT	2.51 ± 0.21	2.50 ± 0.16	2.55 ± 0.20
PBT	1.71 ± 0.15	1.72 ± 0.09	1.73 ± 0.13
TPT	2.50 ± 0.19	2.52 ± 0.18	2.54 ± 0.17
AT	4.01 ± 0.61	4.03 ± 0.53	4.01 ± 0.62
PLM	5.78 ± 0.45	5.85 ± 0.34	5.85 ± 0.68
PBM	9.52 ± 1.12	9.67 ± 1.04	9.73 ± 0.54

*Values are mean ± SD in mm

[a] Statistically significant differences (P<0.05, d>0.2) between coper and healthy

[b] Statistically significant differences between CAI and healthy

[c] Statistically significant differences between coper and CAI

- CSA of selected ankle structures among healthy, coper and CAI

Structures	Healthy	Coper	CAI
PLT	20.5 ± 02.1	21.1 ± 02.9	21.5 ± 02.5
PBT	15.4 ± 01.0	15.5 ± 01.4	15.8 ± 01.6
TPT	17.2 ± 01.6	17.8 ± 01.3	17.7 ± 01.7
AT	51.0 ± 04.5	51.4 ± 02.9	51.9 ± 03.8
PLM	73.9 ± 04.2	74.0 ± 04.1	74.5 ± 05.2
PBM	234.7 ± 31.7	246.2 ± 28.1	256.4 ± 31.5

*Values are mean ± SD in mm^2.

Appendix 13- Ultrasound measurements of selected ankle structures between neutral and tension position

- Length of ATFL in two positions neutral and tension among healthy participants

Structures	Neutral	Tension	P-value	Cohen's d
	mean ± SD	mean ± SD		
ATFL	18.74 ± 1.34	21.36 ± 2.74	0.03	1.21

- Thickness of selected ankle structures among right and left limbs

Structures	Neutral	Tension		
	mean ± SD	**mean ± SD**	**P-value**	**Cohen's d**
ATFL	1.82 ± 0.14	1.80 ± 0.14	0.19	0.14
CFL	1.61 ± 0.07	1.60 ± 0.08	0.51	0.12
PLT	2.50 ± 0.14	2.48 ± 0.17	0.44	0.13
PBT	1.67 ± 0.21	1.68 ± 0.18	0.73	0.05
TPT	2.51 ± 0.15	2.49 ± 0.14	0.44	0.14
AC	3.06 ± 0.20	3.01 ± 0.15	0.41	0.03
PLM	5.80 ± 0.50	5.75 ± 0.55	0.30	0.09
PBM	8.62 ± 1.30	8.59 ± 1.37	0.73	0.02

- CSA of selected ankle structures among right and left limbs

Structures	Neutral	Tension		
	mean ± SD	**mean ± SD**	**P-value**	**Cohen's d**
PLT	20.4 ± 01.70	20.1 ± 01.50	0.83	0.00
PBT	15.2 ± 01.70	15.0 ± 02.20	0.84	0.00
TPT	18.7 ± 02.20	18.6 ± 02.20	0.21	0.15
AC	51.2 ± 03.30	50.8 ± 02.30	0.12	0.11
PLM	76.1 ± 12.4	75.0 ± 12.5	0.32	0.15
PBM	229.30 ± 6.3	224.2 ± 3.9	0.71	0.07

Appendix 14- Ultrasound measurements of selected ankle structures between right and left limbs

- Length of ATFL in two positions (neutral and tension) among right and left limbs

Structures	Right	Left	P-value	Cohen's d
	mean ± SD	mean ± SD		
ATFL N	18.96±1.41	18.75±1.13	0.10	0.16
ATFL T	22.79±1.46	22.58±1.48	0.08	0.14

- Thickness of selected ankle structures among right and left limbs

Structures	Right	Left		
	mean ± SD	mean ± SD	P-value	Cohen's d
ATFL	1.89 ± 0.09	1.88 ± 0.11	0.577	0.13
CFL	1.70 ± 0.11	1.69 ± 0.12	0.330	0.01
PLT	2.61 ± 0.09	2.61 ± 0.08	1.000	0.00
PBT	1.77 ± 0.10	1.77 ± 0.11	1.000	0.00
TPT	2.56 ± 0.17	2.58 ± 0.21	0.453	0.10
AC	3.35 ± 0.50	3.38 ± 0.28	0.083	0.11
PLM	5.80 ± 0.50	5.72 ± 0.42	0.386	0.17
PBM	9.78 ± 0.72	9.92 ± 1.02	0.396	0.16

- CSA of selected ankle structures among right and left limbs

Structures	Right	Left		
	mean ± SD	mean ± SD	P-value	Cohen's d
PLT	21.3 ± 02.1	21.3 ± 01.9	0.83	0.00
PBT	15.7 ± 01.7	15.7 ± 01.6	0.84	0.00
TPT	17.0 ± 01.4	17.2 ± 01.2	0.21	0.15
AC	52.8 ± 03.5	52.4 ± 03.7	0.12	0.11
PLM	73.8 ± 02.5	73.4 ± 02.7	0.32	0.15
PBM	244.0 ± 24.6	246.0 ± 30.7	0.71	0.07

Appendix 15- Ultrasound measurements of selected ankle structures between male and female

- Length of ATFL in two positions (neutral and tension) among female and male

Structures	**Female**	**Male**	**P-value**	**Cohen's d**
	mean ± SD of female	mean ± SD		
ATFL N	18.75 ± 1.55	18.96 ± 1.40	0.69	0.14
ATFL T	22.00 ± 2.51	22.38 ± 2.23	0.57	0.16

- Thickness of selected ankle structures among female and male

Structures	**Female**	**Male**		
	mean ± SD	mean ± SD	**P-value**	**Cohen's d**
ATFL	1.85 ± 0.12	1.86 ± 0.14	0.57	0.08
CFL	1.66 ± 0.14	1.67 ± 0.10	0.87	0.01
PLT	2.51 ± 0.14	2.52 ± 0.23	0.91	0.05
PB T	1.72 ± 0.15	1.72 ± 0.09	0.85	0.00
TP	2.48 ± 0.10	2.50 ± 0.22	0.76	0.11
AT	3.20 ± 0.24	3.24 ± 0.24	0.54	0.17
PL M	5.70 ± 0.35	5.72 ± 0.32	0.83	0.06
PB M	9.22 ±0.80	9.19 ± 1.34	0.93	0.03

- CSA of selected ankle structures among female and male

Structures	**Female**	**Male**	**P-value**	**Cohen's d**
	mean ± SD	mean ± SD		
PLT	20.9 ± 01.8	21.1 ± 01.7	0.72	0.11
PBT	15.2 ± 01.2	15.4 ± 01.3	0.63	0.16
TPT	18.3 ± 02.0	18.6 ± 01.5	0.67	0.17
AT	52.2 ± 03.1	52.7 ± 03.8	0.64	0.14
PLM	73.1 ± 02.6	73.6 ± 03.5	0.63	0.16
PBM	227.0 ± 26.2	230.8 ± 31.2	0.73	0.11

Appendix 16- Ultrasound measurements of selected ankle structures between normal weight and overweight participants

- Length of ATFL in two positions (neutral and tension) among normal weight and overweight

Structures	Normal	Overweight	P-value	Cohen's d
	mean ± SD	mean ± SD		
ATFL N	18.70 ± 1.43	19.33 ± 2.15	0.15	0.44
ATFL T	21.95 ± 2.50	22.82 ± 2.06	0.22	0.38

- Thickness of selected ankle structures among normal and overweight

Structures	Normal	Overweight	P-value	Cohen's d
	mean ± SD	mean ± SD		
ATFL	1.84 ± 0.13	1.94 ± 0.19	0.06	0.61
CFL	1.65 ± 0.16	1.71 ± 0.08	0.20	0.47
PLT	2.47 ± 0.25	2.69 ± 0.12	0.00	1.12
PBT	1.66 ± 0.16	1.75 ± 0.16	0.05	0.65
TPT	2.46 ± 0.22	2.71 ± 0.18	0.00	1.24
AT	3.15 ± 0.35	3.38 ± 0.28	0.02	0.73
PLM	5.68 ± 0.50	5.90 ± 0.53	0.05	0.86
PBM	9.22 ± 1.22	10.15 ± 1.02	0.01	0.83

- CSA of selected ankle structures among right and left limbs

Structures	Normal	Overweight	P-value	Cohen's d
	mean ± SD	mean ± SD		
PLT	20.2 ± 01.7	22.3 ± 02.3	0.00	1.04
PBT	15.2 ± 01.4	16.1 ± 01.6	0.06	0.60
TPT	17.5 ± 02.1	18.7 ± 01.8	0.05	0.61
AT	50.4 ± 04.0	53.1 ± 04.9	0.05	0.60
PLM	73.1 ± 03.1	76.0 ± 04.1	0.01	0.80
PBM	223.0 ± 35.3	261.5 ± 37.4	0.00	1.06

Appendix 17- Score sheet for SEBT & limb length

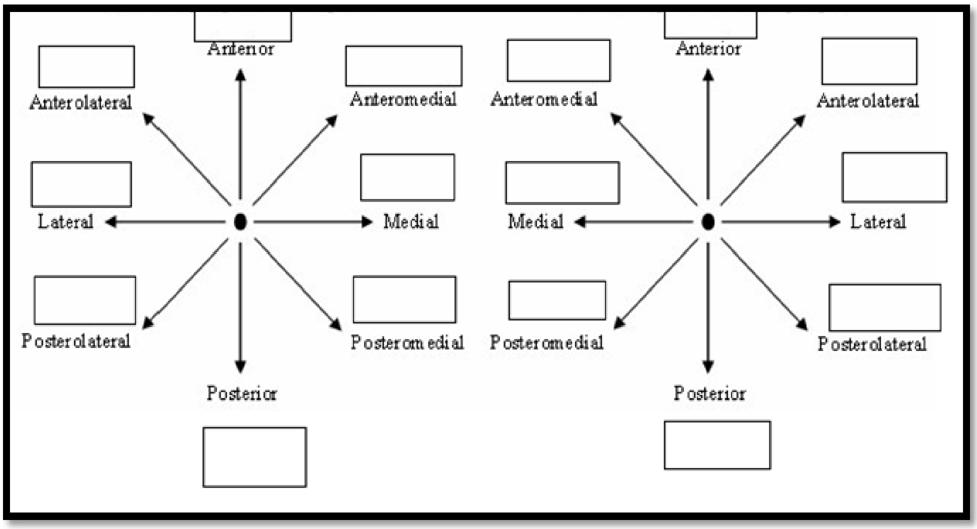
Score Sheet for SEBT & Limb Length

Participant number: ___________

Limb Length: ________________

Stance Leg Right/Reach Leg Left

Stance Leg Left/Reach Leg Right



Appendix 18- Reliability results of SEBT

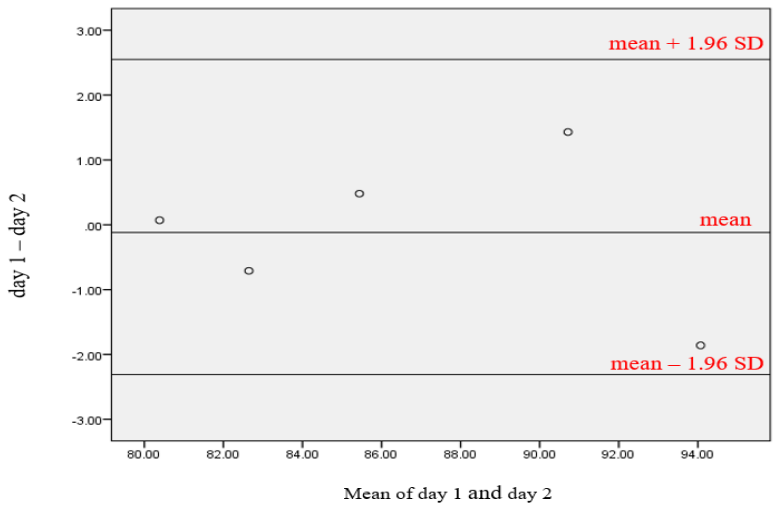
Reliability and limit of agreement results for 8 reach distances of uninvolved limb of healthy participants

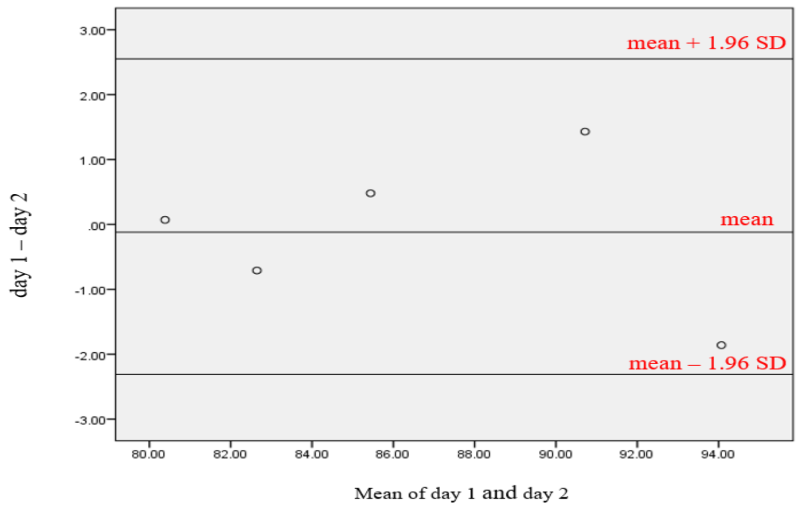
	Day 1 mean (SD)	Day 2 mean (SD)	ICC	95% LoA			LoA %
				lower	upper		
Ant	86.51 (03.64)	85.40 (04.16)	0.95	-1.77	3.99	6.70	1.29
AntLat	93.60 (03.80)	93.00 (02.34)	0.94	-2.48	3.70	6.62	0.65
Lat	93.48 (04.85)	93.00 (04.52)	0.99	-1.94	2.72	5	0.51
PostLat	96.68 (04.43)	96.31 (04.32)	0.99	-0.46	1.18	1.70	0.37
Post	97.05 (03.61)	97.64 (03.58)	0.90	-5.02	3.84	9.10	-0.60
PostMed	90.07 (05.37)	89.60 (05.94)	0.98	-2.42	3.38	6.5	0.53
Med	86.59 (05.56)	86.71 (05.83)	0.99	-2.55	2.31	5.61	-0.14
AntMed	84.11 (6.39)	84.82 (05.88)	0.98	-3.96	2.54	7.70	-0.84

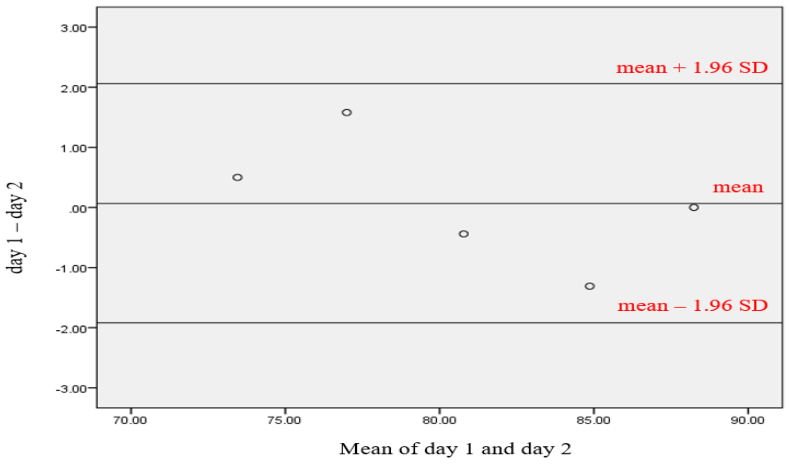
Reliability and limit of agreement results for 8 reach distances of uninvolved limb of injured participants

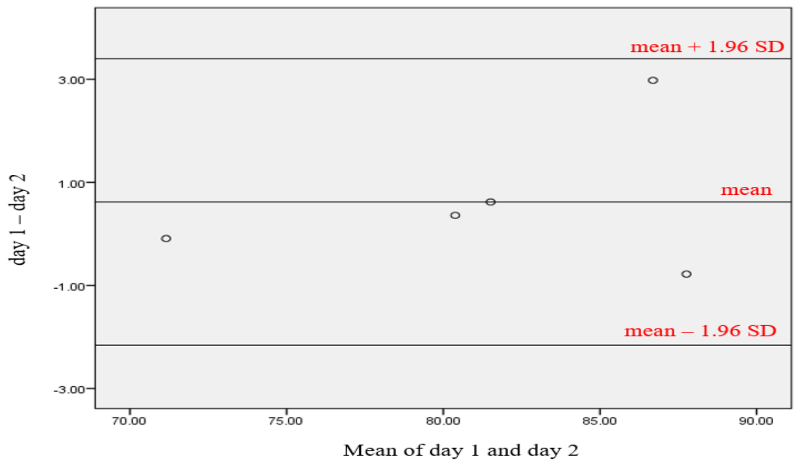
	DAY 1 mean (SD)	Day 2 mean (SD)	ICC	95% LoA			LoA %
				lower	upper		
Ant	78.44 (05.58)	77.29 (06.26)	0.97	-2.17	4.49	8.55	1.50
AntLat	84.52 (04.92)	83.74 (04.72)	0.98	-1.39	2.95	5.16	0.92
Lat	88.47 (06.82)	89.32 (05.04)	0.95	-6.36	4.66	12.4	0.95
PostLat	90.58 (05.13)	89.64 (03.69)	0.93	-3.66	5.54	10.21	1.04
Post	89.07 (04.92)	89.88 (03.58)	0.92	-5.60	3.96	10.68	-0.90
PostMed	87.59 (06.75)	87.23 (07.25)	0.99	-1.87	2.59	5.10	0.41
Med	79.46 (04.18)	79.88 (04.01)	0.98	-2.92	2.08	6.27	-0.52
AntMed	79.32 (04.19)	79.32 (04.28)	0.95	-4.07	4.07	10.26	0.01

Appendix 19- Bland and Altman plots for several directions of healthy and injured participant with representation of limit of agreements









Appendix 20- Differences of kinematics data between the 3 groups in sagittal plane

	Between groups		Between groups
Anterior	P<0.01	**Posterior**	P=0.07
Anterolateral	P<0.01	**Posteromedial**	P<0.01
Lateral	P<0.01	**Medial**	P=0.05
Posterolateral	P=0.01	**Anteromedial**	P=0.01

	Healthy vs Coper	Healthy vs CAI	Coper vs CAI
Anterior	P=0.98 , d=0.31	P<0.01 , d=1.31	P=0.08 d= 0.93
Anterolateral	P=1.00 , d=0.25	P<0.01 , d=1.76	P<0.01 , d=1.28
Lateral	P=1.00 , d=0.31	P<0.01 , d=1.25	P=0.09 , d= 0.82
Posterolateral	P=1.00 , d=0.08	P=0.01 , d=1.11	P=0.06 , d=0.86
Posterior	P=1.00 , d=0.06	P=0.08 , d=0.81	P=0.24 , d=0.61
Posteromedial	P=1.00 , d=0.23	P<0.01, d=1.16	P=0.08 , d=0.86
Medial	P=1.00 , d=0.03	P=0.07 , d=0.80	P=0.13 , d=0.82
Anteromedial	P=1.00 , d=0.01	P=0.01 , d=1.09	P=0.04 , d=1.08

Appendix 21- Differences of kinematics data between the 3 groups in transverse plane

	Between groups		**Between groups**
Anterior	P=0.04	**Posterior**	P=0.03
Anterolateral	P=0.13	**Posteromedial**	P=0.15
Lateral	P=0.05	**Medial**	P=0.05
Posterolateral	P=0.21	**Anteromedial**	P=0.20

	Healthy vs Coper	**Healthy vs CAI**	**Coper vs CAI**
Anterior	P=0.21 , d=0.57	P=0.08 , d=0.84	P=1.00 , d= 0.11
Anterolateral	P=1.00 , d=0.31	P=0.14 , d=0.70	P=1.00 , d=0.38
Lateral	P=0.44 , d=0.47	P=0.05 , d=0.85	P=1.00 , d= 0.33
Posterolateral	P=1.00 , d=0.27	P=0.24 , d=0.63	P=1.00 , d=0.32
Posterior	P=1.00 , d=0.30	P=0.03 , d=0.90	P=0.34 , d=0.63
Posteromedial	P=0.92 , d=0.37	P=0.17 , d=0.61	P=1.00 , d=0.28
Medial	P=0.97 , d=0.32	P=0.03, d=0.92	P=0.50 , d=0.47
Anteromedial	P=0.46 , d=0.43	P=0.40 , d=0.60	P=1.00 , d=0.07

Appendix 22- Differences of kinematics data between the 3 groups in frontal plane

	Between groups		Between groups
Anterior	P=0.80	**Posterior**	P=0.37
Anterolateral	P=0.32	**Posteromedial**	P=0.62
Lateral	P=0.16	**Medial**	P=0.44
Posterolateral	P=0.57	**Anteromedial**	P=0.13

	Healthy vs Coper	Healthy vs CAI	Coper vs CAI
Anterior	P=1.00 , d=0.21	P=1.00 , d=0.16	P=1.00 , d= 0.04
Anterolateral	P=0.49 , d=0.33	P=0.93 , d=0.35	P=1.00 , d=0.14
Lateral	P=0.18 , d=0.64	P=1.00 , d=0.32	P=1.00 , d= 0.32
Posterolateral	P=0.96 , d=0.35	P=1.00 , d=0.21	P=1.00 , d=0.12
Posterior	P=0.50 , d=0.44	P=1.00 , d=0.24	P=0.96 , d=0.27
Posteromedial	P=1.00 , d=0.30	P=1.00 , d<0.01	P=1.00 , d=0.32
Medial	P=1.00 , d=0.17	P=0.62 , d=0.41	P=1.00 , d=0.28
Anteromedial	P=1.00 , d=0.12	P=0.15 , d=0.71	P=0.50 , d=0.54

Appendix 23- Structural and functional studies of ankle sprain

Structural studies	**Functional studies**
Imaging diagnosis for chronic lateral ankle ligament injury: a systemic review with meta-analysis (Cao et al., 2018)	Foot and ankle kinematics in chronic ankle instability subjects using midfoot strike pattern when running, including influence of taping (Deschamps et al., 2018)
Ultrasound guided management of ankle sprain (Sanjay, Babulreddy, & Umamahesh, 2018)	Neuromuscular control of ankle and hip during performance of the star excursion balance test in subjects with and without chronic ankle instability (Jaber et al., 2018)
An Ultrasound Classification of Anterior Talofibular Ligament (ATFL) Injury (Cai, Li, Chen, Hua, & Shan, 2017)	Y-balance test performance and BMI are associated with ankle sprain injury in collegiate male athletes (Hartley, Hoch, & Boling, 2018)
Accuracy of magnetic resonance imaging in diagnosing lateral ankle ligament injuries: A comparative study with surgical findings and timings of scans (Tan, Jing, Teh, & Chee, 2017)	Ankle dorsiflexion range of motion influences Lateral Step Down Test scores in individuals with chronic ankle instability (Grindstaff, Dolan, & Morton, 2017)
Acute and Chronic Lateral Ankle Instability (Shakked & Sheskier, 2017)	Assessment of evertor weakness in patients with chronic ankle instability: Functional versus isokinetic testing (Terrier, Degache, Fourchet, Gojanovic, & Forestier, 2017)
Acute Tears of the Tibialis Posterior Tendon Following Ankle Sprain (Jackson, Dunaway, & Lundeen, 2017)	Effects of chronic ankle instability on kinetics, kinematics and Muscle activity during walking and running: A systematic review (Moisan, Descarreaux, & Cantin, 2017)
Comparison of Magnetic Resonance Imaging and Stress Radiographs in the Evaluation of Chronic Lateral Ankle Instability (Jolman, Robbins, Lewis, Wilkes, & Ryan, 2017)	Hip-ankle coordination during gait in individuals with chronic ankle instability (Yen, Chui, Corkery, Allen, & Cloonan, 2017)
Magnetic Resonance Imaging of Ligamentous Injuries in Ankle Sprain	Hip strength and star excursion balance test deficits of patients with

(Chu et al., 2017)	chronic ankle instability (McCann et al., 2017)
MRI appearance of injured ligaments and/or tendons of the ankle in different positions: study protocol for a single-center, diagnostic clinical trial (Liu et al., 2017)	Movement Strategies among Groups of Chronic Ankle Instability, Coper, and Control (Son, Kim, Seeley, & Hopkins, 2017)
Simultaneous Radiographic Technique to Evaluate Ankle instability (De Aguiar et al., 2017)	A new approach of the Star Excursion Balance Test to assess dynamic postural control in people complaining from chronic ankle instability (Pionnier, Découfour, Barbier, Popineau, & Simoneau-Buessinger, 2016)
Study on the role of ulrasonography in ligament injuries by ankle sprain (Baezegari, Amedfar, Moezzi, Kohandel, & Rafiei, 2017)	Dynamic postural stability differences between male and female players with and without ankle sprain (Dallinga et al., 2016)
The feasibility of point-of-care ankle ultrasound examination in patients with recurrent ankle sprain and chronic ankle instability: Comparison with magnetic resonance imaging (Lee & Yun, 2017)	Kinematics and muscle activities of the lower limb during a side-cutting task in subjects with chronic ankle instability (Koshino, et al. 2016)
Effectiveness of ultrasonography in diagnosing chronic lateral ankle instability : A systematic review (Radwan et al., 2016)	Locomotive biomechanics in persons with chronic ankle instability and lateral ankle sprain copers (Doherty et al., 2016)
MRI in acute ligamentous injuries of the ankle (Martella et al., 2016)	Postural Control Strategies are Dependent on Reach Direction in the Star Excursion Balance Test (Keith, Condon, Phillips, McKeon, & King, 2016)
Ultrasonography Comparison of Peroneus Muscle Cross-sectional Area in Subjects With or Without Lateral Ankle Sprains (Lobo et al., 2016)	Sagittal Plane Gait Kinematics in Individuals With Chronic Ankle Instability (Hoch, Mullineaux, Jeon, & McKeon, 2016)
Increased Ligament Thickness in Previously Sprained Ankles as Measured by Musculoskeletal Ultrasound (Liu et al., 2015)	The ability of modified star excursion balance test to differentiate between women athletes with and without chronic ankle instability (Razeghi, Rahnama, & Shokri, 2016)
Magnetic resonance imaging abnormalities after lateral ankle trauma in injured and contralateral ankles (Van Putte-Katier, Van Ochten, Van Middelkoop, Bierma-Zeinstra, & Oei, 2015)	The Effect of Chronic Ankle Instability (CAI) on Y- Balance Scores in Soccer Athletes (Payne, Mccabe, & Pulliam, 2016)
Musculoskeletal Ultrasound in Common Foot and Ankle Pathologies	Using balance tests to discriminate between participants with a recent

(Badon & Brown, 2015)	index lateral ankle sprain and healthy control participants: A cross-sectional study (Pourkazemi et al., 2016)
Ultrasonography in the Assessment of Lateral Ankle Ligament Injury, Instability, and Anterior Ankle Impingement: A Diagnostic Case Report (Battaglia, Craig, & Kettner, 2015) Ultrasound and radiography	Kinematic predictors of star excursion balance test performance in individuals with chronic ankle instability (Hoch et al., 2016)
Ultrasound Findings of the Painful Ankle and Foot (Artul & Habib, 2014)	Laboratory Measures of Postural Control During the Star Excursion Balance Test After Acute First-Time Lateral Ankle Sprain (Doherty et al., 2015)
Unrecognised Acute Rupture of the Achilles Tendon in Severe Ankle Sprain (Wai & Hing, 2015)	Individuals with both perceived ankle instability and mechanical laxity demonstrate dynamic postural stability deficits (Brown et al., 2015)
MRI in chronic ankle inversion injures (Pashnikova, Trufanov, Fokin, & Pchelin, 2014)	Lower Extremity Muscle Activation in Patients With or Without Chronic Ankle Instability During Walking (Feger, Donovan, Hart, & Hertel, 2015)
Structural abnormalities and persistent complaints after an ankle sprain are not associated: an observational case control study in primary care (Van Ochten et al., 2014)	Multi-segment foot landing kinematics in subjects with chronic ankle instability (De Ridder et al., 2015)
The use of MRI in pre-operative evaluation of anterior talofibular ligament in chronic ankle instability (Kanamoto, Shiozaki, Tanaka, Yonetani, & Horibe, 2014)	Weight-Bearing Dorsiflexion Range of Motion and Landing Biomechanics in Individuals With Chronic Ankle Instability (Hoch, Farwell, Gaven, & Weinhandl, 2015)
Value of ultrasonography for detecting chronic injury of the lateral ligaments of the ankle joint compared with ultrasonography findings (Cheng, Cai, & Wang, 2014)	Acute Ankle Sprain Injury Alters Kinematic and Center of Pressure Measures of Postural Control During The Star Excursion Balance Test (Doherty et al., 2014)
Application of Ultrasound in Sports Injury (Chiang, Wang, & Hsieh, 2013)	Balance failure in single limb stance due to ankle sprain injury: An analysis of centre of pressure using the fractal dimension method (Doherty et al., 2014)
The accuracy of ultrasound evaluation in foot and ankle trauma (Ekinci, Polat, Günalp, Demirkan, & Koca, 2013)	Concurrent and Discriminant Validity of the Star Excursion Balance Test for Military Personnel With Lateral Ankle Sprain (Bastien et al.,

	2014)
The Role of Dynamic Ultrasound and MRI in the poorly resolving ankle sprain (Zietkiewicz, Mercouris, & Marshall, 2013)	Dynamic Postural Control Assessment with Star Excursion Balane Test among Chronic Ankle Instability and Healthy (Khuman, Surbala, & Kamlesh, 2014)
New method of diagnosis for chronic ankle instability: Comparison of manual anterior drawer test, stress radiography and stress ultrasound (Lee et al., 2013)	Postural control strategies during single limb stance following acute lateral ankle sprain (Doherty et al., 2014b)
Ultrasound-assisted triage of ankle trauma can decrease the need for radiographic imaging (Hedelin, Goksör, Karlsson, & Stjernström, 2013)	Postural-Stability Tests That Identify Individuals With Chronic Ankle Instability (Linens, Ross, Arnold, Gayle, & Pidcoe, 2014)
Ultrasound examination of the ankle: most prevalent disease in our environment (Molero et al., 2013)	Ankle dorsiflexion range of motion influences dynamic balance in individuals with chronic ankle instability (Basnett et al., 2013)
Accuracy of MRI findings in chronic lateral ankle ligament injury comparison with surgical finding (Park et al., 2012)	Ankle kinematics of individuals with chronic ankle instability while walking and jogging on a treadmill in shoes (Chinn, Dicharry, & Hertel, 2013).
Chronic ankle instability: diagnosis and treatment (Rodriguez-Merchan, 2012)	Examining the diagnostic accuracy of dynamic postural stability measures in differentiating among ankle instability status (Liu et al., 2013)
Differences in Lateral Ankle Laxity Measured via Stress Ultrasonography in Individuals With Chronic Ankle Instability, Ankle Sprain Copers, and Healthy Individuals (Croy et al., 2012)	Using the Star Excursion Balance Test to Assess Dynamic Postural-Control Deficits and Outcomes in Lower Extremity Injury: A Literature and Systematic Review (Gribble, Hertel, & Plisky, 2012)
Diagnosis and treatment of acute ankle injuries: development of an evidence-based algorithm (Polzer, 2012)	Dorsiflexion range of motion significantly influences dynamic balance (Hoch, Staton, & McKeon, 2011).
Ultrasound examination for the diagnosis of chronic anterior talofibular ligament injury (Hau et al., 2012)	Functional Performance Testing in Athletes with Functional Ankle Instability (Sharma, Sharma, & Sandhu, 2011)
Accuracy of Plain Radiographs Versus 3D Analysis of Ankle Stress Test (Hoffman et al., 2011)	Spatial postural control alterations with chronic ankle instability (Pope et al., 2011)
Accuracy of MRI scan in the diagnosis of ligamentous and chondral	The effect of EMG activity of the lower leg with dynamic balance of

pathology in the ankle (Joshy, Abdulkadir, Chaganti, Sullivan, & Hariharan, 2010)	the recreational athletes with functional ankle instability (Ahn, Kim, & Kim, 2011)
Chronic ankle instability. Which tests to assess the lesions? Which therapeutic options? (Tourné, Besse, & Mabit, 2010)	Difference in balance measures between patients with chronic ankle instability and patients after an acute ankle inversion trauma (de Vries, Kingma, Blankevoort, & van Dijk, 2010)
Evaluation of anterior talofibular ligament injury with stress radiography, ultrasonography and MR imaging (Oae, Takao, Uchio, & Ochi, 2010)	Dynamic Postural Stability in Females with Chronic Ankle Instability (Brown, Bowser, & Orellana, 2010)
Is MRI Adequate to Detect Lesions in Patients with Ankle Instability? (O'Neill, Aman, & Guyton, 2010)	Dynamic postural control but not mechanical stability differs among those with and without chronic ankle instability (Wikstrom et al., 2010)
Value of ultrasonography for detecting ligament damage in athletes with chronic ankle instability compared to computed arthotomography (Guillodo, Varache, & Saraux, 2010)	Postural control differs between those with and without chronic ankle instability (Wikstrom, Fournier, & McKeon, 2010)
Ankle Ligaments on MRI Appearance of Normal and Injured Ligaments (Perrich, Goodwin, Hecht, & Cheung, 2009)	The Effects of Fatigue and Chronic Ankle Instability on Dynamic Postural Control (Hosseinimehr, Daneshmandi, & Norasteh, 2010).
The Value of Ultrasound in Acute Ankle Injury: Comparison With MR (Margetic et al., 2009)	Alterations in knee kinematics and dynamic stability associated with chronic ankle instability (Gribble & Robinson, 2009)
Audit on the use of radiography and the management of ankle sprains in A&E (Borg & Pickard, 2008)	Functional performance deficits in patients with CAI: Validity of the multiple hop test (Eechaute et al., 2008)
	Functional Performance Testing in Participants With Functional Ankle Instability and in a Healthy Control Group (Buchanan, Docherty, & Schrader, 2008)
	Spatiotemporal postural control deficits are present in those with chronic ankle instability (McKeon & Hertel, 2008)

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