The gait features & plantar pressures of people with diabetic peripheral neuropathy & plantar foot ulcers

Thesis submitted by

Malindu Eranga Fernando, B. HSc (Podiatry) Hons

in June 2017

for the degree of Doctor of Philosophy

in the College of Medicine and Dentistry

James Cook University

Acknowledgements

'The beginning of wisdom is this: Get wisdom. Though it cost all you have, get understanding. Plans fail for lack of counsel, but with many advisers they succeed'

-Proverb

First, all glory to God for HIS continued provision, providence and abundant grace I have received from the start to the completion of this PhD journey.

I am earnestly thankful to my brilliant advisory team, who made this PhD journey both bearable and achievable. Foremost, my sincere thanks to Prof. Jonathan Golledge. Thank you for taking me on as a PhD student and for giving me the opportunity to learn from you and achieve what I have during my PhD. Thank you for your constant support, your incredible work-ethic and guidance. To Dr. Robert Crowther, thanks for your patience and for teaching me all you knew about the intricacies of movement analysis research, for challenging me and for all the incredible support, ample time and guidance you provided through this journey. To Dr. Peter Lazzarini, you are an incredible advisor and a great friend. Thank you for your constant encouragement and guidance and for connecting me with numerous opportunities during my PhD journey. To Dr. Sangla, Dr. Tan and Dr. Cunningham, thank you for your much-needed encouragement and support on this journey. Dr. Petra Buttner; you were an incredible mentor during my PhD journey. Your experience and knowledge were crucial to the many outcomes of my PhD, thank you for all the time you invested in me during this PhD. Emeritus Prof. Rhondda Jones, I was lucky to have learnt a great deal from you about biostatistics and your assistance has significantly enhanced the capacity of my PhD, for that I thank you. Prof. Scott Wearing, thank you for your time and assistance with various technical aspects of this thesis.

I would also like to acknowledge and thank, Dr. Melissa Crowe, Dr. Jenni Judd and all my peers from the CMD Doctoral Cohort Program for starting me off on this PhD journey and for the many conversations and ideas we shared during my candidature. Melissa, you have been a great mentor and I thank you for everything you did to support me during my PhD. A special thanks to all my peers and colleagues both past and present from the QRCPVD, who have been, and continue to be incredible, supportive and encouraging people. I also

sincerely thank all the research support staff, from the CMD and the GRS for their support and assistance during my candidature. A special thanks to the HP Research Grant Scheme, the GRS and the NHMRC for project funding, without which this work would not have been possible.

My heartiest thanks to Ms. Liz McDonald, thank you for your kind heart and for your incredible support with my research Liz. Thanks to Toni, Diane, Janine, Eshwar and Safraz for helping with various aspects of this large project as research assistants. Thank you to my amazing mentors and friends Sally and Cliff Kingi for your grace and belief in me and to our incredible adopted family at AUC for your love, encouragement and support throughout this journey.

To my dearest parents Malika and Sriya Fernando, every opportunity that I've had for my education have been credit to you. You scarified much for our benefit so that we can dream big and achieve our dreams. To my brother and Sister, Tharindu and Manasha, thank you for your love and support during my PhD journey. Lastly and most importantly, to my beautiful wife and best friend, Maheesha. My everlasting gratitude and thanks go out to you for your continued love, support and friendship. You are an exemplary example of dedication and are the hardest working person I know. You encourage me and challenge me every day. Your love, encouragement, patience and belief in me were crucial from the start to the end of this PhD journey. We now have an adventure that awaits us!

Finally, my sincere thanks and appreciation go to all the wonderful participants for their time and efforts which has contributed immensely to the research contained in this thesis. You may be the invisible and nameless contributors of this thesis, but your investment in the research contained in this thesis will not be forgotten.

Statement of the Contribution of Others

Nature of Assistance	Contribution	Names, Titles and Affiliations of Co-Contributors
Intellectual support	Proposal writing	***Primary Advisor:*** **Prof. Jonathan Golledge** Director, Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University Townsville, Queensland, Australia ***Co-advisors:*** **Dr. Robert Crowther** Senior Lecturer (Biomechanics) School of Health Sciences University of South Australia Adelaide, South Australia, Australia **Dr. Kunwarjit Singh Sangla** Director, Department of Diabetes and Endocrinology The Townsville Hospital Townsville, Queensland, Australia ***Associate advisor:***

		Dr. Peter Lazzarini Senior Health Practitioner Research Fellow, Allied Health Research Collaborative, Metro North Hospital & Health Service, Queensland Health, Australia Brisbane, Queensland, Australia
	Data Analysis	**Dr. Petra Buttner** Professorial Research Fellow (Biostatistics & Epidemiology) Centre for Chronic Disease Prevention, James Cook University Cairns, Queensland, Australia **Emeritus Prof. Rhondda Jones** Director, Research Development Division of Tropical Health & Medicine James Cook University Townsville, Queensland, Australia **Dr. Margaret Cunningham** Senior Research Fellow, Faculty of Health Sciences and Sport University of Stirling, Stirling, Scotland, United Kingdom
	Statistical support	**Dr. Petra Buttner** Professorial Research Fellow (Biostatistics & Epidemiology) Centre for Chronic Disease Prevention,

		James Cook University Cairns, Queensland, Australia **Emeritus Prof. Rhondda Jones** Director, Research Development Division of Tropical Health & Medicine James Cook University Townsville, Queensland, Australia
	Editorial assistance	***Primary Advisor:*** **Prof. Jonathan Golledge** Director, Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University Townsville, Queensland, Australia ***Co-advisors:*** **Dr. Robert Crowther** Senior Lecturer (Biomechanics) School of Health Sciences University of South Australia Adelaide, South Australia, Australia **Dr. Kunwarjit Singh Sangla** Director, Department of Diabetes and Endocrinology The Townsville Hospital

		Townsville, Queensland, Australia ***Associate advisor:***
		Dr. Peter Lazzarini Senior Health Practitioner Research Fellow, Allied Health Research Collaborative, Metro North Hospital & Health Service, Queensland Health, Australia Brisbane, Queensland, Australia **Dr. Petra Buttner** Professorial Research Fellow (Biostatistics & Epidemiology) Centre for Chronic Disease Prevention, James Cook University Cairns, Queensland, Australia **Dr. Margaret Cunningham** Senior Research Fellow, Faculty of Health Sciences and Sport University of Stirling, Stirling, Scotland, United Kingdom **Dr. Melissa Crowe** Head, Division of Tropical Health & Medicine Cohort Doctoral Studies Program College of Healthcare Sciences James Cook University Townsville, Queensland, Australia

		Prof. Scott Wearing Institute of Health Biomedical Innovation Clinical Sciences, Biomedical Engineering, Human Movement and Sports Science Queensland University of Technology Brisbane, Queensland, Australia
	Thesis formatting	**Ms. Katherine J Fowler** Academic Consultant Narangba, Queensland Australia
Financial support	Research costs	Queensland Government, Health Practitioner Research Grant Scheme (2013-2014) James Cook University Graduate Research School HDR Grant (2013-2014)
	Stipend	Australian Postgraduate Award (APA) scholarship (2013-2015) Top-up scholarship from College of Medicine and Dentistry (2013-2015)
	Write-up Grant	Graduate Research School Write-up grant (2015)
Data collection	Research assistance	**Mrs. Elise Papas** Former Research Officer

		Queensland Research Center for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University Townsville, Queensland, Australia **Mr. Safraz Mohamed Omer** Doctoral research student Queensland Research Center for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University Townsville, Queensland, Australia **Mr. Saiumaeswar Yogakanthi** Medical Student College of Medicine Faculty of Medicine, Nursing and Health Science, Monash University, Melbourne, Victoria, Australia **Ms. Elizabeth McDonald** Former Research Officer College of Medicine and Dentistry, James Cook University Townsville, Queensland, Australia

Statement on Ethics

The research presented in this thesis was conducted within the guidelines for research ethics outlined in the National Statement on the ethics Conduct in Research Involving Human (1999), the joint NHMRC/AVCC Statement and Guidelines on Research Practice (1997), the James Cook University Policy on Experimentation Ethics Standard Practices and Guidelines (2001), and the James Cook University Statement and Guidelines on Research Practice (2001). The research methodology received clearance from the James Cook University Human Research Ethics Committee (approval number H4693) and Townsville Health Service District Human Research Ethics Committee (approval number HREC/12/QTHS/77).

30/06/17

_____

Date

_____

Signature

MALINDU FERNANDO

_____

Name

Consent to use Published Material in Thesis

Title of thesis: **The gait features and plantar pressures of people with diabetic peripheral neuropathy and plantar foot ulcers**

Name of candidate: **Malindu Eranga Fernando**

Chapter #	**Details of publication(s) on which chapter is based**	**Nature and extent of the intellectual input of each author**	**I confirm the candidate's contribution to this paper and consent to the inclusion of the paper in this thesis**
2	Biomechanical characteristics of peripheral diabetic neuropathy: A systematic review and meta-analysis of findings from the gait cycle, muscle activity and dynamic barefoot plantar pressure.	MF designed the protocol, carried out the literature searches, carried out quality review, data entry, analysis and drafted the final manuscript; RC assisted with literature searches, in formulating methodology, gave expert advice on the biomechanical content and assisted with data extraction and edited the final manuscript; PL assisted with independent quality review of articles, and assisted with the methodology of the review, write-up of the manuscript and edited the final manuscript; KS was the subject expert on diabetes mellitus and provided clinical input	Name (please print): MALINDU FERNANANDO (MF) Signature: Name (please print): ROBERT CROWTHER (RC) Signature: Name (please print): PETER LAZZARINI (PL) Signature:

	regarding the condition, the methodology of the paper, selection of key factors for analysis and comparison and edited the final manuscript; MC assisted with the design of the review and study selection, provided critical input of quality review and data analysis and edited the final manuscript; PB was the expert statistician who carried out the data-analysis, constructed the figures and provided critical input of methods and quality review of the studies and edited the final manuscript; JG directed the overall process of the review, assisted in formulating review methods, analysis and write-up and reviewed the final manuscript.	Name (please print): KUNWARJIT SANGLA (KS) Signature: Name (please print): MARGARET CUNNINGHAM (MC) Signature: Name (please print): PETRA BUTTNER (PB) Signature: Name (please print): JONATHAN GOLLEDGE (JG) Signature:

3	Plantar pressure in diabetic peripheral neuropathy patients with active foot ulceration, previous ulceration and no history of ulceration: a meta-analysis of observational studies.	MEF was the primary author and designed the protocol, extracted data, analyzed data and wrote the manuscript for publication. MEF, RGC, EP, PAL, MC ,KSS, PB and JG all conceived and designed the experiments. MEF performed all the experiments and analyzed the data. MEF, RGC, EP, PAL, MC, KSS, PB and JG all contributed to the writing of the manuscript. RC was the subject expert on plantar pressure and assisted in selection of studies for inclusion. Both EP and PAL conducted the quality assessment and assisted with the editing of the manuscript. MC provided expertise on methods, assisted with assessing study inclusion and write-up of the manuscript. Both KSS and JG assisted with subject expertise on diabetes and research methods and assisted with preparation and planning of methods, editing the final publication and critical input regarding analyses. PB was the statistician and subject expert on meta-analysis and assisted MEF with carrying out all analysis and graphical illustrations.	Name (please print): MALINDU ERANGA FERNANDO (MEF) Signature: Name (please print): ROBERT GEORGE CROWTHER (RGC) Signature: Name (please print): ELISE PAPPAS (EP) Signature: Name (please print): PETER ANTHONY LAZZARINI (PAL) Signature:

Name (please print): KUNWARJIT SINGH SANGLA (KSS)

Signature:

Name (please print): MARGARET CUNNINGHAM (MC)

Signature:

Name (please print): PETRA BUTTNER (PB)

Signature:

Name (please print): JONATHAN GOLLEDGE (JG)

Signature:

4	Lower limb biomechanical characteristics of patients with neuropathic diabetic foot ulcers: the diabetes foot ulcer study protocol.	MEF conceived the study, and drafted the first version of the manuscript. RC assisted with developing the methods for the study, trained MEF on how to perform all assessments, assisted with biomechanical outcome definitions and edited the manuscript for publication. MC assisted with the development of the methods and analyses and edited the manuscript for publication. PL provided intellectual input on the design and methods of the study, especially in relation to foot ulcer outcomes and edited the manuscript for publication. KS provided clinical expertise on diabetes participants and research methods and edited the manuscript for publication. JG assisted with planning study methodology and contributed to writing and editing of the manuscript for publication.	Name (please print): MALINDU ERANGA FERNANDO (MEF) Signature: Name (please print): ROBERT CROWTHER (RC) Signature: Name (please print): PETER LAZZARINI (PL) Signature: Name (please print): KUNWARJIT SANGLA (KS) Signature:

Name (please print): MARGARET CUNNINGHAM (MC)

Signature:

Name (please print): JONATHAN GOLLEDGE (JG)

Signature:

5	The reproducibility of acquiring three-dimensional gait and plantar pressure data using established protocols in participants with and without type 2 diabetes and foot ulcers.	MF conceived the study, carried out data collection, led data interpretation and was the primary author of the manuscript. RC assisted with data collection, provided biomechanical expertise in methods and procedures, trained MF in carrying out all biomechanical assessments and edited the manuscript for publication. MC assisted with checking methods and edited the manuscript for publication. PL provided intellectual input regarding study planning and methods and the interpretation and presentation of data and edited the manuscript for publication. KS provided clinical expertise on diabetes participants and research methods, assisted with participant recruitment and edited the manuscript for publication. PB provided statistical and methodological expertise, assisted with sub-group analyses and edited the publication. JG assisted with planning study methodology and implementation, assisted with patient recruitment and interpretation of results and contributed to writing and editing of the manuscript for publication.	Name (please print): MALINDU FERNANDO (MF) Signature: Name (please print): ROBERT CROWTHER (RC) Signature: Name (please print): PETER LAZZARINI (PL) Signature: Name (please print): KUNWARJIT SANGLA (KS) Signature:

Name (please print): MARGARET CUNNINGHAM (MC)

Signature:

Name (please print): PETRA BUTTNER (PB)

Signature:

Name (please print): JONATHAN GOLLEDGE (JG)

Signature:

6	**Gait parameters of people with diabetes-related neuropathic plantar foot ulcers.**	MF conceived the study, carried out subject recruitment and data collection, led data analysis, interpretation and write-up and was the principal investigator of the study. RC assisted with data collection and methods, provided biomechanical expertise in methods and procedures, trained MF in carrying out all biomechanical assessments and assisted in data analyses and interpretation, edited the manuscript for publication and assisted with figures. PL provided intellectual input regarding study planning and methods and the interpretation and presentation of data and edited the manuscript for publication. KS provided clinical expertise on diabetes subjects and research methods, assisted with subject recruitment and edited the manuscript for publication. PB assisted with study planning, sample-size calculations, data analyses and provided expertise on the statistical methodology of the paper and edited the paper. JG assisted with planning study methodology and implementation, assisted with subject recruitment and interpretation of results and contributed to writing and editing of the manuscript for	Name (please print): MALINDU FERNANDO (MF) Signature: Name (please print): ROBERT CROWTHER (RC) Signature: Name (please print): PETER LAZZARINI (PL) Signature: Name (please print): KUNWARJIT SANGLA (KS) Signature:

	publication and provided overall expertise on study methodology.	Name (please print): PETRA BUTTNER (PB) Signature: Name (please print): JONATHAN GOLLEDGE (JG) Signature:

7	**Plantar pressures are higher in cases with diabetic foot ulcers compared to controls despite a longer stance phase duration**	MF was the principal investigator of the study, conceived the study, carried out subject recruitment and data collection, led data extraction, analysis, interpretation and write-up and performed all analyses. RC assisted with data collection methods, trained MF in carrying out all biomechanical assessments, provided expertise in methods and procedures, and assisted in data interpretation, edited the manuscript for publication and assisted with figures. PL provided intellectual input regarding study planning and methods and the interpretation and presentation of data and edited the manuscript for publication. KS provided clinical expertise on diabetes subjects and research methods, assisted with subject recruitment and edited the manuscript for publication. SW assisted with data extraction, interpretation and presentation and provided subject-specific expertise on plantar pressure measurement and edited the paper. PB assisted with study planning, sample-size calculations, data analyses, provided expertise on the statistical methodology of the paper and edited the paper. JG assisted with study	Name (please print): MALINDU FERNANDO (MF) Signature: Name (please print): ROBERT CROWTHER (RC) Signature: Name (please print): PETER LAZZARINI (PL) Signature: Name (please print): KUNWARJIT SANGLA (KS) Signature:

	methodology and implementation, assisted with subject recruitment and data interpretation, provided expertise on data analyses and reporting of results and contributed to writing and editing of the manuscript for publication and provided overall guidance and direction to the study. All authors read and approved the final manuscript.	Name (please print): SCOTT WEARING (SW) Signature: Name (please print): PETRA BUTTNER (PB) Signature: Name (please print): JONATHAN GOLLEDGE (JG) Signature:

8	Plantar pressures remain elevated in people with longstanding diabetes-related foot ulcers during follow-up	MF was the principal investigator of the study, conceived the study, carried out participant recruitment and data collection, and led data extraction, analysis, interpretation and the write-up and presentation. RC assisted with data collection methods, trained MF in carrying out all biomechanical assessments and provided biomechanical expertise in methods and procedures, and assisted in data interpretation and edited the manuscript for publication. PL provided intellectual input regarding study planning and methods and the interpretation and presentation of data and edited the manuscript for publication. SY was a research assistant and assisted with data extraction from plantar pressure software, carried out data-checks and provided assistance with tabulation of data and edited the manuscript for publication. KS provided clinical expertise on diabetes participants and research methods, assisted with participant recruitment and edited the manuscript for publication. PB provided statistical expertise, performed initial sample size calculations and checked the manuscript and	Name (please print): MALINDU FERNANDO (MF) Signature: Name (please print): ROBERT CROWTHER (RC) Signature: Name (please print): PETER LAZZARINI (PL) Signature: Name (please print): SAIUMAESWAR YOGAKANTHI (SY) Signature: Name (please print): KUNWARJIT SANGLA (KS)

	methods and provided expertise on the overall statistical methodology of the paper and edited the manuscript for publication. RJ assisted with writing 'R' codes for statistical analyses, assisted with data analyses and interpretation and modelling and graphing and provided expertise on the methodology of the paper and edited the paper. JG assisted with study methodology and implementation, assisted with participant recruitment and data interpretation, provided expertise on data analyses and reporting of results and contributed to writing and editing of the manuscript for publication and provided overall guidance and direction to the study.	Signature: Name (please print): PETRA BUTTNER Signature: Name (please print): RHONDDA JONES (RJ) Signature: Name (please print): JONATHAN GOLLEDGE (JG) Signature:

Communications, Awards and Grants

Scientific publications not included in thesis

Book chapters

1. Malindu E. Fernando, Robert G. Crowther, Scott Wearing: The Importance of Foot Pressure in Diabetes. Handbook of Human Motion, 01/2016; DOI:10.1007/978-3-319-30808-1_39-1

Peer-reviewed publications

1. Malindu E Fernando, Seneviratne RM, Tan YM, Lazzarini PA, Sangla KS, Margaret Cunningham, Buttner PG, Jonathan Golledge: Intensive versus conventional glycaemic control for treating diabetic foot ulcers (Review). Cochrane database of systematic reviews (Online) 01/2016; 1(1).DOI:10.1002/14651858.CD010764.pub2

2. Vianne Nsengiyumva, Malindu E Fernando, Joseph V Moxon, Smriti M Krishna, Jenna Pinchbeck, Safraz M Omer, Dylan R Morris, Rhondda E Jones, Corey S Moran, Sai W Seto, Jonathan Golledge: The association of circulating 25-hydroxyvitamin D concentration with peripheral arterial disease: A meta-analysis of observational studies. Atherosclerosis 11/2015; 243(2)., DOI:10.1016/j.atherosclerosis.2015.10.011

3. Peter A Lazzarini, Sheree E Hurn, Malindu E Fernando, Scott D Jen, Suzanne S Kuys, Maarten C Kamp, Lloyd F Reed: Prevalence of foot disease and risk factors in general inpatient populations: A systematic review and meta-analysis. BMJ Open 11/2015; 5(11)., DOI:10.1136/bmjopen-2015-008544

4. Jason Warnock, Malindu Fernando: What's happening up north?. Journal of Foot and Ankle Research 09/2015; 8 (Suppl 2-Suppl 2)., DOI:10.1186/1757-1146-8-S2-P17

5. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Jonathan Golledge: Is it how they walk? Biomechanics in diabetic peripheral neuropathy: a review of the literature. Journal of Foot and Ankle Research 05/2013; 6(1)., DOI:10.1186/1757-1146-6-S1-P4

6. Malindu E Fernando, Ridmee M Seneviratne, Margaret Cunningham, Peter A Lazzarini, Kunwarjit S Sangla, Yong Mong Tan, Petra G Buttner, Jonathan Golledge: Intensive versus conventional glycaemic control for treating diabetic foot ulcers (Protocol). Cochrane database of systematic reviews (Online) 10/2013; 1(10)., DOI:10.1002/14651858.CD010764

Scientific publications included in thesis

1. Malindu E Fernando, Robert G Crowther, Peter A Lazzarini, Eshwar Yogakanthi, Kunwarjit S Sangla, Petra Buttner, Rhondda Jones, Jonathan Golledge. Plantar pressures are elevated in people with longstanding diabetes-related foot ulcers during follow-up. PLoS ONE, 08/ 2017 12(8), e0181916.

2. Malindu E Fernando, Robert G Crowther, Peter A Lazzarini, Kunwarjit S Sangla, Scott Wearing, Petra Buttner, Jonathan Golledge: Plantar pressures are higher in cases with diabetic foot ulcers compared to controls despite a longer stance phase duration. BMC Endocrine Disorders 09/2016; 16(1)., DOI:10.1186/s12902-016-0131-9

3. Malindu Eranga Fernando, Robert G. Crowther, Peter A. Lazzarini, Kunwarjit S. Sangla, Petra Buttner, Jonathan Golledge: Gait parameters of people with diabetes-related neuropathic plantar foot ulcers. Clinical biomechanics (Bristol, Avon) 06/2016; 37., DOI:10.1016/j.clinbiomech.2016.06.006

4. Malindu E Fernando, Robert G Crowther, Margaret Cunningham, Peter A Lazzarini, Kunwarjit S Sangla, Petra Buttner, Jonathan Golledge: The reproducibility of acquiring three-dimensional gait and plantar pressure data using established protocols in participants with and without type 2 diabetes and foot ulcers. Journal of Foot and Ankle Research 01/2016; 9(4)., DOI:10.1186/s13047-016-0135-8

5. Malindu Eranga Fernando, Robert George Crowther, Margaret Cunningham, Peter Anthony Lazzarini, Kunwarjit Singh Sangla, Jonathan Golledge: Lower limb biomechanical characteristics of patients with neuropathic diabetic foot ulcers: The diabetes foot ulcer study protocol. BMC Endocrine Disorders 10/2015; 15(1-1)., DOI:10.1186/s12902-015-0057-7

6. Malindu Eranga Fernando, Robert George Crowther, Elise Pappas, Peter Anthony Lazzarini, Margaret Cunningham, Kunwarjit Singh Sangla, Petra Buttner, Jonathan Golledge: Plantar Pressure in Diabetic Peripheral Neuropathy Patients with Active Foot Ulceration, Previous Ulceration and No History of Ulceration: A Meta-Analysis of Observational Studies. PLoS ONE 06/2014; 9(6)., DOI:10.1371/journal.pone.0099050

7. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge: Biomechanical characteristics of peripheral diabetic neuropathy: A systematic review and meta-analysis of findings from the gait cycle, muscle activity and dynamic barefoot plantar pressure. Clinical biomechanics (Bristol, Avon) 08/2013; 28(8)., DOI:10.1016/j.clinbiomech.2013.08.004

Non peer-reviewed communications

1. Preventing foot related complications of diabetes- Reducing foot ulceration risk. Tropical Ear: GP Newsletter for North Queensland General Practitioners TMML May 2013. Malindu Fernando

2. The detrimental effects of walking- Health and Wellbeing Women's Magazine February 2013. Malindu Fernando

Conference presentations

1. Poster Presentation: Biomechanical causes of diabetic foot ulceration- Festival of Life Sciences, School of Medicine, James Cook University, Townsville, October 2012. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Jonathan Golledge.

2. Oral presentation: Is it how they walk? Biomechanics in diabetic peripheral neuropathy: A review of the literature. Australasian Society for Behavioural Health and Medicine 10th Annual Scientific Conference, New Castle, Australia, October 2012. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Jonathan Golledge.

3. Poster Presentation: Foot ulceration in people with type 2 diabetes mellitus: The Townsville Diabetes Foot Ulcer Study. Townsville Hospital Research Week, October 2012 A Meta-Analysis of Observational Studies. Malindu Fernando, Robert Crowther, Elise Pappas, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

4. Poster Presentation: Is it how they walk? Biomechanics in diabetic peripheral neuropathy: A review of the literature. Australasian Podiatry Council Conference, Sydney, Australia August 2013. A Meta-Analysis of Observational Studies. Malindu Fernando, Robert Crowther, Elise Pappas,

Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

5. Poster Presentation: Plantar pressure in diabetes mellitus: Is there a difference from peripheral neuropathy to foot ulceration? Townsville Hospital Research Week, October 2013 A Meta-Analysis of Observational Studies. Malindu Fernando, Robert Crowther, Elise Pappas, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

6. Oral and poster presentation: Plantar pressure in diabetes mellitus: Is there a difference from peripheral neuropathy to foot ulceration? Festival of Life Sciences, September 2013 A Meta-Analysis of Observational Studies. Malindu Fernando, Robert Crowther, Elise Pappas, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

7. Oral Presentation: Plantar pressures are higher in patients with previous diabetic neuropathic ulcers: Results of a meta-analysis. The Australian Wound Management Association National Conference May 2014, Gold Coast, Australia. Malindu Fernando, Robert Crowther, Elise Pappas, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

8. Oral Presentation: Biomechanical characteristics of patients with plantar neuropathic diabetic foot ulcers: Assessing the gait cycle, kinematics, kinetics, electromyography and plantar pressure: Lessons from the Townsville Diabetes Foot Ulcer Study (DFU). Doctoral Cohort Conference, Faculty of Medicine, Health and Molecular Science, James Cook University, Townsville, July 2014. Malindu Fernando, Robert Crowther, Elise Pappas, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

9. Oral Presentation: Biomechanical characteristics of patients with plantar neuropathic diabetic foot ulcers: Assessing the gait cycle, kinematics,

kinetics, electromyography and plantar pressure: Lessons from the Townsville Diabetes Foot Ulcer Study (DFU). Australian Podiatry Association (Qld) Inc, State Conference, Townsville, Queensland, Australia August 2014. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

10. Oral Presentation: Biomechanical characteristics of patients with plantar neuropathic diabetic foot ulcers: Assessing the gait cycle, kinematics, kinetics, electromyography and plantar pressure: Lessons from the Townsville Diabetes Foot Ulcer Study (DFU). Queensland Tropical Health Alliance, Annual Conference, Palm Cove, Cairns, Queensland, September 2014. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

11. Oral Presentation: Biomechanical characteristics of patients with plantar neuropathic diabetic foot ulcers: Asia Diabetes Conference 2014, Kuala Lumpur, Malaysia. October 2014. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

12. Expert panel representative, Australian Diabetic Foot research, Australian Diabetic Foot Conference, Liverpool Hospital, Liverpool, Sydney, Australia, April 2015.

13. Oral Presentation, Biomechanical Characteristics of patients with diabetic peripheral neuropathy and plantar foot ulcers, Australian Diabetic Foot Conference, Liverpool Hospital, Liverpool, Sydney, Australia, April 2015. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

14. Poster presentation, Biomechanical Characteristics of patient with plantar diabetic foot ulcers: A case control study. 7th International symposium on the Diabetic Foot, The Hague, Netherlands, May 2015. Malindu Fernando,

Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

15. Oral Presentation: Biomechanical characteristics of patients with diabetic peripheral neuropathy during active ulceration: A cross sectional analysis Asia Diabetes Conference 2014, Kuala Lumpur, Malaysia. August 2015. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Petra Buttner, Jonathan Golledge.

16. Oral Presentation, Keynote invitation. Does gait change before, during or after ulceration? Annual Queensland Podiatry Research Forum, Herston, Brisbane October 2015. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

17. Oral Presentation: Gait and plantar pressure features in people with diabetic foot ulcers. New Zealand Society for the Study of Diabetes, Annual Conference, Rotorua, New Zealand, May 2016. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

18. Oral Presentation: Gait in diabetic neuropathy. Invited presentation. Lower Extremity Amputation Prevention Conference, Melbourne, September 2016. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Margaret Cunningham, Petra Buttner, Jonathan Golledge.

19. Oral Presentation: A longitudinal evaluation of site specific plantar pressures in people with diabetes related foot ulcers and diabetes controls without ulcers. Australasian Podiatry Conference, Melbourne, May 2017. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Eshwar Yogakanthi, Petra Buttner, Rhondda Jones, Jonathan Golledge.

20. Oral Presentation: Biomechanical Characteristics Of People With Active Diabetes-Related Plantar Foot Ulcers During Ulcer Healing. International Society of Biomechanical Conference, Brisbane, July 2017. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Eshwar Yogakanthi, Petra Buttner, Rhondda Jones, Jonathan Golledge.

21. Oral Presentation: Gait and plantar pressures in people with diabetes-related foot ulcers: relevance of offloading. Plenary Session and Panel Discussion. Diabetic Foot Australia Conference, Gold Coast, September 2017. Malindu Fernando, Robert Crowther, Peter Lazzarini, Kunwarjit Sangla, Eshwar Yogakanthi, Petra Buttner, Rhondda Jones, Jonathan Golledge.

Awards

1. Accommodation and travel grant, Logemas Ltd., Best research award to attend International Society of Biomechanics Conference, Brisbane, July 2017

2. Travel and accommodation grant, Lower Extremity Amputation Prevention Conference, September 2016, Melbourne, Australia.

3. Travel grant, Annual Queensland Podiatry Research Forum October 2015, Allied Health Office of Queensland, Herston, Brisbane, Australia.

4. Best oral presentation Award, THHS Research Week 2015, Allied Health Symposium, Townsville Hospital and Health Service District, Townsville, Australia.

5. University Finalist, Three Minute Thesis Competition 2015, Representing College of Medicine and Dentistry, James Cook University, Australia.

6. Best Oral Presentation Award, Asia Diabetes Conference, August 2015, Kuala Lumpur, Malaysia.

7. Outstanding PhD Candidate Award June 2015, Queensland Research Center for Peripheral Vascular Disease, Appreciation Day, Townsville, Australia.

8. Travel and accommodation grant to attend the Australian Diabetic Foot Conference 2015, Molnlyke Medical, Sydney, Australia.

9. Prof. Mustafa- Young Investigator Award, NADI, Diabetes Asia Conference October 2014, Kuala Lumpur, Malaysia.

10. Highly commended presentation Award, Queensland Tropical Health Alliance, Annual Conference September 2014, Palm Cove, Cairns, Queensland, Australia.

11. Best presentation Award, The Australian Wound Management Association National Conference May 2014, Gold Coast, Australia.

12. Best Paper Presentation Award, Festival of Life Sciences Competition 2013, Faculty of Health, Medicine and Molecular Sciences, James Cook University, Australia.

Funding

Year		Scheme	Grant Body	Amount
2015	Fernando M, Vangavetti V, Malabu U	Research Infrastructure block Grant (RIBG)	James Cook University	$4800
2014	Fernando M, Sangla K, Malabu U, Rasalam R, Blake D, Warnock J, Tan Y M, Vangavetti V, Young D, Joseph A	Research Infrastructure block Grant (RIBG)	James Cook University	$17,400
2014	Fernando M, Golledge J, Sangla K, Lazzarini P, Crowther R, Cunningham M	Graduate Research School Grant Scheme (Round 1)	James Cook University	$1,700
2013	Fernando M, Golledge J, Sangla K, Lazzarini P, Crowther R, Cunningham M	Health Practitioner Research Grant Scheme,	Queensland Health	$19,809
2013	Fernando M, Golledge J, Sangla K, Lazzarini P, Crowther R, Cunningham M	Graduate Research School Grant Scheme (Round 1)	James Cook University	$2,000
2013	Sangla K, Fernando M, Golledge J, Malabu U, Rasalam R, Vangavetti V,	Research Infrastructure block Grant (RIBG)	James Cook University	$28,629

List of Abbreviations

Abbreviation	Name
$AUD	Australian dollars
$US	American dollars
ABPI and abpi	Ankle-brachial pressure index
ANOVA	Analysis of variance
ASIS	Anterior superior iliac spine
BMI	Body Mass Index
CA	Contact Area
CCC	Concordance Correlation Coefficient
CI	Confidence Intervals
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CKD	Chronic Kidney Disease
CV	Coefficient of Variation
CVA	Cerebrovascular Accident
CVD	Cardiovascular Disease
D	Cohen's d
DFU	diabetes-related foot ulcer
DM	diabetes mellitus
DMC	Diabetes mellitus control
DPN	Diabetic peripheral neuropathy
EMBASE	Excerpta Medica database
F_{AP}	Anterior-posterior ground reaction force
F_{ML}	Medial-lateral ground reaction force
F_v	Vertical ground reaaction force
HAV	Hallux Abducto Valgus
HC	healthy control
HREC	Human research ethics committee
HRQoL	health related quality of life

IQR	Inter-quartile range
Kg	Kilogram
LME	Linear mixed-effects
M	Million
Mm	millimeters
m/s	meters per second
MNSI	Michigan Neuropathy Screening Instrument
MPP	mean peak pressure
MSP	maximum sensor pressure
NA	Not Applicable
OR	Odd's ratio
PAD	peripheral arterial disease
PhD	Doctor of Philosophy
PPDFU	people with previous or present diabetes-related foot ulcers
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTI	pressure-time integral
ROM	Range of motion
SD	Standard deviation
SE	Standard error
SPSS	Statistical Package for the Social Sciences
TSP	Temporal-spatial parameter
UTWCS	University of Texas Wound Classification System
VGRF	Vertical ground reaction force

Abstract[1]

Diabetes-related foot ulcers (DFUs) are a leading cause of morbidity and mortality world-wide. DFUs have been associated with lower physical, mental and social health that impacts on the overall functioning of individuals. The most significant contributing factor in the onset of DFUs is the development of diabetic peripheral neuropathy (DPN). DPN has been associated with biomechanical alterations in gait and elevated plantar pressures during gait. Although it was previously thought that elevated plantar pressures in the presence of DPN can lead to the development of DFUs, less was known about gait and plantar pressure in people during active ulceration. Therefore, despite international recommendations to offload plantar pressures to prevent the development of DFUs, the offloading requirements during active ulceration were less clearly established. This thesis sought to address this evidence deficiency though examining gait (joint angular kinematics, kinetics and temporal-spatial parameters) and plantar pressures in patients with active DFUs.

The five aims of the research contained in this thesis were: 1) to evaluate what was previously known about the gait and plantar pressure characteristics of people with DPN by performing a systematic review of observational studies; 2) to evaluate whether differences in plantar pressures exist between people with a history of DFUs (active and previous) compared to people with DPN without a history of DFUs by performing a meta-analysis of observational studies; 3) to determine whether available methodology could be used to obtain gait and plantar pressure data with adequate repeatability in people with active DFUs; 4) to prospectively assess whether gait and plantar pressures differ in people with active DFUs in comparison to healthy and diabetes controls without DFUs in a case-control study; and 5) to longitudinally evaluate whether plantar pressures change in people with active DFUs during follow-up compared to diabetes controls without DFUs. A series of studies was undertaken to achieve the above aims.

[1] Throughout the Abstract the word ‘significant’ refers to the result of statistical tests and implies p-values less than 0.05.

Overall, the results from the systematic reviews and meta-analyses contained in this thesis suggested that people with DPN have a significantly longer duration of time in the stance phase during walking and exhibit significantly elevated plantar pressures at multiple sites under their feet during walking compared to healthy and diabetes controls without DPN. Meta-analyses of previous observational studies suggested plantar pressures during walking were significantly higher in people with DPN with a history of DFUs compared to controls with DPN without a history of DFUs. However, there was noted to be a high level of heterogeneity and small sample sizes in studies comparing plantar pressures during walking in people with active DFUs compared to controls. The later finding suggested further research was needed regarding plantar pressures in patients with active DFUs. The prospective study on measurement reproducibility demonstrated that important gait and plantar pressure measurements could be reliably acquired in people with DFUs. Nearly all measures contributing to three-dimensional gait assessment and almost all plantar pressure measurements were within predefined acceptable limits.

The prospective case-control study demonstrated that when adjusted for age, sex and body mass index, people with active DFUs had a significantly smaller amount of plantar flexion, knee flexion and pelvic obliquity during walking compared to both control groups. People with active DFUs also had a significantly greater range of anterior-posterior ground reaction force, total vertical ground reaction force, slower walking speed and smaller step length compared to controls. The mean peak plantar pressures and pressure-time integrals measured under the toes and mid-foot during walking in people with DFUs were significantly higher than controls. The stance phase duration was also significantly longer in people with DFUs compared to both control groups. The longitudinal study suggested that when adjusted for age, sex, body mass index and the presence of DPN, patients with DFUs experienced higher mean peak plantar pressures under the toes and mid-foot and higher pressure-time integrals under the hallux, metatarsal 1, the mid-foot and rear-foot during walking, compared to controls, throughout follow-up.

An important and novel finding from the research contained in this thesis was that several gait characteristics were significantly different in people with active DFUs compared to

controls. These included distinct joint angular kinematics (angles during the gait cycle) and higher kinetics (vertical and anterior-posterior ground reaction forces) during walking. The main clinical implication of the prospective research contained in this thesis was that plantar pressure offloading should be continued if a DFU develops both during and following an active episode of ulceration. This is on a background of findings from the two meta analyses suggesting that plantar pressures are likely to be significantly higher in people with DPN prior to ulceration and following ulcer healing.

However, further research is needed to identify the required magnitude and most appropriate method of offloading pressure on the plantar surface of the foot during gait in people with active DFUs. Hence the future focus should be to define how to best: 1) optimize the offloading approach; 2) obtain good offloading adherence; and 3) monitor the appropriateness of offloading in people with DFUs.

Table of Contents

List of Tables

List of Figures

List of Plates

Chapter 1 Introduction and Outline

This chapter will review diabetes related foot ulceration, which is one of the key peripheral complications of both type 1 and type 2 diabetes and outline the significance of the research content and format contained in this thesis.

1.1 Background

In 2015, approximately 415 million people worldwide (9.1% of adults aged 18 years and older) were estimated to have diabetes mellitus (IDF, 2016). It is projected that this figure will reach 642 million people, by the year 2040 (see Figure 1.1) (IDF, 2016). The latest Australian statistics for 2017 from the National Diabetes Services Scheme suggested approximately 1.2 million Australians (or approximately 5% of the Australian population) have been diagnosed with diabetes (Diabetes Australia, 2017).

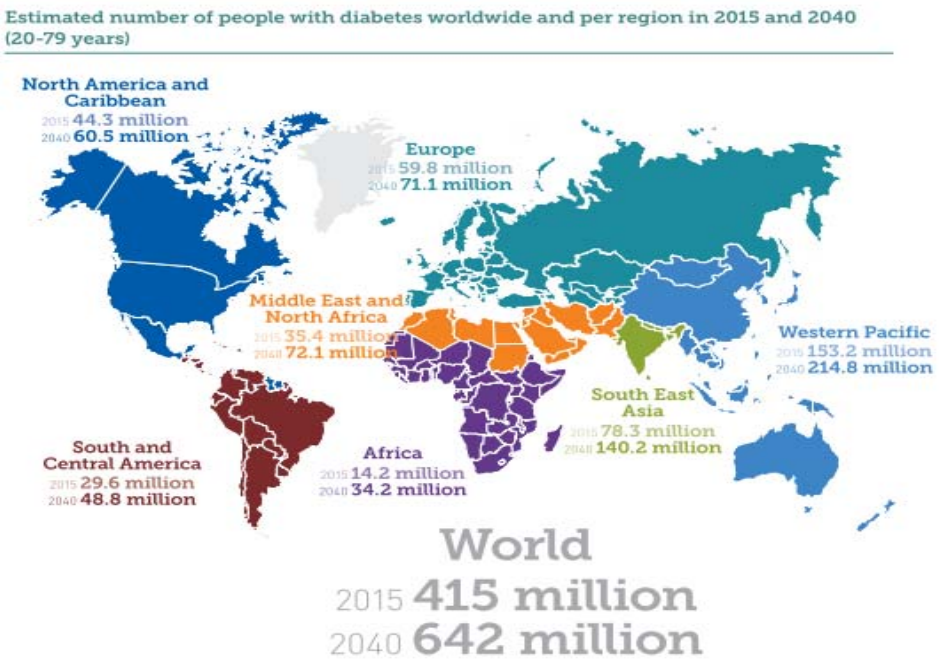


Figure 1.1 Estimated prevalence of diabetes mellitus worldwide: A comparison for data from 2015 and a prediction for 2040 (IDF, 2016, page 11).

Diabetes is a chronic, multi-system disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (Butler, Janson, Bonner-Weir, Ritzel, & Butler, 2003). Hyperglycemia, or raised blood sugar, is the primary effect of uncontrolled diabetes that over time can lead to considerable damage to many of the body's systems, especially the peripheral nervous and vascular systems, resulting in severe complications (Brownlee, 2005).

Diabetes-related foot ulcers (DFUs) are an important example of a complication of diabetes as it places substantial strain on healthcare resources in the developing and developed world and significantly debilitates the quality of life and health status of affected people (Davis, Norman, Bruce, & Davis, 2006; Vileikyte, 2001; Weir, 2010). DFUs have been defined as either a full thickness wound below the ankle in patients with diabetes irrespective of duration (Apelqvist & Larsson, 2000), or a lesion of the foot penetrating through the dermis (see Plate 1.1) (Schaper, 2004).

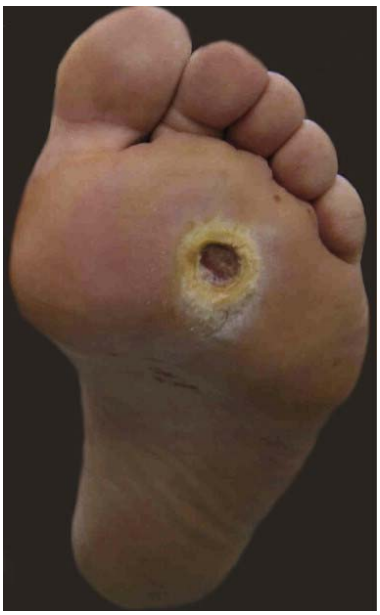


Plate 1.1 Visual illustration of a neuropathic plantar diabetes foot ulcer (Stanley & Collier, 2009, page 8).

Pooled estimates from population-based studies suggests a global prevalence for DFUs of 4.6% (95% Confidence Interval: 3.7–7.6%) for patients with diabetes (Zhang et al., 2017). Although the Australian prevalence for DFUs of 1.5% (95% CI: 0.7–2.4%) reported in the same study (Zhang et al., 2017) has been regarded as the lowest national estimate of all global studies, this is likely to be an under-estimate as it is based on one population-based study from a metropolitan region of Australia (Baba, Davis, & Davis, 2014). Further population-based studies suggest up to 2% of all hospital in-patients with diabetes in Australia have been hospitalised for DFUs (Lazzarini et al., 2016) giving an indication on the severity of the problem in Australia.

A major precursor in the development of DFUs is the onset of diabetic peripheral neuropathy (DPN) in the lower limbs which leads to a lack of protective sensation, motor nerve neuropathy, and loss of proprioception (Armstrong, 2005; Booya et al., 2005; Boulton, 2005; Lavery, Armstrong, Vela, Quebedeaux, & Fleischli, 1998; Shenoy, 2012). DPN manifests in the motor, autonomic and sensory components of the nervous system, causing damage to the intrinsic foot muscles, creating bony prominences and pressure areas which can lead to skin breakdown and the formation of DFUs (Boulton, Vileikyte, Ragnarson-Tennvall, & Apelqvist, 2005; Tesfaye et al., 2010).

DFUs are thought to be caused by the interplay of several factors; including most notably DPN as mentioned above, and also peripheral arterial disease (PAD), tissue ischemia and alterations in blood flow regulation and skin integrity due to autonomic neuropathy as indicated in Figure 1.2 (Boulton, 1998b; Bustos-Saldaña & Prieto-Miranda, 2009; Clayton & Elasy, 2009). The prevalence of PAD can be as high as 50% in patients with DFUs (Hinchliffe et al., 2012). Both DPN and PAD are linked to chronic hyperglycemia and the altered metabolic state in people with diabetes resulting in chronic inflammation (Boulton, 2004a; Ikem, Ikem, Adebayo, & Soyoye, 2010; Ogbera, Osa, Edo, & Chukwum, 2008; Tesfaye & Selvarajah, 2012).

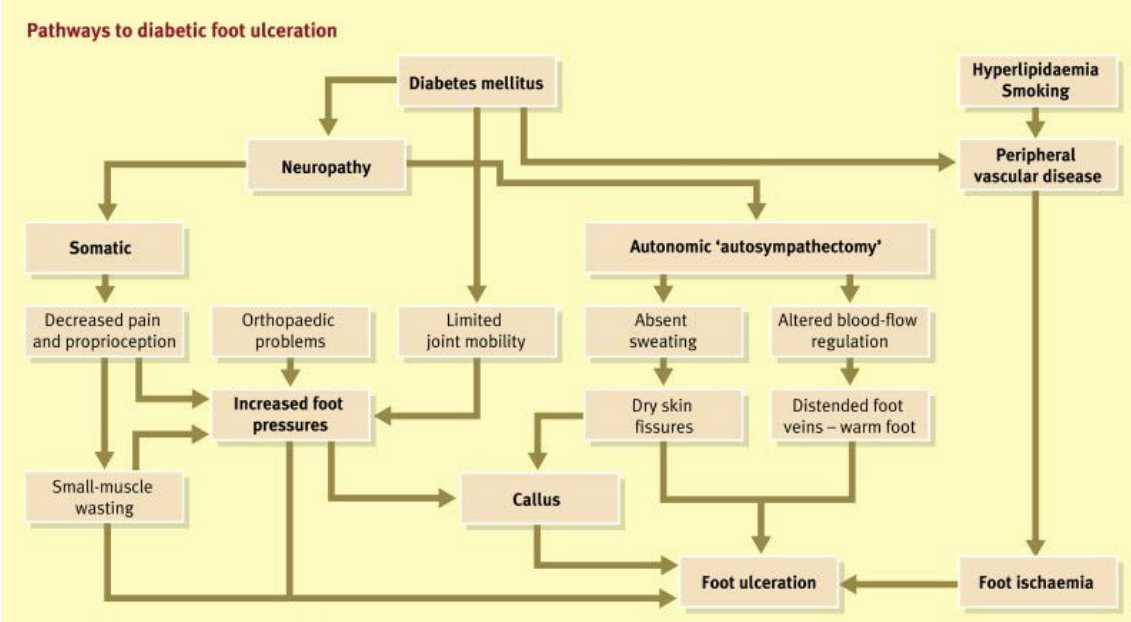


Figure 1.2 Pathways to diabetes foot ulceration (Boulton, 2013, page 16).

A neuropathic foot ulcer is an ulcer that is related to progressive end stage DPN (Boulton, Krisner, & Vileikyte, 2004). Such ulcers have a distinct appearance with a callused border, increased depth and lack of sensation or pain associated with them (Plate 1.1). Hence, neuropathic DFUs are often located on weight bearing areas such as the plantar sole of the foot due to the pressure that the plantar tissues bear (Stanley & Collier, 2009). This is in contrast to other types of foot ulcers that occur in people with diabetes, such as (1) neuro-ischemic foot ulcers that also have a macrovascular or PAD component to their aetiology, and (2) arterial or venous ulcers that are entirely due to peripheral arterial or venous circulatory insufficiency (Heikkinen, Salmenpera, Lepantalo, & Lepantalo, 2007; Miller et al., 2004; Schaper, Prompers, & Huijberts, 2007; Viswanathan, 2007).

In contrast to other types of ulceration, the development of neuropathic DFUs are associated with considerable biomechanical alterations including joint deformities of the foot (Bus, Maas, de Lange, Michels, & Levi, 2005; Cavanagh, Simoneau, & Ulbrecht, 1993; Dinh & Veves, 2005; Ledoux et al., 2003; Ledoux et al., 2005; Mueller et al., 2003), restricted range of motion of joints (Fernando, Masson, Veves, & Boulton, 1991; Giacomozzi, D'Ambrogi, Cesinaro, Macellari, & Uccioli, 2008; Mueller, Minor, Sahrmann, Schaaf, & Strube, 1994a) and increased tissue stress (Najafi, Crews, & Wrobel, 2010a; Wrobel & Najafi, 2010) caused by the effects of DPN during gait.

1.2 The importance of diabetes-related foot ulcers

The implications of a DFU can broadly be categorized as 1) personal implications for the individual with an ulcer and their caregivers; 2) implications for healthcare workers and healthcare services; and 3) broader economic implications. Some of these factors are discussed below;

1) Implications for the individual and their caregivers: It has been estimated that globally there is a limb amputation every 30 seconds due to diabetes (Game, 2012). In the 2000s the estimated likelihood of lower limb amputation was 10 to 30 times higher amongst patients with diabetes and 85% of all amputations were preceded by a DFU (Boulton et al., 2004; Singh, Armstrong, & Lipsky, 2005), making DFUs the leading cause of lower extremity amputation in people with diabetes mellitus worldwide (Singh et al., 2005). The five-year mortality rate after the onset of DFUs ranged from 43% to 55% and was up to 74% for patients with lower limb amputation in the United States of America (Robbins et al., 2008). These mortality rates are higher than those for several types of cancer including prostate, breast, colon, and Hodgkin's disease, which calls for aggressive management of DFUs locally, systemically and psychologically (Robbins et al., 2008).

   Despite best efforts in preventing recurrence, most recent estimates indicate that up to 40% of patients with healed DFUs will develop a subsequent DFU within one year of healing and often this sequale ends in lower limb amputation (Pound, Chipchase, Treece, Game, & Jeffcoate, 2005; Wu, Driver, Wrobel, & Armstrong, 2007; Armstrong et al., 2017). The two most prominent risk factors for lower limb amputation in people with DFUs are ischemia and infection (Prompers et al., 2007) and a higher risk of amputation seems to be associated with male gender and the presence of neuro-ischemic ulceration (Moura Neto, Zantut-Wittmann, Fernandes, Nery, & Parisi, 2013).

Health Related Quality of Life (HRQoL) of patients with DFUs is known to be significantly lower than in people without DFUs (Garcia-Morales et al., 2011; Valensi, Girod, Baron, Moreau-Defarges, & Guillon, 2005). Overall HRQoL improves with the DFU healing as the presence of DFUs can be a large emotional burden on patients and their caregivers (Nabuurs-Franssen, Huijberts, Nieuwenhuijzen Kruseman, Willems, & Schaper, 2005). In a Spanish cohort, the diabetes foot related factors that significantly reduced patients HRQoL were neuropathy, amputation history and poor metabolic control (Garcia-Morales et al., 2011). Hence people with DFUs seem to have lower general health, mental health, physical functioning and social functioning when compared to people with diabetes alone, while a poorer HRQoL during follow-up was associated with poor prognosis (Ribu, Birkeland, Hanestad, Moum, & Rustoen, 2008). Reduced HRQoL and risk of amputation highlight the nature of the condition and are clearly reflective of the profound effect that DFUs can have on patients and their carers.

2) Implications for health care workers and healthcare services: DFUs have a profound impact on healthcare services and healthcare providers both globally and in Australia (Boulton et al., 2005; Lazzarini et al., 2016). Latest figures from Australia estimate that DFUs are the primary reason for 2 out of every 100 hospital beds used in the state of Queensland at any given time (Lazzarini et al., 2016). Between 2005 and 2010, 24,917 diabetes foot-related admissions occurred in Queensland, resulting in the use of 260,085 bed days (Lazzarini, O'Rourke, Russell, Derhy, & Kamp, 2015b). In Australia, foot complications from diabetes ranked third after cardiovascular and chronic kidney disease (CKD) in terms of the number of acute hospital admissions relating to diabetes (Lazzarini, Gurr, Rogers, Schox, & Bergin, 2012). Previous research showed that the average length of stay in hospital was longer for diabetes foot related admissions when compared to all other diabetes complications (Lazzarini et al., 2012). This research concluded that DFU management might be the second largest consumer of acute care resources of all diabetes related complications in Australia (Lazzarini et al., 2012). This data is reflective of the profound impact of DFUs on healthcare services.

3) Economic implications: Foot ulceration is affecting patients with diabetes in populations globally resulting in major economic consequences for the patients, their families, and the societies at large (Boulton et al., 2005). Estimates from the United States of America for people over 65 years indicate that during a 12-month follow-up period, people with DFUs had more hospitalized bed days, more days requiring home health care, more emergency department visits and more outpatient or physician office visits than matched controls (Rice et al., 2014). This resulted in an average cost of $US 11,710 in incremental annual health care costs for Medicare and $US 16,883 for private insurance, compared with matched controls (Rice et al., 2014). It has long been known that patients with DFUs in the United States have a much higher cost implication (up to three times higher) than those without DFUs, due to the associated ongoing costs of DFU treatment and prevention and the management of further complications resulting from DFUs (Harrington, Zagari, Corea, & Klitenic, 2000; Ramsey et al., 1999).

   Increasing severity of the ulcer, also increases average healthcare costs, ranging for example in the early 200s in the United States of America from $US 1,892 for an uncomplicated superficial ulcer to $US 27,721 for an ulcer resulting in gangrene or amputation (Stockl, Vanderplas, Tafesse, & Chang, 2004). The predicted healthcare cost of a DFU in the United States of America in 2010, adjusted to currency ranged from $US 21,259 to $US 37,894 (Cook & Simonson, 2012). Globally, the cost burden to a patient with superficial and non-infected DFUs of six weeks duration varied from a comparison of six days of average income in the United States of America to 126 days of average income in India (Cavanagh et al., 2012).

   In 1998, the expense for DFU related amputations in Australia was estimated to be $AUD 48 million in hospital management and in 1994, the average cost of hospitalization for treatment of a DFU was $AUD 12,474 (Colagiuri, Colagiuri, & Ward, 1998).More recent figures from 2012 demonstrated that a person with type 1 or type 2 diabetes with micro-vascular complications (i.e. DPN) costed $AUD 7000 per year in healthcare and a person with both micro and macro-vascular (PAD) complications

costed $AUD 16,700 per year (Lazzarini et al., 2012). In Australia, chronic wounds were estimated to cost the health care system at least $AUD 3.76 billion per year (Norman et al., 2016) and recent estimates have indicated that the overall 5-year cost savings were in excess of $AUD 12,000 per person, if patients were to receive optimal, evidence based care for DFUs (Cheng et al., 2016). These figures demonstrate the economic burden of DFUs on any healthcare system and emphasize the need to prevent DFUs and to achieve timely wound healing preventing the progression to further complications.

1.3 Statement of the problem

The ability to remain mobile is an essential aspect of a person's quality of life as it is crucial for the preservation of independence and for participating in activities of daily living (Volpato et al., 2002). The presence of diabetes has been associated with substantial reductions in mobility, drastically impaired lower extremity function, an inability to carry out activities of daily living, increased levels of physical disability and increased risk of mortality compared to people without diabetes (Blaum, Ofstedal, Langa, & Wray, 2003; McGuire, Ford, & Ajani, 2006; Volpato et al., 2002; Volpato et al., 2003; Volpato, Maraldi, & Fellin, 2010). In general, walking capacity and walking performance seems to decline with progression of foot complications in people living with diabetes (Kanade, van Deursen, Harding, & Price, 2006). However, little is known regarding the mobility patterns of people with active DFUs. The measurement of plantar pressure and three-dimensional movement analyses provide methods by which the biomechanical function in relation to the mobility patterns of people with DFUs can be evaluated.

1.3.1 Plantar pressures

Plantar pressure measurement refers to the study of pressure fields acting between the plantar surface of the foot and a supporting surface (Orlin & McPoil, 2000). The current biomechanical theory of plantar ulceration states that three mechanisms of pressure development account for the occurrence of abnormal plantar pressures, leading to the formation of neuropathic DFUs (van Schie, 2005). These mechanisms are;

1) Increased duration of plantar pressures - relatively low amounts of pressure applied for a prolonged amount of time, leading to skin ischemia and ulceration due to tissue stress;

2) Increased magnitude of pressures - high pressure acting for a short-period of time, inducing injury to tissue due to lack of sensory perception leading to ulceration;

3) Increased number of pressures - repetition of pressure at same site, resulting in mechanical fatigue and skin trauma leading to ulceration.

The result of elevated plantar pressures on plantar tissue in the presence of DPN is mechanical fatigue. Mechanical fatigue is defined as the failure of a structure or biological tissue to maintain integrity caused by repeated bouts of loading (van Schie, 2005). Injury to tissues of the foot because of mechanical fatigue seems to occur in the insensitive skin and subcutaneous tissue of patients with DPN. It has been proposed that the human body responds to repeated high pressures or micro trauma with callus formation to protect the skin from further damage (Boulton, 1998b).

Hence the current principal biomechanical focus of treatment and prevention of neuropathic DFUs is on the reduction of plantar pressure (Bell, 2008; Bowering, 2001; Bus et al., 2008a; Bus et al., 2016a; Bus, Ulbrecht, & Cavanagh, 2004; Bus et al., 2008b; Bus et al., 2008b; Illgner et al., 2009; Wu, Crews, & Armstrong, 2005). This is achieved through the debridement of any callused areas likely to result in trauma and ulceration (Schaper et al., 2007) and by using various pressure offloading devices such as orthoses, special footwear, cast walkers and various padding methods to relieve any mechanical loading on the foot during standing and during gait (Bus et al., 2016a; Klobučar, 2010; Mueller et al., 2006; Nubé et al., 2006; Raspovic, Newcombe, Lloyd, & Dalton, 2000; Wu, Jensen, Weber, Robinson, & Armstrong, 2008).

Offloading devices are outlined as an essential treatment modality in the majority of international guidelines on DFU management, including the most recent international guidelines (Bus et al., 2016a). The recommended gold-standard in offloading DFUs is total contact casting or the use of irremovable cast-walkers designed to disperse plantar pressures of the foot and ankle, in order to encourage ulcer healing (Armstrong, Lavery, & Bushman, 1998a; Bus et al., 2016a; Hartsell, Brand, Frantz, & Saltzman, 2004;

Lavery, Vela, Lavery, & Quebedeaux, 1997b; Rajbhandari, Jenkins, Davies, & Tesfaye, 2002; Rathur & Boulton, 2005; Shaw et al., 1997). However, these devices are rarely used due to patient noncompliance, contraindications and availability as well as delays with clinician initiation (Fife, Carter, & Walker, 2010; Raspovic & Landorf, 2014; Wu et al., 2008). Irrespective of these limitations, the barefoot plantar pressures of people with active DFUs remains largely unknown as there is a paucity of studies in the field which has previously investigated this. Therefore, identifying the gait parameters in people with active DFUs and longitudinally evaluating plantar pressures would provide much needed data and improve the present understanding regarding the plantar pressure levels and hence the offloading requirements of people with active DFUs to expedite wound healing.

1.3.2 Gait characteristics

Three-dimensional movement analysis systems can be used to assess gait characteristics relating to the lower limb. This involves attaching reflective markers to the participant's skin over the surface of key anatomical landmarks, which enables the assessment of movement characteristics such as joint angular kinematics and descriptors of the gait cycle (GC) (see Appendix A, Appendix Figure 1) termed temporal-spatial parameters (TSPs) (Kirtley, Whittle, & Jefferson, 1985; Whittle, 2012). The simultaneous and synchronized use of force platforms allow for the assessment of kinetics which includes ground reaction forces, joint powers, joint moments, velocities and accelerations of movement. A full description of three-dimensional movement analyses outcomes and their significance in gait analysis is provided in Appendix A.

It can be assumed that gait characteristics are likely to influence the rate of increase of plantar pressure, the duration of high plantar pressure and the frequency of high plantar pressure in people with DFUs during active ulceration (van Schie, 2005). While research to date has focused on plantar pressure and gait characteristics of individuals with diabetes with DPN and people with DPN with a history of DFUs, limited attention has been given to the short- and longer-term observations of gait and plantar pressure in people with chronic neuropathic DFUs (Kanade, Deursen, Harding, & Price, 2006; Katoulis, Boulton, & Raptis, 1996). Therefore, there is a need to systematically review the currently available literature that report on gait and plantar pressure outcomes in

people with DPN and in people with a history of DFUs and to evaluate how DPN manifests in people with active DFUs in the form of altered gait and plantar pressure outcomes when compared to people without DFUs.

Although current clinical guidelines stress the need for optimized off-loading of plantar pressure in people at risk of developing DFUs and in people with active DFUs, a better understanding of gait characteristics and plantar pressures in people with chronic DFUs is likely to improve the approaches to pressure-offloading, given that there are major limitations with current best management approaches (Bus et al., 2016a; Cavanagh, Ulbrecht, & Caputo, 2000; Cavanagh & Bus, 2011). As mentioned earlier, there is a paucity of research on the gait characteristics in people with DFUs, therfore it is likely that current offloading approaches may not have fully considered the implications of gait parameters when considering offloading devices and the impact of offloading devices on the gait parameters in people with active DFUs (Crews et al., 2016). Recent evidence has suggested that postural instability is a powerful predictor of non-adherence to offloading devices (Crews et al., 2016). Given the issues with non-compliance as well as conraindications of gold standard devices, a better understanding of the gait and plantar pressure outcomes in people with DFUs may lead to more focused approaches of offloading and a more streamlined process for the selection of offloading devices for people with DFUs based on biomechanical outcomes. Lastly, given that there is a delay in initiating pressure offloading by clinicians, prospective studies of barefoot plantar pressures and gait outcomes may provide much needed evidence for the importance of offloading plantar pressures in people with active DFUs.

Despite the fact that the current biomechanical theory of mechanical stress via elevated plantar pressure as a potential cause of neuropathic DFUs has been extensively assessed (Armstrong, Peters, Athanasiou, & Lavery, 1998d; Boulton et al., 2004; Boulton, 2004a; Boulton, 2004b; Bus, 2016; Cavanagh, Ulbrecht, & Caputo, 2000; Fernando, et al., 1991; Frykberg, 2002; Lavery, Armstrong, Wunderlich, Tredwell, & Boulton, 2003; van Schie & Boulton, 2002), there is limited understanding regarding plantar pressures and gait characteristics (kinematics, kinetics and TSPs) of people during active ulceration in prospective studies. This lack of knowledge illustrates the importance of further studies identifying the biomechanical function of the lower limb in relation to DFUs to warrant better treatment approaches. It is important to study the full range of

gait outcomes (kinematics, kinetics and TSPs) in conjunction with plantar pressures as plantar pressure outcomes are likely to be the result of altered gait characteristics in this cohort. Therefore, an evaluation of kinematics will provide insight on any restrictions in joint angular kinematics due to motor neuropathy; the evaluation of kinetics will provide specific insight on the ground reaction forces which have direct association with plantar pressures and the evaluation of TSPs is likely to demonstrate whether gait deviations occur in the timing of the gait cycle. All these outcomes could provide important information regarding the biomechanical implications on plantar pressures and foot ulceration.

1.4 Primary Research Questions

Considering the gaps in research stated above, the following five research questions will be examined in this thesis;

- Question 1: What is known about the gait and plantar pressure characteristics of people with DPN?
- Question 2: Do differences in plantar pressures exist between people with DPN: without a history of DFU, with a history of DFU and with active DFUs?
- Question 3: Can currently available methodology be used to obtain gait and plantar pressure data with adequate reproducibility in people with DPN with and without active DFUs?
- Question 4: What are the gait and plantar pressure characteristics of people with DFUs that differentiate them from people with diabetes without DFUs and from healthy people?
- Question 5: Do the plantar pressures of people with DFUs remain different compared to people with diabetes without DFUs over the course of 6 months follow-up?

1.5 Outline of thesis

To address the above research questions, the following thesis is presented by publication and contains nine chapters; an introduction, seven published manuscript chapters and a discussion chapter. A brief outline of each chapter's titles, aims,

hypotheses and the references to resulting peer-reviewed publication is presented in Table 1.1. Each of the seven manuscripts were written to represent specific chapters of the thesis; Chapter 2 and 3 are reviews of the existing literature, Chapters 4 and 5 are the methodology chapters for prospective studies and Chapters 5 to 8 are the results chapters from original prospective studies. Each chapter contains a preface that briefly outlines the manuscript contained within the chapter and the relevance of the work to the entirety of the thesis and any additional pertinent information required to assist the reader with linking the various content presented in in individual chapters.

Table 1.1 General outline of the thesis including aims and hypotheses of individual thesis chapters.

Chapter Number	Title	Research Question In focus	Aim	Hypothesis	Peer-reviewed publication reference
1	**Introduction and outline**	Nil	To review diabetes related foot ulcers (DFUs)*, and outline the significance of the research content and format contained in this thesis.	Nil	Nil
2	**A systematic review and meta-analysis of gait characteristics and dynamic barefoot plantar pressure measurements in people with diabetic peripheral neuropathy**	1	To assess the effect of DPN on gait (temporal-spatial characteristics, joint angular kinematics and kinetics), dynamic electromyography (muscle activation and deactivation patterns) and dynamic barefoot plantar pressures (plantar pressures during gait).	Reductions in temporal-spatial characteristics, increases in kinetics (specifically the vertical ground reaction force and joint moments), and reductions in kinematics of the lower limb (evident as restrictions in the sagittal plane) and altered dynamic electromyography findings in those with DPN may manifest from or contribute towards altered plantar pressure loading in this population	Fernando M, et al. (2013). Biomechanical characteristics of peripheral diabetic neuropathy: A systematic review and meta-analysis of findings from the gait cycle, muscle activity and dynamic barefoot plantar pressure. *Journal of Clinical Biomechanics 2013 Oct;28(8):831-45.*

3	**Meta analysis of plantar pressure measurements in People with diabetic peripheral neuropathy with active foot ulceration, previous ulceration and no** **History of ulceration**	2	To compare plantar pressures in patients with a previous history of or active ulcers (cases) and individuals with DPN without a history of ulceration (controls).	Even though those with active foot ulcers are insensate, they may still alter their movement characteristics to a 'guarded gait strategy' during barefoot gait to compensate for the presence of their active ulcer, which in turn may result in a reduction in plantar pressures during the active ulcer phase.	Fernando M, et al. (2014). Plantar pressure in diabetic peripheral neuropathy patients with active foot ulceration, previous ulceration and no history of ulceration: a meta-analysis of observational studies. *PLoS One. 2014 Jun 10;9(6): e99050.*
4	**General methods and study protocol**	3	To establish the lower limb biomechanical characteristics (TSPs, kinematics, kinetics, muscle activations and plantar pressures) of patients with plantar neuropathic DFUs and to longitudinally evaluate these characteristics at 3 months and 6 months follow-up.	The DFU group will demonstrate significantly poorer TSPs, restricted kinematics at the ankle, knee, hip and pelvis and altered kinetics (anterior posterior, medial-lateral and vertical ground reaction forces) and significantly different plantar pressure compared to diabetes and healthy controls at baseline. The plantar pressures in the DFU group will remain significantly higher at 3 and 6 months follow-up compared to the diabetes controls.	Fernando M, et al. (2015). Lower limb biomechanical characteristics of patients with neuropathic diabetic foot ulcers: the diabetes foot ulcer study protocol. *BMC Endocrine Disorders. 2015; 15: 59.*

5	**The reproducibility of acquiring three dimensional gait and plantar pressure data using established protocols in participants with and without type 2** **Diabetes and foot ulcers.**	3	To describe the methods and the reproducibility of assessments related to three-dimensional gait analysis and plantar pressure assessment in people with DFUs and controls with diabetes without DFUs and healthy controls.	Nil	Fernando M, et al. (2016). The reproducibility of acquiring three-dimensional gait and plantar pressure data using established protocols in participants with and without type 2 diabetes and foot ulcers. *Journal of Foot and Ankle Research. 2016 Jan 29; 9:4. doi: 10.1186/s13047-016-0135-8. eCollection 2016.*
6	**Gait characteristics of people with diabetes-related neuropathic plantar foot ulcers**	4	To comprehensively assess the kinematic, kinetic and TSPs in cases with active DFUs using three-dimensional movement analyses.	Compared to diabetes and healthy controls, people with plantar neuropathic DFUs would display significantly restricted angular kinematic variables in the lower limb; significantly increased kinetic characteristics, leading to a higher planar load distribution; significantly altered TSPs.	Fernando M, et al. (2016). Gait parameters of people with diabetes-related neuropathic plantar foot ulcers. *Clinical Biomechanics. 2016; 37:98-107.*

7	**Plantar pressures are higher in cases with diabetes-related foot ulcers compared to controls despite a longer stance phase duration**	4	To assess whether plantar pressures were higher in patients with active unilateral plantar DFUs of >3 months duration (cases) compared to patients without a foot ulcer history (diabetes controls) and patients without a diabetes or foot ulcer history (healthy controls).	Cases with active DFUs would have higher magnitudes and durations of plantar pressure compared to controls.	Fernando M, et al. (2016). Plantar pressures are higher in cases with diabetic foot ulcers compared to controls despite a longer stance phase duration. *BMC Endocrine Disorders 2016; 16:51 DOI 10.1186/s12902-016-0131-9.*
8	**Plantar pressures are elevated in people with longstanding diabetes-related foot ulcers during follow-up**	5	To investigate plantar pressures at baseline and three and six months later in participants with DFUs (cases) compared to participants without DFUs (controls).	Cases with DFUs would have significantly higher plantar pressures at baseline compared to controls and that these plantar pressure differences would remain during follow-up at three and six months.	Fernando M, et al. Plantar pressures are elevated in people with longstanding diabetes-related foot ulcers during follow-up. *PLOS ONE, 2017 12(8): e0181916.*
9	**General discussion and recommendations**	1,2,3,4,5	To summarize the findings of the numerous studies described in this thesis and provide a discussion of the theoretical implications of the research	Nil	Nil

Legend: DFU = diabetes-related foot ulcer, TSPs = temporal-spatial parameters, DPN = diabetic-peripheral neuropathy

1.6 Significance of the thesis

To the best of my knowledge, this was the first known thesis to investigate the biomechanical characteristics of the lower limb (gait and plantar pressures) in people with active plantar DFUs and evaluate plantar pressure during active ulceration in a longitudinal manner. It was anticipated that findings from this study will advance the understanding of lower limb mobility patterns of people with end stage DPN complications with a focus on DFUs. DFUs are the most common prequel to lower extremity amputation in people with diabetes mellitus, a cause of severe disability and increase of diabetes related mortality worldwide. It was also hoped that the observations and associations found and discussed in this thesis will improve the current understanding of gait characteristics which are likely to impact on elevated plantar pressure in people with active neuropathic DFUs. Results from the research contained in this thesis will deepen the current conceptual understanding of how lower limb mobility changes in response to DPN and DFU and deliver novel insights regarding future approaches to the offloading of DFUs and invoke interest in future research.

Chapter. 2 A Systematic Review and Meta-Analysis of Gait Characteristics and Dynamic Barefoot Plantar Pressure Measurements in People with Diabetic Peripheral Neuropathy

This chapter has been adapted from a publication titled;

Biomechanical characteristics of peripheral diabetic neuropathy: A systematic review and meta-analysis of findings from the gait cycle, muscle activity and dynamic barefoot plantar pressure.

Authors: Fernando M, Crowther R, Lazzarini P, Sangla K, Cunningham M, Buttner P, Golledge J.

Journal of Clinical Biomechanics 2013 Oct; 28(8):831-45.

2.1 Preface

This chapter forms the first part of the literature review component of this thesis and is a systematic review and meta-analysis of observational studies addressing question 1, from Chapter 1. This review was performed by systematically identifying, selecting and appraising previously published studies that investigated the lower limb biomechanical characteristics of patients with DPN compared to healthy and diabetes controls (HC and DMC). Hence this systematic review attempts to outline the gait characteristics (TSPs, joint angular kinematics, kinetics and electromyography findings) and plantar pressures of cases with DPN compared to controls with diabetes without DPN and healthy controls.

2.2 Abstract

Background: Diabetic Peripheral Neuropathy (DPN) is an important cause of foot ulceration and limb loss. This systematic review and meta-analysis investigated the effect of DPN on gait, dynamic electromyography and dynamic plantar pressures.

Methods: Electronic databases were searched systematically for articles reporting the effect of DPN on gait, dynamic electromyography and plantar pressures. Searches were restricted to articles published between January 2000 and April 2012. Outcome measures assessed included temporal-spatial characteristics, lower limb kinematics, kinetics, muscle activation and plantar pressure. Meta-analyses were carried out on all outcome measures reported by ≥3 studies.

Results: Sixteen studies were included consisting of 382 people with DPM, 216 diabetes controls without neuropathy and 207 healthy controls. Meta-analysis was performed on 11 gait variables. A high level of heterogeneity was noted between studies. Meta-analysis results suggested a longer stance time and moderately higher plantar pressures in people with DPN at the rearfoot, midfoot and forefoot compared to controls. Systematic review of studies suggested potential differences in the biomechanical characteristics (kinematics, kinetics, EMG) of people with DPN. However, these findings were inconsistent and limited by small sample sizes.

Conclusions: Current evidence suggests that patients with DPN have elevated plantar pressures and occupy a longer duration of time in the stance-phase during gait. Firm conclusions are hampered by the heterogeneity and small sample sizes of available studies.

2.3 Introduction

One of the many consequences of diabetes is the onset of DPN (Shenoy, 2012). The prevalence of DPN ranges from 13 to 68% in diabetes populations (van Dieren, Beulens, van der Schouw, Grobbee, & Neal, 2010). DPN affects the sensory, motor, and autonomic components of the nervous system, manifesting as a loss of protective sensation, intrinsic foot muscle dysfunction and anhydrosis of the foot (Shenoy, 2012). These manifestations often lead to bony deformities and high plantar pressure areas which result in skin breakdown and ulceration (Boulton et al., 2005). It is believed that the majority of diabetes related foot ulcers develop as a result of the repetitive action of mechanical stress (pressure) during gait, in the presence of peripheral neuropathy or loss of protective sensation (Armstrong et al., 2004). Lower-limb amputations in people with diabetes are typically preceded by diabetes-related foot ulcers (DFUs), suggesting

that better understanding of the mechanisms of ulcer development are of vital importance (Singh, Armstrong, & Lipsky, 2005). This includes better understanding of the biomechanical components (Formosa, Gatt, & Chockalingam, 2013). There is thus a need to collate and summarize the currently existing studies investigating the gait characteristics of people with DPN.

It has been postulated that DPN-related changes in the lower limbs may lead to functional gait variations; predominantly related to reduced range of movement of joints, reduced active muscle power and changes in gait mechanics (Andersen, 2012). The biomechanical changes resulting from DPN may translate to increased plantar pressures in the foot, which contributes to the pathogenesis and development of foot ulcers, especially at the forefoot (van Deursen, 2004). In particular, the first metatarsophalangeal joint has been implicated as a site of biomechanical dysfunction leading to elevated plantar pressures during gait, promoting ulceration at this site (Turner, Helliwell, Burton, & Woodburn, 2007). Therefore, we hypothesised that reductions in temporal-spatial characteristics (TSPs), increases in kinetics (specifically the vertical ground reaction force and joint moments), and reductions in kinematics of the lower limb (evident as restrictions in the sagittal plane) and altered dynamic electromyography findings in people with DPN may manifest from or contribute towards altered plantar pressure loading in this population based on previous research in the field (Cavanagh et al., 2000). Therefore, this systematic review and meta-analysis aimed to assess the effect of DPN on gait (temporal-spatial characteristics (TSPs), joint angular kinematics and kinetics), dynamic electromyography (EMG) (muscle activation and deactivation patterns) and dynamic barefoot plantar pressures (plantar pressures during gait). We sought case-control studies comparing cases with DPN to controls with diabetes mellitus without neuropathy (DMCs) or healthy controls (HCs).

2.4 Methods

2.4.1 Literature search strategy

Electronic databases (Ovid, CINAHL, PubMed, Scopus and Google Scholar) were searched systematically by the first author for articles published between January 2000 to April 2012, reporting studies on people with DPN in the three biomechanical areas of

gait, dynamic EMG and plantar pressure. The initial search was conducted in April 2012. An additional search was conducted in January 2013 to ensure any further articles were also assessed for inclusion prior to publication. No new articles were found. Search results were restricted to articles published between January 2000 and January 2013. Publications prior to the twenty first century were not included to restrict the focus of the review to the most recent findings from studies which assessed gait using current technology, which is more reliable and comprehensive. This is especially true in relation to three-dimensional joint angular kinematic analysis, which was introduced at around this time (Sutherland, 2001; Sutherland, 2002, 2005). The following keywords and MeSH headings were used:

#1 Gait AND diabetes
#2 electromyograph* AND diabetes
#3 EMG AND diabetes
#4 biomechanic* AND diabetes
#5 kinematic AND diabetes
#6 plantar pressure AND diabetes
#7 (diabetes MeSH) AND 1# AND 2# AND 3# AND 4# AND # 5
#8 (diabetic foot MeSH) AND 1# AND 2# AND 3# AND 4# AND # 5
#9 (diabetic neuropathy MeSH) AND 1# AND 2# AND 3# AND 4# AND # 5

2.4.2 Selection of studies

The titles and abstracts retrieved from the initial database search were screened by the first author utilising the question 'Did the study investigate one of the three biomechanical areas of interest?' The full text was obtained for articles that remained relevant after the initial screening. One of the authors then reviewed the full text for the final decision on inclusion utilising the entry criteria. All articles meeting these initial criteria had their full-texts retrieved and were then further evaluated by two authors (MEF and RGC) using the inclusion and exclusion criteria below. All studies meeting the exclusion criteria were removed from the review.

The inclusion criteria were:

- Studies published in the 2000 to 2012-year range as this time-period has seen a substantial increase in biomechanical literature investigating people with DPN, especially utilising 3-dimensional gait models;
- Studies in English language;
- Studies reporting findings in clearly identified DPN groups in comparison to a DMC and/or a HC group using eligible inclusion and/or screening criteria;
- Studies investigating barefoot walking (barefoot investigation was chosen over shod as this was thought to provide insight into biomechanical characteristics in the disease without the influence of shoes);
- Studies in adult populations (≥ 18 years old);
- Study reported findings for at least one outcome measure of interest in the review.

Exclusion criteria were:

- Any study investigating participants gait, EMG or plantar pressure while wearing shoes, inserts or orthotic devices;
- Any study which included participants with current DFUs or a history of DFUs as a part of their DPN or DMC groups;
- Studies that investigated movement on a treadmill;
- Studies where reported outcome measures were not comparable with at least one outcome measure of interest and could not be converted;
- Studies where authors were unable to provide datasets or outcome variables that were compatible for comparison (mean and S.D), in place of missing data.

2.4.3 Outcome measures

Studies were included in the review if they reported at least one of the following outcome measures:

- TSPs- walking speed (m/s) with or without stride length (m);
- Kinetics- reported findings on net joint moments of force (flexion and extension) for at least one lower limb joint (ankle, knee or hip) and/or reported ground reaction force at initial contact and/or toe-off as separate values;

- Kinematics- reported joint range of motion (ROM) findings for at least one lower-limb joint (ankle, knee or hip) in both flexion and extension directions;
- EMG- activation and deactivation durations of any lower limb muscle during walking in % stance or % gait cycle;
- Plantar pressure- reported on at least one site at the rearfoot or midfoot or forefoot or in any other plantar location in either MPP or PTI or both.

2.4.4 Assessment of methodological quality of studies

Two assessors (MEF and PAL) independently evaluated the quality of the studies utilising a modified version of the quality assessment tool by Downs and Black (Downs & Black, 1998). The criteria within the tool that were not applicable to the studies included in this review were omitted from the analysis (see Table 2.1). The total quality scores of each question were then analysed for the level of quality and reported as an average score between the two assessors. As a simplified version of the quality assessment instrument tool by Downs and Black (Downs & Black, 1998) was utilised, the original scoring system for the tool was scaled according to a total score of 18. Therefore, a score of ≤ 7 was considered low quality, 8-11 as fair quality and >11 as good quality (Downs & Black, 1998).

Table 2.1 Assessment of methodological quality of studies.

			Assessor 1 (MEF)																Assessor 2 (PL)															
			Akashi 2008	Bacarin 2009	Caselli 2002	Gomes 2011	Guldemond 2002	Melai 2011	Sacco 2009	Saura 2010	Savelberg 2009	Savelberg 2010	Sawacha 2009a	Sawacha 2009b	Sawacha 2012a	Sawacha 2012b	Uccioli 2001	Yavuzer 2006	Akashi 2008	Bacarin 2009	Caselli 2002	Gomes 2011	Guldemond 2008	Melai 2011	Sacco 2009	Saura 2010	Savelberg 2009	Savelberg 2010	Sawacha 2009a	Sawacha 2009b	Sawacha 2012a	Sawacha 2012b	Uccioli 2001	Yavuzer 2006
1			Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y
2			Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
3			Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
5[a]			P	P	P	P	P	N	P	P	P	P	Y	P	Y	Y	Y	Y	P	P	P	P	P	N	P	P	P	P	Y	P	Y	Y	Y	P
6			Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
7			Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
10			N	N	Y	Y	N	N	Y	N	Y	Y	N	N	N	Y	N	Y	N	Y	Y	Y	N	N	Y	N	Y	Y	N	N	N	N	N	Y
11			-	Y	Y	-	Y	Y	Y	Y	-	Y	Y	Y	Y	Y	-	-	-	Y	Y	-	Y	Y	Y	Y	-	Y	Y	Y	Y	Y	-	-
12			-	-	-	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	-	-	-	-	-
13			-	Y	Y	-	-	Y	Y	Y	Y	Y	Y	-	Y	Y	-	Y	-	-	Y	-	Y	Y	Y	Y	Y	Y	Y	-	Y	Y	-	-
16			Y	-	-	-	Y	Y	Y	-	Y	Y	N	-	Y	-	Y	Y	Y	Y	-	-	Y	Y	Y	-	Y	Y	N	-	Y	Y	Y	Y
18			Y	Y	Y	Y	Y	-	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
20			Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-	Y	Y	Y	Y	Y	Y	Y	Y
21			-	Y	Y	-	Y	Y	Y	-	Y	Y	Y	-	Y	Y	Y	Y	-		Y	-	Y	Y	Y	-	Y	Y	Y	Y	Y	Y	Y	Y
22			-	-	Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-		Y	-	-	-	-	-	-	-	-	-	-	-	-	-
25			Y	Y	Y	N	Y	N	N	N	N	N	Y	N	N	N	-	-	Y	Y	Y	N	Y	N	N	N	N	N	Y	N	N	N	Y	N
27[b]			N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Total				10	12	14	9	12	10	12	10	13	12	13	8	11	13	11	13		10	12	14	8	13	11	13	8	13	13	13	9	12	13	12	11
Mean				*10*	*12*	*14*	*8*	*12*	*10*	*12*	*9*	*13*	*12*	*13*	*8*	*12*	*13*	*11*	*12*																			

Legend: Methodological quality of studies as assessed independently by (MF) and (PL) using a shortened version of the quality assessment tool by Downs and Black (Downs & Black, 1998). 1= (Y) Yes, 0 = (N) No and (-) = unable to determine. The Total score was out of 18. Mean scores were the average of the two assessors rating rounded down to the nearest integer. Q1; hypothesis, aims, objectives, Q2; relates to main outcomes described, Q3; patient characteristics, Q5; addressing confounders, Q6; Description of main findings, Q7; random variability estimates, Q10; reporting probability values, Q11; representative of population (asked), Q12; representative of population (agreed), Q13; methods representative, Q16; mention of data dredging, Q18; use of appropriate statistical test, Q20; accurate outcome measures, Q21; internal validity (selection bias), Q22; recruitment time period, Q25; adjustment for confounding, Q27; Statistical power determined

[a] This question was scored on the basis of P (partial)=1, Y=2 and N=0 and the original scoring criterion was used. [b] This question was converted to a yes/ no answer for ease of comparison.

2.4.5 Data extraction and reporting

Data extraction was performed by the first author (MEF) with statistical assistance from a statistician (PB) for data analysis. Data were entered into tables for ease of comparison and grouping of variables. Only studies that reported the outcome measures of interest were used in the statistical analysis that followed. Descriptive characteristics of participants (age, gender, BMI), entry criteria for diagnosis of DPN, site of participant recruitment, exclusion criteria used by study and diabetes duration of groups were recorded. Where data were missing or unreported, authors from the studies were contacted in an attempt to obtain or clarify results. Where authors did not reply, the studies were excluded from the review. The MOOSE guidelines for reporting meta-analysis of observational studies was utilised in the synthesis of this review (Stroup, Berlin, Morton, & et.al, 2000).

2.4.6 Statistical analysis

Where possible, data were transformed into standardised units of measure for comparison and for statistical analysis. Weighted means (weighted by sample size of the study) were calculated for the reported demographic variables. All data were reported in Tables (see Tables 2.1 to 2.8). Meta-analysis was carried out on individual outcome measures when more than three studies reported on the particular individual outcome measure (see Table 2.9). Difference in mean values divided by pooled standard deviation (SD) was used to compute effect size, utilising Cohen's d (Cohen, 1988). Heterogeneity of studies was calculated using the Q-statistic and I^2 statistic. Results were reported as standardized mean differences with 95% confidence intervals and p-values. In addition to this, the classic fail safe (N) was also computed, as this gives an estimation of studies needed to be published with a null-effect to renounce the effects from the meta-analysis (Persaud, 1996). For purposes of analysis, a Cohen's d score of zero was interpreted as no difference in effect; a result of 0 to 0.2 was interpreted as a small effect, 0.2-0.5 as a medium effect and ≥ 0.8 as a large effect (McGough & Faraone, 2009). All statistical analysis was carried out by a statistician (PB).

2.5 Results

2.5.1 Search yield

Figure 2.1 outlines the process and results of each step of the literature search. Overall, 1,813 unique records were originally identified. However, 1,800 articles were excluded for a variety of reasons, such as inappropriate study design, use of inappropriate comparison groups, unsuitable methods used in data capture, lack of neuropathy classification, and missing data, irrelevant data or because data were unable to be acquired from authors. Thus, 13 articles remained eligible for inclusion; three extra articles were located by hand-searching reference lists of included articles. Therefore, 16 articles were included in the review. Several studies reported on more than one focus area. Gait findings (temporal-spatial characteristics, kinematics and kinetics) were reported in ten studies. Dynamic EMG results were reported in three studies and barefoot dynamic plantar pressure in seven studies. Table 2.2 displays a summary of the characteristics of participants in included studies.

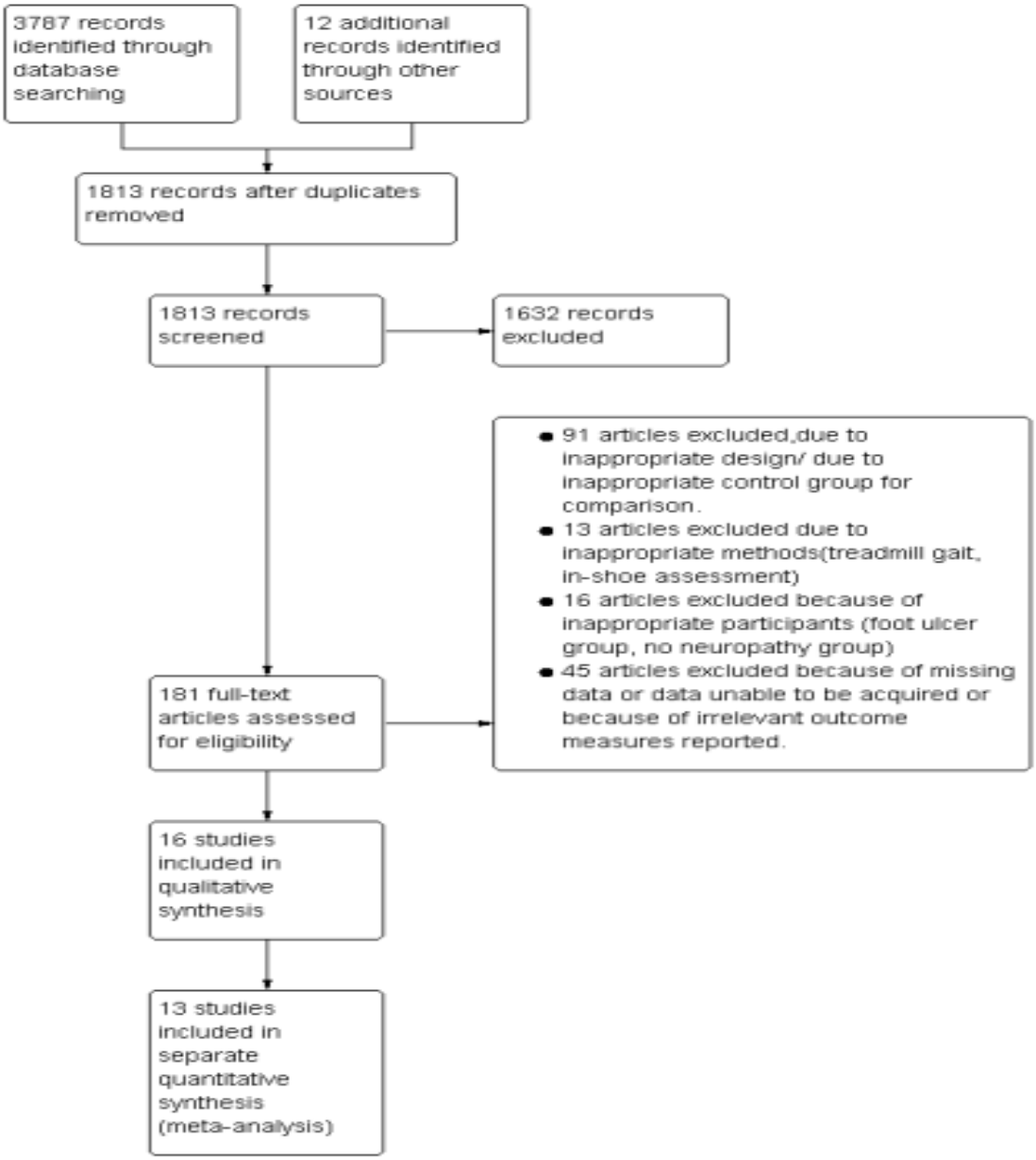


Figure 2.1 Search results.

2.5.2 Study quality

Although there were minor differences in ratings between quality assessors for studies, the overall agreement between quality assessors was good (see Table 2.1). There were no studies which had a score ≤ 7 and therefore seven studies were of fair quality (8-11) and nine studies were of good quality (>11) according to the quality assessment instrument (Downs & Black, 1998). The main difference between the studies that achieved a good quality compared to a fair quality was that they had provided actual probability values (i.e. p =0.004) rather than approximate values (i.e. P <0.05) (reporting) along with a more comprehensive list of confounding variables (reporting). Additionally, the good quality studies described the demographic and recruitment sites of the participants in detail (external validity) and reported the populations of recruitment for groups as being the same or different (internal validity). The majority of good quality studies also used a means of adjustment for confounding with or without using multiple regression analysis.

None of the studies reported sample size calculations. All except two studies had clear aims (Sawacha et al., 2012a; Sawacha et al., 2012b). All studies differed in reporting of confounding variables, especially pertaining to biomechanical research. Important confounders of relevance included diabetes duration, severity of DPN, presence of foot deformity, BMI, gender and presence of claudication pain or presence of peripheral arterial disease (PAD) affecting gait. One study (Melai et al., 2011) did not report on any major confounders. One study did not provide random estimates of variability (either SD or IQR) in their results (Sawacha et al., 2012a). It was difficult to ascertain whether or not the recruited samples were representative of the source population in most studies, however some studies stated the recruitment strategy clearly. Only one study commented on the number of participants who accepted and rejected invitation for the study (external validity) (Savelberg, Schaper, Willems, de Lange, & Meijer, 2009b). Lastly, only one study reported on time frames for recruitment, as it was a part of a larger study (Caselli, Pham, Giurini, Armstrong, & Veves, 2002). For other studies, this could not be determined (internal validity).

2.5.3 Participant characteristics

There were 382 people with DPN (cases) in total from the 16 included studies (see Table 2.2). The mean group size was 25.5 and ranged from 8 to 76 participants. The age range of participants in the DPN groups was 54 to 69 years with a weighted mean age of 61 years. The majority (55%) of subjects were males with a BMI of 24 to 30 kg/m^2 (weighted mean 28 kg/m^2). The weighted mean diabetes duration in the DPN group was 15.2 (range 12 to 27) years.

Studies utilised a variety of participant recruitment sources; community outpatient setting (8/16), hospital settings (3/16), volunteers (2/16), previous studies (1/16) or unspecified (2/16). Thirteen studies utilised a HC group for comparison and were recruited on a voluntary basis from the community or through hospital staff. Nine studies (9/16) used a DMC group, usually from the same setting as DPN patients. A summary of the recruitment sites can be found in Table 2.3.

Table 2.3 presents screening criteria for the diagnosis of DPN and population source of samples used in each study as well as additional exclusion criteria used. A range of methods were used in the diagnosis of DPN in different studies. Eleven studies utilised a validated screening tool to assess sensory neuropathy; the most commonly used was the Michigan Neuropathy Screening Instrument (MNSI). A few studies used only clinical assessment (4/16) and one study utilised nerve conduction testing to assess both motor and sensory neuropathy (Yavuzer, Yetkin, Toruner, Koca, & Bolukbas, 2006). All studies disqualified patients with previous or current DFUs from inclusion in the DPN group and excluded those with additional orthopedic and neurological conditions, rheumatological conditions and disabilities that produce walking constraints. Two studies excluded participants with PAD, assessed on clinical examination or with ankle brachial pressure index (ABPI) values <0.85 (Guldemond et al., 2008; Uccioli et al., 2001). Two studies did not specify exclusion criteria (Caselli et al., 2002; Sawacha et al., 2009a).

Finally, the DMC group comprised of 216 participants with a mean sample size of 24. The age of patients was 50 to 64 years with a weighted mean age of 57 years. The BMI of this group ranged from 25 to 30 kg/m^2 with a weighted mean of 28 kg/m^2. The majority of participants were male (50%) and the total diabetes duration was lower than

for the DPN group; ranging from 8 to 23 years and with a weighted mean of 14 years. The HC group comprised of 207 participants with a mean sample size of 15.9. The age of participants ranged from 46 to 68 years with a weighted mean age of 58 years. The majority of HCs were male (53%) with a BMI range between 24 and 29 kg/m^2 and with a weighted mean BMI of 25 kg/m^2. The findings from the studies are reported in their respective sections below and in Tables 2.4-2.8. Meta-analysis was carried out for 11 separate gait variables (see Table 2.9). Forest-plots of all significant meta-analyses were constructed (see Appendix B, Appendix Figures 1-7).

Table 2.2 Characteristics of study participants in included studies.

Cases					**Diabetes Controls (DMC)**							**Healthy controls (HC)**			
Study	**Focus Area**	**DPN (n=)**	**Age (years)**	**% Male**	**BMI (Kg/m^2)**	**Diabetes Duration (years)**	**DMC (n=)**	**Age**	**% Male**	**BMI (Kg/m^2)**	**Diabetes Duration (years)**	**HC (n=)**	**Age (years)**	**% Male**	**BMI (Kg/m^2)**
Akashi 2008	EMG	**19**	57.6	48	26.6 (4.2)	12.6 (5.3)	*No diabetes control group used*					**16**	51.1(8.3)	50	23.9 (2.9)
Bacarin 2009	P.P	**17**	54.7 (7.8)	47	26.1 (4.6)	13.4 (8.2)	*No diabetes control group used*					**20**	48.7 (9.4)	35	24.3 (2.6)
Caselli 2002	P.P	**57**	59.7 (11.7)	74	28.9 (5.8)	16.4 (10.9)	**20**	50.2 (16.2)	35	27.9 (4.1)	8.4 (10.4)	*No healthy control group used*			
Gomes 2011	Gait and EMG	**23**	56 (8.0)	39	-	14.4 (6.5)	*No diabetes control group used*					**23**	55 (8.0)	39	-
Guldemond 2008	P.P	**44**	58.8 (10.4)	34	29.6 (6.5)	>10	**49**	50.9 (9.5)	39	28.2 (4.8)	>10	*No healthy control group used*			
Melai 2011	P.P	**76**	66.0 (7.2)	-	-	-	**33**	62.8 (7.1)	-	-	-	**19**	68.1 (5.2)	-	-
Sacco 2009	P.P	**15**	57 (6.0)	60	-	>5	*No diabetes control group used*					**16**	46 (11.0)	31	-
Saura 2010	Gait	**16**	63 (3.89)	31	28.78 (2.88)	12.13 (1.26)	**10**	63 (3.92)	30	27.66 (2.66)	12 (1.15)	**10**	62 (3.77)	40	27.35 (2.5)

Savelberg 2009#	P.P &	**8**	68.9 (6.3)	88	28.0 (3.2)	19.0 (13.6)	**10**	60.5 (6.9)	70	29.2 (3.7)	8.1 (0.6)	**10**	72.4(6.0)	80	24.7(2.9)
Savelberg 2010	EMG and	**8**	68.9 (6.3)	88	28.0 (3.2)	19.0 (13.6)	**10**	60.5 (6.9)	70	29.2 (3.7)	8.1 (0.6)	**10**	72.4(6.0)	80	24.7(2.9)
Sawacha 2009 (a)	Gait	**26**	63.2 (6.0)	58	25.6 (2.9)	22.1 (14.3)	**21**	63.8 (5.4)	70	26.3 (2.0)	17.2 (11.7)	**20**	59.0(2.9)	65	24 (2.8)
Sawacha 2009 (b)	Gait	**10**	64.0 (6.8)	70	24.0 (2.8)	-	*No diabetes control group used*					**10**	61.8 (4.3)	80	24.1 (3.0)
Sawacha 2012 (a)	Gait and P.P	**12**	62.0 (6.0)	75	25.2(3.2)	26.7 (10.5)	*No diabetes control group used*					**12**	60.3 (5.2)	83	24.1 (2.6)
Sawacha 2012 (b)	Gait	**20**	61.2 (7.7)	-	26.8 (3.4)	13 (6.5)	**20**	56.53 (13.29)	-	26.4 (2.5)	23.3 (13.7)	**10**	61.2 (5.0)	-	24.4 (2.8)
Uccioli 2001	Gait	**19**	53.7 (10.4)	52	27.0 (4.9)	19.4 (9.3)	**27**	52.7 (12.7)	70	25.3 (3.4)	15.1 (9.3)	**21**	56.6 (11.8)	61	25.0 (3.1)
Yavuzer 2006	Gait	**20**	61.7 (8.5)	60	29.5 (4.5)	18.5 (5.4)	**26**	58.2 (9.5)	46	30.3 (5.9)	14.6 (8.7)	**20**	60.9 (5.9)	50	28.9 (5.1)
TOTAL (N=16)		**382 (mean**	60.8	55.0%	27.7	15.2	**216 (mean**	56.5	50.1%	27.6	13.5	**207 (mean**	57.8	53.2%	25.1

Legend: Reported Means and Standard Deviations (SD) of participants
DPN = Diabetic peripheral neuropathy (cases) compared to DMC= Diabetes Mellitus controls and Healthy controls (HC). Study focus indicates whether the focus was Gait (Gait), Electromyography (EMG) or Plantar Pressure (P.P) or a combination.
Savelberg 2009/2010 was two publications from the same study, therefore only one was used (#) when calculating total sample size and demographic data.
Total: Total sample size per group as well as averages weighted by sample size

Table 2.3 Characteristics of all included studies.

Study	Recruitment source	Neuropathy classification for inclusion	Exclusion criteria
Sawacha 2012a	Cases from outpatient university clinic and controls were from hospital personnel, University of Padova.	A score of >3/15 on the Michigan Neuropathy Screening Instrument and comprehensive neurological examination.	History of ulcers or neurological disorders, Orthopedic problems Lower limb surgery CV disease
Sawacha 2009b	Anti-diabetic unit, University Hospital of Padova, Italy.	Anamnesis and clinical evaluation,	Not specified
Sawacha 2009a	Anti-diabetic unit, University Hospital of Padova, Italy.	A score of >3/15 on the Michigan Neuropathy Screening Instrument. Comprehensive neurological examination.	History of ulcers or neurological disorders, Orthopedic problems, Lower limb surgery, CV disease,
Savelberg 2009 **Savelberg 2010***	Not specified	Clinical examination with sensory testing, tendon reflexes and muscle strength in the lower extremities. Well-regulated metabolic control* BGL 5 -16 mmol/l during trials*	Subjects with foot deformities or active ulceration Limited mobility due to severe joint problems, Angina pectoris or cardiac failure NYHA Class 2 or higher, Myocardial infarction within 1 year, BMI>35 Orthopedic or neuromuscular disease other than diabetic polyneuropathy Severely restricted mobility of lower extremity joints (less than 10 degrees of dorsal flexion or less than 30 degrees of plantar flexion) *

Saura 2010	Cases were patients of the foot and ankle-group, outpatient center, Faculty of medicine, University of Sao Paulo. Controls were community members.	Michigan protocol.	Neuroischemic diabetic foot, Charcot's neuroarthropathy, Inability to mobilise.
Sacco 2009	Cases were recruited through The Brazilian Association for Assistance to Diabetics. The control subjects were employee volunteers from the academic department.	Michigan Neuropathy score >6/15 Type 2 diabetes with >5 year onset. 10 g monofilament absence in 2 plantar areas of the foot. Modified MNSI to assess toe deformities (additional)	Modified MNSI score of >4 (foot deformity) Over 65 years age Hallux amputations or partial foot amputations-except toes Orthopedic problems of the lower limb.
Akashi 2008	Volunteers	At least 5 years post-onset of Type 2 diabetes, At least 2 insensate areas of the foot to the monofilament and a minimal score of 6 on the Michigan neuropathy score. Modified MNSI score of < 4/6.	Age over 65 years, Partial or total amputation, Charcot arthropathy or any other foot deformity confirmed by radiology. Other neurological disease, alcohol abuse, Presence of plantar ulcers, inability to walk.
Melai 2011	Cases from Maastricht University Medical Centre, Maxima Medical Centre, Eindhoven and Veldhoven, St. Anna Hospital Geldrop and Maasland Hospital, Sittard.	Standardized neurological examination.	Severe CVD, renal dysfunction, intermittent claudication, Neurological disorders, RA, severe OA, Foot amputations or deformities, Foot ulcers.
Guldemond 2008	Cases were selected from the outpatient clinic Of the University Hospital Maastricht.	A VPT (vibration perception threshold) >25. Valk score >4.	History of rheumatoid arthritis, Severe foot trauma, severe deformity i.e. which requires orthopedic shoes Surgery of the foot. ABPI

Gomes 2011	Volunteers	Michigan neuropathy screening instrument questionnaire score of >3/13. Michigan Neuropathy Screening Form score of >4/10 >5 years type 2 diabetes duration.	> 65 years of age, Partial or total foot amputation, Charcot arthropathy, Orthopedic foot alteration confirmed by radiography, Any additional central or peripheral nerve disorder, Presence of retinopathy, nephropathy or plantar ulcers Inability to walk.
Caselli 2002	Participants from a large multicenter prospective study,	Neuropathy disability score was used (NDS). Both motor and sensory components measured.	Not defined
Bacarin 2009	Cases recruited through The National Association for Assistance to Diabetics. The control were employee volunteers from the Academic department that conducted this study.	>5 year diabetes duration >6 on Michigan Neuropathy questionnaire At least 2 areas where a 10g monofilament was unfelt.	>65 years of age, Hallux amputation or partial amputation of the foot, major foot shape alterations by visual inspection, Orthopedic disorders of the lower limbs or pain, or use of any assistive devices for walking (walking sticks/canes), Traumatic ulceration that could be immediately recognized by the subject. Charcot arthropathy confirmed by radiography or foot ulcers.
Yavuzer 2006	Outpatient diabetes follow-up clinic of a university hospital	Muscle EMG >2 standard deviations from normal values. Reduced peripheral nerve conduction velocity (peroneal and tibial motor nerves).	Foot ulcers, amputation, Charcot′s joints, Neurological, musculoskeletal or rheumatic disease.

Uccioli 2001	Outpatient clinic	Neuropathy disability score (NDS) > 6/10, Vibration perception threshold >25 and contralateral limb >20 measured with neurothesiometer.	Foot ulcers, > 65 years Inability to walk Neurological, musculoskeletal or rheumatological conditions, PVD, Claudication pain, ABPI <0.85, Charcot joints, amputation.

Legend: Characteristics of participant recruitment sites, assessment of neuropathy and exclusion criteria used according to study.
Caselli 2002 reported findings for three neuropathy groups. The Severe neuropathy group was used in comparison with the diabetes only group in this review.
*= Applies to Savelberg 2010 only.

2.5.4 Temporal-spatial characteristics

There was a marked difference in the walking speeds reported amongst the three groups in different studies (see Table 2.4). Three studies reported that people with DPN walk slower than HCs (Gomes et al., 2011; Savelberg et al., 2010; Sawacha et al., 2009b) and two studies reported slower walking speeds in people with DPN compared to DMCs (Savelberg et al., 2010; Sawacha et al., 2012b). In contrast, two studies reported people with DPN walked faster than both HCs and DMCs (Savelberg, Schaper, Willems, de Lange, & Meijer, 2009b; Yavuzer, Yetkin, Toruner, Koca, & Bolukbasi, 2006).

Meta-analysis results from standardised mean differences, combining data from studies for walking speed (DPN vs. DMC and DPN vs. HC) and stride length (DPN vs. DMC) between the three groups demonstrated no significant difference in walking speed and stride length. There was also a high level of heterogenity present. However, two studies reported stride length findings for DPN vs. HC and both studies reported lower values in people with DPN compared to HCs (Savelberg et al., 2010; Sawacha et al., 2009a).

One study reported stance phase duration as a percentage of the gait cycle (Sawacha et al., 2012b). These findings were consistent with the above findings suggesting people with DPN had longer percentage duration in the stance phase of gait (see Table 2.4). Meta-analysis combining data from three studies (*DPN n=54, DMC n=51*) suggested that people with DPN had a longer stance time at a moderate effect level (standardized mean difference 0.54, 95% CI 0.15-0.93; *P*=0.006). The heterogeneity between studies was minimal I^2= 0.

Table 2.4 Temporal-spatial characteristics.

Study	Walking Speed (m/s)			Stride Length (m)			Stance Time (s)			Stance Phase (% Gait cycle)		
	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC
Sawacha 2009 (a)	1.10 (0.2)	1.10 (0.2)	1.27 (0.1)	1.20 (0.2)	1.23 (0.1)	1.4 (0.1)	0.73 (0.1)	0.66 (0.1)	0.5 (0.1)			
Sawacha 2009 (b)	1.00 (0.2)	/	1.00 (0.1)									
Savelberg 2009	1.40 (0.09)	1.30 (0.13)	1.22 (0.20)									
Sawacha 2012 (b)	1.11 (0.21	1.23 (0.21)	/	1.24 (0.19)	1.33 (0.2)	/	0.7 (0.10)	0.64 (0.0)	/	61.07 (3.14)	59.7 (2.20)	59.4 (2.32)
Gomes 2011	0.9 (0.4)	/	1.03 (0.4)									
Savelberg 2010	1.02 (0.13)	1.06 (0.13)	1.18 (0.22)	1.15(0.15)	1.22 (0.13)	1.28 (0.15)	0.64 (0.5)	0.62 (0.4)	0.61 (0.3)			
Yavuzer 2006	0.94 (0.3)	0.85 (0.2)										

Legend: Mean and standard deviation (SD). Diabetic Neuropathy Subjects (DPN), Diabetes Mellitus controls (DMC); Healthy Controls (HC).
Boxes with / represent missing data or unreported data.

2.5.5 Joint angular kinematics

Only one study (Gomes et al., 2011) reported on kinematics at the hip, knee and ankle in both extension and flexion directions (see Table 2.5). While people with DPN exhibited greater hip flexion (degrees) when compared to HCs, the people with DPN also demonstrated reduced hip extension, knee flexion and knee extension when compared to HCs. Both maximum ankle plantar flexion and ankle dorsiflexion were reduced in people with DPN when compared to HCs. Meta-analysis was not possible for these results.

Table 2.5 Ground reaction forces and joint angular kinematics.

Study	Gomes 2011		Sawacha 2012a	Uccioli 2001	Yavuzer 2006	Saura 2010
Vertical GRF initial contact (N/ Body mass %)	DPN		82.50 (2.87)	87.3 (8.4)	95.0 (5.3)	103.88 (4.82)
	DMC		/	91.3 (9.0)	93.1 (4.9)	91.80 (8.5)
	HC		80.64 (3.17)	93.8 (8.4)	94.2 (5.6)	91.20 (4.4)
Vertical GRF at toe-off (N/ Body mass %)	DPN				95.6 (4.4)	106.38 (8.3)
	DMC				95.0 (4.3)	93.6 (6.9)
	HC				96.5 (5.4)	93.82 (5.3)
Maximum Hip Flexion (deg)	DPN	20.20				
	DMC	/				
	HC	17.97 (3.46)				
Maximum Hip Extension	DPN	5.69				
	DMC	/				
	HC	6.06				
Maximum Knee flexion	DPN	26.30				
	DMC	/				
	HC	29.50				
Maximum Knee extension (deg)	DPN	26.41				
	DMC	/				
	HC	28.53 (16.31				
Maximum Ankle plantar flexion	DPN	9.01 (3.27)				
	DMC	/				
	HC	11.79				
Maximum Ankle dorsiflexion (deg)	DPN	5.63 (2.48)				
	DMC	/				
	HC	5.80 (1.72)				

Legend: Barefoot gait findings. Mean and standard deviation (SD). Diabetic Neuropathy Subjects (DPN), Diabetes Mellitus controls (DMC); Healthy Controls (HC). All joint range of motion (flexion/extension) reported for stance phase duration. GRF = Ground reaction force
Boxes with / represent missing data or unreported data.

2.5.6 Kinetics

Five studies reported kinetic variables (Saura et al., 2010; Savelberg, Schaper, & Meijer, 2009a; Sawacha et al., 2012a; Uccioli et al., 2001; Yavuzer et al., 2006) (see Tables 2.5 and 2.6). Studies reported on both external and internal joint moments. Two studies reported on the force generation components at the ankle, knee and hip (Savelberg et al., 2009b; Yavuzer et al., 2006) (see Table 2.6). According to one study, both the braking and propelling forces were reduced in the DPN group compared to DMC and HC groups (Savelberg et al., 2009a). Both the first maximum support moment and mid stance minimal support moment were elevated in people with DPN compared to DMCs and HCs; however, the second maximum support moment was slightly higher in DMCs when compared to people with DPN (Savelberg et al., 2009a).

The results for maximum ankle plantar flexion moment were inconsistent. One study reported a higher value in people with DPN compared to DMCs (Savelberg et al., 2009a), while another study reported a lower value in DPN when compared to HCs (Yavuzer et al., 2006). Results for knee extension moments were also inconsistent. One study reported reduced extension moments in people with DPN (Savelberg et al., 2009a) and another higher extension moments in people with DPN when compared to both DMCs and HCs (Yavuzer et al., 2006). However, both studies reported greater knee flexion moment in the DPN group compared to both DMC and HC groups respectively (Savelberg et al., 2009b; Yavuzer et al., 2006) (see Table 2.6).

According to a single study, the hip extension moment was greater in the DPN group when compared to both controls (Savelberg et al., 2009a). According to both studies, the hip flexion moment was also reduced in people with DPN compared to both controls (Savelberg et al., 2009b; Yavuzer et al., 2006).

Meta-analysis was only possible for the vertical GRF (first peak) at initial foot-ground contact. Although reported vertical GRF were higher in people with DPN compared to both HCs and DMCs, the results from the meta-analysis were statistically insignificant with a high level of heterogenity. Meta-analysis was not possible for vertical GRF at toe-off (second peak) (see Table 2.5). However, one study reported a higher vertical GRF value in people with DPN at toe-off (Saura et al., 2010) and another a lower value (Yavuzer et al., 2006).

Table 2.6 Joint moments and forces during gait.

Study	Braking Force (N/Kg)			Propelling Force (N/Kg)			Max Ankle Plantar flexion Moment (Nm/Kg)			Max Knee Extension Moment (Nm/Kg)			Max Knee Flexion Moment (Nm/Kg)			Max Hip Extension Moment (Nm/Kg)			Max Hip Flexion Moment (Nm/Kg)			First Max Support Moment (Nm/Kg)			Midstance Min Support Moment (Nm/Kg)			Second Max Support Moment (Nm/Kg)		
	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC
Savelberg 2009*	1.30 (0.31)	1.69 (0.29)	1.74 (0.45)	1.42 (0.35)	2.02 (0.34)	1.78 (0.5)	1.64 (0.26)	1.51 (0.21	1.59 (0.107	0.23 (0.30)	0.42 (0.22)	0.45 (0.36)	0.29 (0.21)	0.16 (0.11)	0.28 (0.27)	1.08 (0.39	1.09 (0.25)	0.85 (0.40)	0.75 (0.36)	0.55 (0.26)	0.70 (0.12)	1.87 (0.32)	1.84 (0.33)	1.52 (0.47)	1.87 (0.32)	0.85 (0.31	0.6 (0.21	1.18 (0.33	1.20 (0.44)	0.86 (0.20)
Yavuzer 2006							1.2 (0.2)	1.1 (0.3)	1.3 (0.2)	0.81 (0.56)	0.52 (0.56)	0.448 (0.63)	0.81 (0.18)	0.43 (0.12)	0.47 (0.19)				0.49 (0.32)	0.50 (0.39)	0.51 (0.20)									

Legend: Mean and standard deviation (SD) of joint moments (external and internal as reported in individual studies) and ground reaction forces during gait. Diabetic Neuropathy Subjects (DPN), Diabetes Mellitus controls (DMC); Healthy Controls (HC). *Study reported values which were normalized to individual body mass of participants.

2.5.7 Dynamic EMG

Muscle activation was reported for several different lower limb muscle groups (see Table 2.7). Two studies reported findings as % stance phase (Akashi, Sacco, Watari, & Hennig, 2008; Gomes et al., 2011) and one study as % gait cycle (Sawacha et al., 2012b). Three studies reported the duration of activity of the tibialis anterior muscle (Akashi et al., 2008; Gomes et al., 2011; Sawacha et al., 2012b). Meta-analysis suggested a non-significant longer duration of muscle activity in the tibialis anterior muscle in people with DPN when compared to HCs.

Meta-analysis was not possible for the other muscle groups due to lack of studies. However, according to two studies, the lateral gastrocnemius muscle had reduced duration of activity in people with DPN (% stance phase) when compared to HCs (Akashi et al., 2008; Sawacha et al., 2012b). On the contrary, assessment of the vastus lateralis muscle suggested a longer duration of activation in the DPN groups when compared to HCs (Akashi et al., 2008; Gomes et al., 2011). Assessment of the peroneus longus muscle produced conflicting results between these two studies (see Table 2.7). One study reported reduced duration of muscle activation in people with DPN (% stance phase) compared to HCs; and another study reported longer duration (% gait cycle) in people with DPN vs. HCs and DMCs.

The findings from the assessment of the gluteus medius muscle, rectus femoris muscle and medial gastrocnemius muscles were from single studies and are highlighted in Table 2.7. Both gluteus medius and rectus femoris muscles were reported to have reduced duration of activity (Sawacha et al., 2012b), whilst the medial gastrocnemius demonstrated longer duration of activity in people with DPN compared to controls (Gomes et al., 2011).

Table 2.7 Electromyography (EMG) peak activation times during stance.

Study	Rectus Femoris (% Support)			Tibialis Anterior (% of Support)			Gluteus Medius (% of Support)			Lateral Gastrocnemius (% of Support)			Medial Gastrocnemius (% of Support			Vastus Lateralis (% of Support)		Peroneus Longus (% of Support)			
Sawacha 2012 (b)	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC
	5.46 (1.26)*	6.82 (1.24)*	11.8 (1.29)*	11.71 (1.13)*	6.96 (1.10)*	9.27 (1.63)*	7.55 (2.68)*	11.7 (1.87)*	13.2 (1.89)*	38.1 (1.66)**	35.9 (1.38)**	41.60 (2.29)**						41.81 (0.09)**		37.2 (0.11)**	33.4 (0.21)**
Akashi 2008	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC
				6.10 (1.68)	/	6.05 (2.15)				62.84 (5.06)	/	63.53 (3.65)				11.97 (2.31)	/	10.82 (3.33)			
Gomes 2011	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC
				3.42 (1.73)		4.33 (1.80)							60.16 (6.99)		54.33 (6.18)	10.37 (3.18)	/	9.02 (3.90)	56.09 (11.22)	/	60.9 (7.98)

Legend: Barefoot dynamic electrography (EMG) during gait. Reported as time of activation peak as per percent (%) of support phase unless otherwise stated. Mean and standard deviation (SD) are reported for both combined left and right measurement unless otherwise specified. Diabetic Neuropathy Subjects (DPN), Diabetes Mellitus controls (DMC); Healthy Controls (HC). Note Sawacha 2012b reported findings as % gait cycle. Findings were reported for initial contact to loading response* Findings were reported for mid-stance * *Boxes with / represent missing data or unreported data.

2.5.8 Plantar Pressure (Peak Pressure and Pressure Time Integral)

Six studies reported plantar pressure data of interest (Bacarin, Sacco, & Hennig, 2009; Caselli et al., 2002; Guldemond et al., 2008; Melai et al., 2011; Sacco et al., 2009b; Sawacha et al., 2012a) (see Table 2.8). The majority of studies reported plantar pressure as MPP while three studies reported PTI (Bacarin et al., 2009; Melai et al., 2011; Sacco et al., 2009b).

Meta-analysis combining data from three studies (DPN n=108, HC n= 55) suggested people with DPN had elevated plantar pressure (both MPP and PTI) at the rearfoot at moderate effect levels (MPP standardised mean difference 0.45, 95% CI 0.09-0.82 $P \leq 0.001$, $I^2=7.0$; and PTI standardised mean difference 0.40, 95% CI 0.05-0.75 p=0.02 $I^2=0$). Both results contained minimal heterogeneity. Despite this, meta-analysis results for MPP at the rearfoot were insignificant for people with DPN when compared to DMCs (see Table 2.9).

Meta-analysis results for the midfoot (DPN n=108, HC n= 55, combining three studies) also revealed greater MPP and PTI in people with DPN (MPP standardised mean difference 0.72, 95% CI 0.37-1.08 $P \leq 0.001$ $I^2=0$; and PTI standardised mean difference 0.50, 95% CI 0.15-0.85 p=0.005 $I^2=7.0$). There was minimal heterogeneity between studies. Meta-analysis for plantar pressure at the forefoot (DPN n=177, DMC n= 102, HC n= 55, combining three studies) demonstrated greater MPP in the forefoot of people with DPN at moderate effect levels compared to HCs (standardised mean difference 0.55, 95% CI 0.20-0.90 p=0.002 $I^2=0$) and DMC (standardised mean difference 0.51, 95% CI 0.24-0.78 $P \leq 0.001$ $I^2=10.1$) respectively. Furthermore, meta-analysis for PTI at the forefoot (DPN n=177, HC n= 55, combining three studies) suggested that forefoot PTI was also elevated in people with DPN at moderate effect levels (standardised mean difference 0.66, 95% CI 0.31-1.02; $P \leq 0.001$; $I^2=0$). There was minimal heterogeneity between studies. Meta-analysis results for the hallux (MPP and PTI) comparing plantar pressure between the three groups revealed non-significant differences (see Table 2.9).

Findings from two studies suggested MPP at the plantar aspect of the first metatarsophalangeal joint was higher in the DPN group compared to DMCs (Guldemond et al., 2008; Melai et al., 2011), while results from one study suggested MPP at the plantar aspect of the first metatarsophalangeal joint was higher in people

with DPN compared to HCs (Melai et al., 2011). According to a single study, the PTI values were higher in people with DPN compared to both DMCs and HCs (Melai et al., 2011). However, according to the same study, there was a lower PTI and MPP for the DPN group in the lesser toes compared to both control groups (Melai et al., 2011) (see Table 2.8).

Table 2.8 Peak plantar pressure and pressure time integral.

Study	Rear-foot			Mid-foot			Metatarsal 1			Fore-foot			Hallux			Lesser toes		
Group	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC	DPN	DMC	HC
Melai 2011**																		
MPP (N/cm²)	42.5 (11.8)	41.9 (10.9)	35.9 (9.3)	15.0 (5.2)	16.5 (6.0)	11.8 (2.4)	44.9 (24.4)	31.5 (12.1)	27.3 (9.2)	50.1 (19.8)	44.8 (13.3)	36.4 (7.5)	46.3 (24.3)	51.4 (28.6)	35.5 (14.9)	15.3 (7.1)	15.4 (8.1)	16.2 (7.2)
PTI Ns/Cm²)	3.4 (0.9)	3.5 (0.7)	3.1 (0.7)	1.2 (0.6)	1.5 (0.6)	1.0 (0.1)	4.7 (2.0)	3.9 (1.0)	3.2 (1.0)	5.9 (1.3)	5.8 (1.4)	5.0 (1.0)	2.6 (1.1)	2.7 (1.1)	2.5 (1.0)	1.0 (0.5)	1.0 (0.6)	1.3 (0.7)
Guldemond 2008#																		
MPP (N/cm²)							48.1 (31.3)	30.8 (13.8)		68.9 (27.9)	55.1 (22.6)		40.5 (25.7)	45.5 (26.4)				
Bacarin 2009*																		
MPP (N/cm²)	34.2 (7.6		33.7 (9.5)	20.5 (11.8)		13.9 (7.6)				36.5 (9.3)		34.7 (8.8)	30.5 (11.1)		30.6 (11.0)			
PTI Ns/Cm²)	9.4 (2.9)		8.3 (2.1)	4.3 (0.9)		3.7 (1.1)				11.0 (2.6)		9.7 (2.3)	7.4 (2.6)		6.8 (2.4)			
Sacco 2009***																		
MPP (N/cm²)	22.0 (4.0)		19.6 (2.7)	11.4 (5.2)		7.5 (3.1)				24.5 (5.6)		21.8 (3.5)						
	2.7 (0.5)		2.5 (0.3)	3.9 (1.7)		3.0 (0.9)				5.3		4.4						
Sawacha 2012a#																		
MPP (N/cm²)	77.5		42.7	51.5		31.2				41.0		58.4						

Caselli 2002#						
MPP (N/cm²)	23.0 (10.0)	32.0 (20.0)		62.0 (40.5)	33.0 (21.0	

Legend: Reported right foot plantar pressures and pressure time integrals normalized to body mass; Mean and standard deviation (SD). Diabetic Neuropathy Subjects (DPN); Diabetes Mellitus controls (DMC); Healthy Controls (HC). *This study reported both medial and lateral plantar pressures for the forefoot. The highest valued pair (medial) was extrapolated for comparison.
** This study reported multiple forefoot plantar pressures; the highest plantar pressure data-set at metatarsal 3 was taken as the forefoot plantar pressure measurements.
***This study reported plantar pressure at initial strike and at toe-off, the initial strike pressures were utilized for rear foot and the toe off pressures for the forefoot and mid-foot as these were the highest pressures reported.
These studies did not report pressure-time integral (PTI).

Table 2.9 Meta-analyses results.

Outcome measure	Comparison	Number of studies	Effect size (95%-CI)	*P* -value	Heterogeneity assessment	Classic fail safe N
Walking speed [m/s]	DPN versus DMC	5	-0.041 (-0.563, 0.482)	*P*=0.879	Q=10.7; *P* =0.030; I^2 = 62.5	**0**
	DPN versus HC	5	-0.270 (-0.936, 0.396)	*P*=0.427	Q=14.6; *P* =0.006; I^2 = 72.6	**0**
Stride length	DPN versus DMC	3	-0.343 (-0.730, 0.044)	*P*=0.083	Q=0.54; *P* =0.763; I^2 = 0	**0**
Stance time	DPN versus DMC	3	0.545 (0.153, 0.937	*P*=0.006	Q=1.4; P =0.496 I2 = 0	2
Vertical GRF Initial Contact	DPN versus DMC	3	0.543 (-0.615, 1.701)	*P* =0.358	Q=17.0; *P* <0.001; I^2 = 88.3	**0**
	DPN versus HC	4	0.614 (-0.601, 1.828)	*P* =0.322	Q=30.6; *P* <0.001; I^2 = 90.2	**0**

Vertical GRF Toe off	DPN versus DMC	3	0.392 (-0.647, 1.430)	*P* =0.460	Q=14.1; *P* =0.001; I^2 = 85.8	**0**
	DPN versus HC	3	0.142 (-1.244, 1.529)	*P* =0.841	Q=22.2; *P* <0.001; I^2 = 91.0	**0**
EMG Tibialis anterior muscle	DPN versus HC	3	0.416 (-0.841, 1.674)	*P* =0.516	Q=19.1; *P* <0.001; I^2 = 89.6	**0**
MPP – rear foot	DPN versus HC	3	0.455 (0.090, 0.821)	*P* =0.015	Q=2.2; *P* =0.341; I^2 = 7.0	**2**
PTI – rear foot	DPN versus HC	3	0.407 (0.058, 0.756)	*P* =0.022	Q=0.115; *P* =0.944; I^2 = 0	**2**
MPP – mid foot	DPN versus HC	3	0.729 (0.373, 1.084)	*P* <0.001	Q=0.33; *P* =0.848; I^2 =0	**10**
PTI – mid foot	DPN versus HC	3	0.503 (0.152, 0.854)	*P* =0.005	Q=0.54; *P* =0.765; I^2 =0	**4**
MPP - forefoot	DPN versus DMC	3	0.510 (0.240, 0.780)	*P* <0.001	Q=2.23; *P* =0.329 I^2 =10.1	**10**
	DPN versus HC	3	0.552 (0.200, 0.903)	*P* =0.002	Q=1.8; *P* =0.415; I^2 =0	**4**
PTI -forefoot	DPN versus HC	3	0.666 (0.313, 1.020)	*P* <0.001	Q=0.22; *P* =0.894; I^2 =0	**8**

Legend: Meta-analyses of studies comparing Diabetic Neuropathy Subjects (DPN); Diabetes Mellitus controls (DMC) and Healthy Controls (HC). Effect size is standardised difference of mean values calculated as DPN – (control group); hence a positive result implies larger value for DPN and negative values reduced values for DPN. Results are from random effect models. Forest Plots for the significant results can be found in Appendix B (Appendix Figures 12-19).

2.6 Discussion

To the best of the authors' knowledge this is the first systematic review and meta-analysis of studies investigating the gait cycle, muscle activation and plantar pressure exclusively in people with DPN compared to DMCs and HCs. The aim of this review and meta-analysis was to assess the gait dissimilarities between the DPN, DMC and HC groups in relation to TSPs and kinetic, kinematic, EMG and plantar pressure variables.

Our findings, within the limitations of the review, indicate gait differences in people with DPN when compared with DMCs and HCs, likely resulting from sensory and motor neuropathy (Andersen, 2012; Kovac, Kovac, Marusic-Emedi, Svalina, & Demarin, 2011). The primary advantage of relating both HC and DMC groups to people with DPN was the ability to appreciate subtle differences within each group for comparison and contrast. However, it must be emphasized that there was a high level of heterogeneity for most variables between studies as highlighted by the Q and I^2 statistics. This high level of heterogeneity was also evident in other systematic reviewers investigating plantar pressures in similar patient groups (Crawford, Inkster, Kleijnen, & Fahey, 2007; Monteiro-Soares, Boyko, Ribeiro, Ribeiro, & Dinis-Ribeiro, 2012).

DPN is a significant complication of diabetes and accounts for significant morbidity and mortality (Boulton, 1998a; Cook & Simonson, 2012). The primary risk factor for DPN is hyperglycemia as it leads to increased oxidative stress, production of advanced glycation end products, increased polyol pathway flux and protein kinase C activation; all these contribute to micro-vascular disease and nerve dysfunction (Park, Park, & Baek, 2004). The end result of DPN can be catastrophic for patients, as this leads to foot ulceration and increased risk of limb amputation, significant healthcare costs, reduced quality of life and reduced mobility (Boulton, 2005; Price, 2004; Singh et al., 2005). Therefore, understanding the impact of DPN on the biomechanical aspects of human locomotion is clinically important (Formosa et al., 2013).

We hypothesised that TSPs would be significantly reduced in people with DPN compared to both controls. The majority of reported findings indicated that people with DPN walked slower and had reduced stride length when compared to both controls; however, meta-analysis results were statistically insignificant. The only significant finding was that people with DPN expended a longer period of time in the stance phase compared to DMCs. We hypothesised that the force generation at the hip, knee and ankle would be significantly increased for both flexion and extension moments in participants with DPN. There were insufficient studies to carry out meta-analysis and the two studies which reported findings demonstrated conflicting results. Regardless of the fact that one study utilised a significantly younger HC group compared to their DPN group, the differences between studies could not be solely explained by a

difference in the age groups of the HC group (Yavuzer et al., 2006). Irrespective of this, increased knee flexion moment in people with DPN was reported by both studies, emphasizing that greater force generation may occur during knee flexion in people with DPN. This alludes to the possibility that knee flexion might be an important compensation strategy due to DPN, as the motor component of DPN manifests in a stocking and glove distribution and affects the distal joints first (Tesfaye & Selvarajah, 2012).

The first maximum support moment (combination of extensor moments at hip, knee and ankle) (Winter, 1980) was higher in the DPN group when compared to DMC and HC groups (Savelberg et al., 2009a). Although reported in a single study, this reveals combined forces at the hip; knee and ankle during the stance phase are greater in people with DPN compared to both controls (Savelberg et al., 2009a). Further studies are needed to evaluate this finding.

Even though meta-analysis of the vertical GRF demonstrated that people with DPN had a higher initial contact force than DMCs and HCs, the level of heterogeneity in studies was high and the meta-analysis results statistically insignificant. While it was anticipated that people with DPN would exhibit higher GRF due to neurological deficit and reduced proprioception; the current findings fail to support this hypothesis. Similarly, we hypothesised that people with DPN would exhibit reductions in joint ROM at the hip, knee and ankle during gait, as a result of motor neuropathy (Andersen, 2012). There were few studies investigating lower limb kinematics of people with DPN during locomotion to support this hypothesis. One study (Sawacha et al., 2012a) reported kinematic variables of the foot which were outside the scope of this review and were not included. Therefore, current findings for joint angle kinematics were drawn from one publication investigating barefoot kinematics in people with DPN (Gomes et al., 2011). With the exception of hip flexion, the findings demonstrated reduced ROM in people with DPN compared to HCs, which was consistent with our hypothesis. A higher proportion of hip flexion is also another possible compensatory mechanism to increase stability in the gait pattern in DPN. Increased hip flexion could also be a compensatory mechanism to adjust for impaired ankle dorsiflexion in patients with DPN. We did not directly examine this possibility in the current review. Further

studies are needed to clarify the cause of greater joint force in knee flexion and greater degree of hip flexion in patients that have DPN.

Dynamic EMG data suggested that the tibialis anterior muscle remained active for a longer duration of time in people with DPN compared to HCs; however meta-analysis revealed that this finding was not statistically significant and demonstrated a high level of heterogeneity. Therefore, it was difficult to ascertain whether this was consistent with our hypothesis of altered muscle activity duration in people with DPN due to misfiring and reduction in neural pathways associated with muscle recruitment and deactivation. It was also challenging to explain the shorter duration of activity of the lateral gastrocnemius muscle and longer duration of activity of the vastus lateralis muscle and the various reported findings of other muscle groups from individual studies. It seems that there are clear differences in muscle activation between the DPN, DM and HC groups however the findings from previous studies were not consistent. It could be possible that these observations were due to changes in action potential amplitude and inconsistency in the number of motor units recruited during electromyography measurement of lower limb muscle activation in people with DPN; however, there is currently insufficient data to support this theory. As hypothesised, the meta-analysis results suggested that people with DPN have higher dynamic plantar pressure at rearfoot, midfoot and forefoot sites when compared to controls. However, there were insufficient studies to carry out a meta-analysis of data collected at the hallux and lesser toe joints and the results from studies were highly contradictory.

Previous reviews have highlighted gait differences in people with diabetes mellitus, but have not concentrated on people with DPN as the main focus (Allet et al., 2008; Wrobel & Najafi, 2010). The limitations of this review were the small number of included studies, the small number of participants in included studies, the high level of heterogeneity between studies, the investigation of barefoot measurements only, the exclusion of kinematic data of the foot and the language limitation to studies written in English.

We can conclude from the current level of evidence that the only biomechanical factors that seems significantly different in people with DPN were elevated plantar pressure and longer stance time, illustrated by a moderate effect sizes from standardised mean differences. Therefore, it is probable that elevated plantar pressure coupled with a

longer period of time spent in stance in people with DPN contributes to the susceptibility for skin damage through prolonged mechanical load on tissue, leading to skin break-down and ulceration (van Dieren et al., 2010). Although it is highly likely that reduced TSPs, elevated vertical GRF, longer muscle duration and reduced joint kinematics contribute to foot ulceration; the current knowledge base is insufficient to evaluate any statistical significance of differences in these variables. There were also significant discrepancies between studies reporting findings. These observations were similar to that of Allet et al and Wrobel and colleagues (Allet et al., 2008; Wrobel & Najafi, 2010).

While all studies in this review utilised procedures for diagnosing DPN in participants, only two studies excluded patients with PAD. PAD has been reported to have significant effects on walking pattern (Crowther, Spinks, Leicht, Quigley, & Golledge, 2007; Crowther et al., 2008). The BMI of all three groups were similar and it is unlikely that this accounted for any difference in gait variables. The mean diabetes duration between the DPN and DMC groups were not significantly different in the studies included. It has been hypothesised that DPN can manifest in people with a diabetes duration greater than 10 years, as it does in those with poor glycaemic control (Kovac et al., 2011; Oguejiofor, Odenigbo, & Oguejiofor, 2010; Valensi, Giroux, Seeboth-Ghalayini, & Attali, 1997). In addition, small foot muscle atrophy resulting from effects of hyperglycemia and small nerve damage has also been confirmed in diabetes patients utilising MRI, before DPN becomes clinically detectable (Wrobel, Mayfield, & Reiber, 2001). Therefore, these factors may also influence gait findings in people with DPN when compared to DMCs. This could be a possible explanation for the similar results in people with DPN vs. DMCs and a lack of statistical significance. However, the scope of this review was also dependent on the sample sizes of original studies, and thus, reported statistical insignificant differences may have been constrained by the small sample sizes.

There is paucity in biomechanical literature investigating the effects of DPN on barefoot gait characteristics, particularly in relationship to the effects of severe neuropathy resulting in foot lesions and its effect on human locomotion. The clinical ramifications from this systematic review and meta-analysis are limited due to the high level of heterogeneity and statistically insignificant results from the meta-analyses.

However, it was evident that people`` with DPN demonstrated greater overall dynamic plantar pressure and forefoot plantar pressure (both MPP and PTI) compared to controls without DPN and expend a longer duration during the stance phase. Both findings potentially contribute towards ulceration in people with DPN. Other biomechanical findings were less clear and we therefore encourage future biomechanical studies in people with DPN to assess factors such as lower limb angular kinematics, kinetics and EMG and to adjust for variables such as PAD, claudication pain and history of foot ulcers in selection of participants with DPN as these factors are highly likely to influence walking patterns.

2.7 Conclusion

Current evidence from the literature indicates that people with DPN exhibit significantly elevated plantar pressures and occupy a longer duration of time in stance-phase during gait compared to healthy and diabetes controls. We encourage future biomechanical studies in people with DPN to assess factors such as lower limb angular kinematics, kinetics and EMG.

Chapter. 3 Meta-Analysis of Plantar Pressure Measurements in People With Diabetic Peripheral Neuropathy With Active Foot Ulceration, Previous Ulceration And No History of Ulceration

This chapter has been adapted from a publication titled;

Plantar pressure in diabetic peripheral neuropathy patients with active foot ulceration, previous ulceration and no history of ulceration: a meta-analysis of observational studies.

Authors: Fernando ME, Crowther RG, Pappas E, Lazzarini PA, Cunningham M, Sangla KS, Buttner P, Golledge J.

PLoS One. 2014 Jun 10;9(6):e99050.

3.1 Preface

This chapter forms the second part of the literature review component of this thesis and is a meta-analysis of observational studies designed to address question 2 from Chapter 1. The study contained in this chapter assessed whether a difference in plantar pressures exist between people with DPN with a history of DFUs (past or present) when compared to controls with DPN without a history of DFUs in meta-analyses using pooled data from a number of previous observational studies. The importance of this study was to determine whether a plantar pressure difference is present in people with active DFUs and in people with a history of DFUs to assist in the planning of the prospective studies reported in Chapters 6-8 of this thesis.

3.2 Abstract

Background: Elevated dynamic plantar pressures are a consistent finding in diabetes patients with peripheral neuropathy with implications for plantar foot ulceration. This meta-analysis aimed to compare the plantar pressures of diabetes patients that had peripheral neuropathy and people with neuropathy with active or previous foot ulcers.

Methods: Published articles were identified from Medline via OVID, CINAHL, SCOPUS, INFORMIT, Cochrane Central EMBASE via OVID and Web of Science via ISI Web of Knowledge bibliographic databases. Observational studies reporting barefoot dynamic plantar pressure in adults with DPN, where at least one group had a history of plantar DFUs were included. Interventional studies, shod plantar pressure studies and studies not published in English were excluded. Overall mean peak plantar pressure (MPP) and pressure time integral (PTI) were primary outcomes. The six secondary outcomes were MPP and PTI at the rear foot, mid foot and fore foot. The protocol of the meta-analysis was published with PROPSERO, (registration number CRD42013004310).

Results: Eight observational studies were included. Overall MPP and PTI were greater in people with DPN with foot ulceration compared to people without ulceration (standardised mean difference 0.551, 95% CI 0.290-0.811, $p<0.001$; and 0.762, 95% CI 0.303-1.221, $p = 0.001$, respectively). Sub-group analyses demonstrated no significant

difference in MPP for people with DPN with active ulceration compared to controls without ulcers. A significant difference in MPP was found for people with neuropathy with a history of ulceration compared to controls without ulcers; (0.467, 95% CI 0.181-0.753, p = 0.001). Statistical heterogeneity between studies was moderate.

Conclusions: Plantar pressures appear to be significantly higher in patients with DPN with a history of foot ulceration compared to people with DPN without a history of ulceration. More homogenous data is needed to confirm these findings.

3.3 Introduction

Raised dynamic plantar pressures are a frequent finding in diabetes patients with DPN (Cavanagh, Ulbrecht, & Caputo, 1996; Savelberg et al., 2009a; Veves, Murray, Young, & Boulton, 1992). Two measures of vertical plantar pressure are most commonly assessed. Mean peak plantar pressure (MPP) represents the maximum amount of pressure during stance and the pressure time integral (PTI) represents the amount of time over which maximum pressure is applied and is measured as the area under the pressure over time graph (Melai et al., 2011). A recent meta-analysis of observational studies by our team demonstrated significantly higher PTI and MPP in DPN patients when compared to HCs and DMCs who did not have neuropathy (Fernando et al., 2013).

There has been extensive interest regarding the role of plantar pressures and pressure offloading in foot ulceration and the ability to determine a plantar pressure cut-off that predicts ulceration (Armstrong, Peters, Athanasiou, & Lavery, 1998c; Bus, Waaijman, Arts, & Manning, 2009; Chevalier, Hodgins, & Chockalingam, 2010; Coles, 2008; Crawford et al., 2007; Lavery et al., 1998; Lavery et al., 2003; Paton, Bruce, Jones, & Stenhouse, 2011; van Deursen, 2008; Viswanathan et al., 2004). Several observational studies have investigated the feasibility of using plantar pressure in identifying people at risk of ulceration, but the reported sensitivities and specificities are below those which are typically accepted for a diagnostic test (Armstrong et al., 1998c; Crawford et al., 2007; Lavery et al., 2003). Nevertheless, if patients with warning signs of impending foot ulceration could be identified using plantar pressures, alongside other

confirmed risk factors, it is possible that clinical management could be improved to avoid DFU development.

Prior to assessing plantar pressure as a screening tool for ulceration, it would appear necessary to determine whether plantar pressures are actually significantly higher in patients with DPN with previous and/or present diabetes-related foot ulceration (PPDFU) compared to patients with DPN without a history of DFUs. The relatively low specificity and sensitivity values obtained in the aforementioned studies raises the question as to whether there is an increase in plantar pressure prior to the onset of ulceration and following ulceration in people with DPN. To the best of the authors' knowledge a meta-analysis to examine these questions has not been previously published.

The primary aim of this meta-analysis was to compare plantar pressures in patients with PPDFU (cases) and individuals with DPN without a history of ulceration (controls). The secondary aims were to assess the quality of studies investigating plantar pressure and to investigate plantar pressure in patients with active and past ulcers.

3.4 Methods

3.4.1 Search strategy and quality assessment

A comprehensive search strategy was utilised, involving MeSH and Emtree terms and relevant keywords for search strings (see Figure 3.1). The databases searched were Medline via OVID (1946 to present), CINAHL (1994-2012), SCOPUS (All years to present), INFORMIT, Cochrane Central (Latest), EMBASE via OVID (1980 to present) and Web of science via ISI Web of Knowledge (1965 to present). One author (MF) and a librarian carried out the searches (using the same search string) independently on two separate occasions in November 2012 to identify all relevant studies published until 18th November 2012. Furthermore, a repeated search was conducted in February 2014 to identify recent studies of interest for inclusion. No new articles of relevance were found after the latter search.

1.Diabetes (exp Diabetes Mellitus, Type 2/ or exp Diabetes Mellitus/ or exp Diabetes Mellitus, Type 1/)
2.Diabetes Neuropathy- Mesh (diabetes autonomic neuropathie) or (neuropathies diabetes autonomic) or (mononeuropathy diabetes) or (diabetes mononeuropathy simplices) or (diabetes neuralgias) or (diabetes autonomic neuropathy) or (autonomic neuropathy diabetes) or(diabetes asymmetric polyneuropathies) or (diabetes polyneuropathy) or (simplex diabetes mononeuropathy) or (mononeuropathy simplices diabetes) or (neuropathies diabetes) or (neuralgia diabetes) or (asymmetric polyneuropathy diabetes) or (diabetes polyneuropathies) or (diabetes mononeuropathies) or (diabetes mononeuropathy) or (autonomic neuropathies diabetes) or (neuropathy diabetes) or (mononeuropathies diabetes) or (polyneuropathy diabetes asymmetric) or (diabetes asymmetric polyneuropathy) or (diabetes mononeuropathy simplex) or (diabetes neuropathy) or (polyneuropathy diabetes) or (diabetes neuropathies) or (asymmetric diabetes proximal motor neuropathy) or (asymmetric polyneuropathies diabetes) or (neuropathy diabetes autonomic) or (painful diabetes neuropathy) or (polyneuropathies diabetes).
3.Diabetes foot- Mesh (diabetes feet) or (diabetes foot) or (feet diabetes) or(foot diabetes) or (foot ulcer diabetes)
4.Foot ulcer- Mesh (foot ulcer) or (foot ulcer) or (ulcer foot) or (plantar ulcer) or (ulcers foot) or (ulcers plantar)or(ulcer plantar)or (plantar ulcers)
5.Plantar pressure
6.Weight-bearing – Mesh (load bearing) or (load bearing) or (weight bearing) or (weight-bearing) or (load-bearing) or (weight bearing)
7.Peak plantar pressure
8.Pressure time integral
9.Force time integral
10.Combine 1 AND 2 AND 3 AND 4
11.Combine 5 AND 6 AND 7 AND 8 AND 9
12.Combine 10 AND 11

Figure 3.1 Search terms.

It has been identified that a single candidate tool for the quality assessment of observational studies does not exist (Sanderson, Tatt, & Higgins, 2007). Assessment of risk of bias was conducted using a quality assessment tool adapted from validated instruments (Pedro and CASP) with the addition of content specific questions concerning plantar pressure and foot ulceration (Critical Appraisal Skills Programme, 2013, 2013 ; Maher, Sherrington, Herbert, Moseley, & Elkins, 2003). Risk of bias was assessed by two blinded authors (PAL and EP) who were given study manuscripts after the removal of author, institutional, title and re-identifiable information. The quality scores were then checked by one author (MEF) for consistency. Where major differences in quality scores existed, these were discussed amongst a second group of authors (MF, MC and PB) and the original blinded quality assessors were asked to independently review any major differences in ratings. The quality assessment tool was trialed by the two assessors prior to use in the meta-analysis. As an adapted quality assessment tool was used a total score of 50 was possible for the 25 questions. Quality scores of ≥45, 30-45, 20-30 and ≤ 20 were defined as excellent, good, fair and poor

respectively (Critical Appraisal Skills Programme, 2013, 2013 ; Maher, Sherrington, Herbert, Moseley, & Elkins, 2003).

3.4.2 Study selection

Studies were included in the meta-analysis if they met all the below inclusion criteria:

- An observational study;
- Subjects included were adults aged 18 years and over;
- The study was reported in or available in the English language;
- The study used a validated method of diagnosing DPN, including one or more of the following methods; a screening questionnaire to assess for DPN; 10g monofilament perception testing; vibration perception threshold; and nerve conduction studies;
- Plantar pressures were reported in two groups of subjects with documented DPN in which at least one group had a previous or active plantar neuropathic foot ulcer;
- Barefoot dynamic plantar pressure during walking was reported without the influence of an offloading intervention or footwear.
- Plantar pressure values were reported as either MPP and/or PTI in any acceptable pressure unit (KPa, N/cm^2 or similar);
- Overall, fore foot, mid foot and/or rear foot MPP or PTI were reported;

Studies were excluded if meeting any of the below criteria:

- The study was an interventional study. The authors considered interventional studies utilising offloading options (such as foot wear, insoles and orthoses) and or/ treatment options (such as podiatry treatment, debridement of callus) as well as the assessment of plantar pressure could potentially alter the natural gait cycle and plantar pressures, and thus were excluded;
- The study did not document DPN status or where all the participants in a single group did not have documented DPN;
- Data could not be extracted for the two groups of interest or computed, or where authors were unable to provide data for the two groups of interest from a larger dataset when requested;

- Only in shoe plantar pressure were reported, as these were considered interventional assessments and the combination of shod and barefoot findings can drastically increase the variability of plantar pressure results (Chevalier et al., 2010) or
- Full text manuscripts could not be acquired.

The two primary outcomes of this study were overall MPP and overall mean PTI. The six secondary outcomes were MPP at the rear foot, MPP at the mid foot, MPP at the fore foot, PTI at the rear foot, PTI at the mid foot and PTI at the fore foot.

Potential studies identified for inclusion were reviewed independently by 3 authors (MF, RC and MC) using the above inclusion criteria. Where there was disagreement in the inclusion of studies, group discussions were held to resolve any differences in opinion. All reference lists of studies meeting the inclusion criteria were browsed for any additional studies for inclusion. Authors of the included studies were likewise contacted via email for identification of other potential studies for inclusion. No further studies of relevance were found.

3.4.3 Data extraction and synthesis

Data extraction was completed by the primary author, using specifically developed data extraction forms. The forms were then checked by 2 authors (EP and PL) for any omissions. Descriptive data, such as age, gender, body mass index (BMI), and disease specific data, such as Diabetes mellitus (DM) duration, type of DM, HbA1c, degree of neuropathy, presence of PAD, and presence of foot deformity were extracted from each study for comparison. Numerical data (mean and standard deviation (SD), for each plantar pressure variable (MPP and/or PTI) were also extracted and included in the analysis. Where anatomical locations were unspecified, or when overall MPP was the only variable reported, authors were contacted for plantar pressure data specific to different plantar locations.

The MOOSE reporting guidelines for meta-analysis of observational studies was used in the synthesis of this manuscript (see Appendix C, Appendix Table 1) (Stroup et al., 2000). The protocol of the meta-analysis was registered and published with PROSPERO database of systematic reviews and meta-analyses prior to completing data extraction (registration number CRD42013004310). Meta-analysis was attempted

where more than three studies reported a given outcome measure. Random effects models were used to analyse the studies based on the prospect there would be between study heterogeneity present.

3.4.4 Statistical methods

Standardised mean differences were used in the computation of meta-analyses of plantar pressure differences utilising Cohen's d (Cohen, 1988). Results were reported as standardised mean differences with 95% confidence intervals (95%-CI) and p-values. Where SD was not reported, standard error (SE), inter-quartile range (IQR) or equivalent was converted to SD values. Since both Boulton et al. and Cavanagh and colleagues (Boulton et al., 1983; Cavanagh, Sims, & Sanders, 1991) did not report SD for the distribution of plantar pressure and were unable to provide information about SD when contacted, these were estimated using linear regression of SD on mean values for MPP. However, as the highest reported aggregate plantar pressure was 83.1 N/cm^2, these estimations are approximations only and sensitivity analyses were conducted excluding these studies. As Stess et al. reported SE (Stess, Jensen, & Mirmiran, 1997), SD was calculated using the formula $SE=SD/\sqrt{n}$. Similarly, as Sauseng et al. reported IQR (Sauseng, Kästenbauer, Sokol, & Irsigler, 1999), SD was estimated using the IQR value and PASS statistical software (NCSS LLC; Kaysville Utah).

Weighted means (according to the sample size of the studies) were calculated for the reported demographic variables. A Cohen's d score of zero was interpreted as no difference in effect; a result of 0 to 0.2 was interpreted as a small effect, 0.2-0.8 as a moderate effect and ≥ 0.8 as a large effect (McGough & Faraone, 2009). All statistical analyses were carried out by an experienced statistician (PB) using the software package Comprehensive Meta-Analysis (www.Meta-Analysis.com, USA).

In order to obtain any missing data or to clarify any discrepancies in data, several attempts were made to contact the corresponding authors by email, using open-ended questions. Where multiple studies were published from the same data set, the study with the most information reported in relation to the outcome measures of interest was used. Where more than one publication reported data from a single study, the publication with the largest data set was used. The primary unit of analysis for the meta-analysis was the patient. Where the unit of analysis were feet instead of patients,

caution was taken in the interpretation of the results and authors were contacted for clarification. Rich et al. reported findings as number of feet instead of number of patients and the authors were unable to provide information specific to the patients (Rich & Veves, 2000).

The Q and I^2 statistics were used to assess statistical heterogeneity between studies. I^2 values of 25%, 50% and 75% were acknowledged as low, moderate and high heterogeneity respectively. In addition to this, the classic fail safe (N) was also computed; as this gives an estimation of studies needed to be published with a null effect to renounce the findings from the meta-analysis (Persaud, 1996). Sub-group analyses and sensitivity analyses were performed to examine the effect of including and excluding several studies. Sensitivity and sub-group analyses comprised of: Analyses including only active foot ulcer patients from the PPDFU group; analyses including only previous foot ulcer patients from the PPDFU group; analyses excluding the two studies for which SD was estimated (Boulton et al., 1983; Cavanagh et al., 1991); analyses excluding Rich et al. due to the difference in unit of analysis (Rich & Veves, 2000); analyses with exclusion of Stess et al. due to the high level of statistical heterogeneity and the inclusion of amputees in the study (Stess et al., 1997); and separate analyses with the exclusion of all three studies listed above.

3.5 Results

3.5.1 Search Results

The systematic search strategy resulted in the identification of 2,730 citations. Figure 3.2 demonstrates a flow diagram representing the inclusion and exclusion of studies. The search strategy identified six studies for inclusion in the meta-analysis. A further two studies were identified and included after browsing through the reference lists of the included articles, resulting in a total of eight observational studies (Armstrong et al., 1998c; Bacarin et al., 2009; Boulton et al., 1983; Brash, Foster, Vennart, Daw, & Tooke, 1996; Cavanagh et al., 1991; Rich & Veves, 2000; Sauseng et al., 1999; Stess et al., 1997).

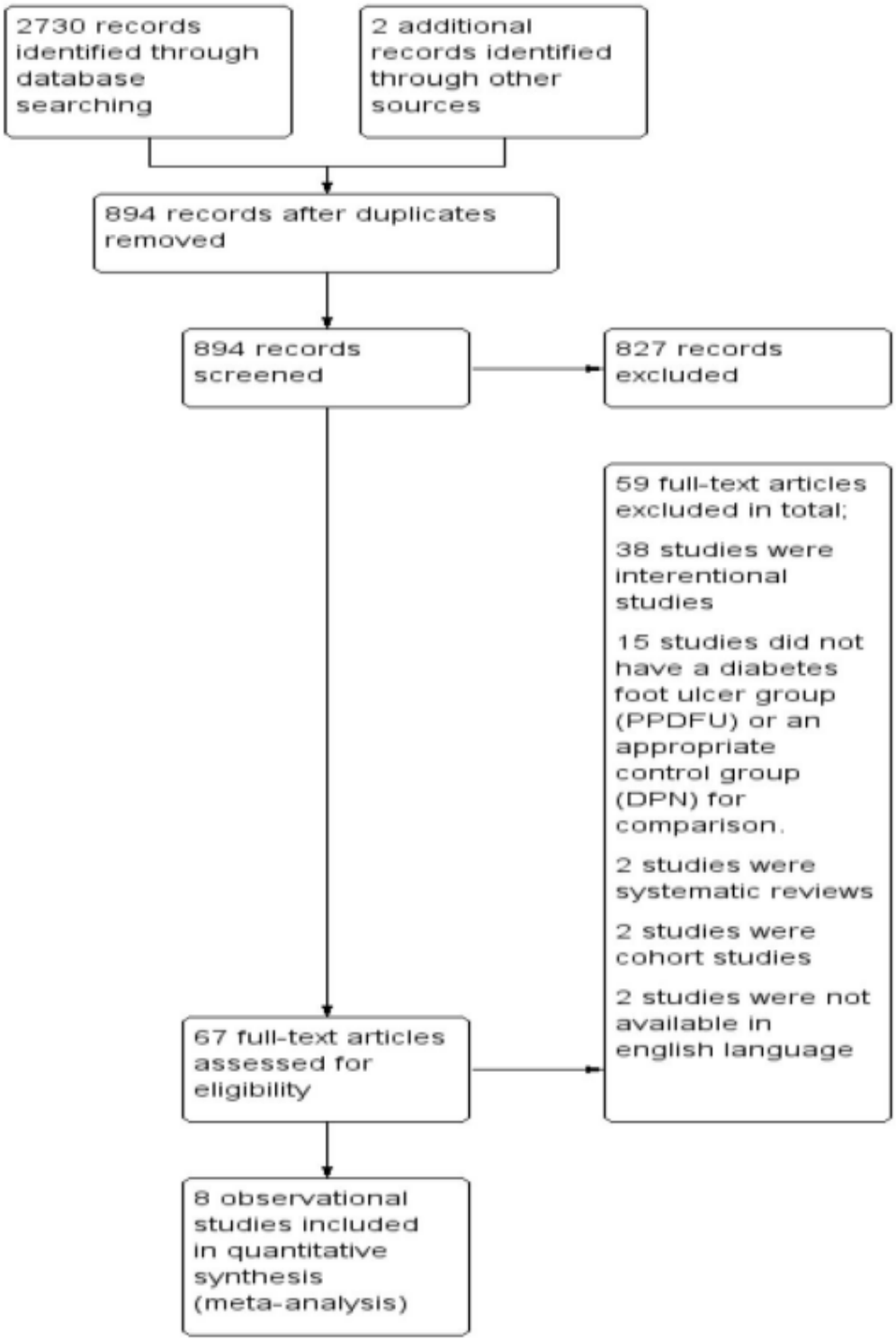


Figure 3.2 Search results from the meta-analysis.

3.5.2 Description of studies

A comprehensive list of study characteristics can be found in Table 3.1. In total, there were 647 diabetes participants from all eight studies. This included 238 PPDFU

patients. The mean sample size of the PPDFU groups was 29.7 and ranged from 9 to 70 patients. The age range of the PPDFU patients was 52.3 to 62.4 years with a weighted mean age of 56.8 years. The majority of patients (77.3%) were men. The BMI of this group ranged from 27 Kg/m^2 to 30.9 Kg/m^2 (weighted mean 30.2 Kg/m^2). The weighted mean duration of DM was 16.4 years with a range of 14.3 to 22.7 years. Sixty six percent of patients had active foot ulcers at the time of data collection and the remaining 34% were patients with a history of foot ulcers. The time period for which ulceration occurred varied between studies and most studies failed to report the time period which had elapsed since the last occurrence of ulceration in people with a history of ulcers. The monofilament perception threshold (MPT) was only reported by one study and was 2.89 (Cavanagh et al., 1991). The mean vibration perception threshold (VPT) in the PPDFU group was 37.6 V with a range of 33.5 to 40 V. Mean HbA1c was only reported by one study and was 8.8% or 73 mmol/mol (Sauseng et al., 1999). Ankle brachial pressure index (ABPI) was reported by three studies and the weighted mean ABPI was 1.06 with a range of 0.96 to 1.44 (Armstrong et al., 1998c; Boulton et al., 1983; Stess et al., 1997).

The group of DPN patients without a history of foot ulcers consisted of 409 subjects. The mean sample size was 51.1 with a range of 9 to 149 patients. Overall the weighted mean age of this group was 54.7 years with a range of 49.9 to 66 years. The majority of subjects (49.7%) were men and the BMI range was 26.1 to 32.3 Kg/m^2 (weighted mean 31.0 Kg/m^2). The weighted mean DM duration was 12.0 years with a range of 9.2 to 22.1 years. The mean MPT value reported in one study was 1.98 (Cavanagh et al., 1991). The weighted mean VPT value was 32.4 V with a range of 28 to 44 V. The mean HbA1c, reported by one study was 8.0%, or 64 mmol/mol (Sauseng & Kästenbauer, 1999). The mean ABPI of this group was 1.04 with a range of 0.99 to 1.30.

The method of diagnosing DPN varied in different studies. One study used monofilament testing alone at six plantar locations to identify DPN (Stess et al., 1997). One study used neuropathic symptoms and an absent ankle reflex for the diagnoses of DPN (Boulton et al., 1983), however this was an acceptable means of diagnosing DPN at the time of the study. A few studies utilised neuropathy questionnaire(s) for the assessments of DPN, in addition to the VPT (Bacarin et al., 2009; Brash et al., 1996;

Rich & Veves, 2000). Only one study commented on the presence/absence of plantar callus on the feet of participants. Brash et al. identified the locations of plantar callus and found no significant difference in callus between the two groups studied (Brash et al., 1996).

Table 3.1 Characteristics of participants in included studies.

Study and (N)			Cases (PPDFU)										Controls (DPN)								
Study	N	DM Type	PPDFU Cases (n=)	Age (Yr.)	% Men	BMI (Kg/m^2)	Diabetes Duration (years)	% Active Ulcer	MPT	VPT (V)	HbA1c (%)	ABPI	DPN Control (n=)	Age	% Men	BMI (Kg/m^2)	Diabetes Duration (years)	MPT	VPT	HbA1c (%)	ABPI
Bacarin 2009	**27**	T1, T2	**10**	58.2 (6.7)	80	27 (5.5)	17.5 (9.3)	0	-	-	-	-	**17**	54.7 (7.8)	47	26.1 (4.6)	13.4 (8.2)	-	-	-	-
Cavanagh 1991	**56**	T1, T2	**14**	62.4	100	-	16.9 (9.2) [a]	100	2.89	39.3	-	-	**42**	57.8	100	-	16.9 (9.2) [a]	1.98	28.0	-	-
Sauseng 1999	**43**	T1, T2	**20**	57.2	-	28.9 (4.3)	19.4 (8.5)	100	-	40 (14.0)	8.8 (1.8)		**23**	65.8 (7.0)	-	28.9 (4.6)	14.0 (7.5)	-	44.0 (6.0)	8.0 (1.2)	
Armstrong 1998	**219**	-	**70**	52.3 (10.3)	74	30.9 (5.7)	14.3 (9.2)	-	-	-	-	0.96 (0.17)	**149**	51.8 (10.4)	33	32.3 (6.2)	9.2 (8.8)	-	-	-	0.99 (0.16)
Boulton 1983	**41**	-	**13**	57.4 (39.0)[a]	76	-	15.2 (15.0) [a]	0	-	35 (12.0)	-	1.44 (0.41)	**28**	49.9 [a] (18.1)	65	-	11.9 (21.1)[a]	-	30.0 (11.0)	-	1.30 (0.27)
Rich 2000[b]	**180**	-	**53**	-	-	-	-	-	-	-	-		**127**	-	-	-	-	-	-	-	
Brash 1996[d]	**18**	-	**9**	52.7 (12.3)	-	-	22.7 (11.1)	100	-	33.5 (4.2)	-	-	**9**	56.5 (9.6)	-	-	22.1 (11.5)	-	31.0 (6.8)	-	-
Stess 1997[c]	**63**	Majority T2	**49**	61.7 (12.4)	-	30.4 (2.7)	16.8	67	-	-	-	1.1 (0.3)	**14**	66.0 (8.9)	-	26.2 (1.1)	15.1	-	-	-	1.1 (0.2)
Total & Weighted averages			**238**	56.8; 52.3 – 62.4	77.3; 67 - 100	30.2; 27.0 – 30.9	16.4; 14.3 – 22.7	66; 0 - 100	2.89	37.6; 33.5 – 40.0	8.8	1.06; 0.96– 1.44	**409**	54.7; 49.9 – 66.0	49.7; 33 - 100	31.0; 26.1 – 32.3	12.0; 9.2 – 22.1	1.98	32.4; 28.0 – 44.0	8.0	1.04; 0.99– 1.30

Legend: Mean and standard deviation (SD) for PPDFU- Past present plantar diabetic foot ulcer (case), DPN- Diabetic peripheral neuropathy (control) groups. Weighted means (weighted by sample size) and ranges provided in final row. MPT is the monofilament perception threshold and VPT is the vibration perception threshold (both used in the diagnosis of DPN), ABPI represents ankle brachial pressure index values. DM Type is diabetes mellitus type (type 1=T1 and type 2=T2). [a] These variables were not described with SD but reported ranges, the difference between the maximum and mean (maximum minus the mean) are reported instead of SD. [b] This study reported findings as number of feet instead of patients. [c] 26% of PPDFU group had digital or metatarsal amputations. Body Mass Index (BMI) was calculated from data provided in non-metric format. MPT= monofilament perception threshold, VPT= vibration perception threshold and ABPI= ankle brachial pressure index. [d] This study used an active ulcer group (33%) for PPDFU but utilised the non-ulcerated foot for measurement of plantar pressure.

3.5.3 Risk of bias in included studies

The overall agreement between the two quality assessors was good; with the range of variation of scores between zero to three points (Table 3.2). In general, all studies used an appropriate study design, accounted for potential confounders and reported data for at least 85% of the participants for a primary outcome measure. However, four studies failed to discuss the main sources of bias within the study (Boulton et al., 1983; Brash et al., 1996; Rich & Veves, 2000; Stess et al., 1997) and two studies did not identify the presence of PAD or exclude people with PAD (Bacarin et al., 2009; Cavanagh et al., 1991). There was not an overall noteworthy difference in the methodological quality of studies. The highest score for the method and participant specific questions were given to two studies which addressed issues such as number of steps used in measurements, number of walking trials and the measurement of factors which potentially affected plantar pressure, such as foot structure (Bacarin et al., 2009; Rich & Veves, 2000).

Table 3.2 Assessment of methodological quality of studies.

	Study	Bacarin et al. 2009		Brash et al. 1996		Armstrong et al. 1998		Stess et al. 1997		Rich et al. 2000		Sauseng et al. 1999		Cavanagh et al. 1991		Boulton et al. 1983	
Q No.	**Question**	**EP**	**PL**	**EP**	**PL**	**EP**	**PL**	**EP**	**PL**	**EP**	**PL**	**EP**	**PL**	**EP**	**PL**	**EP**	**PL**
1	Clear Aim/Purpose	Y	Y	Y	Y	Y	Y	P	Y	P	Y	Y	Y	Y	Y	P	Y
2	Suitable Design was used?	P	P	Y	P	P	P	Y	P	Y	Y	P	P	Y	P	Y	P
3	Efficient recruitment strategy used?	Y	P	Y	P	Y	P	Y	Y	P	P	Y	P	Y	P	Y	P
4	Suitable exclusion and inclusion criteria?	Y	P	Y	P	P	P	P	P	P	P	P	Y	Y	P	Y	P
5	Data reported for at least 85% on a key outcome?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	P
6	Discussed main sources of bias?	Y	Y	N	N	Y	P	N	N	N	N	Y	P	Y	Y	N	N
7	Were the methods justified?	Y	Y	P	Y	P	Y	Y	P	Y	Y	N	N	Y	Y	Y	Y
8	Was a power calculation for sample conducted?	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9	Were Confounders identified?	Y	Y	P	Y	Y	Y	P	P	Y	Y	Y	Y	P	Y	P	Y
10	Confounders accounted for in analysis?	Y	Y	P	P	Y	Y	Y	P	Y	Y	Y	Y	Y	Y	P	Y
11	Between group results reported for at least one key outcome?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	P
12	Compared findings to literature?	Y	Y	Y	Y	Y	P	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
13	Findings relevant to aims?	Y	Y	Y	P	P	P	Y	P	Y	Y	Y	Y	Y	Y	P	P
14	Results statistically appropriate?	Y	Y	N	N	P	Y	P	Y	Y	Y	P	Y	N	N	N	N
15	Are findings applicable to a clinical scenario?	P	Y	Y	P	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	P
16[a]	Assessed degree of neuropathy of participants?	Y	Y	Y	Y	P	P	P	P	Y	Y	Y	Y	Y	Y	Y	Y
17[a]	PAD* identified in participants or excluded?	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y
18[a]	Diabetes duration and type of diabetes identified?	Y	Y	P	P	Y	P	P	P	P	Y	Y	Y	Y	Y	Y	Y
19[a]	Level of glycaemic control of participants was reported (i.e. hbA1c)?	Y	Y	N	N	N	N	N	N	N	N	Y	Y	N	N	N	N
20[a]	Foot structure reported?	P	P	N	N	N	N	N	N	P	P	P	P	P	P	N	N
21[a]	Absence/ presence of current/ past foot ulceration identified?	Y	Y	Y	Y	Y	Y	Y	Y	Y	P	Y	P	Y	P	Y	Y

22 [a]	Does the study specify methods of plantar pressure capture and analysis?	P	P	P	P	P	P	P	P	P	P	Y	P	Y	P	P	P
23 [a]	Does the study state the number of steps used in capture?	Y	Y	Y	Y	Y	Y	P	Y	Y	Y	Y	Y	Y	Y	N	N
24 [a]	Study specifies any verbal instructions given to participants?	Y	Y	N	N	N	N	N	N	Y	Y	N	N	N	N	N	N
25 [a]	Number of walking trials reported?	P	Y	N	N	N	N	Y	Y	Y	Y	N	N	P	Y	N	N
Total	Total independent quality scores	41	41	31	28	33	31	32	31	38	39	38	36	37	34	29	26
Mean	Mean Scores	41 (15)		29 (10)		32 (9)		31 (10)		38 (15)		37 (14)		35 (11)		27 (9)	

Legend: Methodological quality of studies as assessed independently by (EP) and (PL) using a modified quality assessment tool (Fernando et al., 2014b). For the scoring system, 1= (P) partially, 0 = (N) no and 2= (Y) yes. The Total score was out of a possible 50. Mean scores were the average of the two individual scores rounded down to the nearest integer. The mean scores in (brackets) indicate mean scores for the assessment of participant and plantar pressure related methodology out of a total of 22 (Q16-Q25).

*PAD= Peripheral Arterial Disease

[a] These were the subject relevant questions (regarding participant specific characteristics and methods of plantar pressure measurement) that were assessed as suitable by a panel of experts and were added to the quality assessment tool;

- The identification and quantification of DPN-How was neuropathy diagnosed and quantified?
- The identification or exclusion of PAD in participants- Was PAD accounted for appropriately?
- The identification of type of diabetes and diabetes duration in participants- These are important considerations in diabetes foot ulcer pathogenesis.
- Whether the glycaemic control of the participants was reported- This gives guidance as to the level of glycemic control of participants
- Whether the foot structure of participants was reported- An important consideration in the assessment of plantar pressure.
- Whether a history of diabetes foot ulceration or current diabetes foot ulceration was checked in all participants?
- Whether the methods pertaining to plantar pressure capture were reported; this included the general methods, number of steps, verbal instructions and number of walking trials- This was to identify the feasibility for reproducibility of the study using appropriate methods.

3.5.4 Primary outcome measures

3.5.4.1 Overall MPP

Overall MPP was reported by all eight studies. Appendix C, Appendix Table 2 illustrates the reported plantar pressures according to plantar anatomical locations as well as the overall plantar pressures from each study. Meta-analysis combining data from eight studies (PPDFU n= 238; DPN with no foot ulcer history n=409) suggested that patients with PPDFU had greater overall MPP with moderate effect levels (standardised mean difference 0.551, 95% CI 0.290-0.811; p≤0.001). The heterogeneity between studies was moderate I^2= 46.2 (see Figure 3.3 and Table 3.3).

3.5.4.2 Overall PTI

Three studies reported PTI values (see Appendix C, Appendix Table 2). Meta-analysis combining data from all three studies (PPDFU n= 79; DPN with no foot ulcer history n=54) suggested that patients with PPDFU had greater overall PTI with moderate effect levels (standardised mean difference 0.762, 95% CI 0.303-1.221; p= 0.001) (see Figure 3.4 and Table 3.3). The heterogeneity between studies was moderate I^2= 28.4. As only three studies were found, sub-group and sensitivity-analyses were not attempted.

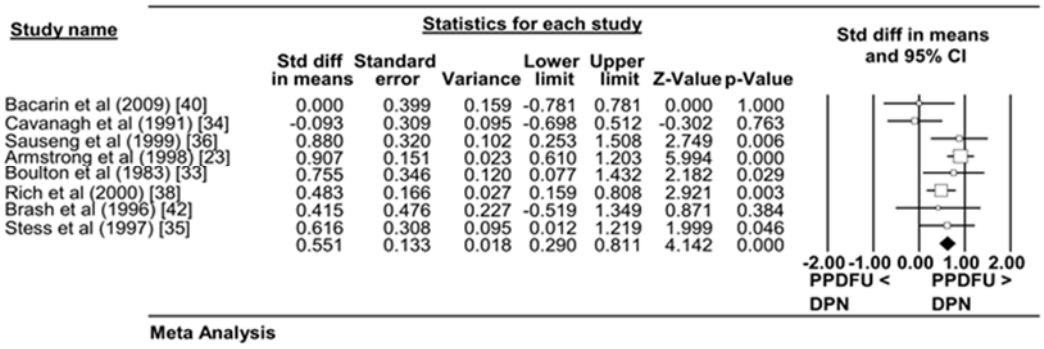


Figure 3.3 Forest Plot. Forest Plot displaying the Overall Peak Plantar Pressure (MPP) between the PPDFU group (cases) and the DPN group (control).

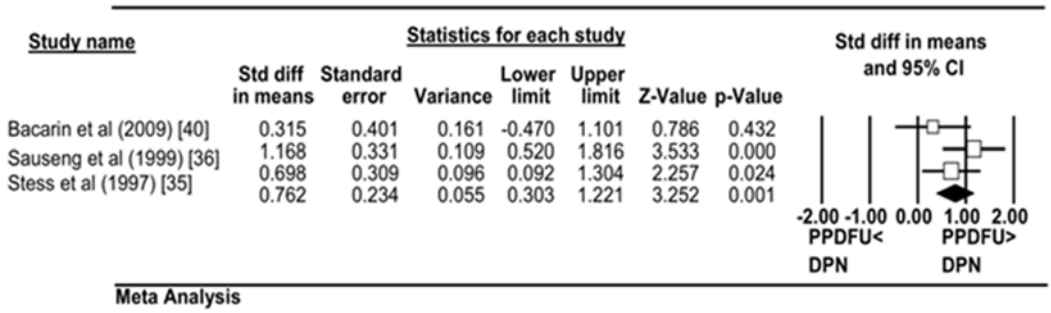


Figure 3.4 Forest Plot displaying the Overall Pressure Time Integral (PTI) between the PPDFU group(cases) and the DPN group(control).

Table 3.3 Meta analyses results.

Outcome measure	Comparison	Number of studies	Effect size (95%-CI)	p-value	Heterogeneity assessment	Classic fail-safe N
Overall Peak Plantar Pressure MPP [N/cm^2]	PPDFU (n=238) versus DPN (n=409)	8[d]	0.551 (0.290, 0.811)	p<0.001	Q=13.0; p=0.072; I^2 = 46.2	63
	PPDFU (n=211) versus DPN (n=339)	6[d2]	0.635 (0.387, 0.884)	p<0.001	Q=7.3; p=0.200; I^2 = 31.4	49
	PPDFU (n=185) versus DPN (n=282)	7[a]	0.553 (0.229, 0.876)	p=0.001	Q=12.2; p=0.058; I^2 = 50.76	41
	PPDFU (n=43) versus DPN (n=74)	3[c]	0.394 (-0.237, 1.026)	p=0.221	Q=4.8; p=0.091; I^2 = 58.3	/
	PPDFU (n=189) versus DPN (n=395)	7[e]	0.534 (0.235, 0.832)	p<0.001	Q=13.0; p=0.043; I^2 =53.9	48
	PPDFU (n=136) versus DPN (n=268);	6[f1]	0.528 (0.143, 0.914)	p=0.007	Q=12.1; p=0.033; I^2 = 58.9	29
	PPDFU (n=76) versus DPN (n=172)	3[g]	0.467 (0.181, 0.753)	p=0.001	Q=2.1; P=0.354; I2 = 3.6	4
Overall Peak Plantar Pressure PTI	PPDFU (n=79) versus DPN (n=54)	3[b]	0.762 (0.303, 1.221)	p=0.001	Q=2.8; p=0.248; I^2 = 28.4	9
Forefoot MPP [N/cm^2]	PPDFU (n=211) versus DPN (n=339)	6	0.635 (0.387, 0.884)	p<0.001	Q=7.3; p=0.200; I^2 = 31.4	49
	PPDFU (n=158) versus DPN (n=212)	5[a]	0.692 (0.392, 0.992)	p<0.001	Q=5.5; p=0.243; I^2 = 26.7	31

	PPDFU (n=162) versus DPN (n=325)	5[e]	0.625 (0.323, 0.927)	p<0.001	Q=7.3; p=0.123; I^2 = 44.9	36
	PPDFU (n=109) versus DPN (n=198)	4[f2]	0.670 (0.273, 1.066)	p=0.001	Q=5.2; p=0.157; I^2 = 42.5	21
Forefoot PTI [Ns/cm^2]	PPDFU (n=79) versus DPN (n=54)	3[b]	0.719 (0.197, 1.242)	p=0.007	Q=3.6; p=0.165; I^2 = 44.4	8

Legend: Random effects model meta-analyses. Effect size is standardised difference of mean values calculated as (DPN – PPDFU). Hence a negative result implies smaller values for DPN.
[a] Rich et al. (2000) excluded because of issues with unit of analysis;
[b] Studies included Bacarin, Sauseng and Stess;
[c] Analysis of studies with 100% active ulcer group [active ulcer only] (Cavanagh et al Sauseng et al and Brash et al);
[d] All studies (n=8) including Cavanagh and Boulton with SD estimated from linear regression;
[d2] Excluding Cavanagh and Boulton with SD estimated from linear regression;
[e] Stess et al (1997) excluded due to high level of heterogeneity and inclusion of amputees;
[f1] Rich et al and Stess et al excluded due to reasons given above;
[f2] Rich et al and Stess et al excluded due to reasons given above;
[g] Analysis with history of ulcers only [excluding active ulceration] (Bacarin et al, Boulton et al and Rich et al).

3.5.5 Sensitivity and sub-group analyses

Meta-analysis combining data from three studies that reported plantar pressure in active ulcer patients (PPDFU [present ulcer only] n= 43; DPN with no foot ulcer history n=74), suggested a non-significant difference for plantar pressure in patients with active foot ulcers compared to DPN participants without foot ulcers (see Table 3.3). Furthermore, an analysis of three studies using patients with a history of foot ulcers [previous ulceration only] suggested that overall MPP was significantly higher in people with a history of ulcers (n=76) versus DPN without a history of ulcers (n=172), (standardised mean difference 0.467, 95% CI 0.181- 0.753; p=0.001) with low heterogeneity I^2 = 3.6.

A sensitivity analysis was performed for overall MPP excluding studies by Cavanagh et al. and Boulton et al. (Boulton et al., 1983; Cavanagh et al., 1991) as their SD was estimated using linear regression (see Appendix C, Appendix Table 2). This resulted in a small increase in effect size (standardised mean difference 0.635, 95% CI 0.387-0.884; p≤0.001), and reduction in heterogeneity I^2= 31.4. Removal of the study by Rich et al. (Rich & Veves, 2000) (see Table 3.3) due to unit of analysis difference resulted in a small increase in effect size but an increase in heterogeneity to I^2= 50.76. Exclusion of the study by Stess et al. (Stess et al., 1997) (due to the inclusion of foot amputees in the PPDFU group) created a minute reduction in effect size and an increase in heterogeneity levels (I^2 =53.9) (see Table 3.3). The exclusion of two major studies potentially causing heterogeneity (Stess et al. and Rich et al. (Rich & Veves, 2000; Stess et al., 1997) resulted in a small reduction in effect size and a substantial increase in heterogeneity I^2 = 58.9 (see Table 3.3).

3.5.6 Secondary outcome measures

3.5.6.1 MPP at various plantar foot regions

As only two studies reported on MPP at the rear foot, meta-analysis was not possible (Bacarin et al., 2009; Rich & Veves, 2000). This was also true with assessment made at the mid foot (Bacarin et al., 2009; Shen et al., 2012). However, six studies reported MPP measures at the fore foot (see Appendix C, Appendix Table 2). Meta-analysis combining data from all six studies (PPDFU n= 211; DPN with no foot ulcer history n=339) suggested

that patients with PPDFU had greater fore foot MPP with moderate effect levels (standardised mean difference 0.635, 95% CI 0.387-0.884; p < 0.001) (see Figure 3.5). The heterogeneity between studies was moderate I^2= 31.4. When excluding the study by Rich et al. due to differences in unit of analysis, the heterogeneity dropped to I^2= 26.7, with a slight increase in the overall effect (see Table 3.3). The exclusion of the study by Stess et al. resulted in a small reduction in effect size and an increase in heterogeneity I^2 = 44.9. The exclusion of the studies by both Rich et al. and Stess et al. resulted in an increase in effect size but also an increase in heterogeneity levels I^2 = 42.5 (see Table 3.3).

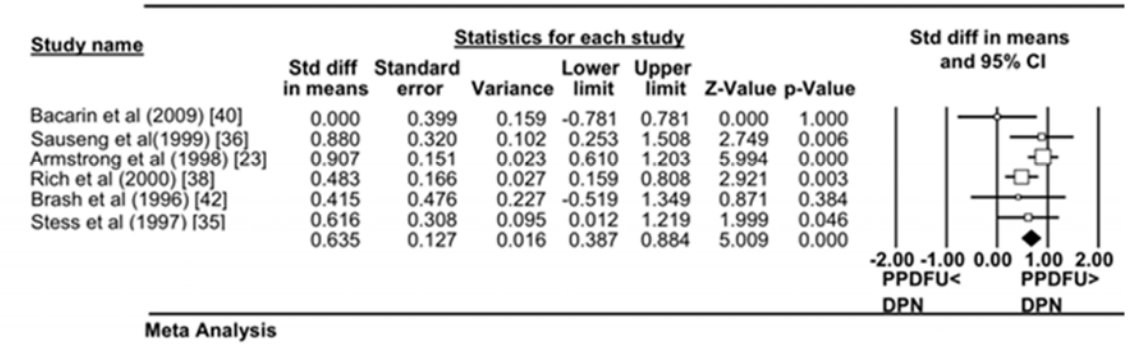


Figure 3.5 Forest Plot displaying the Fore Foot Peak Plantar Pressure (MPP) between PPDFU group (cases) and DPN group (control).

3.5.7 PTI at various plantar foot regions

Meta-analysis was only possible for fore foot PTI, as there were insufficient studies for comparison of rear foot and mid foot PTI. Meta-analysis combining data from three studies (PPDFU n= 79; DPN with no foot ulcer history n=54) revealed higher fore foot PTI in PPDFU patients (standardised mean difference 0.719, 95% CI 0.197-1.242; p= 0.007). The heterogeneity between studies was high I^2= 44.4 (see Table 3.3).

3.5.8 Potential factors affecting plantar pressure measurements in studies

We examined the effect of a number of potential confounding factors including BMI, age and duration of DM on plantar pressure at the aggregate level. Scatterplots of these variables were constructed (see Appendix C, Appendix Figure 20).These analyses suggested that higher BMI was associated with higher MPP in both groups, which was

consistent with previous studies (Shen et al., 2012; Sugimoto, Yasujima, & Yagihashi, 2008). It was not possible to adjust our analyses for BMI since individual data was not available. Therefore, all inferences from aggregate data included in this analysis should be made cautiously.

3.6 Discussion

One of the most detrimental complications of both Type 1 and Type 2 DM is neuropathic foot ulceration (Boulton, 2005). As a majority of neuropathic foot ulcers are likely to form due to mechanical loading on the insensate foot during locomotion, plantar pressure is an important biomechanical consideration in the investigation of the neuropathic foot (Cavanagh et al., 2000; Masson et al., 1989; van Schie, 2005; Veves et al., 1992). This meta-analysis identified that overall MPP was higher in DPN patients with a history of foot ulceration compared to people without a history of foot ulceration, although there was moderate between study heterogeneity. Some patients included in this meta-analysis had active foot ulcers while others had a history of foot ulceration at the time of assessment. Subgroup analyses revealed only people with previous ulceration, and not active ulceration, had significantly greater plantar pressures compared to people with DPN alone, with low between study heterogeneity. The sample sizes for the sub-group analyses were however small. PTI was significantly higher in DPN patients with a history of foot ulceration and this could indicate that both the magnitude and duration of plantar pressures are implicated in the tissue stress placed under the foot during gait in this population. Heterogeneity between studies for this finding was moderate. Sensitivity analyses suggested that heterogeneity between studies assessing MPP was contributed to by a number of factors including study quality and reporting.

Secondary outcome data suggested that patients with PPDFU had higher plantar pressures at the fore foot with moderate between study heterogeneity. Further analysis involving people with previous ulceration only and in people with active ulceration only was not possible. Subgroup analysis was limited by the demographic information available in studies and the overall number of studies. Hence this could not be carried out separately for

Type 1 and Type 2 DM, age, BMI, or DM duration. A sensitivity analysis suggested that heterogeneity was reduced by excluding studies in which the SD had to be estimated (Boulton et al., 1983; Cavanagh et al., 1991). One study included patients with minor pedal amputations which have been reported to increase plantar pressures and may have confounded their assessments (Armstrong & Lavery, 1998b). Therefore, future plantar pressure studies are recommended to adjust for the role of amputation or to include people with a previous minor amputation as a standalone group to determine plantar pressures in this end-stage DPN group. Regardless of these limitation, the finding of elevated fore foot plantar pressures in patients with PPDFU are consistent with the fact that the majority of DFU are found in the forefoot region (Altindas, Kilic, Cinar, Bingol, & Ozturk, 2011). PTI assessed at the forefoot was also greater in patients with PPPDFU. Sensitivity and sub-group analyses were unable to be performed due to the low number of available studies. Hence further studies are needed to assess the plantar pressure characteristics of the different anatomical areas of the foot in active and previous foot ulcer populations.

The MPP of patients with active DFUs was not significantly higher than controls; yet, the MPP of patients with previous DFUs was significantly higher. The between study heterogeneity of factors such as different lengths of time since ulceration and different characteristics of DFUs included in the individual studies may have contributed to this result. Small sample sizes were also present which could have contributed to the non-significant result in the active DFU group. Bacarin et al., 2009 included only patients who had a previous foot ulcer, and no active ulceration, within the 12 months preceding their examination and this was one of the few studies, which reported no difference in plantar pressures between the two groups. However, Sauseng and colleagues utilised active ulcer patients who had a history of at least one ulcer on the contralateral foot (Sauseng et al., 1999). In the study by (Armstrong et al., 1998c), they only included patients who had an existing or recently healed (< 4 weeks) plantar foot ulcer, however the relative number of patients in each category were not defined and this study was unable to be included in subgroup analyses. Boulton et al., 1983 included participants with a history of plantar ulcers with neuropathy and painful bilateral symptoms (paraesthesia, burning, and cramps) and absent ankle jerks for at least twelve months preceding the study. Rich & Veves, 2000 we were unable to distinguish demographic characteristics of the PPDFU and DPN groups

from the reported aggregate data and the authors were unable to provide this information when requested. Brash et al., 1996 studied feet which were ulcerated on the plantar surface of the first metatarsal head up to 2 years prior to the study. Stess et al., 1997 included 67% of patients with active ulcers and 26% of people with active ulcers had some form of lower extremity digital or ray amputation. Ideally, a more homogenous group of DFU participants are needed to confirm plantar pressure changes which accompany previous and active ulceration in people with DPN. Furthermore, better validated and quantifiable methods of diagnosing DPN may have increased homogeneity of this meta-analysis. The accurate and timely diagnosis of DPN is an ongoing clinical concern. The use of a pressure specific sensory device (PSSD) has been reported to provide greater sensitivity in identifying DPN, when compared to methods such as the 10.5g monofilament (Wood et al., 2005). A PSSD has been utilised in identifying DPN (Ferreira, Rodrigues, & Fels, 2004) and in assessing long-term sensory recovery following plastic surgery (Longo, Campanale, Farcomeni, & Santanelli, 2013; Longo, Pagnoni, Ferri, Morello, & Santanelli, 2013). However, none of the studies in this meta-analysis utilised this method of diagnosing DPN.

Although heterogeneity is evident between studies, the results of this meta-analysis seems to suggest that people with DPN and previous ulceration demonstrate an elevation in plantar pressure compared to people with DPN and no ulceration history. However, people with DPN and active ulceration do not demonstrate an elevation in plantar pressures compared to people with DPN and no ulceration history. It could be hypothesised that even though people with active foot ulcers are insensate, they may still alter their movement characteristics to a ‘guarded gait strategy’ during barefoot gait to compensate for the presence of their active ulcer, which in turn may result in a reduction in plantar pressures during the active ulcer phase. This highlights the possibility of an alteration in the gait strategy of these individuals during active ulceration which is contrary to previous findings in the area (Cavanagh et al., 1996; Savelberg et al., 2009a; Veves et al., 1992). Furthermore, it could be postulated that following ulcer healing, it is likely that this guarded gait strategy diminishes over time and plantar pressures return to the high levels that may have either initiated the active ulcer or were experienced during the phase of any previously healed ulcer. This hypothesis would support studies indicating that identifying a plantar pressure cut-off value to predict ulceration is plausible. Although this theory is

consistent with the meta-analysis findings, the current evidence is insufficient to substantiate this hypothesis.

Despite limitations, this meta-analysis supplements the body of research in the area of plantar pressure in patients with DPN and offers evidence for differences in plantar pressure between people with DPN and a history of DFU compared to people with DPN without an ulcer history. This meta-analysis has a number of key limitations including the significant between study heterogeneity, small sample sizes and the lack of available studies. It remains unclear whether screening patients for elevated plantar pressures improves patient outcomes.

3.7 Conclusion

Whilst the potential feasibility of using plantar pressure as a screening tool for ulceration remains viable, more explicit studies, including longitudinal studies involving better defined patient groups are needed to clarify the extent of plantar pressure difference throughout the sequelae of peripheral diabetic complications. Furthermore, while this meta-analysis occurred at an aggregate level, more prospective studies are needed to investigate the role of factors such as BMI, age and DM duration which could potentially influence plantar pressures in people with a history of foot ulcers and active foot ulcers. The use of plantar pressure as a screening tool could be supplemented by further understanding the influence that plantar pressures have in foot ulcer pathogenesis and healing.

Chapter. 4 General Methods and Study Protocol

This chapter has been adapted from a publication titled;

Lower limb biomechanical characteristics of patients with neuropathic diabetes related foot ulcers: the diabetes foot ulcer study protocol.

Authors: Malindu Eranga Fernando, Robert George Crowther, Margaret Cunningham, Peter Anthony Lazzarini, Kunwarjit Singh Sangla, Jonathan Golledge

BMC Endocrine Disorders. 2015; 15: 59.

4.1 Preface

Chapter 2 and 3 reported on systematic reviews of previous observational studies in the field. While most of the research in the field has been focused on assessing the gait and plantar pressures in people with DPN without DFUs and in people after DFUs heal, little attention has been given to the gait and plantar pressures in people with active DFUs. This chapter aims to describe the methods utilised in the observational studies focusing on people with active DFUs reported in this thesis (Chapters 6 to 8). As mentioned in the introduction, there are three prospective studies included in this thesis. The first study (Chapter 6) is a cross-sectional assessment of gait characteristics (TSPs, joint angular kinematics and kinetics) in people with type 2 diabetes with active neuropathic plantar DFUs compared to age and gender matched type 2 diabetes controls without DFUs and healthy controls. Study 2 (Chapter 7) is a cross-sectional investigation of the same cohort of participants but with a focus on plantar pressures. Study 3 (Chapter 8) comprises of a series of follow up observations on plantar pressures over the course of six months carried out on the cohort of patients with type 2 diabetes from the cross-sectional studies. This chapter reports on plantar pressure measurements performed at baseline, three and six months follow-up visits in the same cohort.

4.2 Abstract

Background: Foot ulceration is the main precursor to lower limb amputation in patients with type 2 diabetes worldwide. Biomechanical factors have been implicated in the development of foot ulceration; however, the association of these factors to ulcer healing remains less clear. It may be hypothesised that abnormalities in TSPs (stride to stride measurements), kinematics (joint movements), kinetics (ground reaction forces of the lower limb) and plantar pressures (pressure placed under the foot during walking) contribute to foot ulcer healing. The primary aim of this thesis was to establish the biomechanical characteristics TSPs, kinematics, kinetics and plantar pressures) of cases with plantar neuropathic foot ulcers compared to controls without a history of foot ulcers. The secondary aim was to assess the plantar pressures in people with DFUs and diabetes controls over-time to evaluate whether plantar pressures remain the same or change throughout ulcer healing.

Methods /Design: The design was a case-control study nested in a six-month longitudinal study. Cases were people with active plantar neuropathic foot ulcers (DFU group). Controls consisted of people with type 2 diabetes (DMC group) and healthy controls (HC group) with no history of foot ulceration. Standardised gait and plantar pressure protocols descripted in this chapter were used to collect biomechanical data (gait and plantar pressure) at baseline. Plantar pressures were repeated at three and six-month follow-up. Descriptive variables and primary outcome variables were compared between the three groups at baseline and between the two diabetes groups at follow-up.

Discussion: It was anticipated that the findings from this longitudinal study will provide valuable information regarding the biomechanical characteristic of type 2 diabetes patients with neuropathic foot ulcers. We hypothesised that people with DFUs will demonstrate a significantly compromised gait pattern (reduced TSPs, kinematics and kinetics) at baseline and throughout the follow-up period compared to controls. The findings from the studies contained in this thesis had the scope to provide evidence for the design of gait-retraining approaches, neuro-muscular conditioning and individualized approaches to off-load the

limbs of people with DFUs in order to reduce the mechanical loading on the foot during gait and promote ulcer healing.

4.3 Background

Although biomechanical studies have helped to identify potential triggers of ulceration, whether such triggers change and by how much when DFUs are healing remains largely unknown (Fernando et al., 2013; Fernando et al., 2014b). It is unknown whether patients with DPN can adjust their gait and plantar pressure (i.e. reducing pressure) to account for plantar wounds, as is the case for healthy controls with normal peripheral sensation (Fernando et al., 2014b). It can be hypothesised that due to DPN, patients with active plantar DFUs would continue to demonstrate similar abnormal lower limb biomechanical characteristics as displayed prior to the DFU formation (Fernando et al., 2013). Biomechanical factors associated with DFUs include reduced TSPs, such as speed of walking and stride length; restricted kinematics (movement patterns); delayed muscle activations; and altered forces (kinetics), which may contribute to elevated plantar pressures during gait (Fernando et al., 2014b). It may be possible that the patients who achieve successful DFU healing are able to compensate for the DFU through changes to these biomechanical factors, irrespective of external devices used to offload ulcers. Findings from a recent meta-analysis performed as a part of this thesis (Chapter 3) suggested that patients with active DFUs have reduced plantar pressure compared to controls with DPN without ulcers, contrary to what was previously thought (Fernando et al., 2014b). It was proposed that a 'guarded gait strategy' may be adopted by patients with active DFUs to potentially achieve successful DFU healing leading to reduced plantar pressure (Fernando et al., 2014b). To our knowledge, this concept has not previously been investigated. It is likely that exploring the gait and plantar pressure of patients with DFUs during barefoot walking (without the influence of off-loading devices or shoes) over-time will advance knowledge of how gait changes during DFU healing.

Detailed information about the biomechanical factors in patients with active DFUs may provide evidence for the design of gait-retraining and neuro-muscular conditioning and

more efficient offloading methods to reduce the mechanical loading on the foot during gait (Fernando et al., 2013; Formosa et al., 2013; Sartor et al., 2012). Findings may also provide a means to select the most appropriate patients for different off-loading methods to improve DFU healing. The primary aim of this study was to establish the lower limb biomechanical characteristics (TSPs, kinematics, kinetics, muscle activations and plantar pressures) of people with plantar neuropathic DFUs. The secondary aim was to assess the changes in these characteristics at 3 months and 6 months follow-up. This chapter details the proposed methodology and protocol for this planned study.

4.4 Methods and study design

4.4.1 Study design and participants

A case-control study nested in a six-month longitudinal study was planned as a part of this thesis (Chapters 6 to 8). The study was performed in Townsville, a regional town in north Queensland, Australia with an approximate population of 175,000 and an estimated type 2 diabetes prevalence of 4.4% (see Figure 4.1) (Diabetes Australia, 2017). Figure 4.1 outlines the hospital and health service area from which participants were recruited to the study. Type 2 diabetes patients with active unilateral plantar DFUs (DFU group), type 2 diabetes control patients without a history of DFUs (DMC group) and healthy controls without diabetes or DFUs (HC group) were recruited for this study. The sex of both control groups was matched to cases and the age of controls was matched within a range of five years to cases. A 1:4:2 ratio of cases to controls [DFU: DMC: HC] was planned due to the expected difficulty in recruiting DFU patients in comparison to controls. All assessments were carried out by the principal investigator, who is a trained podiatrist undertaking a PhD. All clinical and anthropometric measures (described below) were carried out three times and averaged. Figure 4.2 outlines the overall study design.

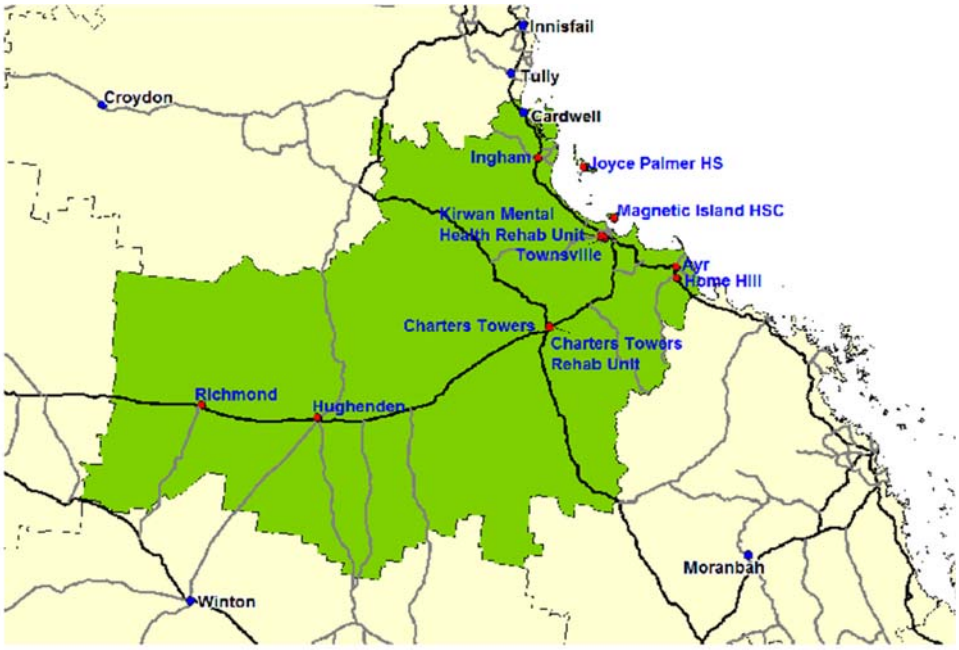


Figure 4.1 Townsville Hospital and Health Service, (highlighted in green) (http://www.health.qld.gov.au/maps/mapto/townsville.asp).

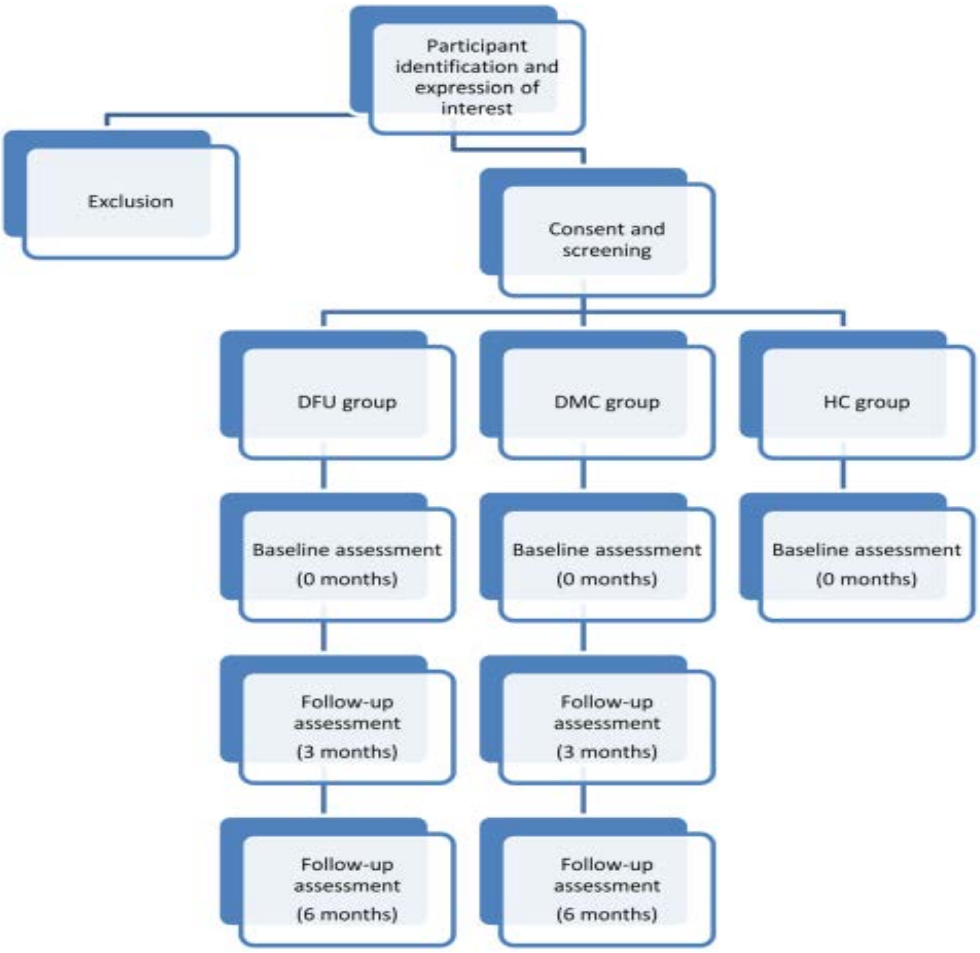


Figure 4.2 Study design.

4.4.2 Definitions of outcome measures and families of biomechanical outcomes

The primary and secondary outcome measures of the case control and longitudinal studies include several biomechanical variables. These variables were grouped into gait and plantar pressure outcomes and contributed four main families of hypotheses. The four families of biomechanical hypotheses were 1) abnormal TSPs, such as speed of walking and stride length; 2) restricted kinematics (movement patterns); 3) altered kinetics, (altered forces), and 4) elevated plantar pressures (Fernando et al., 2014b). The kinematics were further divided into three families; 5) sagittal plane kinematics, 6) frontal plane kinematics and 7) transverse plane kinematics. Therefore, seven families of hypotheses were examined. The main hypothesis was that people with active plantar DFUs have altered

lower limb biomechanical characteristics compared to healthy and diabetes controls. The follow-up hypothesis was that the plantar pressures of people with DFUs will not significantly change over six months follow-up compared to diabetes controls.

Primary outcome measures relating to plantar pressure were:

- Mean peak pressure: The mean peak pressure (average peak pressure), from in N/cm^2 recorded within the plantar aspect of foot at ten anatomical sites;
- Pressure-time integral: The area under the pressure time-curve in Nm/cm2 recorded within the plantar aspect of foot at ten anatomical sites;

Primary outcome measures relating to three-dimensional gait analysis were:

- TSPs (walking speed, cadence, stride time, step time, opposite foot off time, opposite foot contact, foot off time, single support time, double support time, stride length and step length);
- Kinematic variables (angular joint movement characteristics of the pelvis, hip, knee and ankle in the sagittal, transverse and frontal planes during the gait cycle);
- Kinetic variables (ground reaction forces): Reported as the vertical, medial-lateral and anterior-posterior components of ground reaction force during the gait cycle in Newtons (N);

A more detailed description of the variables listed above and their importance in biomechanical analyses can be found in Appendix A. These outcomes were not normalized to body weight as they were adjusted for BMI in statistical analyses.

4.4.3 Sample size calculations

As mentioned above, multiple hypotheses were examined in prospective studies. Sample sizes were calculated to test the hypothesis that forefoot plantar pressures were different in people with active DFUs compared to controls. This hypothesis was chosen for the sample size calculation since previous studies have suggested this measure had large between patient variability and therefore was likely to require the largest sample size (Bacarin et al., 2009). The effect size for this calculation was based on previous research where higher

plantar pressures were documented in people with DPN without a DFU history compared to healthy controls without either DPN or DFUs (Savelberg et al., 2009b). The sample size was based on an expected mean (standard deviation) forefoot plantar pressure of 3.2 (1.0) N/cm^2; 3.9 (1.0) N/cm^2; and 4.7 (2.0) N/cm^2 for the HC, DMC and DFU groups, respectively. It was estimated that people with DFUs would have at least a 20% higher plantar pressure than the control groups due to the observed difference in means of people with DPN vs. healthy controls (Savelberg et al., 2009b). The potential difficulties in recruiting active DFU cases was accounted for in sample size calculations by inflating the control to case ratio. Therefore, a ratio of 1 case; 4 diabetes controls; 2 healthy controls was used. Based on this, 28, 112 and 56 participants were required in the DFU, the DMC and the HC groups, respectively. By using a one-way analysis of variance (ANOVA) with 80% power and an overall significance of 0.05 (maximum of 8 planned hypotheses to be tested). The planned hypotheses to be tested were:

- The DFU group will demonstrate significantly poorer TSPs compared to controls at baseline.
- The DFU group will demonstrate significantly restricted kinematics at the ankle, knee, hip and pelvis compared to controls at baseline.
- The DFU group will demonstrate significantly altered kinetics (anterior posterior, medial-lateral and vertical ground reaction forces) compared to controls at baseline
- The DFU group will demonstrate significantly different plantar pressure. characteristics compared to controls at baseline.
- The plantar pressure characteristics of the DFU group will remain different at 3 and 6 months follow-up compared to the DMC group.

Due to the substantial number of hypotheses, corrections for multiple testing was performed which is described in detail in the statistical analyses section. The sample sizes were inflated further to account for a proposed 10% drop-out rate during the six-month follow-up and therefore 31, 123 and 58 participants were required in the DFU, the DMC and the HC groups, respectively. An interim analysis of the study was planned after recruiting 50% of the estimated number of participants for each group, which was used to determine final sample size numbers. This was done to re-assess the proposed sample sizes

since the initial effect size was based on patients that had DPN but no DFUs. A post-hoc power test was performed to re-assess actual statistical power when recruitment concluded. This was based on plantar pressure values measured in the forefoot. All sample sizes were performed using the G*Power statistical software (G* Power version 3.1, Faul, Erdfelder, Lang, & Buchner, Germany) (Faul, Erdfelder, Lang, & Buchner, 2007).

4.4.4 Sample selection and setting

Cases and controls with diabetes (DFU and DMC groups) were recruited from inpatient wards, outpatient clinics and community health clinics within the Townsville Hospital and Health Service District, Queensland, Australia (see Figure 4.1). The HC group were recruited through community advertising and from staff at the university where the study took place. The study was approved by The Townsville Hospital and Health Service District and the James Cook University human research ethics committees (approval numbers HREC/12/QTHS/77 and H4693). Written informed consent was obtained from all participants at the time of screening for participation in the study and for publication of de-identified data.

4.4.5 Inclusion criteria

Inclusion criteria for the cases (DFU group) were: males and females above the age of 18 years; with a diagnosis of type 2 diabetes (confirmed on review of the patient's hospital or General Practitioner record) and a single active unilateral plantar DFU of >3 months duration which was not completely epithelialized at time of recruitment. The DMC group comprised of males and females above the age of 18 years, with a diagnosis of type 2 diabetes (similarly defined as for the DFU group) and with no prior history of plantar DFUs. The HC group comprised of people without a diagnosis of type 2 diabetes or any foot ulcer history as assessed on interview and examination.

4.4.6 Exclusion criteria

All participants were required to be able to ambulate without any assistance or mobility aids. The exclusion criteria were designed to exclude participants with problems impacting on mobility which would likely mask the impact of plantar DFUs on gait. Exclusion criteria for recruitment to all three groups were: (1) chronic or acute orthopaedic, musculoskeletal, vestibular, visual or neurological problems affecting mobility (other than DPN) at the time of screening; (2) any history of orthopaedic surgical intervention of the lower limb (including knee arthroplasty, ankle reconstruction, bunionectomy and any other orthopaedic surgical intervention of the hip, knee or foot (besides history of surgical debridement of tissue in the DFU group); (3) presence of any form of diabetes other than type 2 diabetes; (4) planned vascular reconstruction; (5) pregnancy; and (6) systemic disease affecting mobility or leading to chronic inflammation of any lower limb joint.

All participants were required to demonstrate visual acuity adequate for walking. This was assessed by asking the participant whether they had visited an optometrist in the last twelve months and whether they had difficulty walking due to visual impairment. If they had not visited an optometrist, participants were required to do this before being considered for the study. Visual acuity was re-assessed prior to group allocation. Participants with diabetic retinopathy were not excluded as this is an associated microvascular complication of diabetes, provided that participants did not disclose limitations in their vision that affected their walking ability. All participants unable to fulfil these criteria were excluded. PAD was excluded as a major contributing cause of foot ulceration by a number of methods. Firstly, the DFU patients were reviewed by a board accredited vascular specialist who obtained a relevant history and performed clinical examinations to exclude significant arterial disease. DFU patients were required to demonstrate an absence of symptoms of intermittent claudication (Mancera-Romero et al., 2013) and an ABPI of >0.8 bilaterally, for inclusion. Participants enrolled in the control groups were also required to demonstrate an absence of symptoms of intermittent claudication (Mancera-Romero et al., 2013) and an ABPI of >0.8 bilaterally. Any participants who demonstrated ABPIs of >1.3 were also referred to the vascular specialist for review of inclusion (Bhasin & Scott, 2007). Participants were not excluded on grounds of a history of peripheral vascular

reconstruction provided they were able to demonstrate adequate peripheral circulation at the time of screening as based on the criteria listed above.

The section below outlines the baseline measurements performed as a part of the studies reported Chapters 6 to 8;

4.4.7 Clinical history and demographic assessment

A previously developed and detailed case report form (see Appendix D) was used by a single researcher for collecting data. A medical history was first obtained from participants via a structured interview using the case report form at baseline and at each follow-up visit. The case report form was used to collect information on: Demographic details; history of significant medical conditions and treatments; medication history; ethnicity, particularly in relation to indigenous status (O'Rourke, Steffen, Raulli, & Tulip, 2013); diabetes history, such as duration of diabetes and family history of diabetes (Fowler, 2008; Hariri et al., 2006); smoking history (Willi, Bodenmann, Ghali, Faris, & Cornuz, 2007); walking and exercise habits (Armstrong et al., 2004); and footwear use (Bus et al., 2008b). The hospital or clinical charts of patients with type 2 diabetes will also be reviewed. Medical conditions recorded will include: hypertension, defined as history of diagnosis or treatment with antihypertensive medication; dyslipidemia, based on history or treatment with hypolipidemic agents; stroke, defined as a history of an ischemic or hemorrhagic stroke; coronary heart disease, defined as history of stable angina or unstable angina or myocardial infarction; chronic heart failure, based on clinical history of congestive heart failure; chronic pulmonary disease, based on clinical history and/ or treatment; chronic liver disease, based on clinical record; chronic kidney disease, defined as an estimated glomerular filtration rate (eGFR) of <60 ml/min/1.73 m^2 for 3 months recorded in the medical records. These risk factors were either known to influence foot ulcer development or healing (Margolis, Hofstad, & Feldman, 2008), were considered as complications or conditions associated with type 2 diabetes (Fowler, 2008; Harris, 2005; Mooradian, 2009), or were related to general health and function (Yentes et al., 2011).

4.4.8 Blood tests

Information regarding blood markers that are obtained as part of clinical care of participants with type 2 diabetes was collected. Blood markers included HbA1c, circulating lipids and eGFR measured in a local pathology department. Ion-exchange high-performance liquid chromatography (HPLC) was used to estimate HbA1c as a measure of long-term glycaemic control with the RIANT II TURBO Link System (Bio-Rad Laboratories, USA) (Little & Roberts, 2009). Serum lipids were measured by separating serum from the whole blood and performing analysis for total cholesterol, triglycerides, low-density lipoprotein (LDL), and high density lipoprotein (HDL) using automated assays on an Abbott Architect ci8200 machine (Abbott Park, IL, USA) (Golledge, van Bockxmeer, Jamrozik, McCann, & Norman, 2010). eGFR was calculated using the Chronic Kidney Disease-Epidemiology Collaboration group (CKD-EPI) formula, which is validated for Australian populations (Golledge, Ewels, Muller, & Walker, 2014; Johnson et al., 2012).

4.4.9 Anthropometric assessments

Physical examination involved the assessment of height, body mass, body mass index (BMI), body fat percentage, and waist and hip circumference. Participant's height was measured using a wall mounted telescopic metal stadiometer (Seca model 220, Seca Scales, Hamburg, Germany). Percentage body fat (% body fat) was estimated using bioelectrical impedance scales (TANITA TBF 521, TANITA Corporation, Arlington heights, Illinois, USA) which also measures body mass (Kg) (Jebb, Cole, Doman, Murgatroyd, & Prentice, 2000). BMI was calculated by dividing the participant's body mass (Kg) by the square of the participant's height (m). A standardized metal measuring tape (KDSF10-02, KDS Corporation, Osaka, Japan) was used to assess hip and waist circumference while the participant was in a relaxed stance position with feet together and arms freely hanging to the side. The measurement were performed at the end of exhalation (Klein et al., 2007). Waist circumference was measured at the natural waist i.e. in between the lowest rib and the top of the iliac crest at the narrowest point of the lower abdomen (Klein et al., 2007). Hip circumference was considered as the widest portion of the buttocks, with the tape parallel to the floor (Esmaillzadeh, Mirmiran, Moeini, & Azizi,

2006). As mentioned earlier, all measurements were repeated three times and averaged (Klein et al., 2007).

4.4.10 Blood pressure and peripheral arterial examination

Systolic and diastolic blood pressure was measured using an electronic blood pressure machine (Connex ProBP 3400 digital blood pressure device; Welch Allyn, New York, USA) mounted on a mobile stand (Luyan, 2014). Systolic and diastolic measurements were taken from both arms while the participant was sitting and relaxed, and repeated three times, two minutes apart (Pickering et al., 2005). The highest averaged value from the two arms were used to represent the participant's blood pressure. Blood pressure measurements were performed using an appropriately sized sphygmomanometer cuff (i.e. using a large-adult cuff size for individuals with an arm circumference of 33 cm or greater) (Wofford et al., 2002).

The peripheral arterial examinations comprised of palpation of the dorsalis pedis and posterior tibial pulses for their presence or absence as well as strength (categorized as normal or reduced) (Kruse & Edelman, 2006). The ABPI was measured in each lower limb using previously validated methods (Stubbing, Bailey, & Poole, 1997). The ABPI was performed with the participant lying on a plinth and after being rested for ten minutes. A 5 MHz Doppler probe (MD 6, Hokanson, Bellevue, USA) angled at 45 degrees to the direction of the blood flow was used to assess the ABPI. First, the brachial artery pulse was manually palpated and the Doppler probe will be used to detect a signal. The sphygmomanometer cuff was then inflated until the Doppler signal disappeared and deflated slowly until the signal returned. The pressure at which the signal returns was recorded as the brachial artery Doppler pressure. Three brachial artery Doppler pressure measurements were taken in each arm using this method. The sphygmomanometer cuff was then placed around the participant's ankles immediately above the medial and lateral malleolus and the Doppler probe was placed on the dorsalis pedis artery after palpation. The above-mentioned process was then repeated three times to assess the dorsalis pedis artery Doppler pressure. A similar process was carried out to assess the posterior tibial artery Doppler pressure. The highest average Doppler pressure measurement from each ankle was divided by the highest average brachial pressure to obtain an ABPI value for

each foot. A combination of ABPI and claudication symptom questionnaire were used to identify the presence of peripheral artery disease as previously reported (Mancera-Romero et al., 2013). This combination was thought to provide a better means of detecting peripheral artery disease, especially since falsely elevated ABPI values are common in patients with diabetes mellitus (Al-Qaisi, Nott, King, & Kaddoura, 2009; Bhasin & Scott, 2007).

4.4.11 Screening for diabetic peripheral neuropathy presence and severity

Neurological examination consisted of a 10g (size 5.07) monofilament sensation test (Wood et al., 2005), a 128 Hz tuning fork sensation perception test (Thivolet, el Farkh, Petiot, Simonet, & Tourniaire, 1990) and administration of the Michigan neuropathy screening instrument (MNSI) (Michigan Diabetes Research and Training Center, 2013) (Moghtaderi, Bakhshipour, & Rashidi, 2006). The MNSI is a tool which has been validated for assessment and quantification of the degree of peripheral sensory and motor neuropathy present in participants with diabetes (Moghtaderi et al., 2006). The MNSI includes the screening of asymptomatic peripheral neuropathy (Hu et al., 2012). The tool contains both a symptom and physical component; hence a score out of 11 was generated for the symptom related questions of the MNSI and a score out of eight was generated for the physical assessment component. (Andersen, 2012; Dyck, Karnes, O'Brien, & Swanson, 1986).

Monofilament sensation was assessed at ten sites (two additional sites compared to previous reports) on each foot including the plantar surfaces of the heel on the medial and lateral sides, hallux, apex of toes 2-5, metatarsal one, metatarsal five and the dorsum of the foot between the first and second metatarsals (Perkins et al., 2010). The monofilament examination was performed with the participant lying supine in a relaxed position with their eyes closed. Participants were given the opportunity to feel the sensation in their index finger prior to testing in the foot. The monofilament was applied for <1s at each site and the participant was requested to verbally acknowledge whether they felt the sensation and to acknowledge the site of sensation (Feng, Schlösser, & Sumpio, 2009). A score of five or less out of eight has been reported to indicate the presence of peripheral neuropathy based on assessing eight sites (Perkins et al., 2010). A similar grading system with the

inclusion of two additional sites was used to encompass the main regions of the whole foot. Where a participant was unable to detect the monofilament at five or less sites they were considered to have DPN (Perkins et al., 2010). Additionally, a 128 Hz tuning fork was applied to the tip of the hallux at the bony prominence of the distal phalanx on both feet to check whether vibration sensation was present (England et al., 2005; Thivolet et al., 1990). Participants were asked to verbally indicate the commencement and cessation of vibration sensation upon application and dampening of vibration four times on each hallux (Thivolet et al., 1990). Vibration sensation was assessed on a scale of 0 to 8 in each leg based on the number of times vibration commencement and cessation was felt accurately (England et al., 2005).

4.4.12 Physical examination of the lower extremities

The lower limb examination comprised of an assessment of the static range of movement of the ankle joints, sub-talar joints and the first metatarsophalangeal joints using previously described methods (Elveru, Rothstein, & Lamb, 1988; Lázaro-Martínez et al., 2011). A goniometer was used while the participant was relaxed and lying on an examination plinth and while the legs were in complete extension (Elveru et al., 1988; Lázaro-Martínez et al., 2011). Ankle and sub-talar joint range of motion were recorded as a categorical outcome where the movement was classified as restricted, normal or hypermobile based on the range of movement of the joint (Fernando et al., 1991). The muscle power of the lower limbs were assessed using the Medical Research Council (MRC) scale (Paternostro-Sluga et al., 2008) while the participant was lying supine (foot segment relative to shank segment). The degree of movement of the foot in the sagittal, coronal and frontal planes were documented during abduction/adduction, plantar flexion/dorsiflexion and inversion/eversion movements. MRC grading was evaluated as a categorical value with a score less than five indicating a restriction in movement (Paternostro-Sluga et al., 2008). Other assessments included examination of the medial longitudinal arch contour (both on stance and during sitting) (Menz, Fotoohabadi, Wee, & Spink, 2012), an examination for lesser toe deformities (claw, hammer and mallet toes) (Bus et al., 2005) and identification of the presence and degree of hallux valgus (HAV) deformity of the first metatarsophalangeal joints (Formosa et al., 2013; Garrow et al., 2001).

4.4.13 Assessment and classification of plantar foot ulcers

The University of Texas Wound Classification System (UTWCS) is a validated tool for the measurement of foot ulcers (Armstrong, Lavery, & Harkless, 1998b). The UTWCS was used to grade the ulcer for severity based on the presence of infection, ischemia and depth. Photographs were taken of each participant's DFU at each visit and wound measurements were conducted to measure depth, width and length of the ulcer in cm where the surface area was calculated using the formula length (cm) x width (cm) = surface area (cm^2) (Piaggesi et al., 2005). Other information such as the ulcer location, the estimated duration of the ulcer (in weeks; recorded at the initial date of presentation within the notes to the time of assessment), the wound exudate level, the type of exudate, the appearance of the wound bed (base of the wound) and wound edge, and the presence of sinus formation were also collected (Lipsky et al., 2012; Wu et al., 2007). If clinical signs of infection were apparent (using Infectious Diseases Society of America Clinical Practice Guideline for the Diagnosis and Treatment of Diabetic Foot Infections (Lipsky et al., 2012) then a wound swab was performed (Apelqvist, 2008; Nelson et al., 2006) to identify the presence of bacterial infection as a part of treatment of the ulcer as reported in current guidelines (Leung, 2007; Lipsky et al., 2012; Nelson et al., 2006). Suitability for continuation in the study was then evaluated on a case-by case basis.

The type of offloading utilised and the type and frequency of wound dressings were also collected (Armstrong & Lavery, 1998a; Frykberg, 2002; Leung, 2007). All patients with DFUs were provided with a standard single-layer generic film wound dressing, which they wore over the wound during gait and plantar pressure examination to minimize the impact of wound dressings on the study results and to standardise the type of dressing during gait assessments. As a precautionary measure, participants with DFUs were only requested to weight bear while gait and plantar pressure assessments were being carried out and were instructed to remain sitting with their feet off the ground as much as possible between assessments.

4.4.14 Assessment of gait characteristics

The three-dimensional movement analysis component of the study involved attaching reflective markers to the participant's skin over the surface of key anatomical landmarks (Davis, Ounpuu, Tyburski, & Gage, 1991). This enabled the assessment of movement characteristics such as TSP's and joint kinematics described in Appendix A (Whittle, 2012). The movement analysis laboratory at James Cook University was used for gait assessments. This lab is equipped with the VICON gait analysis system (VICON, Oxford, United Kingdom) and ten T-40 series infrared cameras positioned around a walking environment capturing data at 100 Hz within the VICON Nexus movement analysis software (version 1.9.1, VICON, Oxford, United Kingdom). The force plates in the laboratory comprise of two 400 x 600 mm OR-6 AMTI force plates and two 900 x 900 mm OR-6 AMTI force plates (AMTI, Watertown, Massachusetts, USA) which were embedded on a 10m long walking surface covered by concrete overlay. The force plates have a maximum excitation range of 10 volts with each occupying six analogue channels (<2% channel cross talk). The force plates was programmed to capture at a rate of 3000 Hz (3000 frames per second), for optimum capture speed whilst utilising all equipment simultaneously. All equipment was linked and synchronized with the VICON system in the laboratory. A similar system was used in a recent study investigating gait of patients with trans-tibial amputation (Eshraghi et al., 2014) and in another study assessing gait in patients with a history of foot ulcers (Raspovic, 2013).

A standard VICON Nexus procedure was used during motion capture (Vicon Motion Systems, Oxford, England) (Vicon, 2010). Ten walking assessments while walking at a self-selected pace were collected from each participant. These ten walking assessments were then averaged and compared across groups. Chapter 5 contains further information about the methodology and repeatability of these assessments and Appendix A provides a detailed account of the importance of biomechanical outcomes.

4.4.15 Assessment of plantar pressure

The Footscan ® pressure plate (RSScan International, Olen, Belgium) was used for plantar pressure assessment with the associated Foot Scan ® processing software (see Appendix

A, Appendix Figure 10). This platform was two meters in length, 0.4 meters in width and contains 16384 sensors capturing at 100 Hz. The plantar pressure platform was freestanding. The platform has been used for previous biomechanical research in participants that have diabetes (Qiu et al., 2013). Ten walking assesments were collected from participants while walking at a self-selected pace using the three step-protocol (Peters, Urukalo, Fleischli, & Lavery, 2002). This involves the participant being trained to approach the plantar pressure platform so as to strike their third step (i.e. contact of the initiating limb) on the pressure platform first followed by the opposite foot second (Peters et al., 2002). The pressure measurement software permitted masking of the foot to enable identification of plantar pressures at a total of 20 anatomical locations in both feet. This allowed for the quantification of plantar pressure based on pre-established anatomical locations (Wolfson, 2001). The locations included the plantar surfaces of the hallux, combined toes one to five, metatarsal one, metatarsal two, metatarsal three, metatarsal four, metatarsal five, the mid foot, the lateral rear foot and the medial rear foot. These measurements were reported by the software as the mean peak pressure (mpp), maximum sensor pressure (msp), pressure time integral (pti) and contact area (ca). A consistent plantar pressure measurement was defined as a walk over the platform in which all ten anatomical locations were visible with a numerical value for mpp, msp, pti and ca in both feet and where lateral or medial deviation of the foot off the pressure platform did not occur (Wolfson, 2001).

4.4.16 Measurements to be repeated at the follow-up assessments

Follow-up assessments were performed on all participants in the DFU and DMC groups at three and six months (see Table 4.1). The HC group was only examined at baseline as it is anticipated that the gait and plantar pressure of these participants will not change during short term follow-up (Ludbrook, 1998). The case report form (see Appendix D) was used for collecting data at each follow-up visit. Follow-up assessment involved a physical examination, including assessment of height, body mass, BMI, % body fat and waist and hip circumference. A questionnaire contained within the case report form was used to assess any changes to participants' medical history or medications. This form was also used to document foot ulcer recurrence, new ulcer formation and ulcer healing, new

treatments and foot ulcer characteristics (if applicable). Plantar pressure measurements were performed at the three and six months follow-up visits to assess any changes to the primary outcome measures relating to plantar pressure. Eligibility to participate was reassessed at each visit and any reasons for discontinuation of the study was recorded and reported.

4.5 Discussion

The methodology of the cross-sectional studies (Chapter 6 and 7) and longitudinal study (Chapter 8) investigating the gait and plantar pressure characteristics of patients with active DFUs is reported in this Chapter. There is a paucity of studies investigating the biomechanical characteristics of patients with active neuropathic DFUs and thus a need to better document these characteristics. Altered biomechanical characteristics are likely to influence the healing of plantar DFUs. It may be possible to address altered biomechanical characteristics to reduce the mechanical loading on the ulcerated foot during gait using gait retraining techniques in addition to off-loading the foot. The design of these interventions is likely to be improved by further understanding of gait during the presence of an ulcer and plantar pressures during ulcer healing.

Table 4.1 Measurements used at each time point of follow-up in observational studies.

Measures	Baseline	3 Month follow-up	6 Month follow-up
Screening and consent	X		
Plantar Pressure Assessment	X	X	X
Lower limb 3-dimensional movement analysis	X		
Blood tests and biochemistry	X	X	X
ABPI	X		
Neuropathy testing	X		
MNSI questionnaire	X		
Ulcer assessment / photograph	X	X	X

Legend: ABPI= ankle brachial pressure index, MNSI= Michigan Neuropathy Screening Instrument.

Chapter. 5 The Reproducibility of Acquiring Three-Dimensional Gait and Plantar Pressure Data Using Established Protocols in Participants with and without Type 2 Diabetes and Foot Ulcers

This chapter has been adapted from a publication titled;

The reproducibility of acquiring three-dimensional gait and plantar pressure data using established protocols in participants with and without type 2 diabetes and foot ulcers.

Authors: Malindu Eranga Fernando, Robert George Crowther, Margaret Cunningham, Peter Anthony Lazzarini, PKunwarjit Singh Sangla, Petra Buttner, Jonathan Golledge

J Foot Ankle Res. 2016 Jan 29; 9:4.

5.1 Preface

The previous Chapter (Chapter 4) reported on the methodology and study protocol for the prospective studies contained in this thesis (Chapters 6-8) and provided the means to report on the detailed procedures and processes for data collection. However, prior to conducting prospective research, it was important to assess the repeatability of measurements using the protocols described in Chapter 4. In particular, it was important evaluate the measurement repeatability of gait and plantar pressure data in people with DFUs as these were the main outcomes arising from the research contained in this thesis and as the proposed protocols had not exclusively been utilised in people with DFUs. This Chapter there reports on the findings of a study which aimed to determine the repeatability of data acquired from three-dimensional movement analysis and plantar pressure measurement and which attempted to answer Question 3 from Chapter 1. This study was carried out in a subset of participants who were initially recruited to the prospective studies reported later (Chapter 6-8).

5.2 Abstract

Background: Several prospective studies have suggested that gait and plantar pressures abnormalities secondary to diabetic peripheral neuropathy contribute to foot ulceration. There are many different methods by which gait and plantar pressures are assessed and currently there is no agreed standardised approach. This study aimed to describe the methods and reproducibility of three-dimensional gait and plantar pressure assessments in a small subset of participants using pre-existing protocols.

Methods: Fourteen participants were conveniently sampled prior to a planned longitudinal study; four patients with diabetes and plantar foot ulcers, five patients with diabetes but no foot ulcers and five healthy controls. The repeatability of measuring key biomechanical data was assessed including the identification of 16 key anatomical landmarks, the measurement of seven leg dimensions, the processing of 22 three-dimensional gait parameters and the analysis of four different plantar pressures measures at 20 foot regions.

Results: The mean inter-observer differences were within the pre-defined acceptable level (<7mm) for 100% (16 of 16) of key anatomical landmarks measured for gait analysis. The intra-observer assessment concordance correlation coefficients were > 0.9 for 100% (7 of 7) of leg dimensions. The coefficients of variations (CVs) were within the pre-defined acceptable level (< 10%) for 100% (22 of 22) of gait parameters. The CVs were within the pre-defined acceptable level (<30%) for 95% (19 of 20) of the contact area measures, 85% (17 of 20) of mean plantar pressures, 70% (14 of 20) of pressure time integrals and 55% (11 of 20) of maximum sensor plantar pressure measures.

Conclusion: Overall, the findings of this study suggest that important gait and plantar pressure measurements can be reliably acquired. Nearly all measures contributing to three-dimensional gait parameter assessments were within predefined acceptable limits. Most plantar pressure measurements were also within predefined acceptable limits; however, reproducibility was not as good for assessment of the maximum sensor pressure. To our knowledge, this is the first study to investigate the reproducibility of several biomechanical methods in a heterogeneous cohort.

5.3 Background

It is well established that biomechanical abnormalities secondary to diabetic peripheral neuropathy (DPN) contribute to the formation of diabetes-related foot ulcers (DFUs) (Boulton, 2004a; Cavanagh et al., 2000; Fernando et al., 2013; Monteiro-Soares et al., 2012). There is limited understanding however regarding how such biomechanical factors influence foot ulcer healing (Fernando et al., 2013; Fernando et al., 2014b; Kanade et al., 2006). Studies examining the biomechanical forces influencing foot ulcer healing need to perform repeated assessments over time in the same patients (Kanade et al., 2006). A prerequisite for such studies are reproducible methods (McGinley, Baker, Wolfe, & Morris, 2009).

Three-dimensional gait and plantar pressure analyses are considered important in assessing the biomechanical characteristics of the foot (Fernando et al., 2013; Kanade et al., 2006; Mueller et al., 1994a; Pham et al., 2000). There are many different methods by which these analyses have been performed and currently there is no agreed standardised approach (Bus & de Lange, 2005; Ferrari et al., 2008; McGinley et al., 2009). The comparison of results within individuals and between different participants can therefore be difficult (Fernando et al., 2013). There is a need to better describe the reproducibility of methods used to acquire gait and plantar pressure data and to define ways to minimize measurement error (Ferrari et al., 2008; Giacomozzi, Keijsers, Pataky, & Rosenbaum, 2012; Leigh, Pohl, & Ferber, 2014; McGinley et al., 2009). This is especially important prior to interpreting results of studies in which gait is being assessed repeatedly over time, since measurement error will need to be taken into account (McGinley et al., 2009).

This study aimed to describe the methods and the reproducibility of measurements performed during three-dimensional gait and plantar pressure assessment. A small group of participants who had diabetes with and without foot ulcers and healthy controls were examined.

5.4 Methods

5.4.1 Study design and participants

Fourteen participants were conveniently selected from a larger group of people enrolled in a longitudinal study (Fernando et al., 2015). Participants were selected on the basis of their availability to attend five extra visits required for the assessment of reproducibility. Four participants with type 2 diabetes and active plantar foot ulcers (DFU group) and five participants with type 2 diabetes without active foot ulcers (DMC group) were recruited from The Townsville Hospital, Queensland, Australia. A further five healthy participants (HC group) were recruited by advertising amongst community groups, hospital and university staff. The HC group did not have diabetes based on their medical history. The study took place between July and December 2012. The study was approved by The Townsville Hospital and Health Service and the James Cook University human research ethics committees (HREC) (approval numbers HREC/12/QTHS/77 and H4693) (see Appendix E). Written informed consent was obtained from all participants prior to commencing the study.

5.4.2 Training prior to reproducibility assessment

The researcher undertaking measurements in this study (MF) initially received extensive training from an expert in biomechanical assessments (RC). RC holds a PhD in biomechanics and is a trained exercise physiologist with more than 10 years' experience in carrying out gait analyses. MF is a trained clinical podiatrist with 3 years clinical experience and limited prior experience in carrying out gait analyses. Approximately 40 hours of customized training was provided by RC to MF prior to the assessments performed in this study. Training involved the placement of reflective markers on correctly identified anatomical landmarks (Leigh et al., 2014), the accurate measurement of limb distances (Vicon, 2010) and the accurate capturing and processing of plantar pressure and gait data based on pre-established protocols (Bus & de Lange, 2005; Cavanagh & Ulbrecht, 1994; Davis et al., 1991; Hafer et al., 2013; Peters et al., 2002; Vicon, 2010). The training was performed on 22 volunteers. All reproducibility assessments were carried out only after training had been completed to an adequate standard assessed by RC.

5.4.3 Biomechanical assessments

One trained investigator (MF) conducted all assessments at the movement analysis laboratory at James Cook University, Townsville, Australia using a standard published protocol (Fernando et al., 2015). A range of approaches were used to assess the single-operator reproducibility of measurements utilising the pre-established protocol. Specifically, we examined the reproducibility of identifying key anatomical landmarks, measuring limb dimensions, processing three-dimensional gait data and measuring plantar pressures as outlined below.

Height and body mass were measured three times per participant and averaged. Height was assessed using a wall mounted telescopic metal stadiometer (Seca model 220, Seca Scales, Hamburg, Germany). Body mass was measured using bioelectrical impedance scales (TANITA TBF 521, TANITA Corporation, and Arlington heights, Illinois, USA). Foot arch type was determined by a podiatrist (MF) by visually examining the participants arch while standing (weight bearing) and the arch was classified as either a pes planus foot type (flat foot), normal arch (neither flat foot or high arched foot) or as a pes cavus foot type (high arched foot).

5.4.4 Identification of key anatomical landmarks

The correct placement of reflective markers on anatomical landmarks is a crucial initial step in obtaining reliable and valid gait data (Della Croce, Leardini, Chiari, & Cappozzo, 2005; Ferrari et al., 2008). We felt it important to firstly assess the degree of measurement error in placing reflective markers. We developed a simple method by which the reproducibility of identifying anatomical landmarks could be examined. This reliability assessment was carried out to assess if examiner one (MF) was able to correctly locate anatomical landmarks for the placement of reflective markers with precision when compared with examiner two, an expert in biomechanics (RC).

Examiner one (MF) was asked to use a black coloured pen to identify and mark the exact positions of the anatomical locations utilised in the standard VICON Nexus plugin-gait analysis protocol (Vicon, 2010). This included 8 locations on both sides of the body (16 in total), following a Helen Hayes marker model (Davis et al., 1991). These locations were

the tibial tuberosity, head of fibula, lateral malleolus, medial malleolus, lateral shin (the mid-point of the leg between the knee and the ankle), the central posterior calcaneus, the head of second metatarsal and the anterior superior iliac spine (ASIS) (see Plate 5.1). After examiner one (MF) had completed this task, examiner two (RC) was asked to place another pen marking of a different colour on where he perceived the anatomical landmarks to be. If examiner two believed that it was at the same location as examiner one, then no pen marking was inserted. After examiner two completed marking the anatomical locations, the difference between the two observers was measured in millimetres (mm).

A difference of more than seven mm was considered important as the base-diameter of the reflective markers used was seven mm. It was reasoned an error above this level in marker placement would likely impact on kinematic (movement) analyses outcomes.

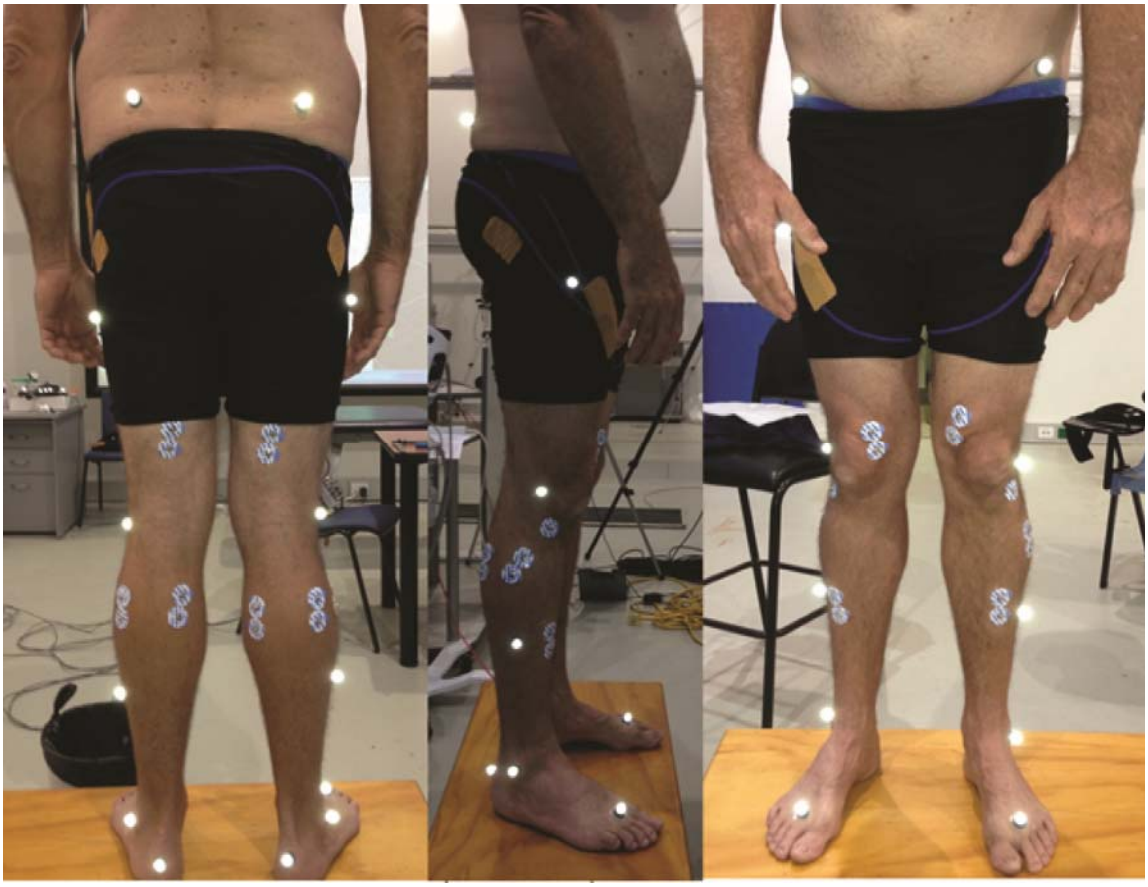


Plate 5.1 Anatomical locations used for reflective marker placement for the plug-in gait model.

5.4.5 Measurements of leg dimensions utilised in constructing a three-dimensional gait model

Accurate measurements of seven anatomical dimensions of the lower limb have to be performed when examining gait using the VICON Nexus plug-in gait model (Davis et al., 1991; Vicon, 2010). These measurements and the reflective markers are used to acquire Euler angles during gait (Davis et al., 1991). We performed measurements using a metal tape measure (KDSF10-02, KDS Corporation, Osaka, Japan) and a handheld Vernier Caliper (Draper Tools Ltd., Hampshire, United Kingdom) in mm as previously described (Vicon, 2010). Anterior superior iliac spine (ASIS) width was defined as the distance from the left to the right ASIS (also known as the inter ASIS distance). Leg lengths were measured from each ASIS to the distal end of each medial malleolus (skeletal leg length). Knee diameter (knee width) was measured as the linear distance (medial to lateral) of the palpable knee joint margin while the knee was in full extension. Ankle diameter (ankle width) was taken as the distance from the anterior lateral edge of the lateral malleolus to the anterior medial edge of the medial malleolus while the participant was in the relaxed stance position (see Plate 5.1). Three measurements of leg dimensions were performed on each participant half an hour apart on the same day by the same examiner (MF).

5.4.6 Processing of gait data

As described in Chapter 4, the movement analysis laboratory at James Cook University is equipped with the VICON motion analysis system (VICON, Oxford, United Kingdom). The system has ten T-40 series infrared cameras positioned around a walking environment capturing data at 100 Hz within the VICON Nexus movement analysis software (version 1.9.1, VICON, Oxford, United Kingdom). The laboratory also comprised of two 400 x 600 mm OR-6 AMTI force plates and two 900 x 900 mm OR-6 AMTI force plates (AMTI, Watertown, Massachusetts, USA) which were embedded on a 10 m long walking surface covered by concrete overlay. The force plates had a maximum excitation range of 10 V with each occupying six analogue channels (<2% channel cross talk) which worked off a strain gauge bridge. The force plates were programmed to capture at a rate of 3000 Hz (3000 frames per second), for optimum capture speed whilst utilising all equipment simultaneously. All equipment was linked and synchronized with the VICON system in the

laboratory. A similar system was used in a recent study investigating the gait of patients with trans-tibial amputation (Eshraghi et al., 2014) and in another study assessing gait in patients with a history of foot ulcers (Raspovic, 2013).

A standard VICON Nexus procedure was used during motion capture (VICON Motion Systems, Oxford, England) (Fernando et al., 2015; Vicon, 2010). Participants were provided with tightly fitting shorts which conformed to the skin. This enabled the appropriate placement of reflective markers in the correct anatomical positions with minimal interference from clothing. Following marker placement, participants were instructed to position their feet against a ruler that was placed on the floor adjacent to the 10-m walking surface. Participants were instructed to keep their feet approximately shoulders length apart prior to walking. Walking commenced with the dominant leg (whichever side the participant selected as their writing hand was on). Participants were advised to practice walking in the assessment surrounding until they felt they had adopted their natural walking rhythm (see Plate 5.2). We sought to establish the reliability of the manual processing of captured gait data. A single walking assessment was recorded from each participant. The same walking assessment was processed on ten occasions over five days (two per day) to establish the reproducibility in processing gait data. We did not examine the variability of gait in this study, but rather the reproducibility of analyzing gait data. The standard VICON pipeline procedure for processing gait data was used (Vicon, 2010).

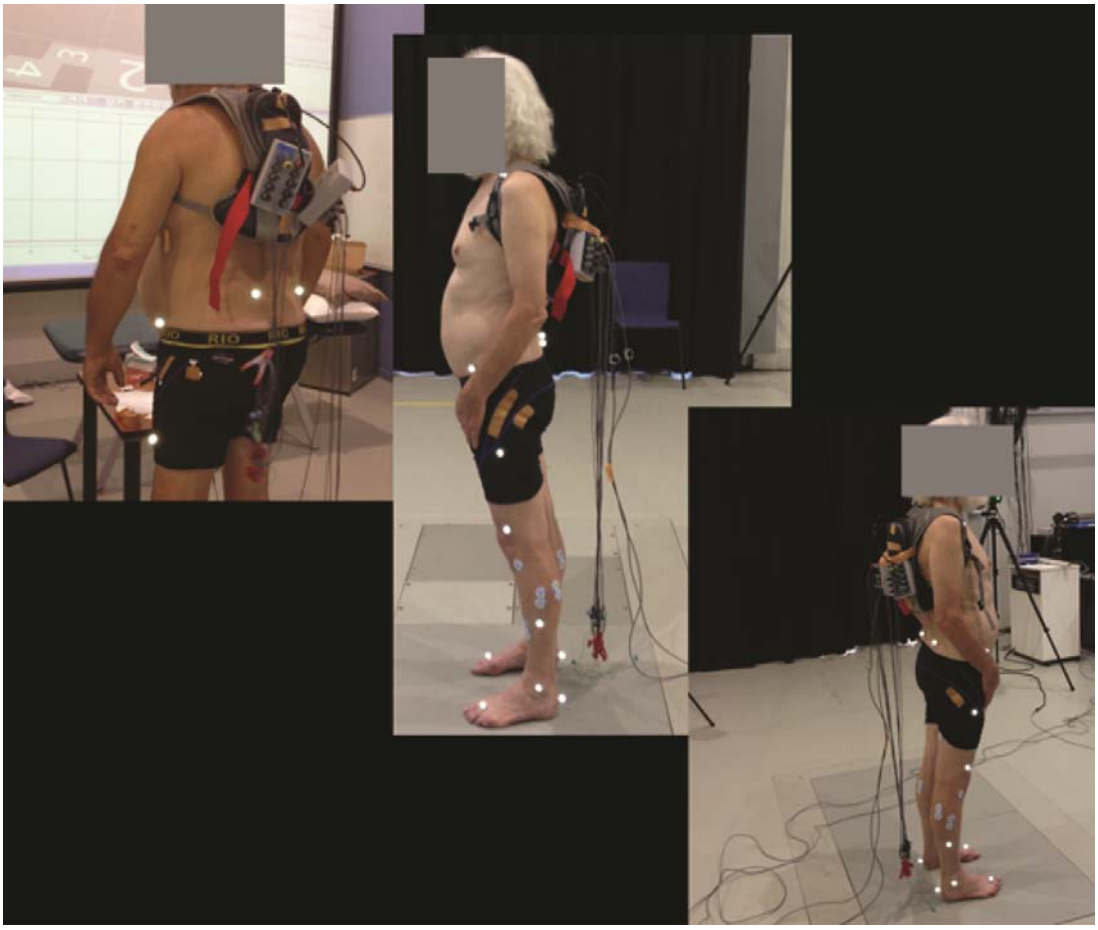


Plate 5.2 Participant getting ready to commence walking assessment.

Temporal-spatial parameters (TSPs) characterize various events, speeds and time intervals related to the gait cycle (see Chapter 4 and appendix A). We examined the variation in selected TSPs since they are imperative for calculating other important outcomes such as kinematics (angles) and kinetics (force) data. The TSPs included a total of 22 parameters measured in both legs, including walking speed, cadence, stride time, step time, opposite foot off time, opposite foot contact, foot off time, single support time, double support time, stride length and step length. These were calculated for the left and right limbs separately. The coefficient of variation (CV) was calculated for each TSP outcome related to each limb using the data outputs from ten processed extracts per participant. This was done by dividing each participant's standard deviation (SD) by the mean and multiplying by 100. The average CV of the population was thus calculated from all individual CVs. The mean CV of each participant group was also determined. This provided a measurement of

disagreement in the processing of gait assessments using standardised gait assessment software.

5.4.7 Plantar pressures

The Footscan ® pressure plate (RSScan International, Olen, Belgium) was used for plantar pressure assessment with the associated Foot Scan ® processing software. This platform was 2000 mm in length, 400 mm in width and contained 16,384 sensors capturing at 100 Hz. The plantar pressure platform was freestanding. The platform has been used for previous biomechanical research in participants that have diabetes (Qiu et al., 2013) and has been confirmed as a reliable platform for plantar pressure measurements (Qiu et al., 2013; Viswanathan, Snehalatha, Sivagami, Seena, & Ramachandran, 2003). The intention of this study was to assess the variation between measurements carried out on five consecutive days utilising a standard protocol (Bus & de Lange, 2005; Peters et al., 2002). We employed the three-step approach which involved the participant being trained to approach the plantar pressure platform so as to strike their third step (i.e. contact of the initiating limb) on the pressure platform first followed by the opposite foot second (Peters et al., 2002). A reference marker was used to keep the starting point consistent for each walking assessment. All participants practiced walking over the platform several times until they were able to establish a comfortable pattern of walking over the platform. Instructions were repeated to obtain a gait pattern where the ipsilateral foot (initiating foot) would make contact with the platform on the third step as required (Peters et al., 2002).

Participants were asked to walk at a self-selected pace over the platform consecutively until ten walking assessments were acquired. A verbal cue was given to commence walking during each assessment. Participants were monitored to check that each assessment commenced at the reference marker placed on the floor. Participants were asked to ‘look straight ahead’ in order to prevent targeting foot placement on the platform. Data capture only commenced when a consistent gait pattern was achieved and the acquired pressure readings were visually consistent (Fernando et al., 2015). A consistent measurement was defined as a walking assessment in which all ten anatomical locations investigated were visible with a numerical pressure value recorded for each of the ten sites in both feet and where lateral or medial deviation of the foot off the pressure platform did

not occur. The foot also had to be contained entirely within the active surface of the sensor array as detailed below (Cavanagh & Ulbrecht, 1994). Walking cadence was not adjusted or standardised and participants were allowed to adopt their natural walking pace (Cavanagh & Ulbrecht, 1994). A degree of intra-participant variability in walking and hence plantar pressures was anticipated due to normal variability in gait (Bacarin et al., 2009). Prior research has suggested that adequate reproducibility can be obtained in measuring plantar pressure (Gurney, Kersting, & Rosenbaum, 2008). The variation between assessments of plantar pressures in healthy controls was reported to be 7% for the lateral aspect of the rear foot and up to 20% for the lateral forefoot for example (Gurney et al., 2008).

We recorded fifty walking assessments per participant over five days. Five walking assessments were then randomly selected per day from the ten captured daily assessments per participant, as previously reported (Hafer et al., 2013). The selection of walking assessments for comparison was performed by one assessor (MF) who randomly selected two assessments from walks one to five, two assessments from walks six to ten and chose one further assessment from the remaining six walking assessments as the fifth included assessment per participant. Each plantar pressure assessment was only used once.

The pressure measurement software permitted masking of the foot to enable identification of plantar pressures at a total of 20 anatomical locations in both feet (Cavanagh & Ulbrecht, 1994). The locations included the plantar surfaces of the hallux, combined toes one to five, metatarsal one, metatarsal two, metatarsal three, metatarsal four, metatarsal five, the mid foot, the lateral rear foot and the medial rear foot. These measurements were reported by the software as the mean peak pressure (mpp), maximum sensor pressure (msp), pressure time integral (pti) and contact area (ca). When comparing the data, the mean and SD were first obtained for the mpp, msp, pti and ca for the ten plantar locations per individual over five days.

5.4.8 Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) for Windows (released 2013, IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, IBM Corp) was used for the

statistical analyses performed in this study. The differences between observers and 95% confidence intervals (95% CI) were computed to assess the accuracy of locating anatomical sites (Du Prel, Hommel, Röhrig, & Blettner, 2009). Lin's concordance correlation coefficients (CCC) were calculated to examine the reproducibility of the three repeated measurements of leg dimensions (Lin, 1989). These were reported with two sided 95% CI using an online statistical program (NIWA, 2013). The CCCs were calculated by comparing the first and the second measurements, the first and the third measurements and the second and third measurements in all participants. We reported three CCCs to represent reproducibility between all three measurements as opposed to reporting an average CCC. CCCs were interpreted as almost perfect (>0.90), substantial (>0.8-0.9), moderate (0.65-0.8) and poor (<0.65) (McBride, 2005). Bland-Altman plots were constructed for the assessments which had the lowest CCC values to illustrate the association between the mean difference in measurements and the mean leg dimension lengths (Altman & Bland, 1983). CVs were used to assess the reproducibility of plantar pressure outcomes and TSPs. CVs are an accepted method of reproducibility evaluation in biomechanical data (Bacarin et al., 2009). CVs were selected as there were far too many measurements to evaluate CCCs. The CVs were calculated by dividing the SD by the mean and multiplying by 100 to acquire a percentage (%). The CVs for the gait data were calculated by selecting one walking assessment from each participant and processing this assessment ten times over five days to obtain ten extracts of TSPs per participant. The individual CVs were calculated from the participant's mean and SD. The mean CV of the population was then calculated by averaging the CVs of all individuals. For the TSPs, a CV of less than 10% was defined as acceptable since it was reasoned that there should be a very low level of variation in the processing of gait data using standardised methods.

The CVs for mpp, msp, pti and ca were calculated by first acquiring a daily mean measurement for each participant from their five walks. The CVs in plantar pressure measurements (mpp, pti, msp and ca) over five days were calculated using the daily means and SDs from each participant. The mean CV of the population was subsequently calculated by averaging the CVs of all individuals. CV values for plantar pressure were considered to have good reproducibility if they were below 30% as it was anticipated that readings between days would have a certain degree of inherent variability (Gurney et al.,

2008). Furthermore, higher CVs may suggest a poor sensitivity to detect changes between conditions. Continuous data were reported as median and interquartile range [IQR] and compared between groups using Kruskal-Wallis and Mann Whitney-U tests. Nominal data were presented as numbers and percentages (%) and compared between groups using Fisher's exact test. We recruited participants from three distinct populations. Given the very different clinical characteristics of these participants, it was envisaged that the reproducibility might vary between groups. Thus the group specific reproducibility was investigated, despite the small sample sizes.

5.5 Results

5.5.1 Participant characteristics

The study population consisted of twenty-eight limbs from fourteen participants (see Appendix F, Supplementary File 1 and Table 5.1).

Table 5.1 Participant characteristics.

Variable	DFU group (n=4)	DMC group (n=5)	HC group (n=5)	*p-value*
Age (yrs)	56.5 [47.0-71.3]	58.0 [52.0-64.0]	64.0 [52.0-72.5]	0.724
Diabetes duration (yrs)	17.0 [8.0-20.0]	3.0 [2.5-14.5]	-	0.138
Height (cm)	176.8 [165.5-179.1]	167.0 [159.5-168.5]	156.0 [154.8-172.5]	0.212
Body mass (Kg)	128.5 [91.3-134.0]	79.4 [77.1-99.6]	73.0 [62.8-89.1]	0.061
Waist circumference (cm)	137.5 [113.0-140.3]	101.0 [91.0-119.0]	87.0 [80.0-98.50]	0.014
Hip circumference (cm)	129.5 [104.5-135.0]	98.0 [93.5-119.5]	89.0 [79.0-97.3]	0.034
Gender ratio [male: female]	3:1	2:3	2:3	0.080
Right foot arch type [pes planus: normal arch: pes cavus]	2:1:1	3:1:1	0:4:1	0.113
Left foot arch type [pes planus: normal arch: pes cavus]	2:1:1	3:1:1	0:4:1	0.113

Legend: The numerical values indicate the median and inter-quartile range [IQR]. The p-values were derived from Kruskal Wallis test for between three group comparisons (DFU vs. DMC vs. HC) and the Mann-Whitney U test for two group comparisons (DFU vs. DMC group). Categorical variables were compared between groups using Fishers exact test. Diabetes duration indicates fractions of years diagnosed with type 2 diabetes mellitus.

5.5.2 The reproducibility of identifying anatomical landmarks

Table 5.2 displays the mean [95% CI], minimum and maximum (absolute) differences in identifying all anatomical landmarks between observers for the entire study population (see Appendix F, Supplementary File 1 and Table 5.2.

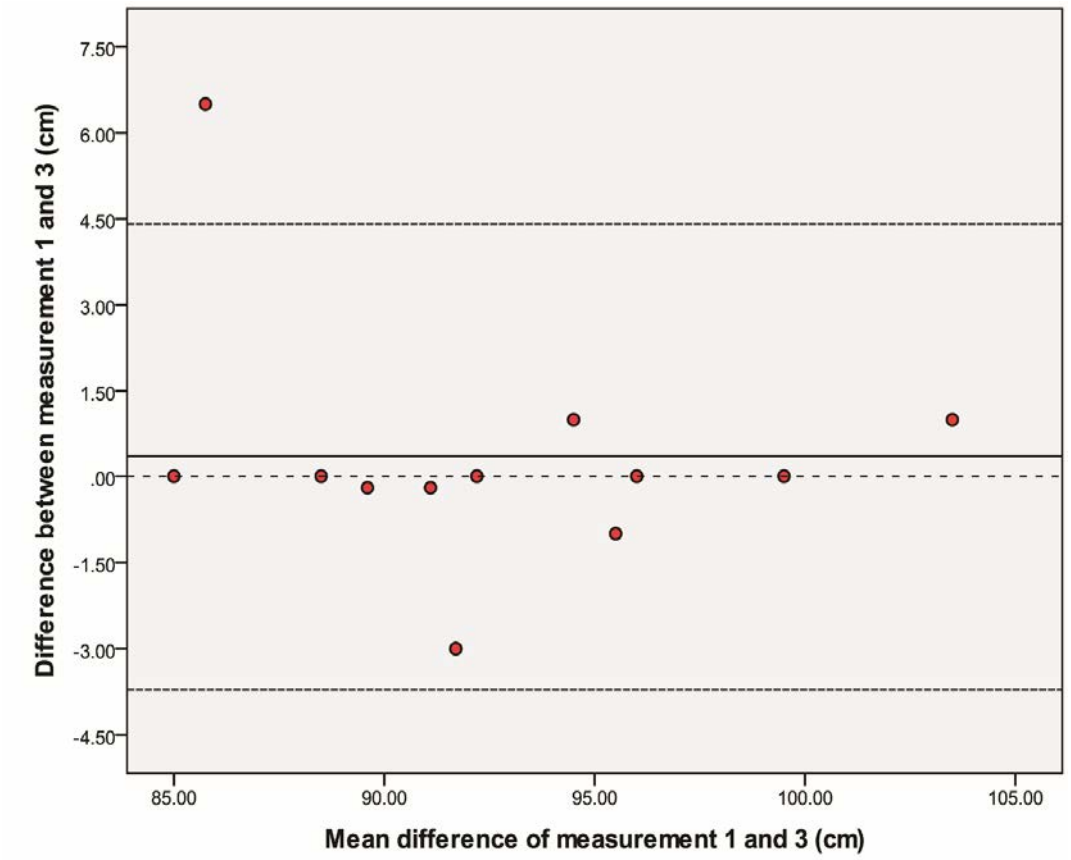
Table 5.2 Average differences between the novice and expert observers in the identification of anatomical sites in participants.

Variable	Mean difference (mm)	SD of difference (mm)	Minimum difference (mm)	Maximum difference (mm)	95% confidence interval
Left Limb					
Tibial tuberosity	3	3	0	15	2-4
Head of fibula	2	2	0	6	2-3
Lateral malleolus	2	2	0	4	1-3
Medial malleolus	2	2	0	5	1-2
Lateral shin	1	2	0	5	0-2
Central posterior calcaneus	2	2	0	4	1-2
Head of second metatarsal	1	1	0	3	0-1
ASIS	5	4	0	10	3-7
Right limb					
Tibial tuberosity	3	3	0	10	2-4
Head of fibula	4	7	0	35	0-7
Lateral malleolus	2	2	0	6	2-3
Medial malleolus	2	2	0	5	1-2
Lateral shin	2	2	0	7	0-2
Central posterior calcaneus	2	2	0	6	1-3
Head of second metatarsal	1	1	0	3	0-1
ASIS	4	3	0	12	3-6

Legend: Data relates to the difference between examiner 1 (MF) and expert examiner 2 (RC) in mm. A 7mm difference was considered acceptable for this analysis. The SD relates to the standard deviation of the mean difference, and the maximum and minimum values indicate the highest and lowest levels of measurement difference between the two observers. The two-sided 95% confidence interval of the difference is also reported. ASIS = anterior superior iliac spine.

5.5.3 The reproducibility of assessing leg dimensions

The group CCC [95% CI] values for limb and joint assessments for measurement 1 vs. 2; measurement 2 vs. 3 and measurement 1 vs. 3 are shown in Table 5.3 (see Appendix F, Supplementary File 1 for a summary of results). Figure 5.1 shows a Bland and Altman plot of the difference between measurement one and measurement three for left leg length.



Legend: A Bland and Altman plot of the difference between measurement one and measurement three for left leg length. The center line of the graph indicates the mean difference between measurement one and three and the upper and lower dashed lines indicate the mean difference ± 2.00 times the standard deviation of the difference. The dashed center line is the zero reference for the y axis. The y axis represents the difference of the two measurements and the x axis represents the mean of two measurements.

Figure 5.1 Bland Altman plot of left leg length [measurement one compared to measurement three].

Table 5.3 Concordance correlation statistics for the reproducibility of assessing leg dimensions on three occasions.

Variable	CCC measurement 1 v 2 [95% CI]	CCC measurement 1 v 3 [95% CI]	CCC measurement 2 v 3 [95% CI]
Left leg length	0.982 [0.949-0.994]	0.919 [0.776-0.972]	0.933 [0.822-0.975]
Left knee diameter	0.965 [0.895-0.988]	0.962 [0.891-0.987]	0.997 [0.990-0.999]
Left ankle diameter	0.961 [0.892-0.985]	0.944 [0.841-0.981]	0.971 [0.910-0.991]
Right leg length	0.994 [0.983-0.998]	0.972 [0.917-0.991]	0.977 [0.931-0.992]
Right knee diameter	0.986 [0.958-0.996]	0.984 [0.955-0.994]	0.980 [0.943-0.993]
Right ankle diameter	0.967 [0.902-0.989]	0.959 [0.878-0.986]	0.957 [0.876-0.985]
ASIS distance	0.994 [0.981-0.998]	0.995 [0.984-0.998]	0.997 [0.992-0.999]
Mass	0.999 [0.999-0.999]	0.999 [0.999-0.999]	0.999 [0.999-0.999]
Height	0.999 [0.999-0.999]	0.999 [0.999-0.999]	0.999 [0.999-0.999]

Legend: Concordance correlation coefficients (CCC) were calculated using http://www.niwa.co.nz/node/104318/concordance. Two sided 95% confidence intervals are presented with the CCC value for each body site. The strength of agreement was considered as: Almost perfect >0.90; Substantial >0.8-0.9; Moderate 0.65-0.8; and Poor <0.65. ASIS = anterior superior iliac spine.

5.5.4 The reproducibility of processing gait measurements

The mean CVs for the repeated processing of gait data were all considerably below the acceptable 10% (see Table 5.4) (see Appendix F, Supplementary File 1 for a summary of results).

Table 5.4 Mean coefficient of variation for gait measurements in the study population.

Variable	Left limb CV (%)	Right limb CV (%)
Cadence	0.1	0.1
Walking speed	0.5	0.6
Stride time	0.2	0.3
Step time	0.5	0.5
Opposite foot off	0.7	0.8
Opposite foot contact	0.1	0.1
Foot off time	0.1	0.2
Single support time	0.8	0.8
Double support time	1.3	1.9
Stride length	0.4	0.6
Step length	0.6	0.9

Legend: CV= Coefficient of variation reported as a percentage. Cadence refers to number of steps per minute. The two measurements reported as <0.1 contained CVs which were below 0.001. A pre-established level of <10% was used as a threshold for determining appropriate variance in gait measures.

5.5.5 The reproducibility of plantar pressure measurements

The calculated mean CVs for plantar pressures are presented in Table 5.5 (see Appendix F, Supplementary File 1 for a summary of results).

Table 5.5 Mean coefficient of variation for plantar pressure measurements in the study population.

Variable	mpp CV (%)	pti CV (%)	ca CV (%)	msp CV (%)	Variable	mpp CV (%)	pti CV (%)	ca CV (%)	msp CV (%)
Left foot					**Right Foot**				
Toe1	32.1	41.3	26.2	32.7	*Toe1*	24.5	31.2	18.2	32.3
Toes 2-5	31.5	38.7	31.5	39.6	*Toes 2-5*	25.9	44.1	28.5	32.7
Metatarsal 1	27.9	34.9	16.2	35.0	*Metatarsal 1*	25.9	26.3	16.2	28.7
Metatarsal 2	22.5	22.0	11.2	31.0	*Metatarsal 2*	20.9	21.1	14.5	24.4
Metatarsal 3	21.4	22.1	9.5	30.5	*Metatarsal 3*	18.3	19.1	13.4	20.6
Metatarsal 4	26.1	28.9	10.3	35.3	*Metatarsal 4*	19.3	21.0	10.5	20.4
Metatarsal 5	31.1	35.7	19.8	41.2	*Metatarsal 5*	20.4	22.9	11.8	21.5
Midfoot	21.8	25.6	16.3	27.4	*Midfoot*	13.7	17.1	7.6	21.4
Medial Heel	19.1	23.1	7.2	24.4	*Medial Heel*	19.3	24.4	7.8	26.5
Lateral Heel	21.4	22.0	8.0	25.2	*Lateral Heel*	19.3	25.0	7.9	26.3

Legend: Mean peak pressure (mpp), pressure time integral (pti), contact area (ca) and maximum sensor pressure (msp) in a plantar anatomical location. CV= Coefficient of variation reported as a percentage. A pre-established level of <30% was used as a threshold for determining appropriate reproducibility.

5.5.6 Influence of group on the reproducibility of measurements

Please see Appendix F, Supplementary File 2, Appendix Tables 3-6 for group data.

5.5.7 The reproducibility of anthropometric and clinical measurements

The CCC [95% CI] values of all other clinical measurements performed in the study are also reported in Appendix F, Supplementary File 2, Appendix Table 7.

5.6 Discussion

This study aimed to describe the reproducibility of one operator in performing key measurements needed for three-dimensional gait and plantar pressure assessments using standard protocols. This study was felt to be an important prerequisite prior to undertaking a longitudinal study investigating the changes in gait and plantar pressures during foot ulcer healing in a cohort of participants with type 2 diabetes (Ferrari et al., 2008; Giacomozzi et al., 2012; McGinley et al., 2009). Overall, the findings suggest that following extensive training, a junior operator can obtain the necessary skills to accurately identify key anatomical landmarks and measure leg dimensions needed for gait analyses. The reproducibility of gait measurements and plantar pressure measurements were mostly within the pre-defined levels of acceptability. The levels of acceptable reproducibility used were set at arbitrary thresholds and are open to criticism. The msp measurement was the most reliable, followed by the pti. Variation in plantar pressure was worse in the left limb. The reasons for this need further clarification.

The assessment of biomechanical parameters requires the reliable identification of anatomical landmarks (Della Croce et al., 2005; Ferrari et al., 2008). One of the methodological challenges lies in the difficulty of placing anatomical markers in precise locations during testing sessions. Errors in marker placement may alter the orientation of body segments, leading to errors in kinematic curves created during gait (McGinley et al., 2009). We assessed the competency of a trained examiner in consistently assessing the locations for reflective marker placement when compared to an expert in the field of biomechanics. Our results suggest that the difference between assessors was acceptable for

all anatomical landmarks. The mean differences and the 95% CIs for almost all landmarks were less than seven mm, which we defined as an important difference. We hypothesised that if the base of the marker measuring 7mm (the attachment point on the skin) was incorrectly placed outside of an anatomical location then it is likely that errors in measurements would result. The maximum differences at a few anatomical sites were however greater than seven mm. Assessments performed at the right head of the fibula was an example where the upper limit of the 95% confidence interval of the mean difference was above the pre-determined limit. This error was due to the large maximum difference noted in one female participant in the HC group with genu recurvatum and a posteriorly displaced head of fibula. Differences in marker placement of greater than 25mm have previously been suggested to be important when examining spine movements, although it is likely smaller differences may impact lower limb assessments (O'Connor, Robinson, Shirley, & Millan, 1993).

A recent study supports our finding that a novice with previous clinical experience is able to learn the operation of three-dimensional gait analyses with good reliability compared to an expert in the field (Leigh et al., 2014). Our participants and methods of reproducibility assessment however differed considerably from this recent study (Leigh et al., 2014). Leigh and colleagues used a much younger cohort of healthy, lean participants and utilised post-processing kinematic data from treadmill walking to assess measurement reproducibility. This method of assessing reproducibility has the potential to be significantly influenced by the natural gait variability of participants which can increase the intra-participant variability level (Ferrari et al., 2008). While Leigh and colleagues concentrated on reflective marker placement, we investigated reflective maker placement and additional parameters such as limb distance measurements and gait data processing to encompass a number of steps that can lead to measurement errors in gait assessments (Leigh et al., 2014). The authors also reported that the inter-observer reproducibility of marker placement was good based on coefficients of correlations between assessors for a number of kinematic measurements exceeding 0.9 (Leigh et al., 2014). The findings from our study, when combined with the findings of Leigh and colleagues, suggest that gait can be assessed reproducibly when standard protocols are used which observers are familiar with.

Accurate assessment of anatomical distances within the lower limbs is required to construct models used in gait assessment. Therefore, we examined the reproducibility of one observer assessing these distances on three occasions. The CCC statistics for repeat assessment of limb dimensions were within an acceptable level of agreement based on our pre-established criteria (Lin, 1989). The lowest CCC was found in the measurement of left knee diameter at 0.644 [95% CI 0.212-0.940] in the HC group. We felt these results still reflected an acceptable level of agreement (McBride, 2005). We also believe this finding reflected an outlier, likely resulting from a recording-error rather than a measurement error. When this outlier was removed the CCC value increased. We are not aware of any other studies which have assessed repeated measurements of anatomical distances used in gait analysis. We did not examine the reproducibility of kinematic outcomes which would have required comparing multiple gait assessments that contain natural intra-participant gait variability (Gurney et al., 2008). Our assessment suggested good reproducibility of TSPs when a standard protocol was used by one operator to process gait data. The highest CV was for double support time, which was above 1%. We considered this level of operator processing error to be insignificant as such a small variation in processing TSPs would have a minor impact on the final outcomes of other kinematic and kinetic variables.

There are nevertheless several other methods by which error can be introduced during gait assessment, which were not assessed in this study (Chiari, Croce, Leardini, & Cappozzo, 2005; Ferrari et al., 2008). These include errors associated with instrumentation, errors caused by the poor placement of cameras, the size of the area and volume being assessed by the cameras and errors caused by the incorrect calibration of cameras before data capture (Chiari et al., 2005). We nonetheless believe that most of these errors should be small when a single trained assessor is conducting measurements based on a standardised protocol (Ferrari et al., 2008).

The CV values for the assessment of plantar pressures suggested that the measurement of msp had the highest variability while mpp, pti and ca had lower overall variability. Our findings suggested greater variation in mpp, pti, ca and msp readings in the DFU group and the HC group compared to the DMC group. The range of CVs in our HC group (4 to 49%) showed greater variability than previously reported (7 to 20%) (Gurney et al., 2008) which

may be because we performed a larger number of measurements. A study by Bacarin and colleagues previously reported CVs of approximately 40% and 50% for pti and mpp assessments, respectively, in participants with a history of DFUs (Bacarin et al., 2009). The range of CVs in our DFU group was lower (13.9 to 38.9% for pti and 13.2 to 31.5% for mpp). We defined a CV of <30% as being reasonably reproducible as this was within the range previously reported (Bacarin et al., 2009; Gurney et al., 2008). Although the CVs for most plantar pressure variables were below our pre-established level, several measurements were also higher than the proposed threshold. It is established that a considerable degree of natural variation occurs during gait and thus in plantar pressure in most healthy participants (Cavanagh et al., 1993; Zammit, Menz, & Munteanu, 2010). Hence this finding was not unexpected in this heterogeneous sample.

Variability in the assessment of plantar pressure could be due to a number of factors. These include variation in participant foot placement and error in the location assignment for plantar sites across walking measurements (Cavanagh & Ulbrecht, 1994; Zammit et al., 2010). The high CVs in plantar pressures that were observed in our DFU group may represent variations in gait due to severe peripheral neuropathy and the presence of DFUs (Fernando et al., 2016c). Our findings are similar to those previously reported (Bacarin et al., 2009). The reasons for this observed variability requires further investigation using a larger cohort. Although a distinctly higher level of variation in plantar pressure measures were noted in the left foot of participants, the cause of this is difficult to ascertain given the small sample size and the design of this study. This result may be related to limb dominance as a majority of our participants were right-limb dominant.

Wearing and colleagues (1999) reported that there were three main factors to be considered when acquiring plantar pressures: (1) the level of reliability required; (2) the foot site being assessed; and (3) the type of pressure measurement studied (for example mpp as opposed to pti) (Wearing, Urry, Smeathers, & Battistutta, 1999). By using the third step from the commencement of walking (i.e. the three-step method) and five walking assessments, it was found that reliable data could be obtained (Wearing et al., 1999). The overall reliability of any variable, however, appeared to be site-dependent (Wearing et al., 1999). It is important to recall that there is natural variation in gait and it is currently

unknown how this variation in gait is important in healthy populations and in disease. Our study highlights that when studying populations with foot pathology such as those with plantar foot ulcers, the reproducibility of plantar pressure assessments may be reduced due to natural gait variation and that these measurements are foot and site dependent (Wearing et al., 1999). On average, the reproducibility of plantar pressure was poorest when measured in the forefoot and toe areas compared to the heel and the mid foot. This finding was similar to that of Wearing and colleagues (Wearing et al., 1999). As the CVs for mpp, pti and ca measurements were the highest at the first toe and the lesser toes, it is highly likely that a smaller surface area in these particular regions may have impacted on the increased variation in plantar pressure. Although the plantar pressure platform contained 16384 sensors which were capturing at 100 Hz, this may still be insufficient to accurately assess plantar pressure in small areas such as the tips of toes. This is a technical limitation and highlights the importance of reporting plantar pressure outcomes according to each individual site, rather than combining plantar pressures or averaging plantar pressures for a given foot region as has been the previous practice.

It is also possible that variation in the placement of the participants' feet on the pressure platform contributed to this finding. The sensor density and sensor number utilised to record each individual pressure measurement may also have influenced these results. The mpp was found to be the most reproducible out of the three plantar pressure measures and is likely the most suitable plantar pressure outcome for the population of interest. We believe that using a three-step protocol and assessing plantar pressure according to its appropriate plantar site and as three distinct measurements are important requirements, especially when evaluating patients with plantar foot ulcers (Armstrong et al., 1998b). Nevertheless, the reported CVs still need to be considered as a limitation in assessing plantar pressures longitudinally. CVs may be reduced by providing adequate practice time for participants and by perhaps increasing the number of steps captured (Wearing et al., 1999). However, the higher CVs may indicate that there is a high level of variability in gait which is unable to be reduced due to a presence of DPN in the population of interest.

This study has a number of limitations. The sample was small and heterogeneous, although it represented the populations of interest. The number of individuals in the different groups

was particularly small and likely affected our ability to compare reproducibility between recruitment groups effectively. The reason for the small sample size was the need for detailed assessments on each participant and the requirement for multiple visits. This limited the ability to recruit a large sample. We attempted to recruit a group of participants that best represented the population who are commonly examined by the techniques assessed. The participants recruited were also representative of those to be investigated in a future prospective cohort study (Fernando et al., 2015). We based our level of acceptability in plantar pressure readings on variances reported in previously published studies (Bacarin et al., 2009; Gurney et al., 2008) and hypothesised an acceptable level of variability for gait trial processing. The arbitrary cut-offs we used as acceptable levels of reproducibility need to be further validated as these have not been investigated previously. The study would have benefitted by blinding assessors or by using a larger number of assessors, particularly in evaluating the reproducibility of identifying anatomical locations. Despite these limitations, we believe the observations outlined in this small study may be useful when considering measurement reproducibility in gait and plantar pressure studies in populations who have known foot ailments. The findings may be useful for others planning to establish biomechanical testing in similar populations.

5.7 Conclusion

The current study reports on the reproducibility of several key measurements needed to examine gait and plantar pressures. Overall, the findings suggest that these measurements can be reliably assessed by a trained observer using currently available standardised protocols. We believe investigating the reproducibility of methods is important prior to initiating longitudinal collection of gait and plantar pressure data and for later comparison of results.

Chapter. 6 Gait Characteristics of People with Diabetes-Related Neuropathic Plantar Foot Ulcers

This chapter has been adapted from a publication titled;

Gait parameters of people with diabetes-related neuropathic plantar foot ulcers

Authors: Malindu Eranga Fernando, Robert George Crowther, Peter Anthony Lazzarini, Kunwarjit Singh Sangla, Petra Buttner, Jonathan Golledge

Clinical Biomechanics 37 (2016) 98–107

6.1 Preface

The previous chapters included in this thesis have focused primarily on reviewing previous studies on the gait characteristics and plantar pressures of people with DPN (Chapter 2) and people with a history of DFUs (Chapter 3). Chapter 4 reported on the methodology and study protocol for measuring the gait characteristics and plantar pressures in the prospective studies contained in this thesis and Chapter 5 reported the protocol was reproducible for acquiring three-dimensional movement analysis data and plantar pressures in healthy people, people with diabetes and people with DFUs. This chapter reports on the gait characteristics in the first prospective case-control study contained in this thesis and focuses on answering Question 4 from Chapter 1. Hence the aim of the study reported below was to determine the gait parameters in people with DPN and active DFUs when compared to diabetes controls and healthy controls without DFUs.

6.2 Abstract

Background: Foot ulceration associated with DPN is a global concern. Biomechanical investigation allows the identification of gait abnormalities that may adversely affect ulcer healing. The objective of this case-control study was to compare the gait parameters of cases with DFUs to controls.

Methods: Three-dimensional movement analyses were performed on 21 people with diabetes-related neuropathic plantar foot ulcers (cases), 69 people with diabetes without a foot ulcer history (diabetes controls) and 56 healthy controls. Outcome data were reported as mean differences, 95% confidence intervals and Cohen's d effect sizes. Binary logistic regressions were used to adjust for age, sex and body mass index.

Findings: People with foot ulcers had a smaller plantar flexion range of motion (Cohen's d= -0.6 vs. diabetes controls and d=-0.8 vs. healthy controls), knee flexion (d=-0.6 vs. diabetes controls and d=-1.0 vs. healthy controls) and pelvic obliquity (d=-0.9 vs. diabetes controls and d=-0.7 vs. healthy controls) (all $P < 0.05$). They also had a significantly greater range of anterior-posterior ground reaction force (d=-0.9 vs. diabetes controls and d=-1.5 vs. healthy controls) and total vertical ground reaction force (d=0.9 vs. diabetes controls and d=1.1 vs. healthy controls), and significantly slower walking speed and smaller step length compared to controls (all $P < 0.05$).

Interpretation: People with plantar foot ulcers have considerably different gait parameters to controls. Whether the observed gait parameters contributed to the ulcer development or are a response to the ulcer is currently unclear and needs further investigation.

6.3 Introduction

Diabetes-related foot ulcers (DFUs) are a leading cause of morbidity and mortality in people with type 2 diabetes (Boulton, 2004a; Lazzarini et al., 2012; Singh et al., 2005). Recent pooled estimates indicate that 2.4% of all hospitalized in-patients worldwide suffer from DFUs at any one time (Lazzarini et al., 2015a). People with DFUs have significantly lower physical, mental and social health status compared to people without DFUs (Ribu et al., 2008). The most significant contributing factor in the development of DFUs is diabetic peripheral neuropathy (DPN) (Boulton et al., 2004). DPN has sensory, motor and autonomic components (Boulton et al., 2004). Sensory DPN has been strongly implicated in DFU formation due to loss of protective sensation (Armstrong, 2005; Wood et al., 2005). Motor DPN also appears important in the development of DFUs via altered gait parameters (Mueller et al., 1994a; Veves et al., 1992). In a systematic review, we

previously demonstrated that people with DPN have different gait parameters than controls including different kinematic (such as increased range of hip flexion and knee extension), kinetic (such as reduced braking and propelling force) and temporal-spatial parameters (TSPs) (such as a longer stance time) (Fernando et al., 2013).The differences in gait parameters are thought to result from DPN which causes restricted lower limb joint range of motion (ROM) and foot-joint deformities which in turn contribute to elevated plantar pressures (Dinh & Veves, 2005; Fernando et al., 1991; Frykberg et al., 1998). Elevated plantar pressures during gait in the presence of sensory DPN increases plantar tissue trauma and predisposes people to DFUs (Boulton, 2004b; Masson et al., 1989; Wrobel & Najafi, 2010).

Most research in the field has focused on assessing plantar pressures before the development, or after the healing, of DFUs (Akashi et al., 2008; Fernando et al., 2013; Raspovic, 2013; Sacco, Akashi, & Hennig, 2010; Savelberg et al., 2009a). This research has suggested that reducing plantar pressures prevents DFUs from occurring and allows optimal healing if they develop (Cavanagh & Bus, 2011; Wrobel & Najafi, 2010). Hence current international guidelines advocate reducing maximum plantar pressure to prevent foot ulcers (Bus et al., 2016a). Although there is information regarding how gait parameters may predispose to ulcer development, there are very few studies that have investigated gait parameters during active ulceration. Hence the gait parameters (kinematics, kinetics and TSPs) of patients with active plantar ulceration remain poorly understood (Fernando et al., 2013). It is important to understand gait parameters during active ulceration as these may differ from those before ulcer development or after ulcer healing (Fernando et al., 2014b; Raspovic, 2013). A comprehensive biomechanical investigation of participants with active DFUs may allow identification of abnormal gait parameters that adversely affect ulcer healing (Formosa et al., 2013). This knowledge may allow for a more precise formulation of tailored treatments that include existing recommendations to reduce plantar pressure in conjunction with novel interventions to promote gait changes (Davis, 1997).

The aim of this case-control study was to comprehensively assess the kinematic, kinetic and TSPs in cases with active DFUs using three-dimensional movement analyses. We had

three overall hypotheses, that compared to diabetes and healthy controls, people with plantar neuropathic DFUs would display:

1) Significantly restricted angular kinematic variables in the lower limb;
2) Significantly increased kinetic parameters, leading to a higher planar load distribution;
3) Significantly restricted TSPs.

6.4 Methods

6.4.1 Study design and setting

This was a case-control study nested in a six-month longitudinal research project, conducted in a single regional Australian site. A full study protocol has been previously published (Fernando et al., 2015), which is described in Chapter 4. In brief, there were three groups of eligible participants: People with type 2 diabetes with an active plantar neuropathic foot ulcer (cases; DFU group); people with type 2 diabetes without a history of foot ulceration (diabetes controls; DMC group); and people without a history of type 2 diabetes or a foot ulcer (healthy controls; HC group). The control groups were matched, based on sex and age range of plus or minus five years, to the cases. The study was approved by two local human research ethics committees, and the approval numbers were HREC/12/QTHS/77 and H4693 (see Appendix E). Written informed consent was obtained from all participants involved in this study prior to initial assessment.

All participants were recruited from the Townville hospital health service region (Queensland, Australia) during the study period July 2012 to May 2014. Participants with type 2 diabetes were recruited from outpatients and inpatients attending the Townsville Hospital and Health Service facilities and via referral from local health practitioners. Healthy controls were recruited via community advertising and amongst university staff where the study took place.

6.4.2 Inclusion and exclusion criteria for cases and controls

Inclusion criteria for cases included adults (18 years or older) with a confirmed diagnosis of type 2 diabetes and a single active unilateral plantar DFU of longer than 3 months duration (Fernando et al., 2015). The DMC group comprised of adults with a confirmed diagnosis of type 2 diabetes without a history of DFUs (Fernando et al., 2015). The HC group comprised of adults without a history of diabetes or DFUs (Fernando et al., 2015). The exclusion criteria were designed to avoid inclusion of people with problems impacting on mobility that would likely mask the impact of a plantar foot ulcer on gait. Exclusion criteria for all groups included: (1) orthopaedic, musculoskeletal, vestibular, visual or neurological problems affecting mobility (other than DPN); (2) previous orthopaedic surgical intervention of the lower limb that potentially altered the participant's original gait; (3) diabetes types other than type 2 diabetes; (4) peripheral arterial disease defined as an ankle-brachial pressure index (ABPI) of < 0.8 in either limb; (5) planned vascular reconstructions in the subsequent 12 months; and (6) pregnancy (Fernando et al., 2015).

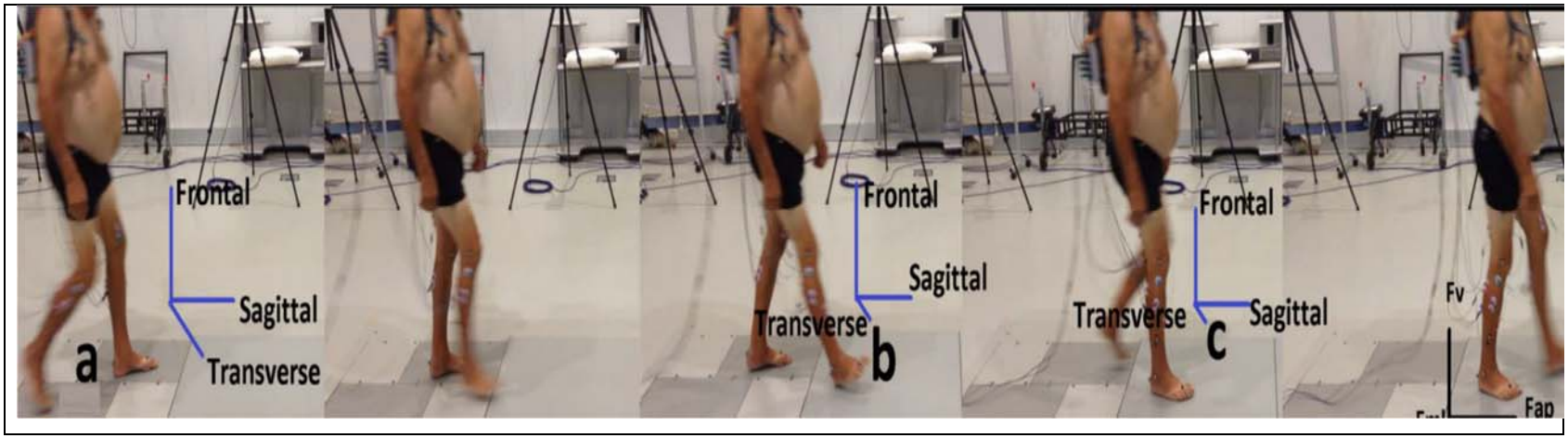
6.4.3 Description of procedures and variables

One trained investigator (MEF) conducted all assessments based on standard protocols. The procedures used for the collection of the outcome variables have been described in detail previously (Fernando et al., 2016a; Fernando et al., 2015) (see Chapter 4). A standard VICON Nexus procedure was used during motion capture (Vicon Motion Systems, Oxford, England) (Vicon, 2010). All methods utilised in the study had good to excellent reproducibility (see Chapter 5) (Fernando et al., 2016a). Based on our overarching hypothesis, the primary outcome variables were grouped into the following three main outcomes: Kinematic parameters, kinetic parameters and TSPs. Appendix A provides a detailed explanation of outcome variables as mentioned previously. We assessed kinematic outcomes throughout the gait cycle, beginning at initial heel contact and ending at the subsequent ipsilateral heel contact (see Plate 6.1).

Kinetic outcomes were expressed according to the vector component of ground reaction force during the stance phase of gait. This accounted for direction and magnitude of force during motion in Newtons (N). Hence there were also three subgroups of kinetic outcomes;

anterior-posterior ground reaction force (F_{AP}), medial-lateral ground reaction force (F_{ML}) and vertical ground reaction force (F_V). These were presented as the initial, final, total, maximum and minimum forces during gait. TSPs were presented as cadence; walking speed; stride time; step time; opposite foot-off time; opposite foot contact time; foot-off time; single support time; double support time; stride length and step length. Therefore, seven separate families of outcomes were examined in this study; sagittal plane kinematics, frontal plane kinematics, transverse plane kinematics, anterior-posterior ground reaction forces (F_{AP}), medial-lateral ground reaction forces (F_{ML}), vertical ground reaction forces (F_V) and STPs.

Anthropometric variables collected included height, body mass, body mass index (BMI), body fat percentage, waist and hip circumference and leg lengths. Clinical variables included ABPI, monofilament score and Michigan Neuropathy Screening Instrument (MNSI) scores (Moghtaderi et al., 2006). The monofilament score was calculated out of a total of 20, measured at ten sites in each foot. MNSI scores indicated the total scores from the neuropathy symptom score and the physical assessment score. The definitions and measurement methods of all these variables have been previously described (Fernando et al., 2016a; Fernando et al., 2015).



Legend: The figure represents a graphical illustration of the biomechanical outcome variables investigated for the right limb. a= toe off, b= initial contact, c=mid stance. The blue lines demonstrate vectors representing kinematic outcomes at toe-off, initial strike and during the gait cycle. Sagittal plane movements represent dorsiflexion and plantarflexion of the ankle joint and extension and flexion of the hip and knee and anterior and posterior pelvis tilt; frontal plane movements represent abduction and adduction of the ankle, knee and hip and left lateral and right lateral rotation of the pelvis (also termed pelvic obliquity) ; transverse plane movements represent inversion and eversion of the ankle (also termed the foot progression angle), medial and lateral rotation of the knee and internal and external rotation of the hip and transverse rotations of the pelvis. These movements occur around the axis demonstrated in the diagram and are quantified as angels occurring during gait events and as minimum, maximum and range of angles throughout. Therefore, all angles are reported for initial strike, toe-off and during the gait cycle. The black lines demonstrate vectors corresponding with the three kinetic outcomes (Fv= vertical ground reaction force, F_{ML}= medial lateral ground reaction force and F_{AP}= anterior posterior ground reaction force. The temporal-spatial parameters (TSPs) are calculated as distances and speeds which relate to the gait cycle. Only the foot progression angle is calculated with respect to the global coordinate system.

Plate 6.1 Diagrammatic representation of how outcome variables were obtained

A pre-defined and detailed case report form (see Appendix D) was used for collecting explanatory variables such as demographic, co-morbidity, anthropometric and clinical information utilising pre-established methodology (Fernando et al., 2015). Demographic variables reported in this study included: Age, sex and ethnicity. Co-morbidity variables included: Diabetes duration, HbA1c, insulin use, hypertension, dyslipidaemia, stroke, coronary heart disease, chronic heart failure, chronic pulmonary disease, chronic liver disease, chronic renal impairment and smoking status.

6.4.4 Sample size calculation

After considering several outcomes, we decided to use plantar pressures as the outcome measure to determine sample sizes as this outcome required the largest samples and as we planned to investigate multiple hypotheses in this study. Sample size calculations suggested that 28 cases, 112 diabetes controls and 56 healthy controls were required (Fernando et al., 2015). A total sample of 196 participants was predicted to achieve 81% power to detect the estimated differences in plantar pressures between groups.

We planned to investigate multiple hypotheses in this study. The overarching hypothesis was that cases with plantar neuropathic DFUs would display significantly lower angular kinematic variables at the ankle, knee, hip and pelvis that resulted in altered kinetic parameters which led to significantly abnormal TSPs (such as slower gait speed and cadence) during self-paced barefoot gait compared to diabetes and healthy controls. A ratio of 1 case to 4 diabetes and 2 healthy controls was proposed due to the predicted difficulty of recruiting adequate number of cases for the study. We inflated our control groups to maintain adequate study power given this limitation. A more detailed account of how the sample size calculations were carried out is reported in our study protocol and in Chapter 4 (Fernando et al., 2015).

6.4.5 Statistical analysis

We used the Statistical Package for the Social Sciences (SPSS) for Windows (released 2013, IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY, IBM Corp) for all analyses. Parametric analyses were selected based on the Shapiro-Wilk test. Continuous variables were presented as mean and standard deviation (SD) and categorical variables as

numbers and percentages (%). Continuous data were compared between the three-groups using one-way analysis of variance (ANOVA). Post hoc comparisons between two groups (cases vs. diabetes controls and cases vs. healthy controls and diabetes controls vs. healthy controls) were performed using Scheffe's method (Scheffé, 1953). The Pearson's chi-square test was used to assess differences in categorical variables between groups. For categorical variables which had less than five expected frequencies, the Fisher's exact test was used. Only post-hoc comparisons between two groups have been reported in the results section although other data have been provided within supplementary tables. A *P* value of less than 0.05 was considered statistically significant throughout.

To test our hypotheses each continuous gait variable assessed in the ulcerated limbs of DFU participants was averaged and compared to data averaged from both limbs of each control group. In order to control for multiple testing, we grouped continuous gait variables into seven "families" with members of each family likely to show strong dependencies (Knudson, 2009). The seven families included the following variables: family 1: sagittal plane kinematics; family 2: frontal plane kinematics; family 3: transverse plane kinematics; family 4: anterior-posterior ground reaction forces (FAP); family 5: medial-lateral ground reaction forces (FML); family 6: vertical ground reaction forces (FV) and family 7: TSPs. Based on previous recommendations (Hopkins, 2002; Knudson, 2009), we used the Holm step-wise correction to minimise the risk of false discovery when assessing the association of DFUs with seven different families of outcomes (Ludbrook, 1998). Where significant differences were identified, the Sidak's post hoc test was used to assess two-way differences in outcomes in the DFU group vs. DMC group, the DFU group vs. the HC group and the DMC group vs. HC group (Hopkins, 2002; Knudson, 2009). These were reported as mean differences with 95% confidence intervals (CI).

A difference in age, sex and BMI were noted between the three groups and these characteristics were identified as possible confounders. Therefore, binary logistic regression was used to adjust all significant post-hoc Sidak's comparison outcomes for age, sex and BMI between the DFU group and the control groups and the DMC group and the HC group. Odds Ratios (ORs) of binary regression outcomes were computed and reported in Appendix G, Supplementary File 1) for all significant Sidak's post-hoc variables. All

variables which were significantly different after adjustment *($p<0.05$)* were flagged in Tables. All outcomes that were significantly different between groups after adjusting for age, sex and BMI are highlighted in Tables 6.2-6.4 and in Appendix G, Supplementary File 1 and within this paper. All other data, including corrected p-values and ORs from logistic regression analyses are reported in Appendix G, Supplementary File 1, Appendix Tables 8-16.

Where significant differences existed between groups, the results were discussed in relation to the mean difference between cases and controls and between controls. As a measure of degree of difference between cases and controls, Cohen's d values with associated 95% CIs were calculated for all variables which remained significant after adjustment and correction, to assess their effect size (standardised difference). This was computed using the formula: Cohen's d = mean 1-mean 2 / pooled SD (Cohen, 1988). The 95% CIs were computed using non-centrality parameters. Cohen's d was calculated for the DFU group compared to control groups (DFU group - control) and for the DMC group compared to the HC group (DMC group - HC). Effect-size magnitudes from previously established biomechanical data were used to define the degree of difference using Cohen's d (Cohen, 1988; Hopkins, 2002). The size of the difference was graded based on Cohen's d as: <0.10 trivial difference; 0.10-0.20 small difference; 0.20-0.60 medium difference; 0.60-1.20 large difference and ≥1.20 a very large difference (Hopkins, 2002). Angular kinematic and kinetic data were aggregated, averaged and time-normalized to represent one complete gait-cycle on the x-axis (0-100%) and were plotted for illustration.

6.5 Results

6.5.1 Participant recruitment

From 208 participants that were screened for the study, 146 were recruited. This included 21 cases in the DFU group, 69 controls in the DMC group and 56 healthy controls in the HC group. Sixty-two participants were excluded for a variety of reasons, the most common being lesser toe or hallux amputation amongst the cases (n=10) and a prior history of orthopedic surgery of the lower limb in the controls (n=16). A post-hoc power test was

performed which suggested the power of the recruited sample size to test our initial hypothesis was in excess of 85% based on the actual sample size. This was likely due to the greater than expected plantar pressures in our population compared to the data used to perform our a priori sample size calculation (Fernando et al., 2015).

6.5.2 Participant characteristics

The DMC group was older than the HC group (see Table 6.1). The DFU and DMC groups had more males than the HC group ($p<0.05$). The DFU group had a longer duration of type 2 diabetes ($p=0.005$) and a greater number of patients using insulin ($p<0.001$) than the DMC group. The DFU group also had a higher prevalence of hypertension, coronary heart disease and chronic renal impairment than both control groups ($p<0.05$) *and* both diabetes groups had a higher prevalence of dyslipidemia compared to the HC group ($p <0.05$).

The DFU group had a larger body mass than the two control groups ($p< 0.05$). Those with diabetes (DFU and DMC group) had a larger BMI and hip and waist circumference compared to the HC group ($p<0.05$) yet there were no significant differences in leg length between the three groups (see Table 6.1). There was no significant difference in ABPI between the three groups. The DFU group had a more severe level of DPN, as represented by a lower monofilament score, higher MNSI symptom score and higher MNSI physical assessment score compared to the DMC group ($p<0.001$). All ulcers in the DFU group occurred on the plantar aspect of the foot. As a majority of the ulcers in cases occurred on, or distal to, the midfoot, we performed sensitivity analyses excluding participants with plantar ulcers proximal to the mid foot. As the overall findings remained largely unchanged we utilised all cases (n = 21) in further analyses.

Table 6.1 Clinical and demographical characteristics of the study cohort.

Variable	DFU (n=21)	DMC (n=69)	HC (n=56)	p-value
Age (years)	63.1 (10.6)	63.4 (9.6) [b]	57.6 (10.3)	0.004
Males	15 (71.4%)[b]	46 (66.7%)[b]	24 (42.9%)	0.011
Ethnicity				0.660
Caucasian	20 (%95.2)	65 (94.2%)	54 (98%)	
Australian Aboriginal/Indigenous/Torres-strait Islander	1 (4.8%)	2 (2.9%)	2 (3.6%)	
Other	-	2 (2.9%)	-	
Diabetes duration [years][#]	16.6 (7.1)	10.7 (8.6)	-	0.005
HbA1c (mmol/l)[#]	58.9 (16.8)	54.8 (13.3)	-	0.284
Uses Insulin [#]	13 (61.9%)	19 (27.5%)	-	<0.001
Smoking Status				0.201
Never Smoked	14 (66.7%)	34 (49.3%)	26 (46.4%)	
Ex-Smoker	6 (28.6%)	29 (42.0%)	29 (51.8%)	
Current Smoker	1 (4.8%)	6 (8.7%)	1 (1.8%)	
History of hypertension	19 (90.5%)[ab]	46 (66.7%)[b]	13 (23.2%)	<0.001
History of dyslipidemia	14 (66.7%)[b]	45 (65.2%)[b]	14 (25.0%)	<0.001
History of stroke*	2 (9.5%)[b]	2 (2.9%)	0 (0.0%)	0.074
History of coronary heart disease	7 (33.3%)[ab]	18 (26.1%)[b]	2 (3.6%)	0.001

History of chronic heart failure	3 (14.3%)	9 (13.0%)	2 (3.6%)	0.148
History of chronic pulmonary disease	4 (19.0%)	14 (20.3%)[b]	4 (7.1%)	0.107
History of chronic liver disease	2 (9.5%)	5 (7.2%)[b]	0 (0.0%)	0.093
History of chronic renal impairment	5 (23.8%)[ab]	10 (14.5%)[b]	0 (0.0%)	0.003
Height [cm]	173.7 (9.8)	169.6 (10.6)	168.9 (9.7)	0.177
Body mass [kg]	102.5 (23.8) [ab]	91.3 (15.2) [b]	74.4 (15.2)	<0.001
BMI [Body Mass Index]	34.0 (8.3) [b]	31.8 (4.80) [b]	26.1 (4.5)	<0.001
Body Fat Percentage [% bf]	28.5 (13.7)	27.8 (12.6)	28.2 (13.5)	0.974
Waist Circumference [cm]	113.5 (17.9) [b]	106.6 (11.2) [b]	86.2 (13.2)	<0.001
Hip Circumference[cm]	110.7 (18.9) [b]	105.8 (10.2) [b]	93.0 (44.7)	0.019
Left leg length [cm]	91.8 (7.1)	90.5 (5.6)	90.9 (4.8)	0.649
Right leg length [cm]	92.9 (8.0)	89.9 (11.4)	91.7 (4.9)	0.323
ABPI^	1.1 (0.2)	1.1 (0.2)	1.2 (0.1)	0.839
Monofilament score	7 (7) [ab]	18 (4)	20 (0)	<0.001
MNSI symptom score[#]	7 (1)	5 (2)	-	<0.001
MNSI physical assessment score[#]	7 (1)	2 (2)	-	<0.001

Legend: All data represents mean and standard deviation (SD) or number and percentages (%). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group HC= healthy control group. The reported p-values indicate main comparison outcomes from one-way ANOVA, Pearson's Chi squared tests or Fishers exact tests between three groups unless indicated by #= DFU vs. DMC comparison only. [a] = $p < 0.05$ vs. DMC group, [b] = $p < 0.05$ vs. HC group on post-hoc tests. A significance level of $p < 0.05$ was used throughout. Diabetes duration indicates fractions of years living with type 2 diabetes mellitus. ^ABPI values represented in the table are for ulcerated limbs of the DFU groups and the lowest reported in the control groups. Monofilament score is out of a total of 20, measured at ten sites for each foot. MNSI scores indicate the total scores from the Michigan Neuropathy Screening Instrument in relation to the neuropathy symptom score and physical assessment score. * Note that the four patients with stroke did not have a history of gait disturbance due to their stroke as the stroke only affected their speech function.

6.5.3 Kinematic outcomes

Kinematic outcomes are reported below (see Table 6.2, Figure 6.2 and in Appendix G, Supplementary File 1).

6.5.3.1 Initial strike

During initial strike, the DFU group demonstrated a significantly lower knee flexion-angle compared to the HC group (Mean difference (Δ) = -4.2 º, 95% CI [-7.3; -1.1], Cohen's d=-0.7). The DFU and the DMC groups also demonstrated a significantly greater degree of anterior pelvic tilt compared to the HC group during initial strike. The mean difference (Δ) was largest between the DFU group and the HC group (Δ=6.8º, [2.1; 11.5], d= 0.7).

6.5.3.2 Toe-off

The increased pelvic angle at initial strike was consistent with a greater anterior pelvic tilt during toe-off in the DFU group compared to the HC group (Δ=6.2 º, [1.6; 10.9], d=0.7). The DFU group also had a pelvic obliquity at toe-off that was significantly lower than the DMC and HC groups (Δ=-1.6º, [-0.2; - 3.0], d=- 0.9 vs. the DMC group, and Δ=-2.2 º [-0.8; -3.7], d=-0.7 vs. the HC group). The DFU group demonstrated a significantly externally rotated foot progression angle during toe-off compared to the HC group (Δ= -13.6º, - [23.0; 4.3], d=-0.8).

6.5.3.3 Gait cycle

The DFU group demonstrated significantly lower maximum ankle-plantar flexion during the gait cycle compared to both control groups (Δ=-3.3 º, [-0.1; -6.6], d=-0.6 vs. the DMC group and Δ=-4.8 º, [-1.5; -8.2], d=-0.8 vs. the HC group). Both the DFU and DMC groups had a total sagittal plane ankle ROM that was significantly lower than the HC group. The DFU group demonstrated a lower maximum knee flexion compared to the DMC and HC groups (Δ=-6.5 º, [-11.6;-1.4], d= -0.6 vs. the DMC group, and Δ=-9.6º, [-14.8; - 4.4], d= -1.0 vs. the HC group). The DFU group and the DMC group had a greater amount of minimum and maximum anterior pelvic tilt compared to the HC group. The difference between the DFU group and the HC group was however much greater than that between the DMC and the HC group; minimum anterior pelvic tilt (Δ=6.4º, [2.0; 10.7], d=0.7 vs.

the HC group and maximum anterior pelvic tilt Δ=6.1º, [2.0; 10.2], d= 0.7 vs. the HC group.

The minimum pelvic obliquity was reduced in the DFU group compared to both control groups and corresponded with a significantly lower total pelvic ROM in the frontal plane compared to the HC group (Δ=-3.7º, [-5.4; - 1.9], d=-0.9). The DMC group also had a significantly lower pelvic ROM compared to the HC group, although the difference was lower (Δ= -2.4º, [-3.7; - 1.2], d= -1.0).

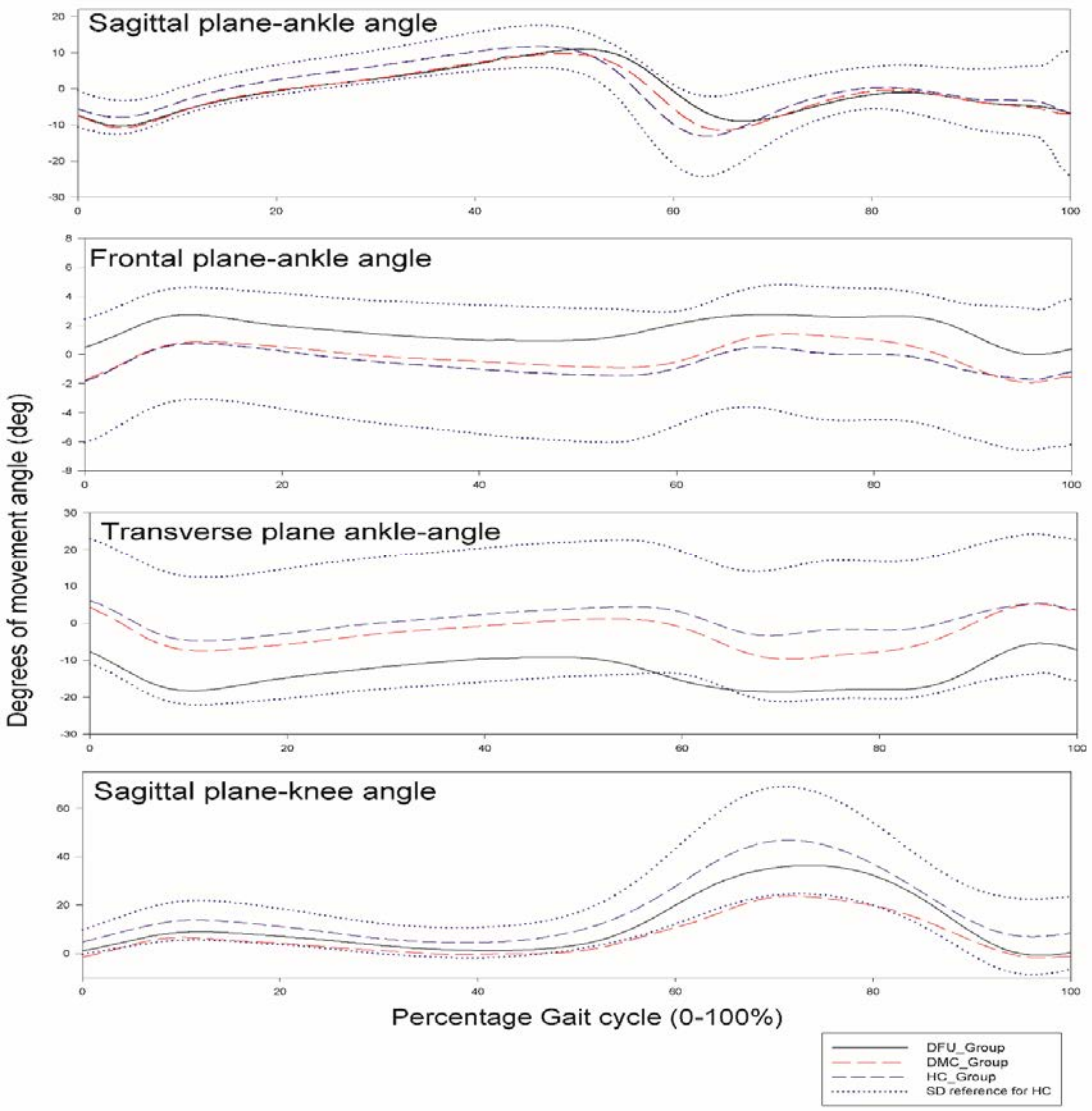
Table 6.2 Joint angles (kinematic) characteristics of participants by group.

Joint angles	DFU (n=21)	DMC (n=69)	HC (n=56)	Corrected p-value	Mean difference DFU vs. DMC [95% CI of difference]	Mean difference DFU vs. HC [95% CI of difference]	Mean difference DMC vs. HC [95% CI of difference]	Cohen's d DFU vs. DMC [95% ci]	Cohen's d DFU vs. HC [95% ci]	Cohen's d DMC vs. HC [95% ci]
Sagittal plane										
Initial strike										
Knee flexion angle (º)	2.4 (6.4) [b]	4.5 (4.4)	6.6 (5.1)	0.042	-2.1 [-5.1;1.0]	-4.2 [-7.3; -1.1] *	-2.1 [-4.3;0.0]	-	-0.7 [0.2;1.3]	-
Anterior pelvic tilt (º)	18.4 (9.5) [b]	15.4 (6.2) [b]	11.6 (8.3)	0.016	2.9 [-1.6;7.5]	6.8 [2.1;11.5] *	3.9 [0.6;7.2] *	-	0.7 [0.3;1.3]	0.6 [0.2;0.9]
Toe-off										
Anterior pelvic tilt (º)	17.6 (8.2) [b]	14.4 (6.4)	11.3 (8.4)	0.042	3.2 [-1.3;7.7]	6.2 [1.6;10.9] *	3.0 [-0.2;6.3]	-	0.7 [0.2;1.3]	-
Gait cycle characteristics										
Maximum ankle plantarflexion (º)	11.8 (4.7) [ab]	15.1 (5.2)	16.6 (5.9)	0.042	-3.3 [0.1;6.6] *	-4.8 [1.5;8.2] *	-1.5 [-0.9;3.8]	-0.6 [0.2;1.2]	-0.8 [0.3;1.4]	-

Maximum ankle dorsiflexion (º)	14.2 (5.3)	12.1 (4.3) [b]	15.4 (5.5)	0.016	2.1 [-0.9;5.1]	-1.2 [-4.2;1.9]	-3.3 [-5.4; -1.1] *	-	-	0.3 [0.0;0.6]
Total ankle rom (º)	26.6 (5.0) [b]	26.8 (5.1) [b]	31.9 (6.2)	0.002	-0.2 [-3.5;3.1]	-5.3 [-8.7; -1.9] *	-5.1 [7.5;2.7] *	-	-0.8 [-0.4; -1.4]	-0.9 [-0.0; -1.3]
Maximum knee flexion (º)	45.6 (10.3) [ab]	52.2 (8.2)	55.3 (7.9)	0.002	-6.5 [-11.6; -1.4] *	-9.6 [-14.8; -4.4] *	-3.1 [-6.8;0.6]	-0.6 [-0.2; -1.2]	-1.0 [-0.6; -1.7]	-
Minimum anterior pelvic tilt (º)	16.4 (8.5) [b]	14.1 (5.0) [b]	10.0 (8.4)	0.002	2.3 [-2.0;6.5]	6.4 [2.0;10.7] *	4.1 [1.1;7.2] *	-	0.7 [0.2;1.2]	0.7 [0.2;0.9]
Maximum anterior pelvic tilt (º)	19.7 (8.1)[b]	17.3 (5.4)[b]	13.6 (7.4)	0.002	2.4[-1.6;6.4]	6.1 [2.0;10.2]*	3.7 [0.8; 6.5]*	-	0.7 [0.3;1.3]	0.6 [0.2;0.9]
Frontal plane										
Toe-off										
Pelvic obliquity (º)	-0.8 (3.3)[ab]	2.4 (1.6)	3.0 (2.6)	0.017	-1.6 [-0.2;-3.0]*	-2.2 [-0.8;-3.7]*	-0.6 [-0.4;1.6]	-0.9 [-0.0;1.1]	-0.7 [0.3;1.3]	-
Gait cycle characteristics										
Maximum ankle valgus (º)	0.7 (3.7)[ab]	-1.3 (2.0)[b]	-3.0 (4.3)	0.002	2.0 [0.0;4.0]	3.7 [1.7;5.7]*	1.7[0.2;3.1]*	-	0.2 [0.0;0.7]	0.6 [0.1;0.8]
Maximum knee varus (º)	4.5 (3.6)[b]	3.8 (2.7)	3.6 (4.1)	0.048	5.4 [-1.8;12.5]	10.0 [2.7;17.3]*	4.6 [-0.5;9.8]	-	0.7 [0.3;1.3]	-

Minimum pelvic obliquity (º)	1.0 (2.5)[ab]	3.2 (2.0)	4.0 (3.5)	0.002	2.2 [0.6;3.9]*	3.1 [1.4;4.8]*	0.9 [-0.3;2.0]	0.9 [0.5;1.6]	0.9 [0.4;1.5]	-
Total pelvic rom (º)	4.9 (2.3)[b]	6.1 (1.6)[b]	8.5 (3.9)	0.002	-1.2 [-2.9;-0.5]	-3.7 [-5.4;-1.9]*	-2.4 [-3.7;-1.2]*	-	-0.9 [-0.5;-1.5]	-1.0 [-0.4;-1.6]
Transverse plane										
Toe-off										
Foot progression angle (º)	-11.3 (18.8)[b]	-2.8 (14.5)	2.4 (14.5)	0.040	-8.5 [-17.6;0.7]	-13.6 [-23.0;-4.3]*	-5.2 [-11.8;1.4]	-	-0.8 [-0.3;-1.4]	-

Legend: Group data represents mean and standard deviation (SD). All data values are in degrees (^{0}). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. ROM= Range of motion. Corrected p-values indicate p-values obtained after Holm correction rounded to 3 decimal places. [a]= $p<0.05$ vs. DMC on Sidak's post hoc test, [b]=$p<0.05$ vs. HC on Sidak's post hoc test. *= Remained significant after adjusting for age, sex and BMI. Binary logistic regression analyses were only performed for variables which were significant on the Sidak's post-hoc test. Cohen's d was only calculated for variables which were significantly different after adjustment. -= Cohen's d not calculated. Cohen's d was calculated for the DFU group compared to control groups (DFU – control) and for the DMC group compared to the HC group (DMC – control). Cohen's d 95% CIs were calculated using non-centrality parameters. See Appendix G, Supplementary File 1 for odds ratios and additional data. Sagittal plane movements represent dorsiflexion and plantarflexion of the ankle joint and extension and flexion of the hip and knee and anterior and posterior pelvis tilt; frontal plane movements represent abduction and adduction of the ankle, knee and hip and left lateral and right lateral rotation of the pelvis ; transverse plane movements represent inversion and eversion of the ankle, medial and lateral rotation of the knee and internal and external rotation of the hip and transverse rotations of the pelvis. All angles are reported for initial strike, toe-off and during stride.



Legend: Figure represents a graphical illustration of kinematics of the ankle and knee during walking. Only some of the significantly different planes of movement are represented due to the large amount of data. DFU represents the diabetic foot ulcer group, DMC represents the diabetes control group and HC represents the healthy control group. The x-axis represents time in reference to percentage gait cycle (0-100%) from heel strike to ipsilateral heel strike. The y-axis represents degrees range of motion. The blue dotted line demonstrates the HC group mean +/- standard deviation (SD).

Figure 6.1 Kinematics of the knee and ankle during the gait cycle.

6.5.4 Kinetic outcomes

Kinetic outcomes are reported below (see Table 6.3, Figure 6.3 and Appendix G, Supplementary File 1. The DFU and DMC groups had significantly higher F_{AP} values than the HC group. All of the differences between the DFU and the HC groups were however much larger than between the DMC and the HC groups. For example, the minimum F_{AP} was significantly higher in the DFU group compared to the DMC group (Δ=-15.2 N, [-27.5; - 2.8], d=-0.9) and compared to the HC group (Δ=-28.5 N, [-41.2; - 15.8], d=-1.5). The DFU group also demonstrated a large difference in the range of F_{AP} compared to both control groups (Δ=24.9 N, [8.4; 41.3], d=1.0 vs. the DMC group and Δ=41.9 N, [25.0; 58.8], d= 1.7 vs. the HC group) as opposed to a smaller difference between the two control groups (Δ= 17.1 N, [5.2; 28.9], d=0.7).

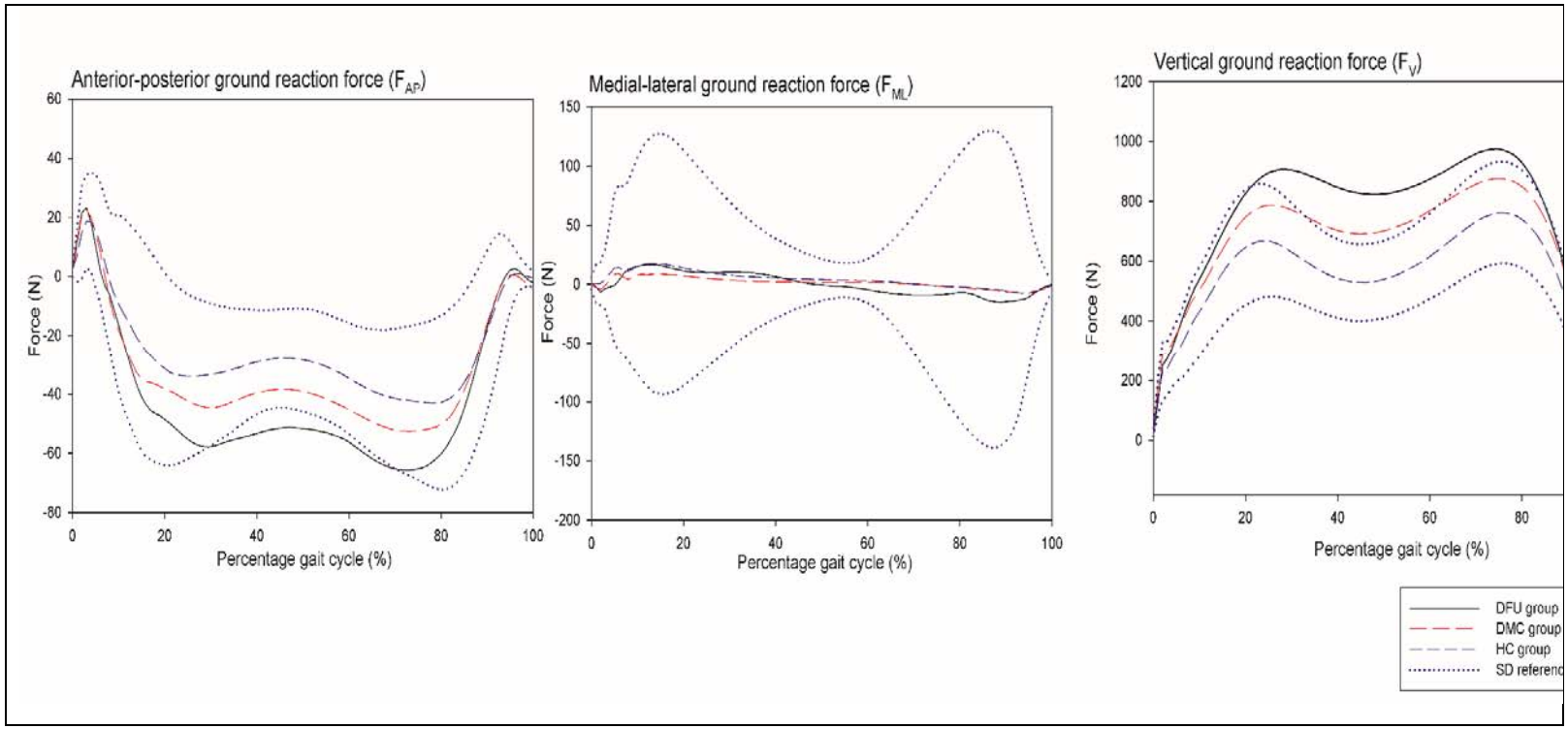
The total F_{ML} in the DFU group was significantly lower than the HC group (Δ=-4.3 N, [-8.3; -0.3], d=-0.6). The total F_{ML} in the DMC group was also significantly lower than the HC group (Δ=-3.5 N, [-6.3; - 0.7], d=-0.5). The total F_V was significantly higher in the DFU group compared to both control groups; (Δ = 89.3 N, [6.61; 71.9], d= 0.9 vs. the DMC group, and Δ = 186.3 N, [101.2; 271.2], d=1.1) vs. the HC group). There was also a large significant difference in total F_V between the DMC group and the HC group (Δ =97.0 N, [37.3; 156.7], d=0.7).

Table 6.3 Kinetic (force) characteristics of participants by group.

Ground reaction forces (Newtons N)	DFU (n=21)	DMC (n=69)	HC (n=56)	Corrected p-value	Mean difference DFU vs. DMC [95% CI of difference]	Mean difference DFU vs. HC [95% CI of difference]	Mean difference DMC vs. HC [95% CI of difference]	Cohen's d DFU vs. DMC [95% CI]	Cohen's d DFU vs. HC [95% CI]	Cohen's d DMC vs. HC [95% CI]
Anterior-posterior ground reaction force F_{AP}										
Min	-77.3 (31.9)[ab]	-62.1 (17.6)[b]	-48.7 (18.5)	0.001	-15.2 [-27.5;-2.8]*	-28.5 [-41.2;-15.8]*	-13.4 [-22.3;-4.5]*	-0.9 [-0.0;-1.0]	1.5 [0.4;1.6]	0.7 [0.4;1.1]
Max	33.0 (18.1)[b]	28.7 (12.4)[b]	21.1 (9.0)	0.001	4.3 [-3.1;11.7]	11.9 [4.3;19.5]*	7.6 [2.3;12.9]*	-	1.2 [0.2;1.3]	0.7 [0.3;1.0]
Range	114.4 (43.7)[ab]	89.5 (23.1)[b]	72.5 (24.2)	0.001	24.9 [8.4;41.3]*	41.9 [25.0;58.8]*	17.1 [5.2;28.9]*	1.0 [0.1;1.1]	1.7 [0.5;1.6]	0.7 [0.4;1.1]
Total	-35.8 (17.8)[b]	-32.8 (10.8)[b]	-26.3 (10.5)	0.024	-3.0 [-10.2;4.2]	-9.4 [-16.8;-2.0]*	-6.5 [-11.7;-1.3]*	-	0.9 [0.1;1.1]	0.6 [0.2;1.0]
Medial-lateral ground reaction force F_{ML}										
Total	0.1 (6.3)[b]	0.9 (6.2)[b]	4.4 (6.7)	0.044	-0.8 [-4.7;3.1]	-4.3 [-8.3;-0.3]*	-3.5 [-6.3;-0.7]*	-	-0.6 [-0.1;-0.2]	-0.5 [-0.2;-0.9]

Vertical ground reaction force Fv											
	Final	25.1 (1.2) [a]	24.5 (0.9) [b]	24.9 (0.5)	0.044	0.6 [0.1;1.1]	0.2 [-0.3;-0.7]	-0.4 [-0.8;0.0]*	1.1 [0.7;1.7]	-	-0.6 [-0.2;-0.9]
	Max	1019.4 (241.2)[b]	914.9 (154.7) [b]	802.7 (195.9)	0.001	104.5 [-7.0;215.9]	216.7 [102.2;331.1]*	112.2 [31.8;192.7]*	-	0.9 [0.5;1.6]	0.6 [0.3;1.0]
	Range	995.1 (241.2)[b]	882.5 (163.3) [b]	772.2 (199.9)	0.001	112.6 [-1.9;227.2]	222.9 [105.3;340.5]*	110.3 [27.6;193.0]*	-	1.0 [0.5;1.6]	0.6 [0.2;1.0]
	Total	736.4 (174.4)[ab]	647.1 (112.4) [b]	550.1 (149.4)	0.001	89.3 [6.6 1;7 1.9]*	186.3 [101.4;271.2]*	97.0 [37.3;156.7]*	0.9 [0.0;1.1]	1.1 [0.7;1.7]	0.7 [0.4;1.1]

Legend: Group data represents mean and standard deviation (SD). All data values are in Newtons (N). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. Corrected p-values indicate p-values obtained after Holm correction rounded to 3 decimal places. [a]= $p<0.05$ vs. DMC on Sidak's post hoc test, [b]=$p<0.05$ vs. HC on Sidak's post hoc test. *= Remained significant after adjusting for age, gender and BMI. Binary logistic regression analyses were only performed for variables which were significant on the Sidak's post-hoc test. Cohen's d was only calculated for variables which were significantly different after adjustment = Cohen's d not calculated. Cohen's d was calculated for the DFU group compared to control groups (DFU – control) and for the DMC group compared to the HC group (DMC – control). Cohen's d 95% CIs were calculated using non-centrality parameters See Appendix G, Supplementary File 1 for odds ratios and additional data. The ground reaction forces in the anterior-posterior, medial-lateral and vertical directions are reported. Initial force indicates the force at initial contact of the ground, final force indicates the force before the limb leaves the ground, the minimal and maximum forces represent the minimal and maximal recorded values during astride and the range and total indicates the range and total force during a stride.



Legend: Figure represents a graphical illustration of the ground reaction forces for the three groups during the gait cycle. DFU= diabetes foot ulcer group DMC= diabetes control group HC= healthy control group. Forces represent the anterior-posterior ground reaction force (F_{AP}), the medial-lateral ground reaction force (F_{ML}) and the vertical ground reaction force (F_V). The x-axis represents time in reference to percentage gait cycle (0-100%) from heel strike to ipsilateral heel strike. The y-axis represents force in Newtons (N). The blue dotted line demonstrates the HC group mean +/- standard deviation (SD).

Figure 6.2 Kinetics during the gait cycle.

6.5.5 Temporal-spatial parameters

The TSPs are reported below (see Table 6.4 and Appendix G, Supplementary File 1).

The cadence of the DFU group was significantly less compared to the HC group (Δ =-8.2 steps/min, [-13.7; - 2.6], d=-1.0). The walking speed was significantly slower in the DFU group compared to both control groups (Δ =-0.1 m/s, [-0.2;0.0], d=-0.5 vs. the DMC group, and Δ =-0.2 m/s, [-0.3; - 0.1], d=-1.6 vs. the HC group). The step length was also significantly smaller in the DFU group compared to both control groups (Δ =-0.1 m, [-0.1; -0.0], d=-0.5 vs. the DMC group and Δ =-0.1 m, [-0.1; 0.0], d=-1.6 vs. the HC group). The stride length was significantly smaller and the opposite foot off time was significantly longer in both the DFU group and the DMC group when compared to the HC group (see Table 6.4)

Table 6.4 TSPs of participants by group.

Variable	DFU (n=21)	DMC (n=69)	HC (n=56)	Corrected p-value	Mean difference DFU vs. DMC [95% CI of difference]	Mean difference DFU vs. HC [95% CI of difference]	Mean difference DMC vs. HC [95% CI of difference]	Cohen′s d DFU vs DMC [95% CI]	Cohen′s d DFU vs. HC [95% CI]	Cohen′s d DMC vs. HC [95% CI]
Cadence (steps/min)	105.7 (7.4) [b]	109.9 (10.2) [b]	113.9 (7.8)	0.007	-4.2 [-9.6;1.3]	-8.2 [-13.7; -2.6] *	-4.0 [-7.9; -1.0]	-	-1.0 [-0.5; -1.6]	
Walking speed (m/s)	0.9 (0.2) [ab]	1.1 (0.2)	1.2 (0.2)	0.001	-0.1[-0.2;0.0] *	-0.2 [-0.3; -0.1] *	-0.1[-0.2;0.0]	-0.5 [-0.1; -1.1]	-1.6 [-0.5; -1.7]	-
Opposite foot-off-time (s)	12.3 (2.2) [b]	11.9 (2.3) [b]	10.4 (1.5)	0.001	0.4 [-0.8;1.7]	1.9 [0.7;3.2] *	1.5 [0.6;2.4] *	-	1.0 [0.6;1.6]	0.8 [0.4;1.1]
Stride length (m)	1.1 (0.2) [b]	1.1 (0.2) [b]	1.3 (0.1)	0.001	-0.1 [-0.2;0.0]	-0.2 [-0.3; -0.1] *	-0.1 [-0.2; -0.0] *	-	-1.7 [-0.5; -1.7]	-0.8 [-0.4; -1.1]
Step length (m)	0.5 (0.1) [ab]	0.6 (0.1) [b]	0.6 (0.1)	0.001	-0.1 [-0.1;0.0] *	-0.1 [-0.1; -0.0] *	0.0 [-0.1;0.0] *	-0.5 [-0.1; -1.1]	-1.6 [-0.5; -1.7]	0.5 [0.1;0.8]

Legend: Group data represents mean and standard deviation (SD). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. Corrected p-values indicate p-values obtained after Holm correction rounded to 3decimal places. [a]= $p<0.05$ vs. DMC on Sidak's post hoc test, [b]=$p<0.05$ vs. HC on Sidak's post hoc test. *= Remained significant after adjusting for age, gender and BMI. Binary logistic regression analyses were only performed for variables which were significant on the Sidak's post-hoc test. Cohen's d was only calculated for variables which were significantly different after adjustment. -= Cohen's d not calculated. Cohen's d was calculated for the DFU group compared to control groups (DFU – control) and for the DMC compared to the HC group (DMC – control). Cohen's d 95% CIs were calculated using non-centrality parameters. See Appendix G, Supplementary File 1 for odds ratios and additional data.

6.6 Discussion

The focus of this case-control study was to identify key differences in the gait of participants with active plantar neuropathic DFUs compared to controls. To our knowledge, this is the first prospective case-control study to assess a comprehensive suite of gait parameters in cases with active DFUs compared with controls. The findings of this study suggest that participants with DFUs have several different kinematic, kinetic and TSP characteristics compared to controls. Several gait parameters were markedly different to previous studies in people with a history of DFUs and in people with DPN without DFUs. We also noted several differences in these characteristics between the DMC and HC groups which have been the focus of a number of previous studies (Akashi et al., 2008; Sacco, Akashi, & Hennig, 2009a; Sacco & Amadio, 2000; Sacco et al., 2009b; Savelberg et al., 2009a; Yavuzer et al., 2006). The distinct gait patterns of patients with DFUs have a number of implications. It may be representative of why people initially developed an ulcer and may also detrimentally influence ulcer healing. Alternatively, the distinct gait may represent a response to the ulcer itself.

The ankle joint is the major joint for controlling sagittal plane movements of the leg relative to the foot and is essential for bipedal ambulation. A reduced amount of plantar flexion in cases would have a significant impact on the ability to move and propel the ulcerated limb off the floor during gait. A significantly higher foot progression angle was observed in our cases during toe-off. The foot progression angle is the angle made by the ankle in the transverse plane with respect to the direction of progression of the foot during gait (Gelber, Isaac, Bohnert, Strube, & Sinacore, 2010; Merriwether, 2014). Previous research suggests that the foot progression angle is a predictor of elevated regional plantar pressure and a proxy measure of dermal injury risk in people with DPN and forefoot DFUs (Gelber et al., 2010). Similarly, a reduction in knee flexion has previously been identified in people with a history of DFUs (Katoulis et al., 1997). The combination of reduced ankle plantar flexion and knee flexion would significantly restrict the freedom by which the ulcerated limb can be propelled effectively after striking the ground in the sagittal plane.

Interestingly, we were unable to demonstrate a reduced amount of dorsiflexion in people with active DFUs (Fernando et al., 1991). This is in line with findings from another study that failed to find the presence of limited ankle dorsiflexion in people with a history of DFUs (Raspovic & Douglas, 2011).

We failed to find any significant differences in hip angular kinematics in patients with DFUs compared to controls, in contrast to previously reported findings (Raspovic, 2013). We did however find an increased amount of pelvic tilt throughout the gait cycle in patients with DFUs compared to controls in addition to a downward pelvic obliquity and reduced total pelvic ROM. The gait parameters of the pelvis are complex as the pelvis progresses the co-ordination between limbs and between the trunk and limbs during gait (Michaud, Gard, & Childress, 2000). An increased pelvic tilt is a known mechanism by which sufficient torque can be generated more proximally to overcome limited torque generation at joints such as the hip (Wilder, Cole, Sobel, & O'Connor, 1994). Whether this is an adaptive gait strategy associated with DPN or due to restrictive movement is not clear from our data. Overall, the kinematic differences observed may lead to an increased risk of falls (Cavanagh et al., 1993). An increased falls risk has already been reported in people with DPN due to postural sway and poor balance (Menz, Lord, St George, & Fitzpatrick, 2004; Richardson & Hurvitz, 1995; Schwartz et al., 2002). It is highly likely that the risk of falls will be further augmented by reduced ankle and knee ROM in people with DFUs (Kanade, Van Deursen, Harding, & Price, 2008).

With regard to ground reaction force, our findings suggest the high anterior-posterior (F_{AP}) and vertical (F_V) ground reaction forces observed in the DMC group were even greater in the DFU group. The presence of high F_{AP} and F_V ground reaction forces adds to previous evidence that the mechanical loading on the ulcerated limb is substantially increased during gait (Cavanagh et al., 1993; Cavanagh et al., 2000; Veves et al., 1992). Likewise, a high F_V has previously been reported in several studies investigating the gait of participants with DPN (Saura et al., 2010; Shaw, Van Schie, Carrington, Abbott, & Boulton, 1998; Uccioli et al., 2001). In contrast, a previous study by Katoulis and colleagues reported that people with a history of DFUs had lower F_V and F_{AP} values compared to people with DPN without a DFU history and to healthy controls (Katoulis et al., 1997). The reduced ground

reaction forces were attributed to reduced walking speed in people with DPN. The different findings in the current study may be due to several factors. We studied people with active DFUs whereas Katoulis and colleagues studied people with healed DFUs (Katoulis et al., 1997). Katoulis and colleagues used a force sampling frequency of 50 Hz whereas we used a more sensitive sampling frequency of 3000 Hz. Therefore, the true magnitude of the peak force may have been overlooked. Katoulis and colleagues also encouraged participants to use their own shoes while their gait was being assessed. Shoes that were specifically designed to reduce peak force for example may have been worn by people with a history of DFUs, thereby reducing the peak force-time profile.

The medial-lateral (F_{ML}) ground reaction force was not significantly higher in our cases, as previously reported by Katoulis and colleagues in people with a history of DFUs (Katoulis et al., 1997). The F_{AP} and F_{ML} ground reaction forces represent the non-vertical force component acting directly in contact with the foot during gait. These may be important in understanding shear stress acting on the ulcerated foot (Yavuz, 2014; Yavuz, Botek, & Davis, 2007a; Yavuz et al., 2007b). Shear can be termed a mechanical force that acts on an area of skin in a direction parallel to the body's surface (i.e. horizontally) (Yavuz, 2014). There is a distinction between shear pressure and shear forces. We measured shear force in this study. Shear may be influenced by the amount of pressure exerted, the surface on which the foot lands and the extent to which the body makes contact with the ground (Yavuz, Tajaddini, Botek, & Davis, 2008). We found that the shear forces in the anterior-posterior direction during gait in people with DFUs were significantly higher than in controls. There have been very few studies investigating shear pressures acting on the neuropathic foot due to the difficulty in measuring this with commercially available platforms (Armstrong et al., 1998c; Yavuz, 2014). Recent investigations suggest that plantar shear pressure is an important factor in predicting plantar DFU (Yavuz, 2014).

In a previous systematic review, we suggested that people with DPN had a longer stance time during gait (Fernando et al., 2013). Data from the current study suggests that stance time is not different in cases with DFUs compared to controls. People with DFUs however walk slower and have a significantly smaller step length compared to diabetes and healthy controls. Interestingly, a longer opposite foot-off time was noted in both the DMC and the

DFU groups. A smaller step-length has previously been suggested to be a protective factor in people with DPN (Mueller, Sinacore, Hoogstrate, & Daly, 1994b) and active DFUs (Kanade et al., 2006). As supported by our findings, it is likely that a slower gait speed and smaller step-length are both protective adaptations to a proprioceptive deficit as both would assist in a more controlled-gait (Katoulis et al., 1997). The presence of protective gait factors questions the traditional viewpoint that patients with DFUs are unable to adjust their mobility patterns to protect plantar ulcers due to sensory and motor DPN unlike people with adequate protective sensation (Cavanagh et al., 1993; Cavanagh et al., 2000). Instead our findings suggest that a guarded gait strategy may be adopted in people with active DFUs (Fernando et al., 2014b). Previous studies have reported an increased peak plantar pressure in the contralateral limb in people with a unilateral foot ulcer (Kanade et al., 2006). This suggests that there may be a shift of weight to the non-ulcerated limb in people with DFUs.

There are some findings in our study which support the presence of adaptive gait patterns to protect the ulcerated foot. These include a smaller step length, increased opposite foot-off time, reduced plantar flexion, knee flexion and a lack of difference in ankle dorsiflexion compared to controls. The lower pelvic obliquity of the DFU group at toe-off which is in the opposite direction to the controls suggest a frontal plane transfer of force away from the ulcerated limb to the contralateral limb. These findings suggest that these may be protective ways to prevent excessive loading of the ulcerated limb. The presence of a guarded gait strategy which potentially reduces plantar pressure may suggest an explanation for why we failed to identify high plantar pressures in people with active DFUs in a previous meta-analysis of observational studies (Fernando et al., 2014b). While the presence of the proposed protective gait parameters may influence ulcer healing by reducing the mechanical load placed on the ulcerated foot, these gait parameters may detrimentally impact gait function as outlined above. The presence of high anterior-posterior ($F_{AP)}$ and vertical (F_V) ground reaction forces however suggest an inadequacy in the guarded gait strategy of people with DFUs and may significantly deter ulcer healing.

There is an important relationship between kinematic and kinetic outcomes during the gait cycle. The presence of higher ground reaction forces in the vertical and anterior-posterior

directions in our study in the presence of a slower walking speed was an unexpected finding. Although we did not evaluate joint moments or muscle activity, the combination of reduced ankle plantar flexion and knee flexion with a slower walking speed and smaller step length highlights that the generation of internal joint moments by the action of muscles is likely significantly impaired in our cohort due to the presence of long standing DPN. Usually as walking speed increases, ground reaction forces and the amount of knee flexion are also expected to increase, although our findings indicate there is an increase in ground reaction forces and a slower walking speed without an increase in knee flexion or ankle plantarflexion. Therefore, it is likely that there is an imbalance between the external and internal moments being generated during gait. External moments are generated by gravitational forces and internal moments are generated by muscles and bones and tendons. It is likely that the relationship between kinematics and kinetics in our cohort is complex. Therefore, we suggest the altered kinematics in cases may potentially have a causal effect on the kinetics. Further studies of gait outcomes including joint moments and muscle activity in people with DFUs would provide further insight on these outcomes in this population.

This study had a number of limitations. This includes the gait analysis system we used which is reliant on the accuracy of the knee flexion-extension axis location based on marker position (Baudet et al., 2014). There are a variety of methods to obtain gait data. Our results are specific to plug in gait model and methodology used to obtain kinematic data. We made use of the movement analysis system and associated procedure which was familiar to us and which was readily available at the time this study was undertaken and assessed measurement reproducibility beforehand. It may be argued that the ABPI cutoff of >0.8 we used was inadequate to exclude peripheral arterial disease altogether. A clinical review of cases by an experienced vascular surgeon was also undertaken. We are therefore confident that clinically significant peripheral arterial disease, which is known to detrimentally influence gait, was excluded in all our participants (Crowther, Spinks, Leicht, Quigley, & Golledge, 2007). The severity of DPN in our DFU group was much worse than that present in the DMC group. As DPN is known to promote ulcer development, it was not possible in our study to match cases and controls based on the severity of DPN. The gait parameters observed in the DFU group may be due to the impact of long-term DPN

alone. It may also be argued that cases with DFUs who are normally encouraged to wear protective footwear may have decisively adjusted their gait during barefoot assessment. Hence whether our findings represent the actual everyday gait of cases with DFUs or their potential walking ability is unclear.

This study had a number of strengths. We published our protocol (Fernando et al., 2015) and carried out reproducibility studies prior to data collection to optimize the methods used in this study (Fernando et al., 2016a). The reproducibility study reported good repeatability for all three-dimensional gait parameters measured in this study and hence we are confident that operator-error was minimized during the process of data collection (Fernando et al., 2016a). We carried out binary logistic regression analyses to adjust for age, sex and BMI differences between groups. The majority of biomechanical outcomes remained significantly different after adjustment. We restricted the number of factors which we adjusted for based on statistical power and on likely confounders. These factors are often overlooked in biomechanical studies (Knudson, 2009).

6.7 Conclusions

People with DFUs demonstrated considerably different gait to controls. The distinguishing gait parameters in cases included restrictions in ankle plantar flexion and knee flexion; a greater amount of anterior pelvic tilt; reduced pelvic obliquity on the ulcerated side; higher vertical and horizontal ground reaction forces acting on the ulcerated foot and slower walking speeds with smaller step lengths. Currently it is unclear whether these characteristics have any integral relationship to ulcer healing.

Chapter. 7 Plantar Pressures are Higher in Cases with Diabetes-Related Foot Ulcers Compared to Controls, Despite a Longer Stance Phase Duration

This chapter has been adapted from a publication titled;

Plantar pressures are higher in cases with diabetic foot ulcers compared to controls despite a longer stance phase duration

Authors: Malindu Eranga Fernando, Robert George Crowther, Peter Anthony Lazzarini, Kunwarjit Singh Sangla, Scott Wearing, Petra Buttner, Jonathan Golledge

BMC Endocrine Disorders (2016) 16:51

7.1 Preface

The previous chapter reported on the first prospective case-control study arising from the work contained in this thesis. Hence Chapter 6 reported on key gait characteristics in people with active plantar DFUs when compared to healthy and diabetes controls without DFUs. The following chapter now reports on the plantar pressure characteristics in the same cohort and is a continuation of this case-control study which aimed to answer Question 4 from Chapter 1. Hence the aim of the study reported below was to determine the plantar pressures in people with DPN and active DFUs when compared to diabetes controls and healthy controls without DFUs. This study was performed in response to findings reported in Chapter 3 from a meta-analysis in a pooled cohort of cases with DFUs which failed to identify any statistically significant difference in plantar pressures (mpp and pti) between people with active DFUs and diabetes controls.

7.2 Abstract

Background: Current international guidelines advocate achieving at least a 30% reduction in maximum plantar pressure to reduce the risk of foot ulcers in people with diabetes.

However, whether plantar pressures differ in cases with foot ulcers to controls without ulcers is not clear. The aim of this study was to assess if plantar pressures were higher in patients with active plantar diabetic foot ulcers (cases) compared to patients with diabetes without a foot ulcer history (diabetes controls) and people without diabetes or a foot ulcer history (healthy controls).

Methods: Twenty-one cases with diabetic foot ulcers, 69 diabetes controls and 56 healthy controls were recruited for this case-control study. Plantar pressures at ten sites on both feet and stance phase duration were measured using a pre-established protocol. Primary outcomes were mean peak plantar pressure, pressure-time integral and stance phase duration. Non-parametric analyses were used with Holm's correction to correct for multiple testing. Binary logistic regression models were used to adjust outcomes for age, sex and body mass index. Median differences with 95% confidence intervals and Cohen's d values (standardised mean difference) were reported for all significant outcomes.

Results: The majority of ulcers were located on the plantar surface of the hallux and toes. When adjusted for age, sex and body mass index, the mean peak plantar pressure and pressure-time integral of toes and the mid-foot were significantly higher in cases compared to diabetes and healthy controls ($p<0.05$). The stance phase duration was also significantly higher in cases compared to both control groups $(p<0.05)$. The main limitations of the study were the small number of cases studied and the inability to adjust analyses for multiple factors.

Conclusions: This study shows that plantar pressures are higher in cases with active diabetic foot ulcers despite having a longer stance phase duration which would be expected to lower plantar pressure. Whether plantar pressure changes can predict ulcer healing should be the focus of future research. These results highlight the importance of offloading feet during active ulceration in addition to before ulceration.

7.3 Background

A large number of studies have suggested that plantar pressures are high in people with diabetic peripheral neuropathy (DPN) and in people with a history of diabetic foot ulcers (DFUs) (Armstrong et al., 1998c; Boulton et al., 1983; Ctercteko, Dhanendran, Hutton, & Le Quesne, 1981; Fernando et al., 2014b; Frykberg et al., 1998; Hafer et al., 2013; Hokkam, 2009; Kanade et al., 2006; Pham et al., 2000; Stokes, Faris, & Hutton, 1975; Veves, Murray, Young, & Boulton, 1992; Waaijman & Bus, 2012; Wrobel & Najafi, 2010). It has been proposed that high plantar pressure predispose people with DPN to develop DFUs (Bus et al., 2016a; Cavanagh, Sims Jr, & Sanders, 1991b; Sauseng et al., 1999). Hence current international guidelines advocate achieving at least a 30% reduction in maximum plantar pressure to reduce the risk of developing DFUs (Bus et al., 2016a). While it is accepted that high plantar pressures in people with DPN lead to DFUs and remain high following DFUs, it is not known if plantar pressures are elevated at the time of active DFUs (Fernando et al., 2014b). To complicate the matter, the few studies which have investigated barefoot plantar pressure in people with active DFUs have major inconsistencies in the populations studied and reported results (Armstrong et al., 1998c; Brash et al., 1996; Cavanagh et al., 1991; Kanade et al., 2006; Sauseng et al., 1999; Stokes et al., 1975). Several studies have investigated heterogeneous cohorts of people either with a history of DFUs or with an active DFU (Armstrong et al., 1998c; Brash et al., 1996; Stokes et al., 1975), whilst another study only investigated male patients (Cavanagh et al., 1991). In addition, some studies have reported plantar pressures in a limited number of sites (Kanade et al., 2006; Sauseng et al., 1999) or alternatively reported aggregated plantar pressure from multiple sites (Boulton et al., 1983; Brash et al., 1996). These inconsistent approaches make it difficult to interpret whether plantar pressures are actually elevated in people with active plantar DFUs. (Fernando et al., 2016a; Wearing et al., 1999)

In a recent meta-analysis of observational studies, we reported that plantar pressures were not significantly different in people with active DFUs compared to controls with DPN without active DFUs (Fernando et al., 2014b). The result from this analysis may have been due to a lack of statistical power to detect a true difference, or due to the fact that plantar pressures are not significantly different in cases with active DFUs compared to controls

DFUs (Fernando et al., 2014b). This prospective study investigated a homogenous cohort of people with active DFUs and assessed a number of plantar pressure measures recorded at multiple sites on the plantar surface of both feet. We hypothesised that cases with active DFUs would have higher magnitudes and durations of plantar pressure compared to controls (Fernando et al., 2014b; Kanade et al., 2006). Therefore, the aim of this study was to assess whether plantar pressures were higher in patients with active unilateral plantar DFUs of >3 months duration (cases) compared to patients without a foot ulcer history (diabetes controls) and patients without a diabetes or foot ulcer history (healthy controls).

7.4 Methods

7.4.1 Study design and criteria for inclusion

The full protocol for this study is published elsewhere (Fernando et al., 2015). Cases with type-2 diabetes mellitus and DFUs (DFU group), type-2 diabetes controls (DMC group) and healthy controls (HC group) were recruited for this case-control study (Fernando et al., 2015) (see Chapter 4).

7.4.2 Sample size calculation and case-control matching

Utilising previous research in patients with DPN without foot ulcers (Savelberg et al., 2009b), we estimated that 28, 112 and 56 participants were required in the DFU, the DMC and the HC groups, respectively. This was determined using a one-way analysis of variance (ANOVA) with 80% power, an overall significance of 0.05 adjusted for multiple tests (maximum of 8) to detect a 20% difference in forefoot plantar pressure, and a ratio of 1 DFU cases: 4 DMC controls: 2 HCs (see Chapter 4).

7.4.3 Anthropometric and clinical assessments

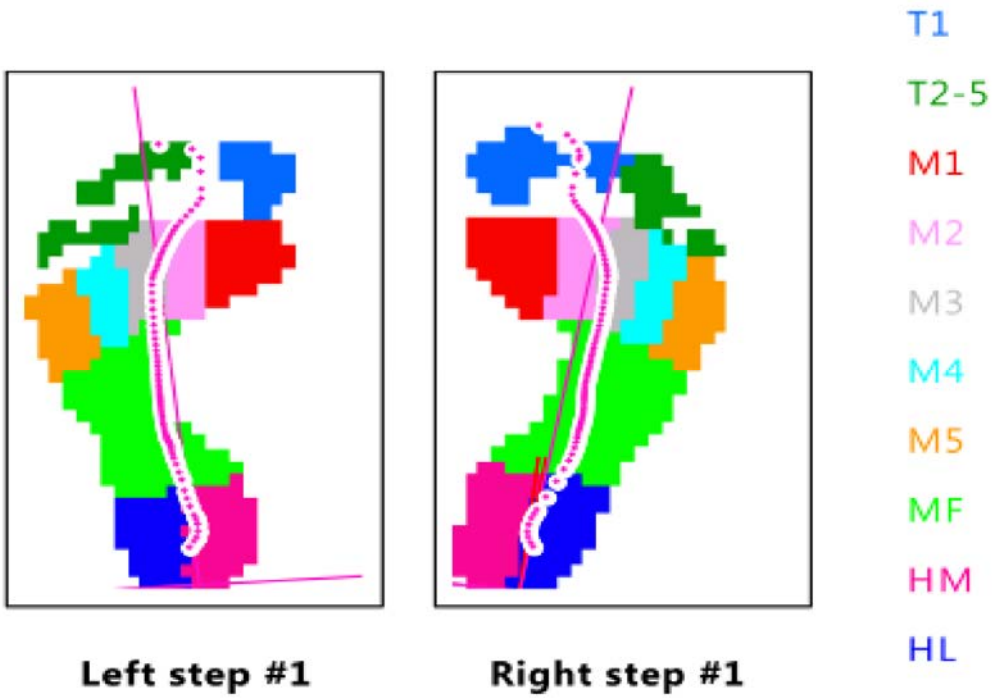
All anthropometric and clinical measurements were performed according to previously published protocols (Fernando et al., 2015) (see Chapter 4) and as described in the previous Chapter (see Chapter 6). The same assessor (MEF) carried out all assessments. Good-to-excellent reproducibility (concordance correlation coefficients between 0.999

[95% Confidence Interval (CI): (0.999-0.999)] and 0.998 [95% CI: (0.995-0.999)]) have been reported for all measurements (Fernando et al., 2016a) (see Chapter 5).

Foot structure and the presence of orthopedic foot abnormalities were assessed in all participants by a trained podiatrist (MEF). Lesser toe deformities, foot abnormalities and arch contours of the feet along with presence and grade of hallux abducto valgus (HAV) deformity were assessed (Garrow et al., 2001), utilising a set protocol and recognized standards of assessment as described previously (Fernando et al., 2015). An extensive account of the methodology used to assess ABPIs, the monofilament score, Michigan Neuropathy Symptom Score (MNSI), Physical Assessment Score and hematological markers, specifically glycated hemoglobin A1C) (HbA1C) and estimated glomerular filtration rate (eGFR) is reported in our published protocol (Fernando et al., 2015) (see Chapter 4).

7.4.4 Assessment of plantar pressure and stance phase duration

A Footscan® pressure plate (RSScan International, Olen, Belgium) was used for plantar pressure assessment. This plate was 2 m in length, 0.4 m in width and contained 16384 sensors, with individual sensor dimensions of 0.0076 m x 0.0051 m. All pressure data were captured at a rate of 100 Hz. A detailed account of the methodology used to capture plantar pressure data is reported in the study protocol (Fernando et al., 2015) (see Chapter 4). A graphical illustration of the anatomical masks (sites of plantar pressure collection) can be seen below (see Figure 7.1).



Legend: T1 = toe 1, T2-5 = toes 2 to 5, M1 = metatarsal 1, M2 = metatarsal 2, M3 = metatarsal 3, M4 = metatarsal 4, M5 = metatarsal 5, MF = mid-foot, HM = medial heel, HL = lateral heel

Figure 7.1 Example of allocation of masks to plantar sites.

The mpp and pti and stance phase duration were considered as primary outcome measures (see Chapter 4 and Chapter 5). The maximum sensor pressure (msp) and contact area (ca) were considered as secondary outcomes. These measurements were reported by the software for the left and right foot for each participant during barefoot gait (Fernando et al., 2015). We have previously reported the reproducibility of plantar pressure assessments (Fernando et al., 2016a). Mpp and pti measurements assessed at most anatomical locations typically resulted in coefficients of variations (cvs) below 30% and were more reproducible than msp measurements (Fernando et al., 2016a) (see Chapter 5).

7.4.5 Statistical Analysis

SPSS 20.0 for Windows (SPSS Inc., Illinois, United States of America) was used for statistical analyses. Non-parametric analyses were selected as the majority of continuous

data were not normally distributed. A p-value of <0.05 was used throughout as an indicator of statistical significance. Descriptive statistics were reported by groups for continuous and categorical variables and consisted of sample size, median and interquartile range [IQR] or numbers and percentages (%). These were statistically compared between the three patient groups initially, followed by *post hoc* (between two-group) tests. Diabetes related outcomes (HbA1c, diabetes duration, overall monofilament score, MSNI scores, eGFR and insulin use) were only compared between the DFU and DMC groups. We used the Kruskal-Wallis test and the Mann-Whitney U test or the Pearson's chi-square tests. We only used the Fishers exact test, if expected frequencies were less than or equal to five for categorical outcomes.

To test our main hypothesis, the plantar pressure data for the ulcerated foot in cases were compared to the average plantar pressures from the left and right foot in the appropriate control group. Initial comparisons of the primary outcomes (mpp, pti and stance phase duration) and secondary outcomes (contact area and msp) were performed using the Kruskal-Wallis test, followed by *post hoc* comparisons using the Mann-Whitney U test. We also carried out paired analyses between the relevant descriptive factors and outcome measures of the ulcerated and non-ulcerated feet of the DFU group using the Wilcoxon Signed Rank and McNemar's tests. As multiple outcomes were tested in this study, we corrected the p-values from primary and secondary outcome test results using the Holm step-wise correction (Ludbrook, 1998). Stance phase duration was not corrected. Between two-group comparisons were reported as estimated differences in the median (Δ) with 95% CIs using Hodges-Lehmann estimates from ranks (Hodges & Lehmann, 1963).

Binary logistic regression analyses were used to adjust all significant post-hoc comparison outcomes for age, sex and BMI. Odds Ratios (ORs) and 95% CIs of binary logistic regression results were computed; significant differences were flagged and reported in in Appendix H. Only outcome data which was significant after correction and adjustment were reported in the results section of the paper and within the main data Tables. All other data (including secondary outcome data) were reported in Appendix H. In addition to estimated differences in the median, Cohen's d (standardised mean difference) was calculated for all variables which remained significant after adjustment and correction, to

assess their effect size using a revised formula for skewed data: Cohen's *d = median 1-median 2 / pooled IQR* (Cohen, 1988). Effect-size magnitudes were used to estimate the degree of difference (Hopkins, 2002). The size of the difference was graded based on Cohen's d as: <0.10 trivial difference; 0.10-0.20 small difference; 0.20-0.60 medium difference; 0.60-1.20 large difference and ≥1.20 a very large difference (Hopkins, 2002).

7.5 Results

7.5.1 Participant recruitment and statistical power

From 208 participants that were screened for the study, 146 were recruited. This included 21 in the DFU group, 69 in the DMC group and 56 in the HC group. See Chapter 6 for a more detailed account of participant recruitment and reasons for exclusion.

7.5.2 Demographic, clinical and foot characteristics

The demographic and clinical characteristics of the participants are displayed in Table 7.1 and are consistent with the characteristics reported in Table 6.1 as it described the same cohort. The DMC group was older and had more males than the HC group *(all $p<0.05$)*. The two diabetes groups had a greater body mass and BMI compared to the HC group *(all $p<0.05$)*. The DFU group also had longer diabetes duration, more insulin use, lower eGFR and higher MNSI neuropathy scores than the DMC group (*all $p < 0.05$*).

Table 7.1 Clinical and demographic characteristics of the study cohort by group.

	DFU Group (n=21)	DMC Group (n=69)	HC Group (n=56)	P value
Age (years)	66.0 [52.0-72.0]	63.0 [58.0-72.0] [b]	56.0 [55.0-73.0]	*P=0.005*
Males [number and %]	15 (71.4%)[b]	46 (66.7%)[b]	24 (42.9%)	*P=0.011*
Ethnicity [number and %]				
Caucasian	20 (95.2%)	65 (94.2%)	54 (96.4%)	*P=0.660*
Australian Aboriginal/Indigenous/ Torres-strait Islander	1 (4.8%)	2 (2.9%)	2 (3.6%)	
Other	-	2 (2.9%)	-	
Diabetes duration [years]	17.00 [14.5-20.5]	7.5 [4.0-16.5]	-	*P=0.008*
Height (cm)	175.1 [164.8-179.0]	170.0 [163.0-177.5]	170.0 [164.0-174.3]	*P=0.199*
Body mass (kg)	99.6 [82.3-125.1] [b]	92.3 [80.1-100.7] [b]	72.9 [64.1-81.6]	*P<0.001*
BMI (Body Mass Index)	32.3 [27.0-37.9[b]	31.0 [29.0-33.4] [b]	25.8 [23.0-29.3]	*P<0.001*
Waist to Hip Ratio	1.0 [0.9-1.1] [b]	0.9 [0.9-1.0]	0.9 [0.9-0.9]	*P=0.099*
Hba1c (mmol/l)	55.5 [46.7-66.5]	51.0 [44.0-61.0]	-	*P=0.514*
Uses Insulin [number and %]	13 (61.9%)	19 (27.5%)	-	*P<0.001*

Smoking Status [number and %]				
Never Smoked	14 (66.7%)	34 (49.3%)	26 (46.4%)	*P=0.201*
Ex-Smoker	6 (28.6%)	29 (42.0%)	29 (51.8%)	
Current Smoker	1 (4.8%)	6 (8.7%)	1 (1.8%)	
Overall lowest ABPI	1.1 [0.9-1.2]	1.1 [1.0 -1.2]	1.0 [1.0-1.1]	*P=0.838*
Overall Monofilament Score (out of 20)	5.00 [2.5-14.0]	20.00 [17.5-20.0]	20.00 [20.0-20.0]	*P<0.001*
MNSI Symptom Score (DPN)	7.00 [6.0-8.0]	5.00 [3.0-6.0]	-	*P<0.001*
MNSI Physical Assessment Score (DPN)	6.00 [6.0-8.0]	2.00 [1.0-3.0]	-	*P<0.001*
eGFR	71.0 [56.2-83.5]	85.0 [71.0-91.0]	-	*P=0.044*

Legend: Data displays median and interquartile range [IQR] and number and percentages (%). All analyses performed were non-parametric. This involved Pearson's chi-squared tests for categorical variables and Kruskal-Wallis test for comparisons between three groups and Man-Whitney U test for between DFU and DMC group comparisons and for post-hoc testing between two groups. A significance level of p=0.05 was used throughout. Diabetes duration indicates fractions of years living with type-2 diabetes mellitus. ABPI= ankle brachial pressure index. ABPI values are for ulcerated limbs of the DFU group and the lowest reported in the control groups (DMC, HC). eGFR= estimated glomerular filtration rate (a marker of renal function). Monofilament score is out of a total of 20, measured at ten sites for each foot. MNSI scores indicate the total scores from the Michigan Neuropathy Screening Instrument in relation to the neuropathy symptom score and physical assessment score. [A]= $p < 0.05$ when compared to the DMC group in post-hoc analysis [b] $p<0.05$ compared to the HC group in post hoc analysis.

The foot morphological characteristics are reported in Table 7.2. No significant difference in foot characteristics existed between the three groups, except hammertoe deformity which was more commonly present in the DFU group compared to the DMC and HC groups ($p < 0.01$). There was also no significant difference in foot characteristics when comparing ulcerated and non-ulcerated feet within the DFU group (see Table 7.2). The plantar ulcer locations of the DFU group were at the lateral heel (n=2), mid-foot (n=3), medial forefoot (n=2), central forefoot (n=1), lateral forefoot (n=1), apex of lesser digits (n=5), and hallux (n=7).

Table 7.2 Foot characteristics of the study cohort by group.

Explanatory measure	**DFU Group (n=21)**		**DMC Group (n=69)**	**HC Group (n=56)**	***P value***	***P value for ulcerated vs. Non-ulcerated feet of cases [paired]***
	Ulcerated feet	**Non-ulcerated feet^**				
Pes planus feet type	14 (66.7%)	12 (60.0%)	29 (42.0%)	19 (33.9%)		
Normal arched feet type	4 (19.0%)	4 (20.0%)	23 (33.3%)	20 (35.7%)	*0.146*	*0.317*
Pes cavus feet type	3 (14.3%)	4 (20.0%)	17 (24.6%)	17 (30.4%)		
First MTPJ RoM (degrees)	30.0 [25.0-45.0]	33.5 [27.5-45.0][ab]	44.0 [30.0-50.0]	45.0 [35.0-60.0]	*0.077*	*0.404*
Ankle Joint RoM (restricted dorsiflexion)	17 (81.0%)	16 (80.0%)	51 (73.9%)	33 (58.9%)	*0.281*	*0.368*
Subtalar Joint RoM (restricted inversion/eversion)	2 (9.5%)	1 (5.0%)	3 (4.4%)	2 (3.6%)	*0.885*	*0.846*
Hallux Abducto Valgus deformity*						
(No deformity)	14 (66.7%)	14 (70.0%)	51 (73.9%)	30 (53.6%)	*0.132*	*0.392*
(Grade 1)	5 (23.8%)	4 (20.0%)	13 (18.8%)	15 (26.8%)		
(Grade 2)	1 (4.8%)	1 (5.0%)	3 (4.3%)	10 (17.9%)		
(Grade 3)	1 (4.8%)	1 (5.0%)	2 (2.9%)	1 (1.8%)		

Claw toe deformity	6 (28.6%)	8 (40.0%)	11 (15.9%)	15 (26.8%)	*0.252*	*0.500*
Hammer toe deformity	12 (57.1%)[ab]	10 (50.0%)[ab]	16 (23.2%)	9 (16.1%)	*0.001*	*0.625*
Mallet toe deformity	3 (14.3%)	5 (25.0%)	14 (20.3%)	8 (14.3%)	*0.630*	*0.500*

Legend: McNemar's test was performed to assess paired significances between the ulcerated and non-ulcerated feet of the DFU group for categorical outcome and the Wilcoxon Signed Rank test was used to assess continuous variables. [A] $p < 0.05$ when compared to the DMC group in post-hoc analysis [b] $p<0.05$ compared to the HC group in post hoc analysis. *Hallux Abducto Valgus (HAV) deformity grades were based on the Manchester scale as reported in the study protocol. ^ These outcomes were calculated with a denominator of 20 due to missing data for the non-ulcerated foot of one participant in the case group.

7.5.3 Primary outcomes

All primary outcome data are reported in Table 7.3 and in more detail in in Appendix H. The DFU group had significantly higher mpp and pti of the toes 2-5 and mid-foot sites compared to the DMC and HC groups (*all* $p < 0.05$). The mpp of metatarsal 1 was also significantly higher in the DFU group compared to the HC group ($p < 0.05$). The DFU also had significantly longer stance phase duration compared to the DMC and HC groups ($p < 0.05$).

Table 7.3 Plantar pressure characteristics of the primary outcome measures by group.

Outcome measure	DFU Group (n=21)	DMC Group (n=69)	HC Group (n=56)	*Corrected P-value*	Median difference DFU vs. DMC [95% CI of	Median difference DFU vs. HC [95% CI of	Cohen's d DFU vs. DMC	Cohen's d DFU vs. HC
Mean peak plantar Pressure (mpp) N/cm²								
Toes 2-5	3.0 [2.4-5.6]	2.5 [1.9-3.1]	2.1 [1.8-2.7]	*P=0.007*	-0.8 [-1.5-(-)0.9]	-1.0 [-1.9-(-)0.5]	0.21	0.40
Metatarsal 1	5.7 [4.5-8.5][b]	5.5 [4.3-6.4]	4.6 [3.8-5.4]	*P=0.008*	-	-1.5 [-2.7-(-)0.5]	-	0.36
Mid-foot	3.8 [3.1-6.5]	3.0 [2.5-3.7]	2.2 [1.8-2.9]	*P<0.001*	-0.9 [-2.0-(-)0.3]	-1.7 [-3.0-(-)1.1]	0.32	0.63
Pressure-time integral (pti) Ns/cm²								
Toes 2-5	0.9 [0.6-1.4]	0.6 [0.5-0.8]	0.5 [0.3-0.6]	*P=0.001*	0.3 [0.1-0.6]	-0.4 [-0.8-(-)0.2]	0.45	0.60
Mid-foot	1.7 [1.2-2.5]	1.0 [0.8-1.3]	0.6 [0.4-0.9]	*P=0.001*	0.6 [0.3-1.0]	-1.0 [-1.3-(-)-0.7]	0.67	1.07
Stance phase duration (ms)	820 [752-960][ab]	739 [699-788]	703 [669-748]	*P<0.001**	-84 [-140-(-)34]	-115 [-163-(-)66]	0.51	0.75

Legend: Data displays median and [IQR] values based on the ulcerated feet of the DFU group compared to the reported maximum values in the DMC and HC groups. Corrected p-values indicate p-values obtained after Holm correction rounded to 3 decimal places. Average stance phase duration indicates the average time the left and right feet were in contact with the ground in milliseconds (ms) from heel contact to toe-off.*Stance phase duration p-values were not corrected as this was analysed as an independent variable. All above reported plantar pressure outcome data were significant on post-hoc two-way tests and remained significant after adjusting for age, sex and BMI. Binary logistic regression analyses were only performed for variables which were significant on the post-hoc test. Cohen's d was only calculated for variables which were significantly different after adjustment. (-)= not computed as this was not significantly different. See Appendix H for odds ratios and additional data.

7.5.4 Secondary outcomes

All secondary outcome results and paired analyses results are reported in Appendix H Appendix Table 18. The DFU group had higher msp of toes 2-5 and higher contact areas of the mid-foot and metatarsal 1 compared to both the DMC group and the HC group *($p<0.05$).* The DFU group had a higher msp of the mid-foot compared to the DMC group (*$p<0.05$*). None of the plantar pressure outcomes were statistically significant between the ulcerated and non-ulcerated feet after correction in paired analyses (see Appendix H, Appendix Tables 22-23).

7.6 Discussion

To the best of our knowledge this is was the first prospective study to have simultaneously examined such an extensive assessment of plantar pressures in a homogenous group of people with active DFUs (Fernando et al., 2015). Our primary results show the mpp (representing magnitudes of plantar pressure) and the pti (representing the duration and magnitude of plantar pressure) at toes 2-5 and the mid-foot were significantly higher in cases with DFUs compared to both control groups. Eight out of twenty-one ulcers in cases occurred at these plantar sites. Secondary results show the msp at the toes and the contact area of the mid-foot were also significantly higher in cases with DFUs compared to both controls. These findings occurred despite a longer stance phase duration representing slower gait speed in cases with DFUs. Consistent with our hypothesis, although cases with DFUs walked slower, their forefoot plantar pressures (especially at toes 2-5) were significantly higher compared to controls without DFUs.

The plantar pressure data in our diabetes control group were similar to that of a previous study that used the same plantar pressure platform (Qiu et al., 2015). We chose not to control gait speed in our participants as it has been reported that people with diabetes walk more cautiously than healthy controls (Ko, Hughes, & Lewis, 2012). However, previous studies have identified that gait speed can significantly alter the distribution and magnitude of plantar pressures (Burnfield, Few, Mohamed, & Perry, 2004; Rosenbaum, Hautmann,

Gold, & Claes, 1994). Faster gait speeds (i.e. shorter stance phase durations) have been shown to increase plantar pressure at the heel, medial and central forefoot and the toes 2-5, while decreasing plantar pressure beneath the mid-foot and lateral forefoot (Burnfield et al., 2004; Rosenbaum et al., 1994). This has been termed a medialization of the loading pattern (Rosenbaum et al., 1994). Conversely, a slower gait speed, as denoted by the longer stance phase duration, would be expected to produce higher plantar pressures beneath the mid-foot and lateral forefoot but lower plantar pressures at all other sites.

While our finding of elevated mid-foot pti and mpp in cases is consistent with a slower walking speed, our finding that mpp was elevated beneath toes 2-5 is counter to this effect. Therefore, despite a longer stance phase, higher plantar pressures still occur in cases with active DFUs. In a previous meta-analysis we proposed a 'guarded gait strategy' may be used by people with active DFUs to reduce plantar pressure acting on the ulcerated foot (Fernando et al., 2014b). Our current findings however are contrary to the presence of a 'guarded gait strategy' which would have resulted in lower plantar pressure in our cases. Our findings suggest that a longer stance phase is inadequate to lower the plantar pressure beneath the ulcerated foot during gait (Fernando et al., 2013). Many factors such as severity of DPN, foot deformity, excess body mass and altered gait patterns have all been implicated as potential causes of elevated plantar pressure in people with DFUs (Cavanagh et al., 1991; Ko et al., 2012).

A study by Stokes et al. (1975) suggested a mechanical aetiology to DFUs in people with DPN, reporting that plantar DFUs may occur at sites of maximal load in people with DPN (Stokes et al., 1975). The authors also reported that there was a lateral shift of the maximum pressure on the forefoot and a decrease in the plantar pressure of the toes in people with DPN (Stokes et al., 1975). Conversely, our study demonstrated higher plantar pressures beneath toes 2-5 in cases with active DFUs. This finding may be due to several reasons including the use of more modern equipment with greater sensitivity and greater spatial resolution in our study, or the differences in the populations studied. Stokes et al. had a heterogeneous cohort with only two participants with active DFUs while all our twenty-one cases had active plantar DFUs (Stokes et al., 1975).

Several DFUs in our cases were located in the toe region. A significantly higher proportion of participants in the DFU group also had a hammer-toe deformity of the lesser toes. A recent study by Barn and colleagues investigated predictors of barefoot plantar pressure in people with DPN with a history of DFUs (Barn, Waaijman, Nollet, Woodburn, & Bus, 2015). Barn and colleagues found that the presence of local deformity (such as toes and foot deformities) were the largest contributing factors to raised barefoot dynamic plantar pressure in their population (Barn et al., 2015). The presence of hammer-toe deformity was the largest single contributor towards elevated plantar pressure at the lesser toes (Barn et al., 2015), consistent with other research (Cavanagh et al., 1991; Mueller et al., 2003). It is possible that the higher plantar pressures seen in the cases in our study may have been associated with the presence of hammer-toe deformity as it has been previously associated with an increased risk of ulceration due to mechanical load placed on toes during gait (Ledoux et al., 2005). Interestingly, no differences in plantar pressures were observed between the ulcerated and non-ulcerated feet in paired analyses and this may have been due the high number of bilateral toe deformities in our cases. In our study, the severity of Hallux Abducto Valgus deformity was not different between groups, which align with Barn and colleague's findings that the presence of Hallux Abducto Valgus was not a predictor of plantar pressure (Barn et al., 2015). Additionally, all other foot characteristics showed no differences between groups or within cases, suggesting that unlike hammer-toe deformities, other foot characteristics may have a lesser effect on plantar pressure (Cavanagh et al., 1991; Molines-Barroso et al., 2016).

The pti is defined as the area under the peak–pressure–time curve and has been used to study ulceration because it incorporates pressure as well as time, both of which are suggested to be important in DFU formation (Sauseng et al., 1999; Stess et al., 1997). In agreement with the view that reporting the pti in addition to the mpp may be counterproductive (Bus & Waaijman, 2013; Keijsers, Stolwijk, & Pataky, 2010; Waaijman & Bus, 2012), the results from our study demonstrate that both the pti and the mpp were significantly higher at toes and at the mid-foot in cases with DFUs compared to either control group. Conversely, the effect-sizes of the differences in pti measurements were much larger compared to those of mpp. This observation is consistent with a recent study in people with DPN without DFUs that identified pti was significantly higher in five out of

ten possible regional comparisons, as opposed to the mp, which was significantly higher only in three out of ten comparisons (Yavuz, 2014).

Another study reported that the difference in pti between the metatarsal heads and the hallux was far greater in people with DFUs compared to controls without DFUs (Sauseng et al., 1999). Bacarin et al. have also previously found that people with DPN and a history of DFUs have a significantly higher pti at the mid-foot after controlling for gait speed (Bacarin et al., 2009). Currently, what the pti represents in the context of ulceration is still uncertain (Bus & Waaijman, 2013). A longitudinal analysis of both the pti and mpp measurements in a cohort of people with healing and non-healing DFUs may provide information regarding the importance of these two parameters on ulcer healing (Fernando et al., 2015; Sauseng et al., 1999). For example, it may be possible that one parameter may be more predictive of ulcer healing. A longitudinal analysis will also provide observations regarding the variability of plantar pressures in cases with DFUs when compared to controls without DFUs over-time (Bus & de Lange, 2005).

In contrast to the findings of the current study, Sauseng et al. found that the maximum plantar pressure and contact area was higher at plantar metatarsal 1 but was lower at metatarsal 4, metatarsal 5 and at the mid-foot in people with DFUs compared to controls (Sauseng et al., 1999). The different findings in the current study may be due to a number of factors. Firstly, in the current study plantar pressures were examined in 10 locations in both feet however Sauseng et al. only studied seven locations (Sauseng et al., 1999). These locations may have been differently defined in the two studies during the masking process of identifying plantar sites. Sauseng and co-workers pooled plantar pressure data from the ulcerated and non-ulcerated feet of cases, debrided plantar callus prior to plantar pressure evaluation, and did not report gait speed or stance phase duration. They also studied a group of patients with very few DFUs occurring at toes 2-5 as a majority of the DFUs were located on the plantar surfaces of the metatarsals in their study (Sauseng et al., 1999). Nevertheless, the results from Sauseng and co-workers are in alignment with our results in indicating that ulcer location is an important predictor of the site of high plantar pressure and that higher plantar pressure may occur at ulcer sites in people with DFUs. Interestingly, Sauseng and co-workers (Sauseng et al., 1999) found that the mpp at the

hallux was not higher in people with DFUs compared to controls, despite the fact that some DFUs occurred at the hallux, which was also the case in our study. We were unable to show any difference in the range of motion of the first metatarsophalangeal joint between groups in our study. The reason why we were unable to see higher plantar pressures at the hallux in people with DFUs may be due to the fact that they were able to limit the amount of loading on the hallux using the available range of motion at the first metatarsophalangeal joint. This is consistent with our finding of higher mpp at this site in our cases compared to healthy controls.

Plantar pressures are theoretically the result of the vertical force exerted on the foot during gait divided by the contact area. Therefore, assuming that the spatial resolution of the sensors were adequate and the plantar skin surface was completely in contact with the pressure platform during measurement (Cavanagh et al., 1991), either vertical ground reaction force has to increase, or the total contact area for a given site has to decrease in order for plantar pressure to be elevated. We have demonstrated increased plantar pressures and larger contact areas in cases with DFUs. These results highlight that vertical ground reaction forces are also likely to be significantly elevated in cases with DFUs. This is consistent with recent findings in the same cohort (Fernando et al., 2016b) (see Chapter 6). To our knowledge, although it is often speculated, increased ground reaction forces have not been previously reported in people with active DFUs until recently (Fernando et al., 2016b). These findings suggest that people with active DFUs experience significantly higher mechanical stresses during gait. On the other hand, while plantar pressures represent only the vertical component of the applied tissue stress, shear-forces are also a crucial consideration in the formation of DFUs (Yavuz, 2014). As the same local area under the foot can experience stresses in opposite directions, investigation of shear forces in cases with DFUs will provide further information regarding tissue stresses (Yavuz et al., 2008). The increased contact areas observed in our cases at several sites is in agreement with the idea that ground reaction forces other than the vertical force (i.e. shear forces) may also be important in DFU formation (Yavuz, 2014). This is consistent with the finding that shear forces in the anterior–posterior direction during gait in the same cohort of people with DFUs was significantly higher than in controls (Fernando et al., 2016b). Future studies should focus on assessing shear-pressures, especially at sites of active ulcers.

This study has a number of limitations and strengths. We were unable to adjust all our analyses for multiple factors such as foot deformities, arch type and neuropathy severity due to relatively small group sizes. We examined barefoot gait rather than shod gait and purposefully did not control gait speed as we wanted to examine the natural gait characteristics of our participants. We believe that by imposing minimal constraints, the observed gait would be consistent with the participant's everyday gait pattern. We used stance phase duration as a surrogate measure of gait speed. We were, however, unable to focus our investigation on individual ulcer sites due to a small sample-size and resultant lack of statistical power and this area still requires investigation. We believe that our findings, however, are consistent with plantar pressures representative of a majority of cases who had DFUs in the forefoot region. There are differences in plantar pressure values obtained using different platforms with different resolutions and various methods of assessment, which is a clear limitation in the field (Ledoux et al., 2013). Our plantar pressure results seem to be lower than other values reported in the literature, but are consistent with others using the same platform to assess participants with diabetes (Qiu et al., 2015). The strengths of our study include the use of reproducible methodology to capture plantar pressure, reporting the reproducibility of plantar pressure acquisition prior to this study (Fernando et al., 2016a) and the use of a conservative statistical approach.

7.7 Conclusion

In summary, this study has demonstrated that plantar pressures are higher in cases with active unilateral diabetic foot ulcers compared to diabetes and healthy controls without ulcers. Higher plantar pressures occurred in cases despite a longer stance phase duration which would be expected to lower plantar pressures. This highlights the importance of offloading feet during active ulceration to overcome the mechanical impact of elevated plantar pressures on ulcerated tissue. Evaluating plantar pressures throughout ulcer progression may provide further clarity on the relationship between plantar pressures and the mechanical stresses experienced by patients with active foot ulcers.

Chapter. 8 Plantar Pressures are Elevated in People with Longstanding Diabetes-Related Foot Ulcers During Follow-Up

This chapter has been adapted from a publication titled;

Plantar pressures are elevated in people with longstanding diabetes-related foot ulcers during follow-up

Authors: Malindu Eranga Fernando, Robert George Crowther, Peter Anthony Lazzarini, Saiumaeswar Yogakanthi, Kunwarjit Singh Sangla, Petra Buttner, Rhondda Jones, Jonathan Golledge

PLoS One (2017) 12(8): e0181916

8.1 Preface

The previous two chapters (Chapter 6 and 7) reported on the baseline findings from the prospective case-control studies contained in this thesis. The following chapter now reports the longitudinal follow-up study of the diabetes cohort from the previous two chapters. The study contained in the following chapter attempted to answer Question 5 from Chapter 1 and focuses on the plantar pressures in the same cohort of people described in Chapter 7. Hence the aim of the study reported below was to determine whether plantar pressures change in people with DFUs during ulceration, when compared to diabetes controls without DFUs at three study visits; baseline, three-months follow-up and six-months follow-up.

8.2 Abstract

Objective: High plantar pressures are implicated in the development of DFUs. Whether plantar pressures remain high in patients with chronic DFUs over time is uncertain. The primary aim of this study was to compare plantar pressures at baseline and three and six months later in participants with chronic DFUs (cases) to participants with diabetes without foot ulcers (controls).

Methods: Standardised protocols were used to measure mean peak plantar pressure and pressure-time integral at 10 plantar foot sites (the hallux, toes, metatarsals 1 to 5, mid-foot, medial heel and lateral heel) during barefoot walking. Measurements were performed at three study visits: baseline, three and six months. Linear mixed effects random-intercept models were utilised to assess whether plantar pressures differed between cases and controls after adjusting for age, sex, body mass index, neuropathy status and follow-up time. Standardised mean differences (Cohen's d) were used to measure effect size.

Results: Twenty-one cases and 69 controls started the study and 16 cases and 63 controls completed the study. Cases had a higher mean peak plantar pressure at several foot sites including the toes ($p=0.005$, Cohen's d =0.36) and mid-foot ($p=0.01$, d=0.36) and a higher pressure-time integral at the hallux ($p<0.001$, d= 0.42), metatarsal 1 ($p=0.02$, d= 0.33) and mid-foot ($p=0.04$, d=0.64) compared to controls throughout follow-up. A reduction in pressure-time integral at multiple plantar sites over time was detected in all participants ($p<0.05$, *respectively).*

Conclusions: Plantar pressures assessed during gait are higher in diabetes patients with chronic foot ulcers than controls at several plantar sites throughout prolonged follow-up. Long term offloading is needed in diabetes patients with diabetes-related foot ulcers to facilitate ulcer healing

8.3 Introduction

High plantar pressures have been implicated in the development of DFU by increasing the mechanical stress experienced by plantar tissue in the presence of diabetic peripheral neuropathy (DPN) (Fernando et al., 2013; Frykberg et al., 1998; Veves et al., 1992). Previous cross-sectional studies have reported that the pressures measured at specific plantar sites and resultant tissue stresses during gait are higher in people with active DFUs than controls (Armstrong et al., 1998c; Fernando et al., 2016c; Kanade et al., 2006). Hence a frequent cause of delayed healing of DFUs is thought to be high plantar pressures during gait (Fernando et al., 2016c; Jeffcoate et al., 2016; Maluf & Mueller, 2003a). Studies have demonstrated much shorter DFU healing times when plantar stresses on ulcerated tissues have been reduced by using gold standard offloading devices (such as total contact casts or removable cast walkers) (Jeffcoate et al., 2016). It has also been suggested that patients with DFUs may adapt to these tissue stresses via the development of an alternative gait strategy to reduce plantar pressures (Fernando et al., 2014b). However, no longitudinal study has examined plantar pressures in patients with chronic DFUs (Fernando et al., 2016c) and hence the role of plantar pressures during ulcer healing is largely unknown. Therefore, measuring plantar pressures in people with DFUs during ulcer healing could provide important insight on whether plantar pressures remain elevated during wound healing or whether plantar pressures reduce over-time.

If plantar pressures are only high during the initial stages of ulceration, then the theory of a guarded gait strategy is likely to explain why plantar pressures do not remain elevated in people with DFUs (Fernando et al., 2014b). However, in order to demonstrate whether plantar pressures remain elevated in people with DFUs throughout ulcer healing, a comparison to a control group is needed. A better understanding of whether plantar pressure differences between cases and controls remain over time would provide much needed guidance to pressure-offloading approaches with the aim of improving wound healing by optimizing the types of offloading devices that are used and the time-frames that these devices should be worn by patients. Therefore, assessing whether differences in plantar pressures between people with active DFUs and controls without DFUs reduces over time is important.

The aim of this study was to investigate plantar pressures at baseline and three and six months later in participants with DFUs (cases) compared to participants without DFUs (controls). We hypothesised that cases with DFUs would have significantly higher plantar pressures at baseline compared to controls and that these plantar pressure differences would remain during follow-up at three and six months.

8.4 Methods

8.4.1 Study design and setting

This was a longitudinal study which was nested in a case-control study (see Chapter 4).The study protocol and the baseline results of the study were previously published (Fernando et al., 2015; Fernando et al., 2016c) (see Chapter 4, 6 and 7). All participants attended the Movement Analysis Laboratory, James Cook University, Townsville, Queensland, Australia on three separate occasions (baseline, first-follow-up at three months and second follow-up at six months) between July 2012 to November 2014.

8.4.2 Participants

Twenty-one participants with active unilateral plantar DFUs of more than 3 months duration (cases) and 69 type 2 diabetes mellitus participants without ulcers (controls) were initially recruited for this longitudinal study (Fernando et al., 2015). Chapter 4 contains a more detailed description of the inclusion and exclusion criteria for this study and this is a continuation of the studies contained in Chapter 6 and 7.

8.4.3 Participant characteristics

All anthropometric, hematological and clinical measurements were performed according to the study protocol (Fernando et al., 2015). This included measuring each participant's body mass, body mass index (BMI), body fat percentage and waist and hip circumference, ulcer area, University of Texas Wound Classification Score (UTWCS) [21], glycated haemglobin A1c (HbA1c) and estimated glomerular filtration rate (eGFR) (Fernando et al., 2015) at each study-visit. Other measures such as age, height, sex, ethnicity, monofilament sensation, the Michigan Neuropathy Symptom and Physical Assessment Scores and ABPIs

were assessed at baseline and were thought to remain unchanged during the six-month follow-up period (Fernando et al., 2015; Fernando et al., 2016b; Fernando et al., 2016c). Both cases and controls were screened for the presence of DPN using several different methods as per recognized guidelines (American Diabetes Association, 2016; Boulton et al., 2008) and as detailed in the study protocol (Fernando et al., 2015).

All participants received standard care between follow up visits external to their involvement in the study (Jeffcoate et al., 2016). For cases, this typically comprised of assessment and treatment of the ulcer by a podiatrist at least once every four weeks (Jeffcoate et al., 2016). Most controls attended an annual foot-check with a podiatrist as per National guidelines and had regular review of their diabetes control with a General Practitioner or Endocrinologist as per standard care (Jeffcoate et al., 2016).

8.4.4 Procedure used to measure plantar pressures

The Footscan® pressure plate (RSscan International, Olen, Belgium) was used for plantar pressure assessment along with the associated Foot Scan ® processing software. Plantar pressures were measured in both feet at baseline, three and six months follow-up visits. All cases with DFUs were given a standard single-layer generic film wound dressing to wear over the wound during gait examination to minimize the impact of wound dressings on the study results and to standardise the type of dressing during gait assessments and to minimize the risk of wound infection as per the study protocol (Fernando et al., 2015). Standardised protocols which have been extensively described before (Fernando et al., 2016a; Fernando et al., 2015; Fernando et al., 2016c) were used in capturing plantar pressure data (see Chapter 4, 5 and 7).

8.4.5 Statistical Analysis

The normality of continuous data was assessed using the Shapiro-Wilk test. Categorical data were reported as numbers and percentages (%) and continuous data were reported as means and standard deviations (SD), mean differences (Δ) or medians and interquartile ranges (IQRs) depending on the distribution of data. Characteristics of cases and controls were compared with Student’s t-test, Pearson's chi-square test or Fisher's exact test when assumptions for Chi-square tests were not met. We compared plantar pressures of ulcerated

feet to plantar pressures averaged from the left and right feet of controls. Differences in mpp and pti over time were examined using linear mixed effects random-intercept models with individual participants as random effects and ulcer presence, months of follow-up, age, sex, body mass index (BMI) and the presence of neuropathy as fixed effects. The fixed effects were selected based on their previously established influence on plantar pressures (Fernando et al., 2015; Fernando et al., 2016b). Within our statistical models, we assessed whether there was an association between 'time' and any changes in plantar pressure in all participants. We used an 'interaction' term within mixed effects models to assess whether any changes in plantar pressure over time differed between cases and controls. Where no significant interaction existed between ulcer presence and plantar pressures over time, the models were repeated excluding the interaction term.

Results of linear mixed effects models were reported using t-values, degrees of freedom (df) and p-values for estimated coefficients. Analysis of variance (ANOVA) was used to assess the overall goodness of fit of the linear mixed models for plantar pressure comparisons and changes over-time (see Appendix I). ANOVA results were reported in the main results as a measure of statistical significance. The statistical significance of outcomes was considered first by assessing the p-value obtained from ANOVA and then by assessing the p-value from the table of coefficients. When both p values were less than 0.05, a result was considered statistically significant. Standardised mean differences (Cohen's d values) were calculated for all outcomes which were significantly different between groups using a previously published formula: standardised mean difference (d) = t $(2/n)^{1/2}$ (Cohen, 1988; Dunlap, Cortina, Vaslow, & Burke, 1996). The size and direction of the difference was graded based on Cohen's d as: <0.10 trivial difference; 0.10-0.20 small difference; 0.20-0.60 medium difference; 0.60-1.20 large difference and ≥1.20 a very large difference (Hopkins, 2002).

In order to assess the impact of ulcer healing during follow-up we performed sensitivity analyses excluding participants with healed ulcers (see Appendix I). These analyses showed similar results to those obtained by analyzing all participants and therefore we have presented the latter results. SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for the statistical comparisons of baseline demographic characteristics. The R (R

Core Team, 2015) software was used for analysis of all longitudinal data with the 'nlme' package (Pinheiro J, Bates D, DebRoy S, Sarkar D, & Team, 2015) for the mixed- effects models and for examining residual plots to check for deviations from homoscedasticity and normality assumptions. Summary plots of pti and mpp at the ten plantar sites were created for each time point (see Figure 8.1 and Figure 8.2).

8.5 Results

8.5.1 Recruitment and attrition of participants

Ninety participants commenced the study and were assessed at baseline (21 cases and 69 controls). Of those, five (24%) cases and six (9%) controls did not complete all follow-up visits. Prior to the three-month follow-up visit [IQR 3-4 months], three cases (two due to orthopedic surgery and one due to acute lower back pain) and two controls (one due to coronary artery bypass and one due to inability to attend) withdrew from the study. Prior to the six-month follow-up visit [IQR 6-11.5 months], another three controls (one due to acute illness and two due to inability to attend and three cases (two due to hospitalisation and one due to inability to attend) also withdrew.

8.5.2 Participant characteristics at baseline

The baseline data from this cohort were reported in an earlier manuscript (Fernando et al., 2016c). Table 8.1 displays the baseline characteristics of the 21 cases and 69 controls that were initially recruited. There were no significant differences in age, sex, ethnicity, BMI, average HbA1c, smoking status or leg length between cases and controls at baseline. The presence of hammer-toe deformity was more common in cases at baseline ($p=0.006$).

Table 8.1 Clinical and demographical characteristics of the enrolled study cohort at baseline.

Variable	Cases (n=21)	Controls (n=69)	*p-value*
Age at enrolment (years)	63.1 (± 10.6)	63.4 (± 9.6)	*0.905*
Males	15 (71.4%)	46 (66.7%)	*0.793*
Ethnicity			*1.000*
Caucasian	20 (95.2%)	65 (94.2%)	
Australian	1 (4.8%)	2 (2.9%)	
Aboriginal/Indigenous/ Torres-strait Islander	-	2 (2.9%)	
Other			
Diabetes duration [years][#]	16.6 (± 7.1)	10.7 (± 8.6)	*0.005*
HbA1c (mmol/l)[#]	58.9 (± 16.8)	54.8 (± 13.3)	*0.284*
Uses Insulin [#]	13 (61.9%)	19 (27.5%)	*0.005*
Smoking Status			*0.443*
Never Smoked	14 (66.7%)	34 (49.3%)	
Ex-Smoker	6 (28.6%)	29 (42.0%)	
Current Smoker	1 (4.8%)	6 (8.7%)	
History of hypertension	19 (90.5%)	46 (66.7%)	*0.049*
History of dyslipidaemia	14 (66.7%)	45 (65.2%)	*1.000*
History of stroke*	2 (9.5%)	2 (2.9%)	*0.231*
History of coronary heart disease	7 (33.3%)	18 (26.1%)	*0.581*
History of chronic heart failure	3 (14.3%)	9 (13.0%)	*1.000*
History of chronic pulmonary disease	4 (19.0%)	14 (20.3%)	*1.000*

History of chronic liver disease	2 (9.5%)	5 (7.2%)	*1.000*
History of chronic renal impairment	5 (23.8%)	10 (14.5%)	*0.506*
Height [cm]	173.7 (± 9.8)	169.6 (± 10.6)	*0.121*
Body mass [kg]	102.5 (± 23.8)	91.3 (± 15.2)	*0.012*
BMI [Body Mass Index] [kg/m^2]	34.0 (± 8.3)	31.8 (± 4.80)	*0.120*
Body Fact Percentage [% bf]	28.5 (± 13.7)	27.8 (± 12.6)	*0.834*
Waist Circumference [cm]	113.5 (± 17.9)	106.6 (± 11.2)	*0.035*
Hip Circumference[cm]	110.7 (± 18.9)	105.8 (± 10.2)	*0.120*
Left leg length [cm]	91.8 (± 7.1)	90.5 (± 5.6)	*0.390*
Right leg length [cm]	92.9 (± 8.0)	89.9 (± 11.4)	*0.266*
ABPI^	1.1 (± 0.2)	1.1 (± 0.2)	*0.913*
Monofilament score	7 (± 7)	18 (± 4)	*<0.001*
MNSI symptom score[#]	7 (± 1)	5 (± 2)	*<0.001*
MNSI physical assessment score[#]	7 (± 1)	2 (± 2)	*<0.001*
Foot-type			*0.166*
Pes planus foot type	14 (66.7%)	29 (42.0%)	
Normal arched foot type	4 (19.0%)	23 (33.3%)	
Pes cavus foot type	3 (14.3%)	17 (24.6%)	
First MTPJ RoM (degrees)	35.8 (± 14.4)	43.1 (± 15.1)	*0.052*
Ankle Joint RoM (restricted dorsiflexion)	17 (81.0%)	51 (73.9%)	*0.897*

Subtalar Joint RoM (restricted inversion/eversion)	2 (9.5%)	3 (4.4%)	*0.703*
Hallux Abducto Valgus deformity	14 (66.7%)	51 (73.9%)	*0.955*
(No deformity)	5 (23.8%)	13 (18.8%)	
(Grade 1)	1 (4.8%)	3 (4.3%)	
(Grade 2)	1 (4.8%)	2 (2.9%)	
(Grade 3)			
Claw toe deformity	6 (28.6%)	11 (15.9%)	*0.213*
Hammer toe deformity	12 (57.1%)	16 (23.2%)	*0.006*
Mallet toe deformity	3 (14.3%)	14 (20.3%)	*0.752*

Legend: All data represents mean ± standard deviation (SD) or number and percentages (%). Cases= foot ulcer group, controls= diabetes mellitus control group without ulcers. The reported test statistic indicates the t-statistic or Pearson's Chi-square or Fisher's exact test values with associated degrees of freedom. The reported p-values indicate main comparison outcomes from student's t-tests, Pearson's Chi squared tests or Fishers exact tests between groups. A significance level of $p < 0.05$ was used throughout. Diabetes duration indicates fractions of years living with type 2 diabetes mellitus. ^ Ankle Brachial Pressure Index =ABPI. ABPI values represented in the table are for ulcerated limbs of the Cases groups and the lowest reported in the control group. Monofilament score is out of a total of 20, measured at ten sites for each foot. MNSI scores indicate the total scores from the Michigan Neuropathy Screening Instrument in relation to the neuropathy symptom score and physical assessment score. * Note that the four patients with stroke did not have a history of gait disturbance due to their stroke as the stroke only affected their speech function. Hallux Abducto Valgus (HAV) deformity grades were based on the Manchester scale (Garrow et al., 2001) as reported in the study protocol (Fernando et al., 2015). RoM= Range of motion, restricted dorsiflexion incorporated people with < 10 degrees dorsiflexion.

8.5.3 Participant characteristics during follow-up

Table 8.2 displays the anthropometric characteristics at baseline and the first and second follow-up visits for cases and controls. All cases had DFUs on the plantar aspect of the foot, including 16 ulcers (76.2%) under the fore-foot and five ulcers (23.8%) under the rear-foot. Most DFUs (81.0%) were superficial with a UTWCS grade of A1 or B1 [n=17 (80.9%)] and the remainder extended to tendon or capsule [A2=3 (14.3%) and B2=1 (4.8%)]. Controls had a slightly longer follow-up period compared to cases (Table 2). There was a small decrease in the mean ulcer area at the first follow-up (Δ =0.4 [SD =0.8] mm^2) and a slight increase in mean ulcer area at the second follow-up (Δ=3.4 [3.6] mm^2). The stance phase duration seemed to be longer in cases compared to controls throughout follow-up. Four (19.0%) DFUs healed during follow-up and remained healed (see Table 8.2). None of the controls developed DFUs during follow-up.

Table 8.2 Clinical and demographic characteristics of the study cohort at each follow-up.

	Cohort at each follow-up					
Variable	**Cases (n=21)**	**Cases (n=19)**	**Cases (n=16)**	**Controls (n=69)**	**Controls (n=66)**	**Controls (n=63)**
	Baseline	***1st follow-up***	***2nd follow-up***	***Baseline***	***1st follow-up***	***2nd follow-up***
Number of months since baseline median [IQR]	-	3.0 [3.0-4.0]	6.0 [6.0-8.0]	-	4.0 [3.0-4.0]	9.0 [6.0-12.0]
Males (%)	15 (71.4%)	13 (68.4%)	12 (75.0%)	46 (66.7%)	45 (68.1%)	44 (69.8%)
Body mass [kg] (SD)	102.6 (± 23.8)	106.9 (± 23.1)	108.5 (± 21.6)	91.3 (± 15.2)	91.0 (± 14.6)	91.4 (± 14.4)
BMI [Body Mass Index] [kg/m^2] (SD)	34.0 (± 8.3)	35.3 (± 8.4)	35.6 (± 8.2)	31.8 (± 4.80)	31.4(± 4.7)	31.7 (± 4.8)
Body Fat Percentage [% bf] (SD)	28.5 (± 13.7)	27.5 (± 13.5)	26.0 (± 14.3)	27.8 (± 12.6)	27.5 (± 1.3)	29.6 (± 13.0)
Waist Circumference [cm] (SD)	113.5 (± 17.9)	106.0 (± 17.8)	109.0 (± 22.1)	106.6 (± 11.2)	105.0(± 10.0)	105.0 (± 10.2)
Hip Circumference[cm] (SD)	110.7 (± 18.9)	104.7 (± 9.7)	108.0 (± 11.3)	105.8 (± 10.2)	103.0 (± 9.3)	103.0 (± 9.3)
Stance phase duration [ms] (SD)	836 (± 115)	747 (± 99)	799 (± 137)	749 (± 93)	743 (± 57)	736 (± 56)

	Cases (n=21)	Cases (n=19)	Cases (n=16)
	Baseline	***1st follow-up***	***2nd follow-up***
Ulcer grade (UTWCS)			
A0	-	2 (10.5%)	4 (25.0%)
A1	16 (76.2%)	14 (73.7%)	8 (50.0%)
A2	3 (14.2%)	1 (5.3%)	1 (6.2%)
A3	-	-	-
B1	1 (4.7%)	2 (10.5%)	3 (18.8%)
B2	1 (4.7%)	-	-
Average ulcer area (mm^2) *	20.3 (± 18.8)	19.9 (± 18.0)	23.3 (± 21.6)

Legend: Data represents mean ± standard deviation SD) or number and percentages (%), or median and [inter-quartile range; IQR]. Cases= foot ulcer group, controls= diabetes mellitus control group without foot ulcers. UTWCS= University of Texas Wound Classification Score. A0= healed ulcer with complete epithelization, A1= superficial ulcer, A2 = ulcer which is down to the level of soft tissue, A3 = ulcer which is down to the level of bone, B1 = infected superficial ulcer, B2 = infected ulcer which is down to the level of soft tissue (Lavery, Armstrong, & Harkless, 1996). * Ulcer area was calculated excluding healed ulcers.

8.5.4 Plantar Pressure Outcomes

8.5.4.1 Mean peak pressure

Cases had a significantly higher mpp at toes 2-5 ($p=0.005$, d =0.36) and the mid-foot ($p=0.01$, d=0.36) throughout follow-up compared to controls (see Table 8.3 and Appendix I). Conversely, cases had a significantly lower mpp at metatarsal 4 compared to controls throughout follow-up ($p=0.017$, d=-0.38) (see Table 8.3 and Appendix I). Mpps decreased during follow-up at some sites including toes 2-5 ($p<0.001$, d=-0.38), metatarsal 1 ($p=0.005$, d=-0.18) and the mid-foot ($p<0.001$, d=-0.36) in all participants (see Table 8.3 and Figure 8.1). The interaction term was insignificant between cases and controls.

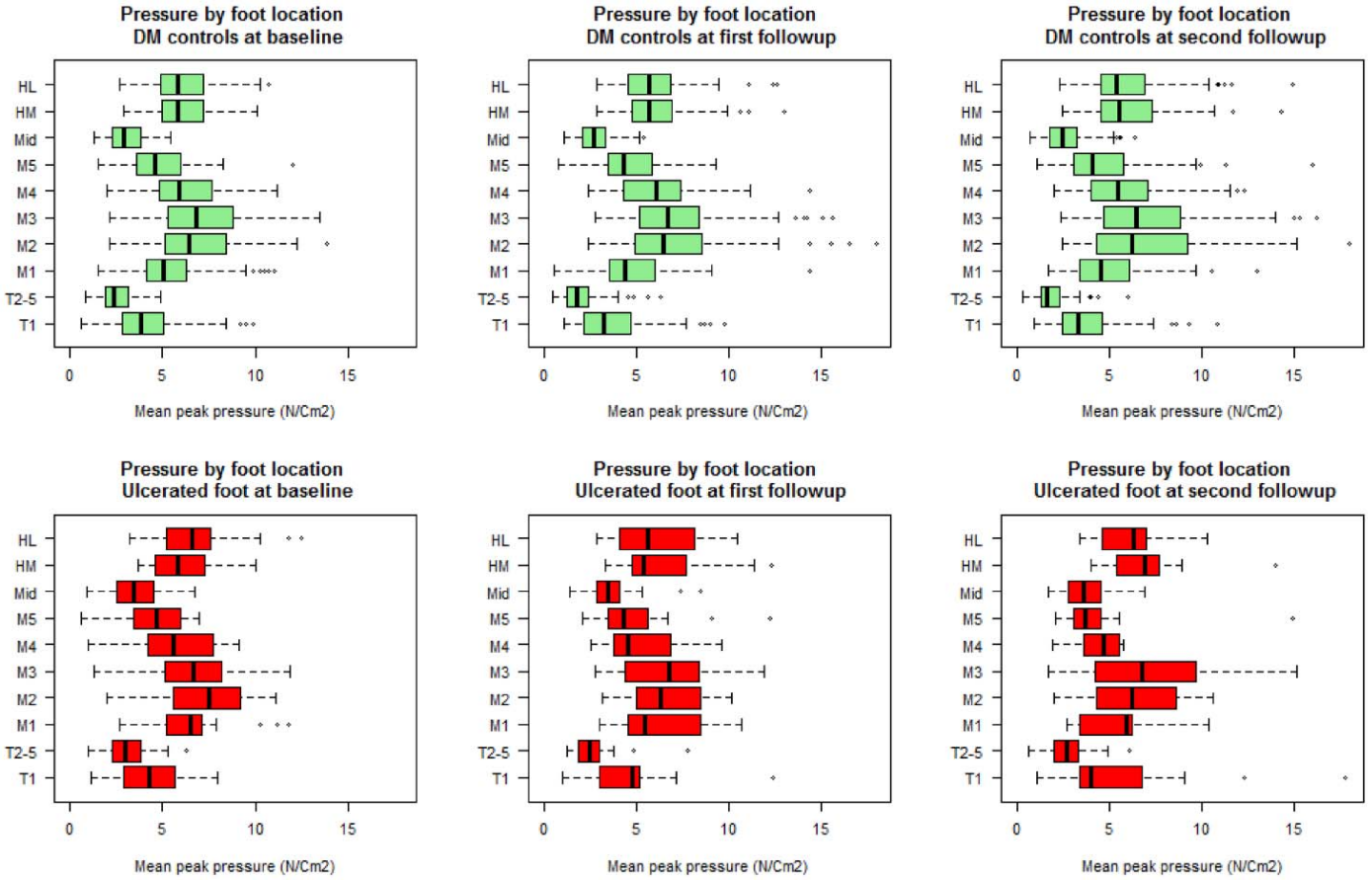


Figure 8.1 Site-specific mean peak pressures over time in participants with DFUs and participants without DFUs.

Legend: Figure indicates the mean peak pressure at ten plantar sites in cases (red) and diabetes controls (DM controls) (green) at each visit. The x-axis has been scaled to allow for better data visualization. All values are in N/Cm^2 and are reported for 10 plantar foot sites. T1 = hallux (big-toe), T2-5 = toes two to five, M1= metatarsal one, M2= metatarsal two, M3= metatarsal three, M4 =metatarsal four, M5= metatarsal five, Mid= mid-foot, HM= medial heel and HL= lateral heel.

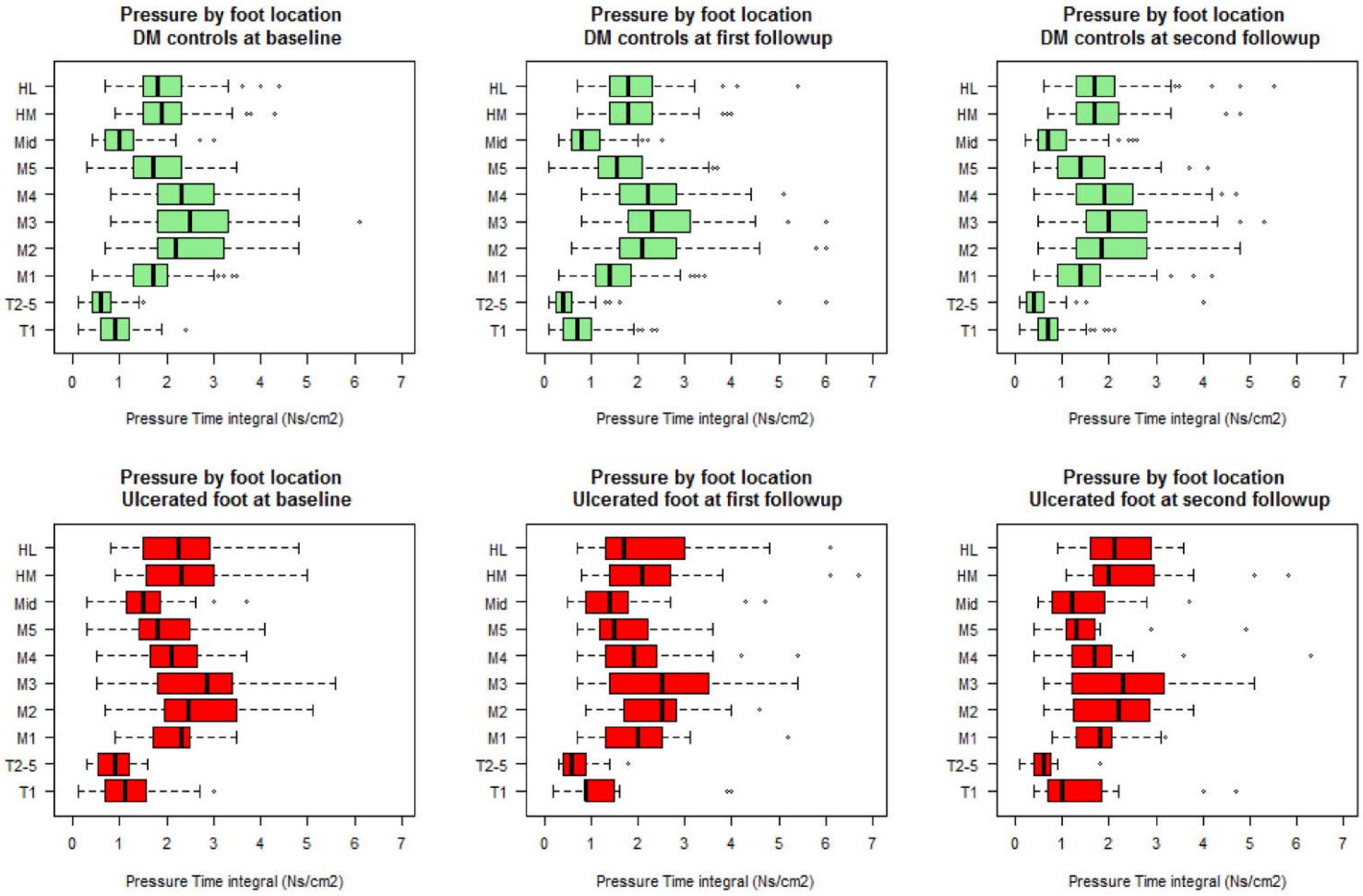
Table 8.3 Mean peak pressure by group at baseline and during follow-up.

	Cases	Cases	Cases	Controls	Controls	Controls	ANOVA p-value for model Ulcer	t-value Ulcer presence (degrees of freedom	p-value Ulcer presence	Cohen's d Ulcer presence	ANOVA p-value for model Change over time	t-value Change over time (degrees of freedom	p-value Change over time	Cohen's d Change over time
Mean peak pressure (mpp)N/cm²	***Baseline***	***1st follow-up***	***2nd follow-up***	***Baseline***	***1st follow-up***	***2nd follow-up***								
Toe 1/ Hallux	4.2 (± 1.9)	4.7 (± 2.7)	5.8 (± 4.4)	4.1 (± 1.8)	3.6 (± 1.8)	3.8 (± 1.8)	0.12	0.61 (86)	0.54	-	0.29	-1.40 (330)	0.16	-
Toes 2-5	3.1 (± 1.4)	2.8 (± 1.6)	2.9 (± 1.4)	2.5 (± 0.9)	1.9 (± 0.9)	1.9 (± 0.8)	0.005	2.37 (86)	0.02	0.36	<0.001	-4.88 (332)	<0.001	-0.38
Metatarsal 1	6.5 (± 2.4)	6.1 (± 2.4)	5.7 (± 2.5)	5.4 (± 1.9)	4.8 (± 1.9)	4.9 (± 2.1)	0.09	2.00 (86)	0.04	-	0.005	-2.28 (334)	0.02	-0.18
Metatarsal 2	7.2 (± 2.4)	6.7 (± 2.3)	6.4 (± 2.8)	6.9(± 2.4)	7.0 (± 3.1)	6.9 (± 3.1)	0.41	0.05 (86)	0.95	-	0.50	-0.28 (334)	0.77	-
Metatarsal 3	6.7 (± 2.5)	6.7 (± 2.7)	7.1 (± 3.7)	7.2 (± 2.6)	7.2 (± 2.7)	7.0 (± 3.1)	0.39	-1.12 (86)	0.26	-	0.65	-0.68 (333)	0.49	-
Metatarsal 4	5.6 (± 3.7)	5.3 (± 2.2)	5.3 (± 4.0)	6.2 (± 2.0)	6.1 (± 2.0)	5.7 (± 2.3)	0.017	-2.48 (86)	0.01	0.38	0.30	-1.36 (334)	0.17	-
Metatarsal 5	4.6 (± 1.7)	4.9 (± 2.6)	4.5 (± 3.0)	4.8 (± 1.6)	4.7 (± 1.7)	4.6 (± 2.2)	0.51	-1.22 (86)	0.22	-	0.64	-0.85 (330)	0.39	-
Mid Foot	3.7 (± 1.5)	3.9 (± 1.8)	3.7 (± 1.5)	3.1 (± 1.0)	2.9 (± 1.0)	2.6 (± 1.1)	0.01	2.35 (86)	0.02	0.36	<0.001	-4.59 (331)	<0.001	-0.36
Medial Heel	6.3(± 2.0)	6.5 (± 2.5)	6.9 (± 2.4)	6.1 (± 1.6)	6.0 (± 1.8)	6.0 (± 1.9)	0.63	0.16 (86)	0.87	-	0.59	0.60 (333)	0.54	-
Lateral Heel	6.8(± 2.5)	6.1 (± 2.5)	6.3 (± 2.1)	6.1 (± 1.7)	5.9 (± 1.8)	5.9 (± 2.1)	0.66	1.06 (86)	0.29	-	0.23	-0.73 (332)	0.46	-

Legend: All data represents mean peak pressures (mpp) and the reported values indicate the mean ± standard deviation (SD) and df= degrees of freedom as indicated. Cases= foot ulcer group, controls= diabetes mellitus control group without ulcers. -= not computed as this was not significantly different. The ANOVA p-value indicates values obtained an overall goodness of fit of statistical models and represent the overall significance of outcomes in the model. The p-values indicate the individual p-values obtained from the correlations tables from linear mixed effects models and the p-value for the effect of ulcer presence on plantar pressure and effect of time on plantar pressure.

8.5.4.2 Pressure-time integral

Cases had significantly higher ptis at the hallux (*p<0.001*, d = 0.42), plantar metatarsal 1 (*p=0.02,* d= 0.33), the mid-foot (*p=0.04*, d= 0.64*)*, the medial heel (*p=0.*02, d=0.20) and the lateral heel (*p=0.03,* d= 0.31*)* throughout follow-up compared to controls (see Table 8.4 and Appendix I). The pti at the hallux (*p=0.02*, d=-0.20), all metatarsals, including metatarsal 1 (*p<0.001*, d=-032) and the mid-foot (*p<0.001*, d=-0.33) decreased during follow-up in all participants (see Table 8.4, Appendix I and Figure 8.2).



Legend: Figure indicates the pressure-time integral at ten plantar sites in cases (red) and diabetes controls (DM controls) (green) at each visit. The x-axis has been scaled to allow for better data visualization. All values are in Ns/Cm^2 and are reported for 10 plantar foot sites. T1 = hallux (big-toe), T2-5 = toes two to five, M1= metatarsal one, M2= metatarsal two, M3= metatarsal three, M4 =metatarsal four, M5= metatarsal five, Mid= mid-foot, HM= medial heel and HL= lateral heel.

Figure 8.2 Site-specific pressure-time integrals over time in participants with DFUs and participants without DFUs.

Table 8.4 Pressure-time integrals by group at baseline and during follow-up

	Cases	Cases	Cases	Controls	Controls	Controls	ANOVA p-value for model Ulcer	t-value Ulcer presence (degrees of freedom	p-value Ulcer presence	Cohen's d Ulcer presence	ANOVA p-value for model Change	t-value Change over time (degrees of freedom [df])	p-value Change over time	Cohen's d Change over time
Pressure-time integral (pti) Ns/cm²	*Baseline*	*1st follow-up*	*2nd follow-up*	*Baseline*	*1st follow-up*	*2nd follow-up*								
Toe 1/ Hallux	1.2 (± 0.8)	1.4 (± 1.1)	1.5 (± 1.3)	0.9 (± 0.4)	0.8 (± 0.4)	0.8 (± 0.4)	<0.001	2.77 (86)	0.006	0.42	0.02	-2.54 (325)	0.011	-0.20
Toes 2-5	0.9 (± 0.4)	0.7 (± 0.4)	0.6 (± 0.4)	0.6 (± 0.3)	0.5 (± 0.8)	0.5 (± 0.8)	0.11	1.53 (86)	0.12	-	0.21	-0.96 (327)	0.33	-
Metatarsal 1	2.2 (± 0.7)	2.1 (± 1.1)	1.8 (± 0.8)	1.8 (± 0.7)	1.5 (± 0.7)	1.4 (± 0.7)	0.02	2.19 (86)	0.03	0.33	<0.001	-4.12 (328)	<0.001	-0.32
Metatarsal 2	2.7 (± 1.1)	2.4 (± 1.1)	2.1 (± 1.0)	2.5 (± 0.9)	2.3 (± 1.0)	2.1 (± 1.0)	0.69	0.87 (86)	0.38	-	<0.001	-3.41 (334)	0.007	-0.26
Metatarsal 3	2.7 (1.3)	2.6 (± 1.4)	2.4 (± 1.4)	2.6(± 1.0)	2.5 (± 1.0)	2.2 (± 1.0)	0.55	0.51 (86)	0.61	-	0.001	-3.68 (333)	<0.001	-0.29
Metatarsal 4	2.1 (± 0.9)	2.2 (± 1.3)	2.0 (± 1.4)	2.4 (± 0.9)	2.3 (± 0.8)	2.0 (± 0.9)	0.21	-1.53 (86)	0.12	-	0.004	-3.69 (333)	<0.001	-0.29
Metatarsal 5	1.9 (± 0.9)	1.8 (± 0.9)	1.6 (± 1.1)	1.8 (± 0.6)	1.7 (± 0.7)	1.5 (± 0.7)	0.97	-0.07 (86)	0.94	-	0.003	-2.84 (331)	0.004	-0.22
Mid Foot#	1.6 (± 0.9)	1.7 (± 1.2)	1.5 (± 0.9)	1.0 (± 0.4)	0.9 (± 0.4)	0.9 (± 0.5)	0.04	4.21 (86)	<0.001	0.64	<0.001	-4.26 (328)	<0.001	-0.33
Medial Heel	2.5 (± 1.2)	2.5(± 1.6)	2.5 (± 1.4)	2.0 (± 0.7)	1.9 (± 0.6)	1.8 (± 0.7)	0.02	1.24 (77)	0.21	0.20	0.18	-1.28 (77)	0.203	-
Lateral Heel	2.3 (± 1.1)	2.3 (± 1.5)	2.6 (± 2.1)	1.9 (± 0.7)	1.9 (± 0.6)	1.8 (± 0.8)	0.03	2.02 (86)	0.04	0.31	0.12	-1.37 (324)	0.171	-

Legend: All data represents pressure time integral (pti) and the reported values indicate the mean ± standard deviation (SD) and df= degrees of freedom as indicated. Cases= diabetic foot ulcer group, Controls= diabetes mellitus control group. -= not computed as this was not significantly different. The ANOVA p-value indicates values obtained an overall goodness of fit of statistical models and represent the overall significance of outcomes in the model. The p-values indicate the individual p-values obtained from the correlations tables from linear mixed effects models and the p-value for the effect of ulcer presence on plantar pressure and effect of time on plantar pressure. #= The interaction-term was significant for the DFU group for the mid-foot over-time; ANOVA p=0.04, t=-2.05, df=328, correlation p=0.04, d=0.16.

8.6 Discussion

The main finding from this study was that plantar pressures (mpp and pti) were higher at multiple sites in cases with chronic DFUs compared to diabetes controls throughout a six-month follow-up period. Overall, mpps and ptis at several sites significantly reduced over time in all participants. Although shorter wound healing times have been achieved by reducing plantar stresses on ulcerated tissue using gold standard offloading devices (Jeffcoate et al., 2016), whether plantar pressures actually remain elevated in people with chronic DFUs was largely unknown. The results from this study provide convincing evidence that plantar pressures remain elevated in people with DFUs and emphasize the need for long term pressure offloading in patients with chronic neuropathic DFUs to expedite and encourage ulcer healing, which is an important clinical consideration.

A number of longitudinal studies have previously assessed the association of plantar pressures with the subsequent risk of developing DFUs (Boulton, Betts, Franks, Ward, & Duckworth, 1987; Ledoux et al., 2013; Pham et al., 2000; Veves et al., 1992). However, we are not aware of any longitudinal studies that have prospectively investigated plantar pressures in patients with existing active DFUs as reported here. Our findings suggest that patients with active DFUs have on-going higher plantar pressures at multiple sites by comparison to controls (Fernando et al., 2016c). Boulton et al. (1987) reported that changes in the levels of plantar pressure may occur during a relatively short time in participants with DPN; however measurement repeatability needs to be considered in interpreting such data (Boulton et al., 1987).

Our findings also suggest that overall plantar pressures reduced during repeated assessment. One possible reason for this is a familiarisation effect with repeated plantar pressure assessment. A previous study reported reductions in plantar pressure from baseline to follow-up for a majority of plantar sites in healthy participants (Zammit et al., 2010). This may indicate that as participants become more familiar with the walking environment and the plantar pressure protocol (Fernando et al., 2016a; Fernando et al., 2015), their plantar pressures show a relative reduction. These findings have some implications for researchers collecting plantar pressure data and may

indicate that collecting plantar pressure data over several observations may lead to small gradual reductions in measurements. However, as the reductions in pressures occurred overall and as the sites identified to have the highest plantar pressures in cases compared to controls were similar throughout follow-up, whether there is added value in repeated plantar pressure measurements is uncertain in comparative studies, but are likely important for intervention studies (Bus & Lange, 2005; Fernando et al., 2016a; Gurney et al., 2008; McPoil, Cornwall, Dupuis, & Cornwell, 1999).

Our results support the need for sustained pressure offloading in patients with a history of chronic DFUs. Whether long term monitoring of plantar pressures can improve management of DFUs is controversial. Bus et al. (2011) suggested the use of in-shoe plantar pressure assessments to better inform pressure off-loading in participants with DPN at risk of developing DFU (Bus, Haspels, & Busch-Westbroek, 2011). Despite this, a number of studies have suggested that plantar pressures are not routinely assessed in clinical practice (Quinton, Lazzarini, Boyle, Russell, & Armstrong, 2015; Wu et al., 2008). At present, very few centers around the world routinely utilize plantar pressures to ascertain levels of required offloading for patients at risk of DFUs (Bus et al., 2016a; Bus et al., 2008b). The high level of intra participant, intra-device and inter-device variability of plantar pressure measurements is considered a deterrent to its routine use in clinical practice (Fernando et al., 2016a; Frykberg & Banks, 2015) and also indicates limited sensitivity to detect change longitudinally. This is a major limitation which needs further attention and improvement. Irrespective of this, plantar pressure measurements may indirectly assist in improving patient compliance with pressure offloading. One of the challenges for clinicians when communicating the importance of offloading with patients is the inability to demonstrate the need visually. The visualization of plantar pressures using pressure measurements, in addition to quantitatively assessing the level of pressure, may provide an incentive to obtain better patient compliance with off-loading (Bus et al., 2013; Waaijman et al., 2013).

A key area of future research focus in the field should be to characterize the important predictors of elevated plantar pressures in people with active DFUs. Recent work has outlined that foot-deformity and foot structure may be predictors of higher mid-foot plantar pressure in people with DPN and a history of DFUs (Barn et al., 2015). Although we did not observe a statistical difference in the foot types between cases and

controls, the higher mid-foot plantar pressures in cases may have been due to the presence of pes-planus foot-type. Other work has outlined that an increase in the viscoelasticity of plantar soft tissues, especially at the first metatarsophalangeal joint may be a crucial factor for elevated peak plantar pressures in people with DPN (Jan, Lung, Cuaderes, Rong, & Boyce, 2013). More recent work has outlined that new measures of plantar pressure such as the pressure gradient angle which quantifies the time-varying directions of plantar pressure may provide more valuable information regarding the plantar stressors experienced by people with DPN (Lung, Hsiao-Wecksler, Burns, Lin, & Jan, 2016). Whether measuring the viscoelasticity of plantar tissue and pressure gradient angles in people with active DFUs provides more insight on the biomechanical mechanisms underlying delayed wound healing is uncertain.

The limitations of our study include a small sample size in combination with a rather large number of statistical comparisons, the limited number of co-variates able to be used in statistical analyses and the inability to assess the association of plantar pressure and ulcer healing due to a small number of healed ulcers. It is also likely that reductions in plantar pressures that were observed were more representative of controls rather than cases as we had more controls than cases. The poor healing rate observed in our study (4 DFUs or 19% during six months follow-up) is representative of the inclusion criteria we used (i.e. people with DFUs of more than 3 months duration). Hence our results are representative of people with chronic DFUs regardless of their healing capacity and supports our earlier finding that following DFU healing, plantar pressures remain higher in people with a history of DFUs (Fernando et al., 2014a). Therefore, our results may not be applicable to people with DFUs of less than 3 months duration. A small increase in ulcer size at the second follow-up may have been due to cases with healing DFUs dropping out of the study, whereas cases with poorly healing DFUs remained. The length of follow-up varied between individuals and between the two groups; however, this was adjusted for in our analyses. A longer follow-up period may have provided further clarity on the relationship between elevated plantar pressures and ulcer healing, although this would have been limited by attrition. Lastly, we were unable to demonstrate that plantar pressures reduce during ulcer healing so that the differences in plantar pressures in cases reduce during follow-up in comparison to controls. Therefore, there is no evidence to warrant a step-down approach in offloading during ulcer healing. Our findings suggest that plantar pressures remain significantly higher in

people with active DFUs. However, this should be further assessed in future studies using a longer follow-up period.

As mentioned earlier, site specific mpp and pti have a variable level of reproducibility with repeated observations which may have also influenced our follow-up results (Fernando et al., 2016a). We assessed barefoot plantar pressure rather than in-shoe pressure, as we wanted to investigate the foot-ground interaction in patients with DFUs without the influence of footwear. Our plantar pressure results seem to be lower than other values reported in the literature, but are consistent with other data obtained using the same pressure measurement system in participants with diabetes (Qiu et al., 2013). We assessed whether the reductions in plantar pressures over time were due to attrition of participants with higher plantar pressures at baseline, however excluding participants who were lost to follow-up did not influence the reductions of plantar pressure over time.

The strengths of this study include the longitudinal design, the reporting of reproducibility prior to data collection (Fernando et al., 2016a) and the use of statistical models to adjust plantar pressure outcomes for a number of key confounding factors including the presence of neuropathy. Our attrition rate was also significantly lower than the rate thought to be acceptable within the field. Our analyses accounted for numbers lost to follow-up and any differences in outcomes due to differences in follow-up times and the sample composition over time.

Our results highlight the importance of offloading in the long-term management of people with DFUs (Jeffcoate et al., 2016). As the ideal percentage of plantar pressure reduction required to facilitate ulcer healing is yet to be determined (Cavanagh & Bus, 2010), offloading efforts should aim to reduce plantar pressures as much as possible throughout ulceration, using a 30% reduction recommended to prevent DFU development as a guide (Bus et al., 2016a). Given our results indicating that plantar pressures may show natural reductions during follow-up, it is imperative that clinicians should appreciate that natural changes in plantar pressures could occur with time. Future work should focus on how best to utilise plantar pressures in managing and preventing DFUs and in identifying alternate methods of reducing plantar pressure during gait (Fernando et al., 2016b).

8.7 Conclusion

The findings from this study suggest that plantar pressures assessed during gait are higher in diabetes patients with chronic DFUs than controls throughout prolonged follow-up at several plantar sites. Long term offloading is needed in diabetes patients with chronic DFUs to facilitate ulcer healing.

Chapter. 9 General Discussion and Recommendations

This concluding chapter provides a brief summary of chapter findings (Section 9.1) before discussing the key findings of this thesis that answer the original research questions reported in Chapter 1. Limitations and strengths of the thesis are then discussed with consideration given to the key findings (Section 9.3 and 9.4). Finally, the key clinical and research recommendations arising from this thesis are outlined (Section 9.5) and conclusions are drawn (Section 9.6).

Broadly, the research contained in this thesis has contributed original knowledge in three forms by; 1) comprehensively synthesizing and summarizing existing literature on the biomechanical outcomes in people with DPN and DFUs in systematic reviews and meta-analyses, 2) evaluating the reproducibility and application of available protocols to measure gait and plantar pressures in people with DFUs and finally and most importantly 3) contributing novel knowledge regarding gait and plantar pressure characteristics of people with active DFUs in cross sectional and longitudinal studies.

9.1 Summary findings from the research

A summary of the aims, hypotheses and main findings from the research contained in this thesis in relation to the original research questions of this thesis are reported in Table 9.1.

Table 9.1 Summary of key findings from the research contained in the thesis.

Chapter	Title	Research Question	Aim	Key findings	Conclusions
2	***A systematic review and meta-analysis of gait characteristics and dynamic barefoot plantar pressure measurements in people with diabetic peripheral neuropathy*** Based on the publication; *Biomechanical characteristics of peripheral diabetic neuropathy: A systematic review and meta-analysis of findings from the gait cycle, muscle activity and dynamic barefoot plantar pressure. Journal of Clinical Biomechanics 2013 Oct;28(8):831-45.*	What is known about the gait and plantar pressure characteristics of people with DPN?	To assess the effect of DPN on gait (temporal-spatial characteristics (TSPs), joint angular kinematics and kinetics), dynamic electromyography (EMG) (muscle activation and deactivation patterns) and dynamic barefoot plantar pressures (plantar pressures during gait).	A systematic review of studies suggested potential differences in the biomechanical characteristics (kinematics, kinetics, EMG) of cases with DPN. However, these findings were inconsistent and limited by small sample sizes. However, cases with DPN have elevated plantar pressures at the forefoot and rear foot and occupy a longer duration of time in the stance-phase during gait.	People with DPN exhibit significantly elevated plantar pressures and occupy a longer duration of time in stance phase during gait compared to controls.

3	***Meta-analysis of plantar pressure measurements in*** ***People with diabetic peripheral neuropathy with active foot ulceration, previous ulceration and no history of ulceration*** Based on the publication; *Plantar pressure in diabetic peripheral neuropathy patients with active foot ulceration, previous ulceration and no history of ulceration: a meta-analysis of observational studies. PLoS One. 2014 Jun 10;9(6): e99050.*	Do differences in plantar pressures exist between people with DPN: without a history of DFU, with a history of DFU and with active DFUs?	To compare plantar pressures in patients with a previous history of or active ulcers (cases) and individuals with DPN without a history of ulceration (controls).	Meta-analyses suggested plantar pressures were significantly higher in people with DPN with a history of DFUs compared to controls with DPN without a history of DFUs, but whether plantar pressures were higher in people with active DFUs was unclear.	Although plantar pressures were higher in people with a history of DFUs, alterations in gait characteristics may occur in people with DPN with active DFUs which reduces the plantar pressures experienced during active ulceration.

4	***General methods and Study protocol*** Based on the publication; *Lower limb biomechanical characteristics of patients with neuropathic diabetic foot ulcers: the diabetes foot ulcer study protocol. BMC Endocrine Disorders. 2015; 15: 59.*	Can currently available methodology be used to obtain gait and plantar pressure data with adequate reproducibility in people with DPN with and without active DFUs?	To establish the lower limb biomechanical characteristics (TSPs, kinematics, kinetics, muscle activations and plantar pressures) of patients with plantar neuropathic DFUs and to longitudinally evaluate these characteristics at 3 months and 6 months follow-up.	The design was a case – control study nested in a six-month longitudinal study. Cases were participants with active plantar neuropathic foot ulcers. Controls were patients with type 2 diabetes and healthy participants with no history of foot ulceration. Standardised gait and plantar pressure protocols were used to collect biomechanical data at baseline, three and six months.	It was anticipated that the findings from the research studies contained in this thesis will provide valuable information regarding the biomechanical characteristic of type 2 diabetes patients with neuropathic foot ulcers.

5	***The reproducibility of acquiring three dimensional Gait and plantar pressure data using established Protocols in participants with and without type 2 diabetes and foot ulcers.*** Based on the publication; *The reproducibility of acquiring three-dimensional gait and plantar pressure data using established protocols in participants with and without type 2 diabetes and foot ulcers. Journal of Foot and Ankle Research. 2016 Jan 29; 9:4. doi: 10.1186/s13047-016-0135-8. eCollection 2016.*	Can currently available methodology be used to obtain gait and plantar pressure data with adequate reproducibility in people with DPN with and without active DFUs?	To describe the methods and the reproducibility of assessments related to three-dimensional gait analysis and plantar pressure assessment in people with DFUs and controls with diabetes without DFUs and healthy controls.	There was no clear association between participant group and the reproducibility of assessing anatomical locations and leg dimensions. Plantar pressure measurements appeared to vary little between groups and were generally greatly varied for all groups of participants for maximum sensor pressure and the pressure-time integral. The between-day variability of kinematic data was not assessed.	Important gait and plantar pressure measurements can be reasonably reliably acquired in people with DFUs when compared to controls without DFUs. Nearly all measures contributing to three-dimensional gait parameter assessments were within predefined acceptable limits. Most plantar pressure measurements were also within predefined acceptable limits; however, reproducibility was not as good for assessment of the

					maximum sensor pressure.
6	***Gait characteristics of people with diabetes-related neuropathic plantar foot ulcers*** Based on the publication; *Gait parameters of people with diabetes-related neuropathic plantar foot ulcers. Clinical Biomechanics. 2016; 37:98-107.*	What are the gait characteristics of people with DFUs that differentiate them from people with diabetes without DFUs and from healthy people?	To comprehensively assess the kinematic, kinetic and TSPs in cases with active DFUs using three-dimensional movement analyses.	When adjusted for age, sex and body mass index, cases with DFUs had a smaller amount of plantar flexion, knee flexion and pelvic obliquity compared to both control groups through the gait cycle. Cases also had a significantly greater range of anterior-posterior ground reaction force and total vertical ground reaction force compared to controls along with a significantly slower walking speed and smaller step length.	Whether the observed gait parameters contributed to the ulcer development or are a response to the ulcer is currently unclear and needs further investigation.

7	***Plantar pressures are higher in cases with diabetes-related foot ulcers compared to controls despite a longer stance phase duration*** Based on the publication; *Plantar pressures are higher in cases with diabetic foot ulcers compared to controls despite a longer stance phase duration. BMC Endocrine Disorders 2016; 16:51 DOI 10.1186/s12902-016-0131-9.*	What are the plantar pressure characteristics of people with DFUs that differentiate them from people with diabetes without DFUs and from healthy people?	To assess whether plantar pressures were higher in patients with active unilateral plantar DFUs of >3 months duration (cases) compared to patients without a foot ulcer history (diabetes controls) and patients without a diabetes or foot ulcer history (healthy controls).	When adjusted for age, sex and body mass index, the mean peak plantar pressure and pressure-time integral of toes and the mid-foot were significantly higher in cases with DFUs compared to diabetes and healthy controls, whereas the stance phase duration was also significantly higher in cases compared to both control groups.	Plantar pressures are higher in cases with DFUs despite having a longer stance phase duration which would be expected to lower plantar pressure. These results highlighted the importance of offloading feet during active ulceration in addition to before ulceration.

8	***Plantar pressures are elevated in people with longstanding diabetes-related foot ulcers during follow-up*** Based on the publication; *Plantar pressures are elevated in people with longstanding diabetes-related foot ulcers during follow-up. PLOS ONE (2017) 12(8): e0181916*	Do the plantar pressures of people with DFUs remain different compared to people with diabetes without DFUs over the course of 6 months follow-up?	To investigate plantar pressures at baseline and three and six months later in participants with DFUs (cases) compared to participants without DFUs (controls).	Cases with DFUs had a higher mean peak plantar pressure at several foot sites including the toes and mid-foot and a higher pressure-time integral at the hallux, metatarsal 1 and the mid-foot and rearfoot compared to controls during each visit. Over time both cases and controls demonstrated a reduction in pressure-time integral at multiple plantar sites.	Plantar pressures assessed during gait are higher in diabetes patients with chronic DFUs than controls at several plantar sites throughout prolonged follow-up. Long term offloading is needed in diabetes patients with DFUs to facilitate ulcer healing.

Legend: DFU = diabetes-related foot ulcer, TSPs = temporal-spatial parameters, DPN = diabetic-peripheral neuropathy

9.2 Discussion of key findings arising from the research contained in the thesis

The section below discusses the key findings from the research contained in this thesis with reference to the original research questions from Chapter 1.

Question 1; What is known about the gait and plantar pressure characteristics of people with DPN?

9.2.1 Altered gait characteristics and higher plantar pressures are evident in people with DPN compared to people with diabetes without DPN and healthy controls

The research contained in this thesis has been able to demonstrate that people with DPN have a different gait pattern to controls without DPN and it was postulated that an altered mobility pattern was likely to contribute towards increased risk of falls and higher risk of tissue trauma predisposing to DFUs in this population (Fernando et al., 2013). Other studies have demonstrated that changes in gait due to early DPN-related sensory-motor changes are likely to manifest early in the disease progression (Picon et al., 2012; Yi, Sartor, Souza, & Sacco, 2016). From the systematic review (Chapter 2) it was evident that alterations to gait and plantar pressure in people with DPN occurs well before the onset of DFUs which is consistent with other work in the area (Katoulis et al., 1996; Mueller et al., 1994a; Raspovic, 2013).

Since the systematic review (Chapter 2) was published (Fernando et al., 2013), several newer studies have reported on the functional outcomes during gait in people with DPN and concluded that perturbations due to DPN during gait are likely to lead to an increased risk of falls (Brown, Handsaker, Bowling, Boulton, & Reeves, 2015; Timar et al., 2016), increased utilization of muscle activity and increased energy expenditure (Brown et al., 2014; Handsaker et al., 2014) and greater difficulties with stair-ascent (Brown et al., 2016; Handsaker et al., 2014). More recently, an observational study evaluating self-perceived unsteadiness was able to show that people with DPN have low awareness of their unsteadiness but actually attempt to self-regulate their unsteadiness by walking more

slowly and taking shorter steps (Reeves, Brown, Petrovic, Boulton, & Vileikyte, 2017; Timar et al., 2016).

These findings are consistent with our findings of an altered gait due to DPN. Therefore, although a slower walking speed might be a defensive gait strategy in people with DPN, our findings indicate that an increased stance phase duration and increased peak plantar pressures and pressure-time integrals especially in the fore-foot of people with DPN are two of the modifiable factors that may be targeted to reduce the risk of ulceration.

Question 2; Do differences in plantar pressures exist between people with active ulcers, people with a history of ulcers and people with DPN without ulcers?

9.2.2 People with a history of DFUs have higher plantar pressures compared to people with DPN without DFUs

The key findings from the research contained in this thesis outline the implications of altered lower limb biomechanics (gait and plantar pressure) in people with diabetes through the sequale of complications arising due to DPN (Chapters 2 to 3 and 6 to 8). The most pertinent finding was that plantar pressures remained elevated in people with active ulcers and in people with DFUs following ulcer healing. This signifies the important role of pressure offloading in people with DPN. The findings demonstrate that once people with diabetes develop DPN, their plantar pressures profiles are higher compared to their diabetes counterparts without DPN (Chapter 2) (Fernando et al., 2013). Similarly, once DFUs develop, plantar pressure profiles are higher compared to people with DPN without a history of DFUs (Chapter 7) (Fernando et al., 2016c). Furthermore, plantar pressures continue to remain elevated in people with a history of DFUs even after the wounds themselves have healed (Chapter 3) (Fernando et al., 2014b). The higher plantar presssures observed in people with DPN are likely to be due to a combination of altered gait characteristics as well as altered tissue properties of the foot (Wrobel & Najafi, 2010). Hence, the findings from the studies contained in this thesis have suggested that once DPN has developed, plantar pressures are significantly higher in people with DPN and does not

seem to reduce during or after the development or healing of DFUs. The findings seem to outline that gait characteristics and hence plantar pressures worsen with worsening DPN and especially leading up to and following the development of DFUs.

Question 3; Can currently available methodology be used to obtain gait and plantar pressure data with adequate repeatability in people with active DFUs?

9.2.3 Gait and plantar pressure characteristics can be reliably acquired in people with diabetes, DPN and DFUs when using standard protocols

As mentioned in Chapter 4, the protocol used for collecting gait and plantar pressures data was trialed prior to initiating data collection in prospective studies and the repeatability of plantar pressure and gait assessment protocols was assessed (Chapter 5). There are no known previous studies which have focused on the protocol-related considerations regarding collecting plantar pressure and gait data in people with active DFUs and therefore the protocols from this study provide novel insight for future studies in the field. However, the application of the protocol should occur with consideration of the limitations reported in Chapter 5, such as the variability of plantar pressure measurements and difficulties in placing reflective markers on participants who are obese or have a larger body mass. Therefore, the findings from the measurement reproducibility study indicated that standard protocols can be utilised to study the gait and plantar pressures in people with DFUs (Fernando et al., 2016a; Fernando et al., 2015). Importance has to be given however to the adherence to a standardised protocol to obtain repeatable and valid data.

Question 4; What are the gait and plantar pressure characteristics of cases with DFUs that differentiate them from controls with diabetes without DFUs and from healthy controls?

9.2.4 People with active DFUs have altered gait characteristics that are likely to contribute to their higher plantar pressures compared to people without DFUs

The results from the prospective studies of this thesis demonstrated that whilst some gait features in people with DFUs were similar to gait features in people with DPN without DFUs, other gait features were markedly different and likely related to the progression or worsening of DPN in people with DFUs (Fernando et al., 2016b). However, despite these findings, whether the gait features in people with DPN changes before or after the development of DFUs remained unclear.

Several studies have previously attempted to evaluate why plantar pressures were elevated in people with DPN (Armstrong & Lavery, 1998b; Barn et al., 2015; Fernando et al., 1991; Goldsmith, Lidtke, & Shott, 2002; Hastings et al., 2000; Mueller et al., 1994b; Orendurff, Rohr, Sangeorzan, Weaver, & Czerniecki, 2006; Payne, Turner, & Miller, 2002; Rao, Saltzman, & Yack, 2006; Savelberg et al., 2009b; Stess et al., 1997). These studies have outlined that restrictions in the joint range of motion of the ankle and first metatarsophalangeal joints (Armstrong & Lavery, 1998b; Fernando et al., 1991; Goldsmith et al., 2002; Hastings et al., 2000; Mueller et al., 1994b; Orendurff et al., 2006; Payne et al., 2002; Rao et al., 2006); foot structure and foot deformities (Barn et al., 2015; Bus et al., 2005); altered radiological alignment of the foot (Cavanagh et al., 1997); increased plantarflexion joint moments (Savelberg et al., 2009b); intrinsic foot muscle atrophy and muscle imbalances (Stess et al., 1997); glycation of collagen-rich tissues due to long-standing hyperglycemia particularly in the plantar fascia of the foot and the posterior muscle compartments of the ankle (Couppe et al., 2016; D'Ambrogi et al., 2003; Giacomozzi, D'Ambrogi, Uccioli, & Macellari, 2005) and an increase in the viscoelasticity of plantar soft tissues, especially at the first metatarsophalangeal joint (Jan et al., 2013) as being contributors of elevated plantar pressures in people with DPN.

However, most of these studies, with the exception of one study (Savelberg et al., 2009b), did not investigate the gait characteristics of their participants. Plantar pressures are most relevant when walking or standing for prolonged periods of time (Najafi et al., 2010a; Wrobel & Najafi, 2010). Thus, it is likely that higher plantar pressures in people with DFUs during walking occur due to an altered gait pattern. Additionally, localized foot deformities only explain a proportion of the variance in plantar pressures at various sites under the foot in recent statistical models (Barn et al., 2015), which indicates functional outcomes such as altered gait features may be more important factors associated with higher plantar pressure. Hence altered gait characteristics and individual variation in gait due to DPN are more likely to have the greatest influences on peak plantar pressures during walking than foot structure (Cavanagh et al., 1997).

Despite some of the findings obtained from three-dimensional movement analysis indicating the presence of adaptive gait patterns to protect the ulcerated foot (Fernando et al., 2016b), the presence of high anterior–posterior (FAP) and vertical (FV) ground reaction forces suggest significant impairments in the gait strategy of people with DFUs. Our results also demonstrate that the kinematics of the pelvis, knee and ankle joints were altered in people with DFUs (Fernando et al., 2016b). It is likely that altered gait due to altered kinematics is a likely factor which is associated with elevated plantar pressures. This indicates that the higher ground reaction forces experienced by people with active DFUs are likely to be at least in part related to their altered kinematics. Hence, the gait characteristics of people with DFUs, particularly through kinematic alterations and kinetic alterations in the vertical ground reaction force is likely to result in elevated plantar pressures.

9.2.5 People with active DFUs have higher plantar pressures compared to people with diabetes without DFUs and healthy controls

Findings from the research in Chapter 7 also showed that plantar pressures at several specific plantar locations are significantly higher in people with active DFUs compared to

controls. It is well known that a higher magnitude or a longer duration of plantar pressure can either individually or cumulatively contribute to increased plantar tisssue stresses which results in DFUs in people with DPN (van Schie, 2005). Yet it was unknown why foot ulceration only occurs in selected individual's with DPN with high plantar pressures (Boulton et al., 1987; Bus, 2016; Ledoux et al., 2013). There is some indication that the location of peak pressure is an important predictor of ulceration (Ledoux et al., 2013). A plausible explanation for this is that overall high plantar pressures alone in the presence of DPN is insufficient to cause ulceration of tissue (Masson et al., 1989).

As plantar pressures are a result of the vertical ground reaction force divided by the contact area of the foot, it is likely that people with DFUs experience higher plantar pressures due to an increased vertical ground reaction force profile coupled with altered tissue properties of the contact area (plantar tissue). Although we did not investigate tissue properties in our study, findings from other studies have reported the presence of soft-tissue glycation (Couppe et al., 2016; D'Ambrogi et al., 2003; Giacomozzi et al., 2005) and altered viscoelasticity of plantar soft tissues (Jan et al., 2013) in people with DPN. The fact that ulcer development seems to be site specific (Ledoux et al., 2013) coupled with the results of gait alterations reported in this thesis provides a more comprehensive view that both determinants of pressure (i.e. vertical ground reaction force and contact area) are altered in people with DPN. This may also be the explanation for why elevated plantar pressures are sustained in people with DFUs during follow-up.

Question 5; Do the plantar pressures of cases with DFUs remain different compared to diabetes controls without DFUs over the course of 6 months follow-up

9.2.6 Plantar pressures remain higher in people with active DFUs throughout follow-up when compared to people without DFUs

A large number of retrospective and prospective studies have suggested that plantar pressures are elevated in patients with DPN at high risk of ulceration (Boulton et al., 1983;

Frykberg et al., 1998; Pham et al., 2000; Veves et al., 1992; Wrobel & Najafi, 2010). However, what was previously not established was whether plantar pressures were elevated throughout active ulceration and following ulcer healing.

However, the offloading requirements during active ulceration were poorly understood, in part, because there has been little study of plantar pressures in people with active DFUs. The results from the longitudinal study contained in this thesis (Chapter 8) suggest that plantar pressures (both mpp and pti) during walking are higher in people with active DFUs compared to controls throughout a six month follow-up period. It was well known that the risk of recurrence is high after DFUs heal (Armstrong et al., 2017; Singh et al., 2005; Waaijman et al., 2014; Wu et al., 2007). Recent research indicates that 40% of patients with healed DFUs will develop a subsequent DFU within one year of healing (Armstrong et al., 2017) and often this sequale results in lower limb amputation (Wu et al., 2007). Given the high recurrence rates of DFUs, some have coined the term 'remission' rather than 'cure' when referring to healed DFUs (Armstrong & Mills, 2013). Studies have shown that offloading can be effective at reducing the risk of initial ulceration as well as in preventing ulcer recurrence (Bus et al., 2008a; Bus et al., 2016a; Bus et al., 2011; Bus et al., 2004; Bus et al., 2008b; Bus et al., 2013; Lavery, 2011; Lavery et al., 1997a; Lavery et al., 1997b; van Netten et al., 2016).

However, the specific offoading requirements for people with active ulcers were poorly understood as the plantar pressures in people with active ulcers during healing was previously unknown. The results from the longitudinal study contained in this thesis (Chapter 8) has been able to show that plantar pressures (both mpp and pti) remain elevated in people with active DFUs compared to controls without DFUs throughout a six month follow-up period. The locations of these pressures seemed to be specific to the type of plantar pressure outcome i.e pti or mpp.

Specifically, the plantar locations which had a higher mpp in people with DFUs compared to controls were the toes and the mid-foot. However, a greater number of plantar locations had higher ptis in people with DFUs compared to controls and included several forefoot,

mid-foot and rear-foot sites. This presumbly indicates that the magnitude (mpp) and duration of pressure (pti) are both important measures of tissue stress in people with active DFUs and therefore the offloading requirements in people with DFUs is likely to be site dependent and specific to the individual. Although further evaluation of the significance of this finding was not possible due to the small sample size in the studies contained in this thesis, this rudimentary finding indicates that measuring both plantar pressure outcomes may be important in people with active DFUs when considering offloading options which are site specific (i.e specific to a an ulcer site).

9.3 Limitations of the research contained in this thesis

The findings of this thesis need to be considered within the context of limitations of the research design, methods and the generalizability of the data from the various studies (Aronson, 1993; Moher, Liberati, Tetzlaff, & Altman, 2009; Stroup et al., 2000).

The main limitations of the systematic reviews and meta-analyses (Chapter 2 and 3) were that they contained heterogeneous groups of studies, which contained small sample sizes and not all studies included in the review accounted for the influence of confounding variables in participant selection and data analysis. Similarly, the selection of controls (i.e. types of controls) also differed in the studies included in reviews and this was likely why there were differences in the definition of outcome measures from individual studies. Importantly the data collection approaches and protocols used to collect gait and plantar pressure data in the individual studies included in the systematic review differed and the validity of these protocols was not always examined. Studies included in the systematic reviews also differed in the approach used to screen and diagnose DPN and in the characteristics of participants with DFUs (i.e. DFUs with differing aetiologies) and therefore the ‘case-definitions’ used in studies varied. These factors were likely to have influenced the consistency and homogeneity of the results. These heterogeneities may explain why some gait characteristics were not statistically significant between people with DPN and people with diabetes without DPN, yet remained statistically significant between people with DPN and healthy controls.

It is also likely that a statistically significant difference in plantar pressures between the sub-group of people with active DFUs and controls with DPN without DFUs was unable to be demonstrated in a meta-analysis due to a small number of people with active DFUs from the studies pooled in the meta-analysis rather than due to the absence of an effect between groups. As there were very few case-control studies on plantar pressures in people with active DFUs, there was a risk of type-2 error. The levels of statistical heterogeneity found in the in meta-analyses ranged from low to moderate and therefore the statistical uncertainty of findings from the meta-analyses was an important limitation of the outcomes from these analyses. However, the levels of statistical heterogeneity from the meta-analyses was evaluated and appropriate subgroup and exclusion analyses were performed to adjust for higher levels of statistical heterogeneity.

The focus of the reviews was on more recent case-control studies investigating the gait and plantar pressures in people with DPN which did not include all studies on the topic. Although this was done purposefully to focus on the more recent developments in the field obtained using novel technology to obtain gait and plantar pressure data, this can be seen as a limitation.

Although we carried out a reproducibility study prior to data collection (Chapter 5), this was limited by the small number of participants and the specific types of outcome measures investigated. A decision was made to only use a conveniently sampled cohort of participants and assess key outcomes. Whether this small sample represented a cross-section of people truly representative of the group to be prospectively recruited was unclear as recruitment to the longitudinal study (Chapter 8) had not concluded at the time the repeatability study was performed. However, for the study on measurement reproducibility, it was preferable to have a mixed cohort of participants with varying characteristics and the characteristics of people in the prospective studies were similar to the demographic of the study reported in Chapter 5. With reference to variability in biomechanical outcomes, the intra-limb gait variability of people with DPN has been the focus of more recent work in the field (Yi et al., 2016). Although not assessed in the reliability study, it is also likely that the intra and inter-participant gait measurements, intra-walk variability and intra-limb variability were sources of variability in kinematic and kinetic data outcomes between

participants and between groups and is a limitation of the findings as this was unaccounted for.

The prospective case-controls studies (Chapter 6 and 7) were likely subjected to selection bias. Given the difficulties of recruiting patients with active DFUs, it may be possible that the sample recruited were not entirely representative of the case population of interest. Similarly, whether the controls that were recruited were reflective of a broader population of diabetes controls and healthy controls from the community is difficult to establish. Even though participant recruitment continued over two years for these studies, the difficulties of recruiting patients with DFUs in prospective studies has previously been demonstrated (Idris, Game, & Jeffcoate, 2005). As this was a novel study which required patients to walk barefoot despite having an active DFU, although in a safe and controlled environment, this was a likely deterrent to participant recruitment (Mueller et al., 2006; Schaper et al., 2007). It must also be noted that patients with active DFUs are already burdened by multiple outpatient medical appointments and are often hospitalized for infection and other complications, therefore their ability to commit to a study was limited (Fernando et al., 2016d; Idris et al., 2005).

Nevertheless, the preliminary findings from these studies can be used as pilot data for a bigger study on people with DFUs. The design of the study did not allow for determining whether the elevated plantar pressures and altered gait characteristics observed in cases preceded ulcer development or occurred as a response to ulceration. This is a well-recognized limitation of a case-control study design which uses prevalent cases (Aronson, 1993). As we recruited people with chronic DFUs, this may have been a limitation. Analyses were un-adjusted for the presence of DPN at baseline, which is a key confounding factor between the diabetes groups. Therefore, the findings from the case control studies may have been more representative of worsening DPN severity in the DFU group, rather than due to the effect of the DFU itself, which was difficult to ascertain. The results from the longitudinal study on plantar pressures was however adjusted for the presence of DPN.

The longitudinal follow-up study also had a number of well recognized limitations (Caruana, Roman, Hernández-Sánchez, & Solli, 2015). Although the attrition in the longitudinal follow-up was within an expected level for a study on people with DFUs (Jeffcoate et al., 2016), the relatively small sized sample meant that inferences about ulcer site and ulcer healing could not be evaluated during longitudinal plantar pressure assessment. One of the key assumptions of the prospective studies was that people with differing ulcer locations could be combined in a group. However, in reality, the location of a plantar ulcer, the duration of an ulcer occurrence and the depth of the ulcer are all likely to have significant impact on plantar pressure distributions (Ledoux et al., 2013). Although we reported on ulcer locations, we were unable to carry out any further sub-group analyses. Another limitation was the inability to simultaneously measure in-shoe plantar pressures. Keeping in mind that patients with DFUs are recommended to wear footwear and prescribed offloading gear, the results of the longitudinal follow-up are not necessarily representative of what occurs in clinical settings in this population. Rather, the findings are representative of what would occur if offloading was not being utilised (i.e. during barefoot gait).

Lastly, the findings were likely to be influenced by information bias and the quality of the equipment that was used to study plantar pressures and gait. The data collection approaches were carried out with the instrumentation available at the James Cook University movement analysis laboratory and therefore the measurements are representative of the quality and validity of the instruments used. Although we used previously validated instruments (Fernando et al., 2016a; Fernando et al., 2015; Qiu et al., 2013), our plantar pressure results were on average lower than previously reported pressure values obtained using other measurement systems (Armstrong et al., 1998c; Boulton et al., 1983). Despite this, previous work has identified that despite differences between measurement systems used, relative differences in plantar pressure could be observed between people with DPN and without DPN (Mueller, Zou, Bohnert, Turtle, & Sinacore, 2008). The results reported in this thesis are consistent with this finding. Lastly, the importance of shear-pressures in the development and progression of DFUs has received considerable interest in the field (Hamatani et al., 2016; Pai & Ledoux, 2012; Yavuz, 2014;

Yavuz et al., 2008). However, shear pressures were not measured as an outcome of the studies included in this thesis.

Similarly, limitations in the three-dimensional movement analysis system used, particularly with reference to the Helen Hayes reflective marker system and errors due to the orientation of the coordinate systems resulting in a cross-talk effect and soft tissue artefacts, known to impact on knee joint angles are likely to have influenced the results (Baker, 2006; Baudet et al., 2014; Collins, Ghoussayni, Ewins, & Kent, 2009; Żuk & Pezowicz, 2015). The use of cluster markers on each segment of the lower limb and the use of calibrated anatomical segment techniques and data reduction techniques for cross-talk correction methods could have improved the validity of gait outcomes, especially at the knee-joint (Baudet et al., 2014; Żuk & Pezowicz, 2015). However, this was beyond the scope of the study. Therefore, whether the findings from the research contained can be directly transfered to other populations is uncertain but the data provides important preliminary observations for future work and are specific to the population studied.

9.4 Strengths of the research contained in this thesis

The strengths of the systematic reviews were the use of a comprehensive search strategy; considering appropriate search criteria; appropriate peer assessment of study quality and the evaluation of statistical heterogeneity and the use of clearly established protocols to perform the reviews (see Chapter 2 and 3). The risk of bias was minimized by using available guidelines for the reporting of systematic reviews and carrying out meta-analyses using a conservative approach (Moher et al., 2009; Stroup et al., 2000). The inclusion of studies from various countries in the systematic reviews also provided a broader cross-section of participants and a more representative global source population.

Important strengths of the prospective research included in this thesis were; the case-control study design nested in a longitudinal follow-up study; the generation of appropriate hypothesis to be tested prior to initiating data collection; the use of standard protocols that were previously validated and specifically tested for reliability in this thesis; adjusting results for important confounders including age, sex and BMI differences between groups

and for the presence of DPN where possible; and reporting results based on appropriate statistical approaches and the reporting of limits of agreement in results. The observational nature of the studies meant that all participants received standard care between study visits, which was an important consideration and strength of the research.

The small number of previous studies which evaluated barefoot plantar pressures in people with active DFUs have been limited by cross sectional designs (Armstrong et al., 1998c; Brash et al., 1996; Cavanagh et al., 1991; Kanade et al., 2006; Sauseng et al., 1999) and longitudinal studies have previously only focused on evaluating plantar pressures leading to ulceration and not plantar pressures during ulceration (Boulton et al., 1987; Ledoux et al., 2013; Pham et al., 2000; Veves et al., 1992). There are no known studies that simultaneously assessed both plantar pressures and the extent of gait variables examined in the research contained in this thesis in people with active DFUs. Hence a significant strength of the research contained in thesis is its longitudinal design and number of outcome measures which were evaluated in people with active DFUs. The findings provide the most recent and comprehensive analysis of gait patterns and plantar pressures in people with active DFUs to date.

9.5 Future work and recommendations

9.5.1 Recommendations for future work

In the context of the above key findings, several key future recommendations for research are evident. Firstly, the findings of this thesis indicate that future research should focus on modifying detrimental gait features preceding ulcer development. Although several clinical trials have attempted to modify gait in people with DPN through exercise-training, muscle strengthening and simple gait-retraining interventions (Allet et al., 2010a; Allet et al., 2010b; Salsabili, Bahrpeyma, & Esteki, 2015; Sartor et al., 2014) with limited success at improving walking speed and reducing forefoot pressure loading patterns and addressing peak muscle activation, whether sustainable clinically relevant outcomes can be achieved in people with DPN through gait-retraining remains questionable (Sartor et al., 2014).This is especially true as gait characteristics can revert to baseline, even following gait

retraining at six months follow-up (Sartor et al., 2014). Based on current international guidelines, the recommended offloading goal is to reduce plantar pressure up by at least 30% compared to standard footwear in people with a history of DFU (Bus et al., 2016a; Bus et al., 2016b). This offloading goal has been specifically recommended for prescribing therapeutic footwear that demonstrates this reduction; however, whether similar 30% recommended reductions in plantar pressure can be the outcome used to help target gait re-training approaches is currently unknown. The focus of future research should be on developing methods aimed at preventing the development of DFUs. Hence more healthcare resources and funding should be dedicated towards pressure offloading using any proven modality in people with diabetes when a diagnosis of DPN is made to mitigate the risk of developing plantar DFUs related to increased plantar pressures during altered gait (van Netten et al., 2016).

Secondly, while progress has been made in the field, previously proposed pressure thresholds to indicate risk of tissue ulceration have been inadequate (Armstrong et al., 1998c; Frykberg et al., 1998; Veves et al., 1992). The reality may be that there is no such single factor threshold which is able to be applied to all individuals with DPN. Hence the pressure magnitude and duration responsible for ulcer development likely varies between individual to individual, based on body composition, sex, lower limb joint range of motion, foot morphology, the extent of altered plantar tissue structure and most importantly based on the gait characteristics of the individual as demonstrated by the research contained in this thesis. Therefore, beter models to predict facotrs related to ulcer development and ulcer healing are urgently needed, incorporating biomechanical outcomes such as plantar pressure measurements, gait variables and measures of offloading adherence and physical activity as these are likely crucial predictors of ulcer healing. Other potential explanations of ulcer development such as shear pressure locations also need to be considered. While shear pressures were not a focus of the research contained in this thesis, the anterior-posterior ground reaction forces were significantly higher in people with DFUs when compared to controls, highlighting that shear forces were significantly higher in people with DFUs (Fernando et al., 2016b). Hence future work should attempt to develop and include methods of measuring shear pressure and it's role on ulcer healing (Yavuz, 2014).

Furthermore, although the studies contained in this thesis failed to focus on the full scope of gait variables (moments, acclerations, velocities, powers, EMG) in people with DFUs, future studies should investigate these important areas. In relation to future research on gait and plantar pressures in people with DFUs, there are a number of key focus areas that should be addressed. Particularly, whether gait characteristics of people with active DFUs change throughout ulcer healing remains unknown and should be a focus of future work. Future studies investigating the impact of ulcer site on plantar pressure distribution should be adequately powered with a suitable number of participants with similar ulcer sites and could be based on findings from this thesis. Similarly, future studies which aims to investigate plantar pressure differences between healing and non-healing ulcers should recruit a larger number of patients over a longer time frame and have a longer follow-up period. Future studies should also focus on the gait variability of people with active DFUs to determine whether stride to stride variability in people with DFUs is simillar to that of people with DPN without DFUs (Yi et al., 2016).

Thirdly, although currrent international guidelines have provided much needed evidence for best clinical practice, a major limitation with the current international guidelines (Bus et al., 2016a) is that it fails to recognise the influence of site specific absolute peak pressure values in people with DFUs. Whether a 30% reduction in an individual with extremely high peak pressures at a particular ulcerated site, or a pressure below 200 kPa is sufficient to reduce the risk of tissue injury in people with active DFUs is currently unclear. The current general guideline on a 30% pressure target to prevent ulcer recurrence was based on data from people with plantar metatarsal head DFUs and to prevent forefoot ulcer recurrence (Bus et al., 2016a). Therefore, further work is needed to determine the required levels of offloading in people with a history of DFUs at other plantar sites. The limitation with a proportional pressure reduction (i.e 30%) target in people with active DFUs is that it does not take into account the absolute site specific pressure experienced by an individual. Furthermore, the degree of pressure reduction needed for wound healing may be much higher compared to that of preventing wound recurrence.

Hence, given a lack of pressure targets in offloading actively ulerated sites, site specific plantar pressures values should be established in future research to guide offloading requirements for DFUs on specific areas of the foot. Therefore, future studies should attempt to evaluate methods of individualising offloading in people with DFUs, with particular focus on evaluating offloading requirements. In-shoe plantar pressures should be considered when evaluating people with active DFUs in future studies as barefoot pressure measurements have been performed in the studies contained in this thesis. An important area of future research will be to assess whether the use of plantar pressures to guide offloading in people with active DFUs is benficial, reliable and cost-effective as it has been in people with a history of DFUs.

9.5.2 Recommendations for clinical practice

Based on the findings of the research contained in this thesis, three recommendations for clinical practice in the field are given below;

Recomendation 1- Better approaches to offloading modalities should be developed and utilized when treating people with active DFUs taking into account site specific plantar pressures

A major finding from the research contained in this thesis was that plantar pressures remain elevated in people with active DFUs throughout ulceration. Such elevated plantar pressures are at least in part due to an altered gait strategy from longstanding DPN in people with DFUs. This illustrates that despite the outcome of an active DFU i.e healing or non-healing, plantar pressures are likely to remain elevated in people with DPN. Therefore, the treatment target should always be to achieve optimal pressure offloading despite the presence or absence of a DFU. This calls for a number of clinical considerations with regards to improving pressure offloading during and following an episode of active ulceration. These considerations can broadly be classified into three areas of priority

concerning pressure offloading; 1) optimising the offloading approach, 2) improving offloading adherence and 3) monitoring the appropriateness of offloading.

1) Optimising the offloading approach; Current guidelines indicate that people with active forefoot DFUs without ischemia or uncontrolled infection should be offloaded using a non-removable knee-high device with an appropriate foot-device interface (Bus et al., 2016a). A limitation with the use of non-removable knee-high device has been a lack of initiation by clinicians and a poor level of acquiescence by patients (Fife et al., 2010; Raspovic & Landorf, 2014; Wu et al., 2008). However, due to a paucity of literature which support a superior efficacy of conventional or standard therapeutic shoes to heal neuropathic plantar foot ulcers, compared to knee-high devices, the international guidelines currently do not encourage the use of therapeutic footwear in people with active DFUs (Bus et al., 2016a). Hence the offloading guidelines recommend as the first line of treatment non-removable knee-high devices such as total contact casts and irremovable cast walkers in people with active DFUs, which regrettably are not always utilized in routine clinical practice (Bus et al., 2016a). This indicates that there is a need to evaluate other offloading devices used in people with active DFUs in context of the guideline recommendations. Considering that the sites of higher pti and mpp were site specific in people with DFUs from the research findings of this thesis, targeted approaches to selecting offloading devices may be required during active ulceration. Given the sustained, higher plantar pressures in people with DFUs compared to controls throughout active ulceration, the highest attainable level of offloading should be attempted initially with all patients and individual offloading requirements need to be evaluated to encourage timely wound healing.

2) Improving offloading adherence; The greatest predictor of succesful offloading is likely to be patient adherence to the prescribed treatment. Off-loading adherence is associated with the amount of DFU healing, while postural instability is a predictor of non-adherence (Crews et al., 2016). Although removable walking devices have shown similar plantar pressure reduction efficacy to total contact casting (Piaggesi

et al., 2007), the total contact cast or removable cast walker rendered irremovable is suggested to be a superior treatment as it guarantees continuous adherence (Armstrong et al., 2001; Crews et al., 2016). In the instance that people with active DFUs wear their off-loading devices for only a minority of steps taken each day, the treatment goal is unlikely to be achieved (Armstrong, Lavery, Kimbriel, Nixon, & Boulton, 2003; Najafi et al., 2016). Although not a direct focus of the research contained in this thesis, given the relationship of adherence and plantar pressure reduction outcomes, developing better methods to measure offloading adherence in patients and strategies to improve offloading is important and this should become a routine clinical measure in patients with active DFUs.

3) Monitoring the appropriateness of offloading; Just as adherence to offloading interventions should be evaluated in patient's with DFUs, it should also be the role of the clinician to monitor whether the offloading modalities used by patients are continually suitable for their clinical problem. In light of the results of this thesis indicating that plantar pressures can be reasonably reliably measured and that mpp and pti outcomes provide different information at varying plantar sites, approrpriate site-specific offloading should be recommended following the development of DFUs and regularly monitored thereafter (i.e every 3 months) using quantitative plantar pressure measurments where possible especially when DFUs heal, as the higher plantar pressures which preceeded ulceration remain (Bus et al., 2008a; Owings, Woerner, Frampton, Cavanagh, & Botek, 2008).

Plantar pressure measurement allows the quantification of mechanical pressure and pressure distribution on the foot during walking using shod or platform-based measurement systems (Boulton, Vileikyte, Ragnarson-Tennvall, & Apelqvist, 2005b; Maluf & Mueller, 2003b; Schaper et al., 2016). As demonstrated in the studies included in this thesis, the measurement of plantar pressure distributions of patients with diabetes mellitus has been a topic of interest for a number of decades (Armstrong et al., 1998c; Boulton et al., 1987; Boulton et al., 1983; Cavanagh et al., 1993; Cavanagh et al., 2000; Lavery et al., 2003; Masson et al., 1989; Pham et

al., 2000; Veves et al., 1992). The technical capabilities of plantar pressure devices have improved overtime and a major focus now is on in-shoe pressure measurements which is more representative of everday activity as mentioned above (Abdul Razak, Zayegh, Begg, & Wahab, 2012; Bus, 2016; Orlin & McPoil, 2000).

Whilst the use of plantar pressure measurements in guiding plantar pressure offloading in people at risk of re-developing DFUs has previously been demonstrated to be effective (Bus et al., 2016a; Bus et al., 2011; Owings et al., 2008) there is still a need to test the use of plantar pressure measurements in guiding offloading approaches in people with active DFUs, to optimise wound healing. Despite recent international recommendations encouraging the use of in-shoe plantar pressure measurements to guide offloading (Bus et al., 2016a), plantar pressure measurements are currently seldom used to guide pressure offloading in people with DFUs due to a lack of access to pressure measurement systems by clinicians and a lack of knowledge regarding the use of pressure measurement devices (Bus, 2016). However, recent developments have demonstrated thaat the use of plantar pressure measurements may assist offloading selection and adherence (Gurney et al., 2017) and requires further investigation in clinical settings in people with active DFUs.

Recomendation 2- Priority should be given to evaluating plantar pressures in clinical settings and in developing novel technologies to map plantar pressure

Within the context of the findings reported in this thesis, plantar pressure measurements may assist in determining the required levels of offloading to encourage wound healing and assist in re-distributing pressures using data-driven, objective approaches to offloading. However, an important limitation of plantar pressure measurements is it's dependence on the operator's knowledge in how to translate pressure measurements into treatments for patients with varying presentations. The latter aspect is dependent on the experience of the operator and the types of interventions that are available to them. Therefore, there is a need

to formulate standardised decision making to offloading which are individudalised and based on the offloading needs of the individual.

Plantar pressure measurements could also be used to improve adherence to offloading by acting as a visual prompt for patients. A fitting example of this application is in newer technologies using smart-sensors that provides bio-feedback in the form of an alarm or cue when plantar pressure values exceed expected values for an individual (Najafi et al., 2010b; Najafi et al., 2017). Such applications may hold promise for the future management of people with DFUs but requires a number of considerations such as the repetability of various plantar pressure measurements, individual thresholds for ulceration and sensors capable of withstanding the tempreatures and forces associated with regular use over-time and the co-operation of patients. Therefore, the findings from the research contained in this thesis indirectly outlines the usefulness of plantar pressure measurments in guiding offloading in people with DFUs and in people at risk of developing DFUs. Regular monitoring of plantar pressure measurements should be a future focus in clinical settings.

Reccomendation 3- A 30% pressure reduction target should be utilised to offload plantar pressure in people with active DFUs in the absence of a better threshold and care should be taken when considering offloading options in patients with recently healed DFUs

Just as DFU prevention requires a larger focus on the use of appropriate therapeutic footwear and offloading devices (Cavanagh, 2004; Cavanagh & Bus, 2011; van Netten et al., 2016), people with active plantar DFUs require a focused approach to offloading. It is well known that if DFUs can be healed quickly and effectively, up to 80% of the morbidity, mortality and cost outcomes can be prevented (Boulton et al., 2005; Schaper et al., 2016).

As discussed previously, although current international guideline recommendations are apt for preventing ulcer recucrrence, they do not provide a pressure offloading target for active ulceration (Bus et al., 2016b). In the absence of a plantar pressure target to achieve wound

healing, perhaps a 30% pressure reduction target should also be used in people with active DFUs to guide treatment, although with the limitations of this target discussed above.

Simillary, offloading approaches should aim to offload plantar presssure during active ulceration and such approaches should be adjusted based on the requirements of pressure offloading and the sites of eleated peak pressure and elevated pressure-time integral. An important transition point is following ulcer healing. A major contributor of ulcer recurrence may be a significant increase in pressure when patients transition from irremovable knee-high devices to footwear or simillar offloading approaches following ulcer healing which is associated with a lower level of offloading (Wu et al., 2008). Given these considerations in offloading, focused solutions to tailoring offloading approaches that are completely individualised and are approriate for a patient's requirements after DFU healing is vital in clinical settings within the context of the results contained in this thesis.

9.6 Conclusion

The studies contained in the thesis have addressed several gaps in research and summarized observational research findings that are pertinent to addressing the problem of DFUs worldwide. The purpose of this thesis was to improve the previous gap in knowledge regarding lower limb mobilty (gait features and plantar pressures) in people with active plantar DFUs. The studies included in this thesis have demonstrated that gait function and capacity are significantly impaired in people with DPN as characterised by longer stance phase durations and higher plantar pressures. The findings also demonstrate that once DFUs develop, the higher plantar pressures that were likely to be present prior to ulceration continue to detrimentally influence the plantar tissues, likely contributing to delayed ulcer healing. This was characterised by a significantly higher ground reaction force profile, restricted joint angular kinematics of the ankle and knee and more proximal kinematic changes of higher pelvic tilt and obliquity and higher plantar pressures in people with active DFUs compared to controls. Higher plantar pressures continued throughout ulceration and were evident in people with DFUs at baseline, three-months and six-months follow-up when compared to controls without DFUs. The locations of higher plantar

pressures differed in reference to the pti and mpp where some locations experienced higher magnitudes (mpp) and others higher durations of pressure (pti). Furthermore, plantar pressures remained higher in people with a history of ulceration following ulcer healing, suggesting that the risk of re-ulceration is high due to the pre-existing alterations in gait leading to elevated plantar pressure demonstrated in the studies contained in this thesis.

The main recommendation arising from this thesis was that sustained, individualised offloading is required in people with DFUs given their altered lower limb mobility and sustained higher plantar pressures. The various findings from the research contained in this thesis has significant impact on the future clinical approaches to optimizing pressure offloading in people with DFUs. It is hoped that the findings and recommendations arising from this work will be used to guide future research in the field and to improve the biomechanical outcomes of people affected by DFUs.

Bibliography

Abdul Razak, A. H., Zayegh, A., Begg, R. K., & Wahab, Y. (2012). Foot Plantar Pressure Measurement System: A Review. *Sensors, 12*(7), 84-98.

Akashi, P. M., Sacco, I. C., Watari, R., & Hennig, E. (2008). The effect of diabetic neuropathy and previous foot ulceration in EMG and ground reaction forces during gait. *Clin Biomech (Bristol, Avon), 23*(5), 584-592.

Al-Qaisi, M., Nott, D. M., King, D. H., & Kaddoura, S. (2009). Ankle Brachial Pressure Index (ABPI): An update for practitioners. *Vascular Health and Risk Management, 5*, 833-841.

Allet, L., Armand, S., Aminian, K., Pataky, Z., Golay, A., de Bie, R. A., & de Bruin, E. D. (2010a). An exercise intervention to improve diabetic patients' gait in a real-life environment. *Gait and Posture, 32*(2), 185-190.

Allet, L., Armand, S., de Bie, R. A., Golay, A., Monnin, D., Aminian, K., . . . de Bruin, E. D. (2010b). The gait and balance of patients with diabetes can be improved: a randomised controlled trial. *Diabetologia, 53*(3), 458-466.

Allet, L., Armand, S., Golay, A., Monnin, D., de Bie, R., & de Bruin, E. (2008). Gait characteristics of diabetic patients: a systematic review. *Diabetes/Metabolism Research & Reviews, 24*(3), 173-191.

Altindas, M., Kilic, A., Cinar, C., Bingol, U. A., & Ozturk, G. (2011). The Epidemiology of Foot Wounds in Patients with Diabetes: A Description of 600 Consecutive Patients in Turkey. *The Journal of Foot & Ankle Surgery, 50*(2), 146-152.

Altman , D. G., & Bland, J. M. (1983). Measurement in Medicine: the Analysis of Method Comparison Studies. *The Statistician, 32*, 307-317.

American Diabetes Association. (2016). 9. Microvascular Complications and Foot Care. *Diabetes Care, 39*(Supplement 1), S72-S80.

Andersen, H. (2012). Motor dysfunction in diabetes. *Diabetes Metab Res Rev, 28 Suppl 1*, 89-92.

Apelqvist, J. (2008). The foot in perspective. *Diabetes Metab Res Rev, 24 Suppl 1*, S110-115.

Apelqvist, J., & Larsson, J. (2000). What is the most effective way to reduce incidence of amputation in the diabetic foot? *Diabetes Metab Res Rev, 16 Suppl 1*, S75-83.

Armstrong, D. G. (2005). Detection of diabetic peripheral neuropathy: Strategies for screening and diagnosis. *Advanced Studies in Medicine, 5*(10 D), S1033-S1037.

Armstrong, D. G., Boulton, A. J. M., & Bus, S. A. (2017). Diabetic Foot Ulcers and Their Recurrence. *New England Journal of Medicine, 376*(24), 2367-2375.

Armstrong, D. G., & Lavery, L. A. (1998a). Evidence-based options for off-loading diabetic wounds. *Clinics in Podiatric Medicine & Surgery, 15*(1), 95-104.

Armstrong, D. G., & Lavery, L. A. (1998b). Plantar pressures are higher in diabetic patients following partial foot amputation. *Ostomy Wound Management, 44*(3), 30-32, 34, 36 passim.

Armstrong, D. G., Lavery, L. A., & Bushman, T. R. (1998a). Peak foot pressures influence the healing time of diabetic foot ulcers treated with total contact casts. *Journal of Rehabilitation Research & Development, 35*(1), 1-5.

Armstrong, D. G., Lavery, L. A., & Harkless, L. B. (1998b). Validation of a Diabetic Wound Classification System- The contribution of depth, infection, and ischemia to risk of amputation. *Diabetes Care, 21*(5), 855-859.

Armstrong, D. G., Lavery, L. A., Holtz-Neiderer, K., Mohler, M. J., Wendel, C. S., Nixon, B. P., & Boulton, A. J. (2004). Variability in activity may precede diabetic foot ulceration. *Diabetes Care, 27*(8), 1980-1984.

Armstrong, D. G., Lavery, L. A., Kimbriel, H. R., Nixon, B. P., & Boulton, A. J. (2003). Activity patterns of patients with diabetic foot ulceration: patients with active ulceration may not adhere to a standard pressure off-loading regimen. *Diabetes Care, 26*(9), 2595-2597.

Armstrong, D. G., & Mills, J. L. (2013). Toward a change in syntax in diabetic foot care: prevention equals remission. *Journal of the American Podiatric Medical Association, 103*(2), 161-162.

Armstrong, D. G., Nguyen, H. C., Lavery, L. A., van Schie, C. H. M., Boulton, A. J. M., & Harkless, L. B. (2001). Off-Loading the Diabetic Foot Wound. *A randomized clinical trial, 24*(6), 1019-1022.

Armstrong, D. G., Peters, E. J., Athanasiou, K. A., & Lavery, L. A. (1998c). Is there a critical level of plantar foot pressure to identify patients at risk for neuropathic foot ulceration? *Journal of Foot & Ankle Surgery, 37*(4), 303-307.

Aronson, S. M. (1993). The language of epidemiology (XV): Biases in case-control studies. *R I Med, 76*(1), 4-5.

Baba, M., Davis, W. A., & Davis, T. M. E. (2014). A longitudinal study of foot ulceration and its risk factors in community-based patients with type 2 diabetes: The Fremantle Diabetes Study. *Diabetes research and clinical practice*, 106(1), 42-49.

Bacarin, T. A., Sacco, I. C., & Hennig, E. M. (2009). Plantar pressure distribution patterns during gait in diabetic neuropathy patients with a history of foot ulcers. *Clinics (Sao Paulo), 64*(2), 113-120.

Baker, R. (2006). Gait analysis methods in rehabilitation. *J Neuroeng Rehabil, 3*(4), 1-10.

Barn, R., Waaijman, R., Nollet, F., Woodburn, J., & Bus, S. A. (2015). Predictors of barefoot plantar pressure during walking in patients with diabetes, peripheral neuropathy and a history of ulceration. *PLoS ONE, 10*(2), e0117443.

Baudet, A., Morisset, C., d'Athis, P., Maillefert, J.-F., Casillas, J.-M., Ornetti, P., & Laroche, D. (2014). Cross-Talk Correction Method for Knee Kinematics in Gait Analysis Using Principal Component Analysis (PCA): A New Proposal. *PLoS ONE, 9*(7), e102098.

Bell, D. (2008). Evidence-based rationale for offloading treatment modalities. *Surgical Technology International, 17*, 113-117.

Bhasin, N., & Scott, D. J. A. (2007). Ankle Brachial Pressure Index: identifying cardiovascular risk and improving diagnostic accuracy. *Journal of the Royal Society of Medicine, 100*(1), 4-5.

Blaum, C. S., Ofstedal, M. B., Langa, K. M., & Wray, L. A. (2003). Functional status and health outcomes in older americans with diabetes mellitus. *Journal of the American Geriatrics Society, 51*(6), 745-753.

Booya, F., Bandarian, F., Larijani, B., Pajouhi, M., Nooraei, M., & Lotfi, J. (2005). Potential risk factors for diabetic neuropathy: A case control study. *BMC Neurology, 5*(25), 1-5.

Boulton, A. J. (1998a). Guidelines for diagnosis and outpatient management of diabetic peripheral neuropathy. European Association for the Study of Diabetes, Neurodiab. *Diabetes & Metabolism, 24 Suppl 3*, 55-65.

Boulton, A. J. (1998b). Lowering the risk of neuropathy, foot ulcers and amputations. *Diabetic Medicine, 15 Suppl 4*, S57-59.

Boulton, A. J. (2013). The pathway to foot ulceration in diabetes. *Med Clin North Am, 97*(5), 775-790.

Boulton, A. J., Betts, R. P., Franks, C. I., Ward, J. D., & Duckworth, T. (1987). The natural history of foot pressure abnormalities in neuropathic diabetic subjects. *Diabetes Res, 5*(2), 73-77.

Boulton, A. J., Hardisty, C. A., Betts, R. P., Franks, C. I., Worth, R. C., Ward, J. D., & Duckworth, T. (1983). Dynamic foot pressure and other studies as diagnostic and management aids in diabetic neuropathy. *Diabetes Care, 6*(1), 26-33.

Boulton, A. J., Krisner, R., S, & Vileikyte, L. (2004). Neuropathic Diabetic Foot Ulcers. *New England Journal of Medicine 351*, 48-55.

Boulton, A. J., Vileikyte, L., Ragnarson-Tennvall, G., & Apelqvist, J. (2005). The global burden of diabetic foot disease. *Lancet, 366*(9498), 1719-1724.

Boulton, A. J. M. (2004a). The diabetic foot: From art to science. The 18th Camillo Golgi lecture. *Diabetologia, 47*(8), 1343-1353.

Boulton, A. J. M. (2004b). Pressure and the diabetic foot: clinical science and offloading techniques. *American Journal of Surgery, 187*(5A), 17S-24S.

Boulton, A. J. M. (2005). Management of Diabetic Peripheral Neuropathy. *Clinical Diabetes, 23*(1), 9-15.

Boulton, A. J. M., Armstrong, D. G., Albert, S. F., Frykberg, R. G., Hellman, R., Kirkman, M. S., . . . Wukich, D. K. (2008). Comprehensive Foot Examination and Risk Assessment: A report of the Task Force of the Foot Care Interest Group of the American Diabetes Association, with endorsement by the American Association of Clinical Endocrinologists. *Diabetes Care, 31*(8), 1679-1685.

Bowering, C. K. (2001). Diabetic foot ulcers: Pathophysiology, assessment, and therapy. *Canadian Family Physician, 47*(May), 1007-1016.

Brash, P. D., Foster, J. E., Vennart, W., Daw, J., & Tooke, J. E. (1996). Magnetic resonance imaging reveals micro-haemorrhage in the feet of diabetic patients with a history of ulceration. *Diabet Med, 13*(11), 973-978.

Brown, S. J., Handsaker, J. C., Bowling, F. L., Boulton, A. J., & Reeves, N. D. (2015). Diabetic peripheral neuropathy compromises balance during daily activities. *Diabetes Care, 38*(6), 1116-1122.

Brown, S. J., Handsaker, J. C., Bowling, F. L., Maganaris, C. N., Boulton, A. J., & Reeves, N. D. (2014). Do patients with diabetic neuropathy use a higher proportion of their maximum strength when walking? *J Biomech, 47*(15), 3639-3644.

Brown, S. J., Handsaker, J. C., Maganaris, C. N., Bowling, F. L., Boulton, A. J. M., & Reeves, N. D. (2016). Altered joint moment strategy during stair walking in diabetes patients with and without peripheral neuropathy. *Gait Posture, 46*, 188-193.

Brownlee, M. (2005). The pathobiology of diabetic complications: a unifying mechanism. *Diabetes, 54*(6), 1615-1625.

Burnfield, J. M., Few, C. D., Mohamed, O. S., & Perry, J. (2004). The influence of walking speed and footwear on plantar pressures in older adults. *Clin Biomech (Bristol, Avon), 19*(1), 78-84.

Bus, S., van Deursen, R., Kanade, R., Wissink, M., Manning, E., van Baal, J., & Harding, K. (2008a). Plantar pressure relief in the diabetic foot using forefoot offloading shoes. *Gait Posture, 29*(4), 618-622.

Bus, S. A. (2016). Innovations in plantar pressure and foot temperature measurements in diabetes. *Diabetes Metab Res Rev, 32 Suppl 1*, 221-226.

Bus, S. A., Armstrong, D. G., Deursen, R., Lewis, J., Caravaggi, C., & Cavanagh, P. R. (2016a). IWGDF guidance on footwear and offloading interventions to prevent and heal foot ulcers in patients with diabetes. *Diabetes Metab Res Rev, 32 Suppl 1*, 25-36.

Bus, S. A., & de Lange, A. (2005). A comparison of the 1-step, 2-step, and 3-step protocols for obtaining barefoot plantar pressure data in the diabetic neuropathic foot. *Clin Biomech (Bristol, Avon), 20*(9), 892-899.

Bus, S. A., Haspels, R., & Busch-Westbroek, T. E. (2011). Evaluation and optimization of therapeutic footwear for neuropathic diabetic foot patients using in-shoe plantar pressure analysis. *Diabetes Care, 34*(7), 1595-1600.

Bus, S. A., Maas, M., de Lange, A., Michels, R. P., & Levi, M. (2005). Elevated plantar pressures in neuropathic diabetic patients with claw/hammer toe deformity. *J Biomech, 38*(9), 1918-1925.

Bus, S. A., Ulbrecht, J. S., & Cavanagh, P. R. (2004). Pressure relief and load redistribution by custom-made insoles in diabetic patients with neuropathy and foot deformity. *Clin Biomech (Bristol, Avon), 19*(6), 629-638.

Bus, S. A., Valk, G. D., van Deursen, R. W., Armstrong, D. G., Caravaggi, C., Hlavacek, P., . . . Cavanagh, P. R. (2008b). The effectiveness of footwear and offloading interventions to prevent and heal foot ulcers and reduce plantar pressure in diabetes: a systematic review. *Diabetes Metab Res Rev, 24 Suppl 1*, S162-180.

Bus, S. A., van Deursen, R. W., Armstrong, D. G., Lewis, J. E., Caravaggi, C. F., & Cavanagh, P. R. (2016b). Footwear and offloading interventions to prevent and heal foot ulcers and reduce plantar pressure in patients with diabetes: a systematic review. *Diabetes Metab Res Rev, 32 Suppl 1*, 99-118.

Bus, S. A., & Waaijman, R. (2013). The value of reporting pressure-time integral data in addition to peak pressure data in studies on the diabetic foot: a systematic review. *Clin Biomech (Bristol, Avon), 28*(2), 117-121.

Bus, S. A., Waaijman, R., Arts, M., de Haart, M., Busch-Westbroek, T., van Baal, J., & Nollet, F. (2013). Effect of custom-made footwear on foot ulcer recurrence in diabetes: a multicenter randomized controlled trial. *Diabetes Care, 36*(12), 4109-4116.

Bus, S. A., Waaijman, R., Arts, M., & Manning, H. (2009). The efficacy of a removable vacuum-cushioned cast replacement system in reducing plantar forefoot pressures in diabetic patients. *Clin Biomech (Bristol, Avon), 24*(5), 459-464.

Bustos-Saldaña, R., & Prieto-Miranda, S. (2009). Foot ulceration risk factors in type 2 diabetes mellitus. *Factores de riesgo de ulceración de los pies en diabéticos tipo 2., 47*(5), 467-476.

Butler, A., E, Janson, J., Bonner-Weir, S., Ritzel, R., & Butler, P., C. (2003). B-Cell Deficit and Increased B-Cell Apoptosis in Humans With Type 2 Diabetes. *Journal of Diabetes52*(1).

Caruana, E. J., Roman, M., Hernández-Sánchez, J., & Solli, P. (2015). Longitudinal studies. *Journal of Thoracic Disease, 7*(11), E537-E540.

Caselli, A., Pham, H., Giurini, J. M., Armstrong, D. G., & Veves, A. (2002). The forefoot-to-rearfoot plantar pressure ratio is increased in severe diabetic neuropathy and can predict foot ulceration. *Diabetes Care, 25*(6), 1066-1071.

Cavanagh, P., Attinger, C., Abbas, Z., Bal, A., Rojas, N., & Xu, Z.-R. (2012). Cost of treating diabetic foot ulcers in five different countries. *Diabetes Metab Res Rev, 28*, 107-111.

Cavanagh, P., Ulbrecht, J., & Caputo, G. (2000). New developments in the biomechanics of the diabetic foot. *Diabetes Metab Res Rev, 16*(Suppl 1), S6 - S10.

Cavanagh, P. R. (2004). Therapeutic footwear for people with diabetes. *Diabetes Metab Res Rev, 20 Suppl 1*S51-S55.

Cavanagh, P. R., & Bus, S. A. (2010). Off-loading the diabetic foot for ulcer prevention and healing. *Journal of Vascular Surgery, 52 Supplement*(3), 37S-43S.

Cavanagh, P. R., & Bus, S. A. (2011). Off-loading the diabetic foot for ulcer prevention and healing. *Plastic & Reconstructive Surgery, 127 Suppl 1*, 248S-256S.

Cavanagh, P. R., Morag, E., Boulton, A. J., Young, M. J., Deffner, K. T., & Pammer, S. E. (1997). The relationship of static foot structure to dynamic foot function. *J Biomech, 30*(3), 243-250.

Cavanagh, P. R., Simoneau, G. G., & Ulbrecht, J. S. (1993). Ulceration, unsteadiness, and uncertainty: the biomechanical consequences of diabetes mellitus. *J Biomech, 26 Suppl 1*, 23-40.

Cavanagh, P. R., Sims, D. S., Jr., & Sanders, L. J. (1991). Body mass is a poor predictor of peak plantar pressure in diabetic men. *Diabetes Care, 14*(8), 750-755.

Cavanagh, P. R., & Ulbrecht, J. S. (1994). Clinical plantar pressure measurement in diabetes: Rationale and methodology. *Foot, 4*(3), 123-135.

Cavanagh, P. R., Ulbrecht, J. S., & Caputo, G. M. (1996). Biomechanical aspects of diabetic foot disease: aetiology, treatment, and prevention. *Diabet Med, 13 Suppl 1*, S17-22.

Cheng, Q., Lazzarini, P. A., Gibb, M., Derhy, P. H., Kinnear, E. M., Burn, E., . . . Norman, R. E. (2016). A cost-effectiveness analysis of optimal care for diabetic foot ulcers in Australia. *International Wound Journal, 10.1111/iwj.12653*.

Chevalier, T. L., Hodgins, H., & Chockalingam, N. (2010). Plantar pressure measurements using an in-shoe system and a pressure platform: A comparison. *Gait Posture, 31*(3), 397-399.

Chiari, L., Croce, U. D., Leardini, A., & Cappozzo, A. (2005). Human movement analysis using stereophotogrammetry: Part 2: Instrumental errors. *Gait Posture, 21*(2), 197-211.

Clayton, W. J., & Elasy, T., A. (2009). A Review of the Pathophysiology, Classification, and Treatment of Foot Ulcers in Diabetic Patients. *Journal of Clinical Diabetes, 27*(2), 52-58.

Cohen, J. A. (1988b). *Statistical power analysis for the behavioral sciences* (2nd edition ed. Vol. 1). Mahwah, NJ, United States: Lawrence Erlbaum Associates.

Colagiuri, S., Colagiuri, R., & Ward, J. (1998). National Diabetes Strategy And Implementation Plan Australia. *Diabetes Australia, Canberra., An initiative of the Ministerial Advisory Committee on Diabetes* online.

Coles, S. (2008). Footwear and offloading for patients with diabetes. *Nursing Times, 104*(3), 40, 42-43.

Collins, T. D., Ghoussayni, S. N., Ewins, D. J., & Kent, J. A. (2009). A six degrees-of-freedom marker set for gait analysis: repeatability and comparison with a modified Helen Hayes set. *Gait Posture, 30*(2), 173-180.

Cook, J., & Simonson, D. (2012). *Epidemiology and Health Care Cost of Diabetic Foot Problems* (Vol. Contemporary Diabetes): Springer.

Couppe, C., Svensson, R. B., Kongsgaard, M., Kovanen, V., Grosset, J. F., Snorgaard, O., . . . Magnusson, S. P. (2016). Human Achilles tendon glycation and function in diabetes. *J Appl Physiology 120*(2), 130-137.

Crawford, F., Inkster, M., Kleijnen, J., & Fahey, T. (2007). Predicting foot ulcers in patients with diabetes: A systematic review and meta-analysis. *QJM, 100*(2), 65-86.

Crews, R. T., Shen, B. J., Campbell, L., Lamont, P. J., Boulton, A. J., Peyrot, M., . . . Vileikyte, L. (2016). Role and Determinants of Adherence to Off-loading in Diabetic Foot Ulcer Healing: A Prospective Investigation. *Diabetes Care, 39*(8), 1371-1377.

Critical Appraisal Skills Programme. (2013). Critical Appraisal Skills Programme (CASP) Making sense of evidence: 12 questions to help you make sense of cohort study. Retrieved from http://media.wix.com/ugd/dded87_e37a4ab637fe46a0869f9f977dacf134.pdf

Critical Appraisal Skills Programme. (2013). Critical Appraisal Skills Programme (CASP) Making sense of evidence: 11 questions to help you make sense of case control study. Retrieved from http://media.wix.com/ugd/dded87_63fb65dd4e0548e2bfd0a982295f839e.pdf

Crowther, R., G, Spinks, W., L, Leicht, A., S, Quigley, F., & Golledge, J. (2007). Relationship between temporal-spatial gait parameters, gait kinematics, walking performance, exercise capacity, and physical activity level in peripheral arterial disease. *Journal of Vascular Surgery, 45*(6), 1172-1178.

Crowther, R. G., Spinks, W. L., Leicht, A. S., Sangla, K., Quigley, F., & Golledge, J. (2008). Effects of a long-term exercise program on lower limb mobility, physiological responses, walking performance, and physical activity levels in patients with peripheral arterial disease. *Journal of Vascular Surgery, 47*(2), 303-309.

Ctercteko, G. C., Dhanendran, M., Hutton, W. C., & Le Quesne, L. P. (1981). Vertical forces acting on the feet of diabetic patients with neuropathic ulceration. *British Journal of Surgery, 68*(9), 608-614.

D'Ambrogi, E., Giurato, L., D'Agostino, M. A., Giacomozzi, C., Macellari, V., Caselli, A., & Uccioli, L. (2003). Contribution of plantar fascia to the increased forefoot pressures in diabetic patients. *Diabetes Care, 26*(5), 1525-1529.

Davis, R., B, Ounpuu, S., Tyburski, D., & Gage, J., R. (1991). A gait analysis data collection and reduction technique. *Human Movement Science 10*(5), 575-587.

Davis, R. B. (1997). Reflections on clinical gait analysis. *J Electromyogr Kinesiol, 7*(4), 251-257.

Davis, W. A., Norman, P. E., Bruce, D. G., & Davis, T. M. (2006). Predictors, consequences and costs of diabetes-related lower extremity amputation complicating type 2 diabetes: the Fremantle Diabetes Study. *Diabetologia, 49*(11), 2634-2641.

Della Croce, U., Leardini, A., Chiari, L., & Cappozzo, A. (2005). Human movement analysis using stereophotogrammetry. Part 4: assessment of anatomical landmark misplacement and its effects on joint kinematics. *Gait Posture, 21*(2), 226-237.

Diabetes Australia. (2017, 17/05/2017). NDSS National Diabetes Services Scheme Map Retrieved from http://www.ndss.com.au/en/Australian-Diabetes-Map/Map/

Dinh, T. L., & Veves, A. (2005). A review of the mechanisms implicated in the pathogenesis of the diabetic foot. *Int J Low Extrem Wounds, 4*(3), 154-159.

Downs, S. H., & Black, N. (1998). The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *Journal of Epidemiology and Community Health, 52*(6), 377-384.

Du Prel, J. B., Hommel, G., Röhrig, B., & Blettner, M. (2009). Confidence interval or p-value? Part 4 of a series on evaluation of scientific publications. *Deutsches Arzteblatt, 106*(19), 335-339.

Dunlap, W. P., Cortina, J. M., Vaslow, J. B., & Burke, M. J. (1996). Meta-analysis of experiments with matched groups or repeated measures designs. *Psychological Methods, 1*(2), 170-177.

Dyck, P. J., Karnes, J., O'Brien, P. C., & Swanson, C. J. (1986). Neuropathy Symptom Profile in health, motor neuron disease, diabetic neuropathy, and amyloidosis. *Neurology, 36*(10), 1300-1308.

Elveru, R. A., Rothstein, J. M., & Lamb, R. L. (1988). Goniometric Reliability in a Clinical Setting: Subtalar and Ankle Joint Measurements. *Phys Ther, 68*(5), 672-677.

England, J. D., Gronseth, G. S., Franklin, G., Miller, R. G., Asbury, A. K., Carter, G. T., . . . Sumner, A. J. (2005). Distal symmetrical polyneuropathy: a definition for clinical research. A report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. *Arch Phys Med Rehabil, 86*(1), 167-174.

Eshraghi, A., Abu Osman, N. A., Karimi, M., Gholizadeh, H., Soodmand, E., & Abas, W. A. B. W. (2014). Gait Biomechanics of Individuals with Transtibial Amputation: Effect of Suspension System. *PLoS ONE, 9*(5), e96988.

Esmaillzadeh, A., Mirmiran, P., Moeini, S. H., & Azizi, F. (2006). Larger hip circumference independently contributed to reduced metabolic risks in Tehranian adult women. *International journal of cardiology, 108*(3), 338-345.

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods, 39*(2), 175-191.

Feng, Y., Schlösser, F. J., & Sumpio, B. E. (2009). The Semmes Weinstein monofilament examination as a screening tool for diabetic peripheral neuropathy. *Journal of Vascular Surgery, 50*(3), 675-682.

Fernando, D. J., Masson, E. A., Veves, A., & Boulton, A. J. (1991). Relationship of limited joint mobility to abnormal foot pressures and diabetic foot ulceration. *Diabetes Care, 14*(1), 8-11.

Fernando, M., Crowther, R., Cunningham, M., Lazzarini, P., Sangla, K., Buttner, P., & Golledge, J. (2016a). The reproducibility of acquiring three dimensional gait and plantar pressure data using established protocols in participants with and without type 2 diabetes and foot ulcers. *Journal of Foot and Ankle Research, 9*(1), e collection.

Fernando, M., Crowther, R., Lazzarini, P., Sangla, K., Cunningham, M., Buttner, P., & Golledge, J. (2013). Biomechanical characteristics of peripheral diabetic neuropathy: A systematic review and meta-analysis of findings from the gait cycle, muscle activity and dynamic barefoot plantar pressure. *Clin Biomech (Bristol, Avon), 28*(8), 831-845.

Fernando, M. E., Crowther, R. G., Cunningham, M., Lazzarini, P. A., Sangla, K. S., & Golledge, J. (2015). Lower limb biomechanical characteristics of patients with neuropathic diabetic foot ulcers: the diabetes foot ulcer study protocol. *BMC Endocr Disord, 15*(59).

Fernando, M. E., Crowther, R. G., Lazzarini, P. A., Sangla, K. S., Buttner, P., & Golledge, J. (2016b). Gait parameters of people with diabetes-related neuropathic plantar foot ulcers. *Clin Biomech (Bristol, Avon), 37*(1), 98–107

Fernando, M. E., Crowther, R. G., Lazzarini, P. A., Sangla, K. S., Wearing, S., Buttner, P., & Golledge, J. (2016c). Plantar pressures are higher in cases with diabetic foot ulcers compared to controls despite a longer stance phase duration. *BMC Endocrine Disorders, 16*(1), 1-10.

Fernando, M. E., Crowther, R. G., Pappas, E., Lazzarini, P. A., Cunningham, M., Sangla, K. S., . . . Golledge, J. (2014a). Plantar pressure in diabetic peripheral neuropathy patients with active foot ulceration, previous ulceration and no history of ulceration: a meta-analysis of observational studies. *PLoS ONE, 9*(1).

Fernando, M. E., Crowther, R. G., Pappas, E., Lazzarini, P. A., Cunningham, M., Sangla, K. S., . . . Golledge, J. (2014b). Plantar Pressure in Diabetic Peripheral Neuropathy Patients with Active Foot Ulceration, Previous Ulceration and No History of Ulceration: A Meta-Analysis of Observational Studies. *PLoS ONE, 9*(6), e99050.

Fernando, M. E., Seneviratne, R. M., Tan, Y. M., Lazzarini, P. A., Sangla, K. S., Cunningham, M., . . . Golledge, J. (2016d). Intensive versus conventional glycaemic control for treating diabetic foot ulcers. *Cochrane Database Syst Rev, 1*, CD010764.

Ferrari, A., Benedetti, M. G., Pavan, E., Frigo, C., Bettinelli, D., Rabuffetti, M., . . . Leardini, A. (2008). Quantitative comparison of five current protocols in gait analysis. *Gait and Posture, 28*(2), 207-216.

Ferreira, M. C., Rodrigues, L., & Fels, K. (2004). New method for evaluation of cutaneous sensibility in diabetic feet: preliminary report. *Revista do Hospital das Clinicas; Faculdade de Medicina Da Universidade de Sao Paulo, 59*(5), 286-290.

Fife, C. E., Carter, M. J., & Walker, D. (2010). Why is it so hard to do the right thing in wound care? *Wound Repair & Regeneration, 18*(2), 154-158.

Formosa, C., Gatt, A., & Chockalingam, N. (2013). The importance of clinical biomechanical assessment of foot deformity and joint mobility in people living with type-2 diabetes within a primary care setting. *Prim Care Diabetes, 7*(1), 45-50.

Fowler, M. J. (2008). Microvascular and Macrovascular Complications of Diabetes. *Clinical Diabetes, 26*(2), 77-82.

Frykberg, R. G. (2002). Diabetic foot ulcers: Pathogenesis and management. *American Family Physician, 66*(9), 1655-1662.

Frykberg, R. G., & Banks, J. (2015). Challenges in the Treatment of Chronic Wounds. *Adv Wound Care (New Rochelle), 4*(9), 560-582.

Frykberg, R. G., Lavery, L. A., Pham, H., Harvey, C., Harkless, L., & Veves, A. (1998). Role of neuropathy and high foot pressures in diabetic foot ulceration. *Diabetes Care, 21*(10), 1714-1719.

Game, F. (2012). Choosing life or limb. Improving survival in the multi-complex diabetic foot patient. *Diabetes Metab Res Rev, 28 Suppl 1*, 97-100.

Garcia-Morales, E., Lazaro-Martinez, J. L., Martinez-Hernandez, D., Aragon-Sanchez, J., Beneit-Montesinos, J. V., & Gonzalez-Jurado, M. A. (2011). Impact of diabetic foot related complications on the Health Related Quality of Life (HRQol) of patients--a regional study in Spain. *Int J Low Extrem Wounds, 10*(1), 6-11.

Garrow, A. P., Papageorgiou, A., Silman, A. J., Thomas, E., Jayson, M. I., & Macfarlane, G. J. (2001). The grading of hallux valgus. The Manchester Scale. *Journal of the American Podiatric Medical Association, 91*(2), 74-78.

Gelber, J. R., Isaac, E. J., Bohnert, K. L., Strube, M. J., & Sinacore, D. R. (2010). Foot progression angle and medial loading in individuals with diabetes mellitus, peripheral neuropathy, and a foot ulcer. *Gait and Posture, 32*(2), 237-241.

Giacomozzi, C., D'Ambrogi, E., Cesinaro, S., Macellari, V., & Uccioli, L. (2008). Muscle performance and ankle joint mobility in long-term patients with diabetes. *BMC Musculoskeletal Disorders, 9*(99), 1-8.

Giacomozzi, C., D'Ambrogi, E., Uccioli, L., & Macellari, V. (2005). Does the thickening of Achilles tendon and plantar fascia contribute to the alteration of diabetic foot loading? *Clin Biomech (Bristol, Avon), 20*(5), 532-539.

Giacomozzi, C., Keijsers, N., Pataky, T., & Rosenbaum, D. (2012). International scientific consensus on medical plantar pressure measurement devices: technical requirements and performance. *Ann Ist Super Sanita, 48*(3), 259-271.

Goldsmith, J. R., Lidtke, R. H., & Shott, S. (2002). The effects of range-of-motion therapy on the plantar pressures of patients with diabetes mellitus. *Journal of the American Podiatric Medical Association, 92*(9), 483-490.

Golledge, J., Ewels, C., Muller, R., & Walker, P. J. (2014). Association of chronic kidney disease categories defined with different formulae with major adverse events in patients with peripheral vascular disease. *Atherosclerosis, 232*(2), 289-297.

Golledge, J., van Bockxmeer, F., Jamrozik, K., McCann, M., & Norman, P. E. (2010). Association between serum lipoproteins and abdominal aortic aneurysm. *Am J Cardiol, 105*(10), 1480-1484.

Gomes, A. A., Onodera, A. N., Otuzi, M. E. I., Pripas, D., Mezzarane, Rinaldo Andre, & Sacco, I. C. N. (2011). Electromyography and kinematic changes of gait cycle at different cadences in diabetic neuropathic individuals. *Muscle and Nerve, 44*(2), 258-268.

Guldemond, N. A., Leffers, P., Walenkamp, G. H. I. M., Schaper, N. C., Sanders, A. P., Nieman, F. H. M., & van Rhijn, L. W. (2008). Prediction of peak pressure from clinical and radiological measurements in patients with diabetes. *BMC Endocrine Disorders, 8*(16).

Gurney, J. K., Kersting, U. G., & Rosenbaum, D. (2008). Between-day reliability of repeated plantar pressure distribution measurements in a normal population. *Gait and Posture, 27*(4), 706-709.

Gurney, J. K., Kersting, U. G., Rosenbaum, D., Dissanayake, A., York, S., Grech, R., . . . Sarfati, D. (2017). Pedobarography as a clinical tool in the management of diabetic feet in New Zealand: a feasibility study. *Journal of Foot and Ankle Research, 10*(1).

Hafer, J. F., Lenhoff, M. W., Song, J., Jordan, J. M., Hannan, M. T., & Hillstrom, H. J. (2013). Reliability of plantar pressure platforms. *Gait and Posture, 38*(3), 544-548.

Hamatani, M., Mori, T., Oe, M., Noguchi, H., Takehara, K., Amemiya, A., . . . Sanada, H. (2016). Factors Associated With Callus in Diabetic Patients, Focused on Plantar Shear Stress During Gait. *Journal of Diabetes Science & Technology, 1*(10), 1353-1359.

Handsaker, J. C., Brown, S. J., Bowling, F. L., Cooper, G., Maganaris, C. N., Boulton, A. J., & Reeves, N. D. (2014). Contributory factors to unsteadiness during walking up and down stairs in patients with diabetic peripheral neuropathy. *Diabetes Care, 37*(11), 3047-3053.

Hariri, S., Yoon, P. W., Qureshi, N., Valdez, R., Scheuner, M. T., & Khoury, M. J. (2006). Family history of type 2 diabetes: A population-based screening tool for prevention? *Genet Med, 8*(2), 102-108.

Harrington, C., Zagari, M. J., Corea, J., & Klitenic, J. (2000). A cost analysis of diabetic lower-extremity ulcers. *Diabetes Care, 23*(9), 1333-1338.

Harris, E. H. (2005). Elevated Liver Function Tests in Type 2 Diabetes. *Clinical Diabetes, 23*(3), 115-119.

Hartsell, H. D., Brand, R. A., Frantz, R. A., & Saltzman, C. L. (2004). The effects of total contact casting materials on plantar pressures. *Foot and Ankle International, 25*(2), 73-78.

Hastings, M., Mueller, M., Sinacore, D., Salsich, G., Engsberg, J., & Johnson, J. (2000). Effects of a tendo-achilles lengthening procedure on muscle function and gait characteristics in a patient with diabetes mellitus. *Journal of Orthopaedic & Sports Physical Therapy, 30*(2), 85-90.

Heikkinen, M., Salmenpera, M., Lepantalo, A., & Lepantalo, M. (2007). Diabetes care for patients with peripheral arterial disease. *Eur J Vasc Endovasc Surg, 33*(5), 583-591.

Hinchliffe, R. J., Andros, G., Apelqvist, J., Bakker, K., Friederichs, S., Lammer, J., . . . Schaper, N. C. (2012). A systematic review of the effectiveness of revascularization of the ulcerated foot in patients with diabetes and peripheral arterial disease. *Diabetes Metab Res Rev, 28 Suppl 1*, 179-217.

Hodges, J. L., & Lehmann, E. L. (1963). Estimates of Location Based on Rank Tests. *Ann. Math. Statist., 34*(2), 598-611.

Hokkam, E. N. (2009). Assessment of risk factors in diabetic foot ulceration and their impact on the outcome of the disease. *Prim Care Diabetes, 3*(4), 219-224.

Hopkins, W. G. (2002). A scale of magnitudes for effect statistics. A new view of statistics. Retrieved from (available online at: http://sportsci.org/resource/stats/effectmag.html).

Hu, H., Li, H., Zheng, F. P., Cheng, Y., Miao, J., & Zhang, W. (2012). [A comparison of clinical effectiveness of different neuropathy scoring systems in screening asymptomatic diabetic peripheral neuropathy]. *Zhonghua Nei Ke Za Zhi, 51*(1), 13-17.

IDF, I. D. F. (2016). IDF Diabetes atlas 7th edition 2015. The global burden. Retrieved from http://www.diabetesatlas.org/

Idris, I., Game, F., & Jeffcoate, W. (2005). Does close glycaemic control promote healing in diabetic foot ulcers? Report of a feasibility study. *Diabetic Medicine, 22*(8), 1060-1063.

Ikem, R., Ikem, I., Adebayo, O., & Soyoye, D. (2010). An assessment of peripheral vascular disease in patients with diabetic foot ulcer. *Foot (Edinb), 20*(4), 114-117.

Illgner, U., Podella, M., Rümmler, M., Wühr, J., Büsch, H. G., & Wetz, H. H. (2009). Reconstructive surgery for Charcot foot: Long-term 5-year outcome. *Rekonstruktive Chirurgie beim Charcot-Fu: 5-Jahres-Langzeitoutcome, 38*(12), 1180-1186.

Jan, Y. K., Lung, C. W., Cuaderes, E., Rong, D., & Boyce, K. (2013). Effect of viscoelastic properties of plantar soft tissues on plantar pressures at the first metatarsal head in diabetics with peripheral neuropathy. *Physiol Meas, 34*(1), 53-66.

Jebb, S. A., Cole, T. J., Doman, D., Murgatroyd, P. R., & Prentice, A. M. (2000). Evaluation of the novel Tanita body-fat analyser to measure body composition by comparison with a four-compartment model. *Br J Nutr, 83*(2), 115-122.

Jeffcoate, W. J., Bus, S. A., Game, F. L., Hinchliffe, R. J., Price, P. E., & Schaper, N. C. (2016). Reporting standards of studies and papers on the prevention and management of foot ulcers in diabetes: required details and markers of good quality. *Lancet Diabetes Endocrinol, 4*(9), 781-788.

Johnson, D. W., Jones, G. R., Mathew, T. H., Ludlow, M. J., Doogue, M. P., Jose, M. D., . . . Usherwood, T. (2012). Chronic kidney disease and automatic reporting of estimated glomerular filtration rate: new developments and revised recommendations. *Medical Journal Australia, 197*(4), 224-225.

Kanade, R. V., Deursen, R. W., Harding, K., & Price, P. (2006). Walking performance in people with diabetic neuropathy: benefits and threats. *Diabetologia, 49*(8), 1747-1754.

Kanade, R. V., Van Deursen, R. W. M., Harding, K. G., & Price, P. E. (2008). Investigation of standing balance in patients with diabetic neuropathy at different stages of foot complications. *Clin Biomech (Bristol, Avon), 23*(9), 1183-1191.

Katoulis, E. C., Boulton, A. J., & Raptis, S. A. (1996). The role of diabetic neuropathy and high plantar pressures in the pathogenesis of foot ulceration. *Hormone & Metabolic Research, 28*(4), 159-164.

Katoulis, E. C., Ebdon-Parry, M., Lanshammar, H., Vileikyte, L., Kulkarni, J., & Boulton, A. J. (1997). Gait abnormalities in diabetic neuropathy. *Diabetes Care, 20*(12), 1904-1907.

Keijsers, N. L., Stolwijk, N. M., & Pataky, T. C. (2010). Linear dependence of peak, mean, and pressure-time integral values in plantar pressure images. *Gait and Posture, 31*(1), 140-142.

Kirtley, C., Whittle, M. W., & Jefferson, R. J. (1985). Influence of walking speed on gait parameters. *Journal of Biomechanical Engineering, 7*(4), 282-288.

Klein, S., Allison, D. B., Heymsfield, S. B., Kelley, D. E., Leibel, R. L., Nonas, C., & Kahn, R. (2007). Waist circumference and cardiometabolic risk: a consensus statement from Shaping America's Health: Association for Weight Management and Obesity Prevention; NAASO, The Obesity Society; the American Society for Nutrition; and the American Diabetes Association. *The American Journal of Clinical Nutrition, 85*(5), 1197-1202.

Klobučar, H. (2010). Foot biomechanics and off-loading. *Biomehanika i "off-loading" stopala, 64 Suppl 1*, 27-32.

Knudson, D. (2009). Significant and meaningful effects in sports biomechanics research. *Sports Biomech, 8*(1), 96-104.

Ko, M., Hughes, L., & Lewis, H. (2012). Walking speed and peak plantar pressure distribution during barefoot walking in persons with diabetes. *Physiotherapy Research International, 17*(1), 29-35.

Kovac, B., Kovac, B., Marusic-Emedi, S., Svalina, S., & Demarin, V. (2011). Clinical and electrophysiological signs of diabetic polyneuropathy --effect of glycemia and duration of diabetes mellitus. *Acta Clinica Croatica, 50*(2), 149-157.

Kruse, I., & Edelman, S. (2006). Evaluation and treatment of diabetic foot ulcers. *Clinical Diabetes, 24*(2), 91-93.

Lavery, L. A. (2011). Discussion: Off-loading the diabetic foot for ulcer prevention and healing. *Plastic & Reconstructive Surgery, 127 Suppl 1*, 257S-258S.

Lavery, L. A., Armstrong, D. G., & Harkless, L. B. (1996). Classification of diabetic foot wounds. *Journal of Foot & Ankle Surgery, 35*(6), 528-531.

Lavery, L. A., Armstrong, D. G., Vela, S. A., Quebedeaux, T. L., & Fleischli, J. G. (1998). Practical criteria for screening patients at high risk for diabetic foot ulceration. *Archives of Internal Medicine, 158*(2), 157-162.

Lavery, L. A., Armstrong, D. G., Wunderlich, R. P., Tredwell, J., & Boulton, A. J. M. (2003). Predictive value of foot pressure assessment as part of a population-based diabetes disease management program. *Diabetes Care, 26*(4), 1069-1073.

Lavery, L. A., Vela, S. A., Fleischli, J. G., Armstrong, D. G., & Lavery, D. C. (1997a). Reducing plantar pressure in the neuropathic foot. A comparison of footwear. *Diabetes Care, 20*(11), 1706-1710.

Lavery, L. A., Vela, S. A., Lavery, D. C., & Quebedeaux, T. L. (1997b). Total contact casts: pressure reduction at ulcer sites and the effect on the contralateral foot. *Archives of Physical Medicine & Rehabilitation, 78*(11), 1268-1271.

Lázaro-Martínez, J., Aragón-Sanchez, F., Beneit-Montesinos, J., González-Jurado, M., Morales, E., & Hernández, D. (2011). Foot biomechanics in patients with diabetes

mellitus: doubts regarding the relationship between neuropathy, foot motion, and deformities. *Journal of the American Podiatric Medical Association, 101*(3), 208-214.

Lazzarini, P. A., Gurr, J. M., Rogers, J. R., Schox, A., & Bergin, S. M. (2012). Diabetes foot disease: the Cinderella of Australian diabetes management? *J Foot Ankle Res, 5*(24), epub.

Lazzarini, P. A., Hurn, S. E., Fernando, M. E., Jen, S. D., Kuys, S. S., Kamp, M. C., & Reed, L. F. (2015a). Prevalence of foot disease and risk factors in general inpatient populations: a systematic review and meta-analysis. *BMJ Open, 5*(11), epub.

Lazzarini, P. A., Hurn, S. E., Kuys, S. S., Kamp, M. C., Ng, V., Thomas, C., . . . Reed, L. F. (2016). The silent overall burden of foot disease in a representative hospitalised population. *International Wound Journal, epub*.

Lazzarini, P. A., O'Rourke, S. R., Russell, A. W., Derhy, P. H., & Kamp, M. C. (2015b). Reduced Incidence of Foot-Related Hospitalisation and Amputation amongst Persons with Diabetes in Queensland, Australia. *PLoS ONE, 10*(6), e0130609.

Ledoux, W. R., Shofer, J. B., Ahroni, J. H., Smith, D. G., Sangeorzan, B. J., & Boyko, E. J. (2003). Biomechanical differences among pes cavus, neutrally aligned, and pes planus feet in subjects with diabetes. *Foot & Ankle International, 24*(11), 845-850.

Ledoux, W. R., Shofer, J. B., Cowley, M. S., Ahroni, J. H., Cohen, V., & Boyko, E. J. (2013). Diabetic foot ulcer incidence in relation to plantar pressure magnitude and measurement location. *Journal of Diabetes and its Complications, 27*(6), 621-626.

Ledoux, W. R., Shofer, J. B., Smith, D. G., Sullivan, K., Hayes, S. G., Assal, M., & Reiber, G. E. (2005). Relationship between foot type, foot deformity, and ulcer occurrence in the high-risk diabetic foot. *Journal of Rehabilitation Research & Development, 42*(5), 665-672.

Leigh, R. J., Pohl, M. B., & Ferber, R. (2014). Does tester experience influence the reliability with which 3D gait kinematics are collected in healthy adults? *Physical Therapy in Sport, 15*(2), 112-116.

Leung, P. (2007). Diabetic foot ulcers - A comprehensive review. *Surgeon, 5*(4), 219-231.

Lin, L. I.-K. (1989). A Concordance Correlation Coefficient to Evaluate Reproducibility. *Biometrics, 45*(1), 255-268.

Lipsky, B. A., Berendt, A. R., Cornia, P. B., Pile, J. C., Peters, E. J., Armstrong, D. G., . . . Infectious Diseases Society of, A. (2012). Executive summary: 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis, 54*(12), 1679-1684.

Little, R. R., & Roberts, W. L. (2009). A review of variant hemoglobins interfering with hemoglobin A1c measurement. *Journal of Diabetes Science & Technology, 3*(3), 446-451.

Longo, B., Campanale, A., Farcomeni, A., & Santanelli, F. (2013). Long-term sensory recovery of nipple-areola complex following superolateral pedicled reduction mammaplasty. *Plastic & Reconstructive Surgery, 132*(5), 735e-742e.

Longo, B., Pagnoni, M., Ferri, G., Morello, R., & Santanelli, F. (2013). The mushroom-shaped anterolateral thigh perforator flap for subtotal tongue reconstruction. *Plastic & Reconstructive Surgery, 132*(3), 656-665.

Ludbrook, J. (1998). Multiple comparison procedures updated. *Clin Exp Pharmacol Physiol, 25*(12), 1032-1037.

Lung, C.-W., Hsiao-Wecksler, E. T., Burns, S., Lin, F., & Jan, Y.-K. (2016). Quantifying Dynamic Changes in Plantar Pressure Gradient in Diabetics with Peripheral Neuropathy. *Frontiers in Bioengineering and Biotechnology, 19*(4), epub.

Luyan, W. (2014). GW25-e4192 Comparison of Digital Blood Pressure Device and Mercury Sphygmomanometers. *Journal of the American College of Cardiology, 64*(16), C180.

Maher, C. G., Sherrington, C., Herbert, R. D., Moseley, A. M., & Elkins, M. (2003). Reliability of the PEDro Scale for Rating Quality of Randomized Controlled Trials. *Phys Ther, 83*(8), 713-721.

Maluf, K., & Mueller, M. (2003a). Comparison of physical activity and cumulative plantar tissue stress among subjects with and without diabetes mellitus and a history of recurrent plantar ulcers. *Clin Biomech, 18*(7), 567-575.

Maluf, K. S., & Mueller, M. J. (2003b). Novel Award 2002. Comparison of physical activity and cumulative plantar tissue stress among subjects with and without diabetes mellitus and a history of recurrent plantar ulcers. *Clinical Biomechanics (Bristol, Avon), 18*(7), 567-575.

Mancera-Romero, J., Rodriguez-Morata, A., Angel Sanchez-Chaparro, M., Sanchez-Perez, M., Paniagua-Gomez, F., Hidalgo-Conde, A., & Valdivielso, P. (2013). Role of an intermittent claudication questionnaire for the diagnosis of PAD in ambulatory patients with type 2 diabetes. *Int Angiol, 32*(5), 512-517.

Margolis, D. J., Hofstad, O., & Feldman, H. I. (2008). Association between renal failure and foot ulcer or lower-extremity amputation in patients with diabetes. *Diabetes Care, 31*(7), 1331-1336.

Masson, E. A., Hay, E. M., Stockley, I., Veves, A., Betts, R. P., & Boulton, A. J. M. (1989). Abnormal foot pressures alone may not cause ulceration. *Diabetic Medicine, 6*(5), 426-428.

McBride, G. B. (2005). A proposal for strength-of-agreement criteria for Lin's Concordance Correlation Coefficient. *NIWA Client Report: HAM2005-062.* Retrieved from https://www.medcalc.org/download/pdf/McBride2005.pdf

McGinley, J. L., Baker, R., Wolfe, R., & Morris, M. E. (2009). The reliability of three-dimensional kinematic gait measurements: A systematic review. *Gait and Posture, 29*(3), 360-369.

McGough, J. J., & Faraone, S. V. (2009). Estimating the size of treatment effects: moving beyond p values. *Psychiatry (Edgmont), 6*(10), 21-29.

McGuire, L. C., Ford, E. S., & Ajani, U. A. (2006). The impact of cognitive functioning on mortality and the development of functional disability in older adults with diabetes: the second longitudinal study on aging. *BMC Geriatrics, 6*, 8.

McPoil, T. G., Cornwall, M. W., Dupuis, L., & Cornwell, M. (1999). Variability of plantar pressure data. A comparison of the two-step and midgait methods. *Journal of the American Podiatric Medical Association, 89*(10), 495-501.

Melai, T., Ijzerman, T. H., Schaper, N. C., de Lange, T. L. H., Willems, P. J. B., Meijer, K., . . . Savelberg, H. H. C. M. (2011). Calculation of plantar pressure time integral, an alternative approach. *Gait and Posture, 34*(3), 379-383.

Menz, H. B., Fotoohabadi, M. R., Wee, E., & Spink, M. J. (2012). Visual categorisation of the Arch Index: a simplified measure of foot posture in older people. *J Foot Ankle Res, 5 Suppl 1*, P15-P15.

Menz, H. B., Lord, S. R., St George, R., & Fitzpatrick, R. C. (2004). Walking stability and sensorimotor function in older people with diabetic peripheral neuropathy. *Arch Phys Med Rehabil, 85*(2), 245-252.

Merriwether, E. N. (2014). *Foot Progression Angle in Individuals with Diabetes Mellitus and Peripheral Neuropathy.* (PhD), Washington University
http://openscholarship.wustl.edu/cgi/viewcontent.cgi?article=2251&context=etd. (All Theses and Dissertations (ETDs). Paper 1251)

Michaud, S. B., Gard, S. A., & Childress, D. S. (2000). A preliminary investigation of pelvic obliquity patterns during gait in persons with transtibial and transfemoral amputation. *Journal of Rehabilitation Research & Development, 37*(1), 1-10.

Miller, D. R., Enoch, S., Conway, K., Harding, K. G., Price, P. E., Pataky, Z., & Golay, A. (2004). Relationship between peripheral vascular disease and higher plantar pressures in diabetic neuro-ischaemic patients. *Diabetes and Metabolism, 30*(2), 201-202.

Moghtaderi, A., Bakhshipour, A., & Rashidi, H. (2006). Validation of Michigan neuropathy screening instrument for diabetic peripheral neuropathy. *Clinical Neurology and Neurosurgery, 108*(5), 477-481.

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS medicine, 6*(7), e1000097.

Molines-Barroso, R. J., Lázaro-Martínez, J. L., Aragón-Sánchez, F. J., Álvaro-Afonso, F. J., García-Morales, E., & García-Álvarez, Y. (2016). Forefoot ulcer risk is associated with foot type in patients with diabetes and neuropathy. *Diabetes research and clinical practice, 114*, 93-98.

Monteiro-Soares, M., Boyko, E., Ribeiro, J., Ribeiro, I., & Dinis-Ribeiro, M. (2012). Predictive factors for diabetic foot ulceration: a systematic review. *Diabetes Metab Res Rev, 28*(7), 574-600.

Mooradian, A. D. (2009). Dyslipidemia in type 2 diabetes mellitus. *Nat Clin Pract End Met, 5*(3), 150-159.

Moura Neto, A., Zantut-Wittmann, D. E., Fernandes, T. D., Nery, M., & Parisi, M. C. (2013). Risk factors for ulceration and amputation in diabetic foot: study in a cohort of 496 patients. *Endocrine, 44*(1), 119-124.

Mueller, M., Minor, S., Sahrmann, S., Schaaf, J., & Strube, M. (1994a). Differences in the gait characteristics of patients with diabetes and peripheral neuropathy compared with aged-matched controls... including commentary by McPoil T and Cavanaugh PR with author response. *Phys Ther, 74*(4), 299-313.

Mueller, M. J., Hastings, M., Commean, P. K., Smith, K. E., Pilgram, T. K., Robertson, D., & Johnson, J. (2003). Forefoot structural predictors of plantar pressures during walking in people with diabetes and peripheral neuropathy. *J Biomech, 36*(7), 1009-1017.

Mueller, M. J., Lott, D. J., Hastings, M. K., Commeam, P. K., Smith, K. E., & Pilgram, T. K. (2006). Efficacy and mechanism of orthotic devices to unload metatarsal heads in people with diabetes and a history of plantar ulcers. *Phys Ther, 86*(6), 833-842.

Mueller, M. J., Sinacore, D. R., Hoogstrate, S., & Daly, L. (1994b). Hip and ankle walking strategies: Effect on peak plantar pressures and implications for neuropathic ulceration. *Arch Phys Med Rehabil, 75*(11), 1196-1200.

Mueller, M. J., Zou, D., Bohnert, K. L., Turtle, L. J., & Sinacore, D. R. (2008). Plantar stresses on the neuropathic foot during barefoot walking. *Phys Ther, 88*(11), 1375-1384.

Nabuurs-Franssen, M. H., Huijberts, M. S., Nieuwenhuijzen Kruseman, A. C., Willems, J., & Schaper, N. C. (2005). Health-related quality of life of diabetic foot ulcer patients and their caregivers. *Diabetologia, 48*(9), 1906-1910.

Najafi, B., Crews, R. T., & Wrobel, J. S. (2010a). Importance of time spent standing for those at risk of diabetic foot ulceration. *Diabetes Care, 33*(11), 2448-2450.

Najafi, B., Grewal, G. S., Bharara, M., Menzies, R., Talal, T. K., & Armstrong, D. G. (2016). Can't Stand the Pressure. *Journal of Diabetes Science and Technology, epub*.

Najafi, B., Horn, D., Marclay, S., Crews, R. T., Wu, S., & Wrobel, J. S. (2010b). Assessing postural control and postural control strategy in diabetes patients using innovative and wearable technology. *Journal of Diabetes Science & Technology, 4*(4), 780-791.

Najafi, B., Ron, E., Enriquez, A., Marin, I., Razjouyan, J., & Armstrong, D. G. (2017). Smarter Sole Survival. *Journal of Diabetes Science and Technology, epub*.

Nelson, E. A., O'Meara, S., Craig, D., Iglesias, C., Golder, S., Dalton, J., . . . Powell, J. (2006). A series of systematic reviews to inform a decision analysis for sampling and treating infected diabetic foot ulcers. *Health Technol Assess, 10*(12), 1-221.

NIWA. (2013). Statistical calculators. *The National Institute of Water and Atmospheric Research.* Retrieved from http://www.niwa.co.nz/node/104318/concordance

Norman, R. E., Gibb, M., Dyer, A., Prentice, J., Yelland, S., Cheng, Q., . . . Graves, N. (2016). Improved wound management at lower cost: a sensible goal for Australia. *International Wound Journal, 13*(3), 303-316.

Nubé, V. L., Molyneaux, L., Bolton, T., Clingan, T., Palmer, E., & Yue, D. K. (2006). The use of felt deflective padding in the management of plantar hallux and forefoot ulcers in patients with diabetes. *Foot (Edinb), 16*(1), 38-43.

O'Connor, P. D., Robinson, M. E., Shirley, F. R., & Millan, M. M. (1993). The effect of marker placement deviations on spinal range of motion determined by video motion analysis. *Phys Ther, 73*(7), 478-483.

O'Rourke, S., Steffen, C., Raulli, A., & Tulip, F. (2013). Diabetic major amputation in Far North Queensland 1998-2008: what is the Gap for Indigenous patients? *Aust J Rural Health, 21*(5), 268-273.

Ogbera, O. A., Osa, E., Edo, A., & Chukwum, E. (2008). Common clinical features of diabetic foot ulcers: perspectives from a developing nation. *Int J Low Extrem Wounds, 7*(2), 93-98.

Oguejiofor, O. C., Odenigbo, C. U., & Oguejiofor, C. B. (2010). Evaluation of the effect of duration of diabetes mellitus on peripheral neuropathy using the United Kingdom screening test scoring system, bio-thesiometry and aesthesiometry. *Niger J Clin Pract, 13*(3), 240-247.

Orendurff, M. S., Rohr, E. S., Sangeorzan, B. J., Weaver, K., & Czerniecki, J. M. (2006). An equinus doformity of the ankle accounts for only a small amount of the increased forefoot plantar pressure in patients with diabetes. *J Bone Joint Surg Br., 88*(1), 65-68.

Orlin, M., & McPoil, T. (2000). Plantar pressure assessment. *Phys Ther, 80*(4), 399-409.

Owings, T. M., Woerner, J. L., Frampton, J. D., Cavanagh, P. R., & Botek, G. (2008). Custom therapeutic insoles based on both foot shape and plantar pressure measurement provide enhanced pressure relief. *Diabetes Care, 31*(5), 839-844.

Pai, S., & Ledoux, W. R. (2012). The shear mechanical properties of diabetic and non-diabetic plantar soft tissue. *J Biomech, 45*(2), 364-370.

Park, T. S., Park, J. H., & Baek, H. S. (2004). Can diabetic neuropathy be prevented? *Diabetes research and clinical practice, 66, Supplement*(0), S53-S56.

Paternostro-Sluga, T., Grim-Stieger, M., Posch, M., Schuhfried, O., Vacariu, G., Mittermaier, C., . . . Fialka-Moser, V. (2008). Reliability and validity of the Medical Research Council (MRC) scale and a modified scale for testing muscle strength in patients with radial palsy. *J Rehabil Med, 40*(8), 665-671.

Paton, J., Bruce, G., Jones, R., & Stenhouse, E. (2011). Effectiveness of insoles used for the prevention of ulceration in the neuropathic diabetic foot: a systematic review. *Journal of Diabetes & its Complications, 25*(1), 52-62.

Payne, C., Turner, D., & Miller, K. (2002). Determinants of plantar pressures in the diabetic foot. *Journal of Diabetes & its Complications, 16*(4), 277-283.

Perkins, B. A., Orszag, A., Ngo, M., Ng, E., New, P., & Bril, V. (2010). Prediction of incident diabetic neuropathy using the monofilament examination: a 4-year prospective study. *Diabetes Care, 33*(7), 1549-1554.

Persaud, R. (1996). Misleading meta-analysis. "Fail safe N" is a useful mathematical measure of the stability of results. *BMJ, 13*(312(7023)), 125.

Peters, E. J., Urukalo, A., Fleischli, J. G., & Lavery, L. A. (2002). Reproducibility of gait analysis variables: one-step versus three-step method of data acquisition. *Journal of Foot & Ankle Surgery, 41*(4), 206-212.

Pham, H., Armstrong, D. G., Harvey, C., Harkless, L. B., Giurini, J. M., & Veves, A. (2000). Screening techniques to identify people at high risk for diabetic foot ulceration: a prospective multicenter trial. *Diabetes Care, 23*(5), 606-611.

Piaggesi, A., Macchiarini, S., Rizzo, L., Palumbo, F., Tedeschi, A., Nobili, L. A., . . . Del Prato, S. (2007). An off-the-shelf instant contact casting device for the management of diabetic foot ulcers: a randomized prospective trial versus traditional fiberglass cast. *Diabetes Care, 30*(3), 586-590.

Piaggesi, A., Palumbo, F., Tedeschi, A., Ambrosini, L., Macchiarini, S., Scatena, A., . . . Rizzo, L. (2005). Measurements in the diabetic foot. *Wounds, 17*(9), 247-254.

Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J., Hill, M. N., . . . Roccella, E. J. (2005). Recommendations for Blood Pressure Measurement in Humans and Experimental Animals: Part 1: Blood Pressure Measurement in Humans: A Statement for Professionals From the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Hypertension, 45*(1), 142-161.

Picon, A. P., Sartor, C. D., Roveri, M. I., Pássaro, A. C., Ortega, N. R., & Sacco, I. C. N. (2012). Diabetic patients with and without peripheral neuropathy reveal different hip and ankle biomechanical strategies during stair descent. *Rev Bras Fisioter, 16*(6), 528-534.

Pinheiro J, Bates D, DebRoy S, Sarkar D, & Team, a. R. C. (2015). nlme: Linear and Nonlinear Mixed Effects Models. *R package version 3.1-12.* Retrieved from https://cran.r-project.org/web/packages/nlme/nlme.pdf

Pound, N., Chipchase, S., Treece, K., Game, F., & Jeffcoate, W. (2005). Ulcer-free survival following management of foot ulcers in diabetes. *Diabetic Medicine*, 22(10), 1306-1309.

Price, P. (2004). The Diabetic Foot: Quality of Life. *Clinical Infectious Diseases, 39 Suppl 2*, S129-S131.

Prompers, L., Huijberts, M., Apelqvist, J., Jude, E., Piaggesi, A., Bakker, K., . . . Schaper, N. (2007). High prevalence of ischaemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. Baseline results from the Eurodiale study. *Diabetologia, 50*(1), 18-25.

Qiu, X., Tian, D. H., Han, C. L., Chen, W., Wang, Z. J., Mu, Z. Y., . . . Liu, K. Z. (2013). Risk Factors Correlated with Plantar Pressure in Chinese Patients with Type 2 Diabetes. *Diabetes Technology & Therapeutics, 15*(12), 1025-1032.

Qiu, X., Tian, D. H., Han, C. L., Chen, W., Wang, Z. J., Mu, Z. Y., & Liu, K. Z. (2015). Plantar Pressure Changes and Correlating Risk Factors in Chinese Patients with Type 2 Diabetes: Preliminary 2-year Results of a Prospective Study. *Chin Med J (Engl), 128*(24), 3283-3291.

Quinton, T. R., Lazzarini, P. A., Boyle, F. M., Russell, A. W., & Armstrong, D. G. (2015). How do Australian podiatrists manage patients with diabetes? The Australian diabetic foot management survey. *J Foot Ankle Res, 8*(8), epub.

Rajbhandari, S., Jenkins, R., Davies, C., & Tesfaye, S. (2002). Charcot neuroarthropathy in diabetes mellitus. *Diabetologia, 45*(8), 1085-1096.

Ramsey, S. D., Newton, K., Blough, D., McCulloch, D. K., Sandhu, N., Reiber, G. E., & Wagner, E. H. (1999). Incidence, outcomes, and cost of foot ulcers in patients with diabetes. *Diabetes Care, 22*(3), 382-387.

Rao, S., Saltzman, C., & Yack, H. J. (2006). Ankle ROM and stiffness measured at rest and during gait in individuals with and without diabetic sensory neuropathy. *Gait and Posture, 24*(3), 295-301.

Raspovic, A. (2013). Gait characteristics of people with diabetes-related peripheral neuropathy, with and without a history of ulceration. *Gait and Posture, 38*(4), 723-728.

Raspovic, A., & Douglas, R. (2011). Is there a relationship between static and dynamic ankle joint dorsiflexion during gait in people with a history of neuropathic ulceration? *Journal of Foot and Ankle Research, 4 Suppl 1*, p47.

Raspovic, A., & Landorf, K. B. (2014). A survey of offloading practices for diabetes-related plantar neuropathic foot ulcers. *Journal of Foot and Ankle Research, 7*(1), 1-8.

Raspovic, A., Newcombe, L., Lloyd, J., & Dalton, E. (2000). Effect of customized insoles on vertical plantar pressures in sites of previous neuropathic ulceration in the diabetic foot. *Foot (Edinb), 10*(3), 133-138.

Rathur, H. M., & Boulton, A. J. M. (2005). Pathogenesis of foot ulcers and the need for offloading. *Hormone and Metabolic Research, 37 Suppl 1*, S61-S68.

Reeves, N. D., Brown, S. J., Petrovic, M., Boulton, A. J. M., & Vileikyte, L. (2017). How Does Self-Perceived Unsteadiness Influence Balance and Gait in People With Diabetes? Preliminary Observations. *Diabetes Care, 40*(5), e51.

Ribu, L., Birkeland, K., Hanestad, B. R., Moum, T., & Rustoen, T. (2008). A longitudinal study of patients with diabetes and foot ulcers and their health-related quality of life: wound healing and quality-of-life changes. *Journal of Diabetes & its Complications, 22*(6), 400-407.

Rice, J. B., Desai, U., Cummings, A. K., Birnbaum, H. G., Skornicki, M., & Parsons, N. B. (2014). Burden of diabetic foot ulcers for medicare and private insurers. *Diabetes Care, 37*(3), 651-658.

Rich, J., & Veves, A. (2000). Forefoot and Rearfoot Plantar Pressures in Diabetic Patients: Correlation to Foot Ulceration. *Wounds, 12*(4), 82-87.

Richardson, J. K., & Hurvitz, E. A. (1995). Peripheral neuropathy: a true risk factor for falls. *Journals of Gerontology Series A-Biological Sciences & Medical Sciences, 50*(4), M211-215.

Robbins, J. M., Strauss, G., Aron, D., Long, J., Kuba, J., & Kaplan, Y. (2008). Mortality rates and diabetic foot ulcers: is it time to communicate mortality risk to patients with diabetic foot ulceration? *Journal of the American Podiatric Medical Association, 98*(6), 489-493.

Rosenbaum, D., Hautmann, S., Gold, M., & Claes, L. (1994). Effects of walking speed on plantar pressure patterns and hindfoot angular motion. *Gait and Posture, 2*(3), 191-197.

Sacco, I. C., Akashi, P. M., & Hennig, E. (2009a). Re: The vertical component of the ground reaction force does not reflect horizontal braking or acceleration per se. *Clinical biomechanics (Bristol, Avon), 24*(7), 595.

Sacco, I. C., Akashi, P. M., & Hennig, E. M. (2010). A comparison of lower limb EMG and ground reaction forces between barefoot and shod gait in participants with diabetic neuropathic and healthy controls. *BMC Musculoskeletal Disorders, 11*(24), epub.

Sacco, I. C., & Amadio, A. C. (2000). A study of biomechanical parameters in gait analysis and sensitive cronaxie of diabetic neuropathic patients. *Clin Biomech (Bristol, Avon), 15*(3), 196-202.

Sacco, I. C., Hamamoto, A. N., Gomes, A. A., Onodera, A. N., Hirata, R. P., & Hennig, E. M. (2009b). Role of ankle mobility in foot rollover during gait in individuals with diabetic neuropathy. *Clin Biomech (Bristol, Avon), 24*(8), 687-692.

Salsabili, H., Bahrpeyma, F., & Esteki, A. (2015). The effects of Task-Oriented Motor Training on gait characteristics of patients with type 2 diabetes neuropathy. *Journal of Diabetes and Metabolic Disorders, 15*(14), epub.

Sanderson, S., Tatt, I. D., & Higgins, J. P. (2007). Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: a systematic review and annotated bibliography. *International Journal of Epidemiology, 36*(3), 666-676.

Sartor, C. D., Hasue, R. H., Cacciari, L. P., Butugan, M. K., Watari, R., Pássaro, A. C., . . . Sacco, I. C. (2014). Effects of strengthening, stretching and functional training on foot function in patients with diabetic neuropathy: results of a randomized controlled trial. *BMC Musculoskeletal Disorders, 15*(1), 1-13.

Sartor, C. D., Watari, R., Passaro, A. C., Picon, A. P., Hasue, R. H., & Sacco, I. C. (2012). Effects of a combined strengthening, stretching and functional training program versus usual-care on gait biomechanics and foot function for diabetic neuropathy: a randomized controlled trial. *BMC Musculoskeletal Disorders, 13*(1), epub.

Saura, V., dos Santos, A. L. G., Ortiz, R. T., Parisi, M. C., Fernandes, T. D., & Nery, M. (2010). Predictive factors of gait in neuropathic and non-neurophatic diabetic patients. *Acta Ortopedica Brasileira, 18*(3), 148-151.

Sauseng, S., & Kästenbauer, T. (1999). Influence of limited joint mobility in plantar pressure in patients with type 1 diabetes mellitus. *Acta Medica Austriaca, 26*(5), 178-181.

Sauseng, S., Kästenbauer, T., Sokol, G., & Irsigler, K. (1999). Estimation of risk for plantar foot ulceration in diabetic patients with neuropathy. *Diabetes, Nutrition and Metabolism - Clinical and Experimental, 12*(3), 189-193.

Savelberg, H. H., Schaper, N. C., & Meijer, K. (2009a). The vertical component of the ground reaction force does not reflect horizontal braking or acceleration per se. *Clin Biomech (Bristol, Avon), 24*(6), 527-528.

Savelberg, H. H., Schaper, N. C., Willems, P. J., de Lange, T. L., & Meijer, K. (2009b). Redistribution of joint moments is associated with changed plantar pressure in diabetic polyneuropathy. *BMC Musculoskeletal Disorders, 10*(16), epub.

Savelberg, H. H. C. M., Ilgin, D., Angin, S., Willems, P. J. B., Schaper, N. C., & Meijer, K. (2010). Prolonged activity of knee extensors and dorsal flexors is associated with adaptations in gait in diabetes and diabetic polyneuropathy. *Clin Biomech (Bristol, Avon), 25*(5), 468-475.

Sawacha, Z., Cristoferi, G., Guarneri, G., Corazza, S., Dona, G., Denti, P., . . . Cobelli, C. (2009a). Characterizing multisegment foot kinematics during gait in diabetic foot patients. *J Neuroeng Rehabil, 23*(6), 37.

Sawacha, Z., Gabriella, G., Cristoferi, G., Guiotto, A., Avogaro, A., & Cobelli, C. (2009b). Diabetic gait and posture abnormalities: A biomechanical investigation through three dimensional gait analysis. *Clin Biomech (Bristol, Avon), 24*(9), 722-728.

Sawacha, Z., Guarneri, G., Cristoferi, G., Guiotto, A., Avogaro, A., & Cobelli, C. (2012a). Integrated kinematics-kinetics-plantar pressure data analysis: A useful tool for characterizing diabetic foot biomechanics. *Gait and Posture, 36*(1), epub.

Sawacha, Z., Spolaor, F., Guarneri, G., Contessa, P., Carraro, E., Venturin, A., . . . Cobelli, C. (2012b). Abnormal muscle activation during gait in diabetes patients with and without neuropathy. *Gait and Posture, 35*(1), 101-105.

Schaper, N. C. (2004). Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. *Diabetes Metab Res Rev, 20 Suppl 1*, S90-95.

Schaper, N. C., Prompers, L. M., & Huijberts, M. S. P. (2007). Treatment of diabetic foot ulcers. *Immunology, Endocrine and Metabolic Agents in Medicinal Chemistry, 7*(1), 95-104.

Schaper, N. C., Van Netten, J. J., Apelqvist, J., Lipsky, B. A., Bakker, K., & on behalf of the International Working Group on the Diabetic, F. (2016). Prevention and management of foot problems in diabetes: a Summary Guidance for Daily Practice 2015, based on the IWGDF Guidance Documents. *Diabetes/Metabolism Research And Reviews, 32*, 7-15.

Scheffé, H. (1953). A method for judging all contrasts in the analysis of variance*. *Biometrika, 40*(1-2), 87-110.

Schwartz, A. V., Hillier, T. A., Sellmeyer, D. E., Resnick, H. E., Gregg, E., Ensrud, K. E., . . . Cummings, S. R. (2002). Older Women With Diabetes Have a Higher Risk of Falls: A prospective study. *Diabetes Care, 25*(10), 1749-1754.

Shaw, J. E., Hsi, W. L., Ulbrecht, J. S., Norkitis, A., Becker, M. B., & Cavanagh, P. R. (1997). The mechanism of plantar unloading in total contact casts: implications for design and clinical use. *Foot & Ankle International, 18*(12), 809-817.

Shaw, J. E., Van Schie, C. H., Carrington, A. L., Abbott, C. A., & Boulton, A. J. (1998). An analysis of dynamic forces transmitted through the foot in diabetic neuropathy. *Diabetes Care, 21*(11), 1955–1959.

Shen, J., Liu, F., Zeng, H., Wang, J., Zhao, J. G., Zhao, J., . . . Jia, W. P. (2012). Vibrating perception threshold and body mass index are associated with abnormal foot plantar pressure in type 2 diabetes outpatients. *Diabetes Technology & Therapeutics, 14*(11), 1053-1059.

Shenoy, A. M. (2012). Guidelines in practice: Treatment of painful diabetic neuropathy. *Continuum (Minneap Minn), 18*(1), 192-198.

Singh, N., Armstrong, D., & Lipsky, B. (2005). Preventing foot ulcers in patients with diabetes. *JAMA, 293*, 217 - 228.

Stanley, J. C., & Collier, A. M. (2009). The diabetic foot and ankle. *Orthopaedics and Trauma, 23*(1), 61-68.

Stess, R. M., Jensen, S. R., & Mirmiran, R. (1997). The role of dynamic plantar pressures in diabetic foot ulcers. *Diabetes Care, 20*(5), 855-858.

Stockl, K., Vanderplas, A., Tafesse, E., & Chang, E. (2004). Costs of lower-extremity ulcers among patients with diabetes. *Diabetes Care, 27*(9), 2129-2134.

Stokes, I. A., Faris, I. B., & Hutton, W. C. (1975). The neuropathic ulcer and loads on the foot in diabetic patients. *Acta Orthop Scand, 46*(5), 839-847.

Stroup, D. F., Berlin, J. A., Morton, S. C., Olkin, I., Williamson, G. D., Rennie, D., . . . Thacker, S. B. (2000). Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA, 283*(15), 2008-2012.

Stubbing, N. J., Bailey, P., & Poole, M. (1997). Protocol for accurate assessment of ABPI in patients with leg ulcers. *Journal of Wound Care, 6*(9), 417-418.

Sugimoto, K., Yasujima, M., & Yagihashi, S. (2008). Role of advanced glycation end products in diabetic neuropathy. *Curr Pharm Des, 14*(10), 953-961.

Sutherland, D. E. (2001). The evolution of clinical gait analysis part l: kinesiological EMG. *Gait and Posture, 14*(14), 61–70.

Sutherland, D. H. (2002). The evolution of clinical gait analysis. Part II kinematics. *Gait Posture, 16*(2), 159-179.

Sutherland, D. H. (2005). The evolution of clinical gait analysis part III--kinetics and energy assessment. *Gait Posture, 21*(4), 447-461.

Tesfaye, S., Boulton, A. J. M., Dyck, P. J., Freeman, R., Horowitz, M., Kempler, P., R., . . . Valensi, P. (2010). Diabetic Neuropathies: Update on Definitions, Diagnostic Criteria, Estimation of Severity, and Treatments. *Diabetes Care, 33*(10), 2285–2293.

Tesfaye, S., & Selvarajah, D. (2012). Advances in the epidemiology, pathogenesis and management of diabetic peripheral neuropathy. *Journal of Diabetes Metab Res Rev, 1*(1), 8-14.

Thivolet, C., el Farkh, J., Petiot, A., Simonet, C., & Tourniaire, J. (1990). Measuring vibration sensations with graduated tuning fork. Simple and reliable means to detect diabetic patients at risk of neuropathic foot ulceration. *Diabetes Care, 13*(10), 1077-1080.

Timar, B., Timar, R., Gaiță, L., Oancea, C., Levai, C., & Lungeanu, D. (2016). The Impact of Diabetic Neuropathy on Balance and on the Risk of Falls in Patients with Type 2 Diabetes Mellitus: A Cross-Sectional Study. *PLoS ONE, 11*(4), e0154654.

Turner, D. E., Helliwell, P. S., Burton, A. K., & Woodburn, J. (2007). The relationship between passive range of motion and range of motion during gait and plantar pressure measurements. *Diabetic Medicine, 24*(11), 1240-1246.

Uccioli, L., Caselli, A., Giacomozzi, C., Macellari, V., Giurato, L., Lardieri, L., & Menzinger, G. (2001). Pattern of abnormal tangential forces in the diabetic neuropathic foot. *Clin Biomech (Bristol, Avon), 16*(5), 446-454.

Valensi, P., Girod, I., Baron, F., Moreau-Defarges, T., & Guillon, P. (2005). Quality of life and clinical correlates in patients with diabetic foot ulcers. *Diabetes & Metabolism, 31*(3), 263-271.

Valensi, P., Giroux, C., Seeboth-Ghalayini, B., & Attali, J.-R. (1997). Diabetic peripheral neuropathy: Effects of age, duration of diabetes, glycemic control, and vascular factors. *Journal of Diabetes and its Complications, 11*(1), 27-34.

van Deursen, R. (2004). Mechanical Loading and Off-Loading of the Plantar Surface of the Diabetic Foot. *Clinical Infectious Diseases, 1 Suppl 2*(39), 87-91.

van Deursen, R. (2008). Footwear for the neuropathic patient: Offloading and stability. *Diabetes Metab Res Rev, 24 Suppl 1*, S96-S100.

van Dieren, S., Beulens, J. W., van der Schouw, Y. T., Grobbee, D. E., & Neal, B. (2010). The global burden of diabetes and its complications: an emerging pandemic. *Eur J Cardiovasc Prev Rehabil, 17 Suppl 1*, S3-8.

van Netten, J. J., Price, P. E., Lavery, L. A., Monteiro-Soares, M., Rasmussen, A., Jubiz, Y., . . . on behalf of the International Working Group on the Diabetic, F. (2016). Prevention of foot ulcers in the at-risk patient with diabetes: a systematic review. *Diabetes Metab Res Rev, 32*, 84-98.

van Schie, C. H. (2005). A review of the biomechanics of the diabetic foot. *Int J Low Extrem Wounds, 4*(3), 160-170.

van Schie, C. M., & Boulton, A. J. (2002). Biomechanics of the Diabetic Foot: The Road to Foot Ulceration. In A. Veves, J. Giurini, & F. LoGerfo (Eds.), *The Diabetic Foot* (pp. 147-161): Humana Press.

Veves, A., Murray, H. J., Young, M. J., & Boulton, A. J. (1992). The risk of foot ulceration in diabetic patients with high foot pressure: a prospective study. *Diabetologia, 35*(7), 660-663.

Vicon. (2010). Plugin NexusPluginGait ProductGuide. *Foundation_Rev2.0_2010April.* Retrieved from http://www.metrics.co.uk/support/downloads/cef70cc8dbcc7149988652bf7dff8ff84cc6bc77302948.73654482/Plugin_Nexus_PluginGait_ProductGuide_Foundation_Rev2.0_2010April

Vileikyte, L. (2001). Diabetic foot ulcers: a quality of life issue. *Diabetes Metab Res Rev, 17*(4), 246-249.

Viswanathan, V. (2007). The diabetic foot: Perspectives from Chennai, South India. *International Journal of Lower Extremity Wounds, 6*(1), 34-36.

Viswanathan, V., Madhavan, S., Gnanasundaram, S., Gopalakrishna, G., Das, B. N., Rajasekar, S., & A, R. (2004). Effectiveness of different types of footwear insoles for the diabetic neuropathicfoot: a follow-up study. *Diabetes Care, 27*(2), 474-474.

Viswanathan, V., Snehalatha, C., Sivagami, M., Seena, R., & Ramachandran, A. (2003). Association of limited joint mobility and high plantar pressure in diabetic foot ulceration in Asian Indians. *Diabetes Research & Clinical Practice, 60*(1), 57-61.

Volpato, S., Blaum, C., Resnick, H., Ferrucci, L., Fried, L. P., Guralnik, J. M., . . . Aging, S. (2002). Comorbidities and impairments explaining the association between diabetes and lower extremity disability: The Women's Health and Aging Study. *Diabetes Care, 25*(4), 678-683.

Volpato, S., Ferrucci, L., Blaum, C., Ostir, G., Cappola, A., Fried, L. P., . . . Guralnik, J. M. (2003). Progression of lower-extremity disability in older women with diabetes: the Women's Health and Aging Study. *Diabetes Care, 26*(1), 70-75.

Volpato, S., Maraldi, C., & Fellin, R. (2010). Type 2 diabetes and risk for functional decline and disability in older persons. *Current Diabetes Reviews, 6*(3), 134-143.

Waaijman, R., & Bus, S. A. (2012). The interdependency of peak pressure and pressure-time integral in pressure studies on diabetic footwear: no need to report both parameters. *Gait and Posture, 35*(1), 1-5.

Waaijman, R., de Haart, M., Arts, M. L. J., Wever, D., Verlouw, A. J. W. E., Nollet, F., & Bus, S. A. (2014). Risk Factors for Plantar Foot Ulcer Recurrence in Neuropathic Diabetic Patients. *Diabetes Care, 37*(6), 1697-1705.

Waaijman, R., Keukenkamp, R., de Haart, M., Polomski, W. P., Nollet, F., & Bus, S. A. (2013). Adherence to wearing prescription custom-made footwear in patients with diabetes at high risk for plantar foot ulceration. *Diabetes Care, 36*(6), 1613-1618.

Wearing, S. C., Urry, S., Smeathers, J. E., & Battistutta, D. (1999). A comparison of gait initiation and termination methods for obtaining plantar foot pressures. *Gait and Posture, 10*(3), 255-263.

Weir, G. (2010). Diabetic foot ulcers – evidence-based wound management. *Continuing Medical Education CME, 28*(4), epub.

Whittle, J. (2012). *Whittle's Gait Analysis* (Fifth Edition ed. Vol. One). China: Elsevier.

Wilder, R. P., Cole, A. J., Sobel, J., & O'Connor, F. G. (1994). Overuse injuries of the hip and pelvis in sport. *J Back Musculoskelet Rehabil, 4*(3), 236-247.

Willi, C., Bodenmann, P., Ghali, W. A., Faris, P. D., & Cornuz, J. (2007). Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA, 298*(22), 2654-2664.

Winter, D. A. (1980). Overall principle of lower limb support during stance phase of gait. *J Biomech, 13*(1), 923-927.

Wofford, M. R., Harkins, K. G., King, D. S., Habeeb, G. E., Wyatt, S. B., & Jones, D. W. (2002). P-175: Accurate cuff size in blood pressure measurement. *American Journal of Hypertension, 15 Suppl 3*, 92A.

Wolfson, L. (2001). Gait and balance dysfunction: a model of the interaction of age and disease. *Neuroscientist, 7*(2), 178-183.

Wood, W. A., Wood, M. A., Werter, S. A., Menn, J. J., Hamilton, S. A., Jacoby, R., & Dellon, A. L. (2005). Testing for loss of protective sensation in patients with foot ulceration: a cross-sectional study. *Journal of the American Podiatric Medical Association, 95*(5), 469-474.

Wrobel, J. S., Mayfield, J. A., & Reiber, G. E. (2001). Geographic variation of lower-extremity major amputation in individuals with and without diabetes in the Medicare population. *Diabetes Care, 24*(5), 860-864.

Wrobel, J. S., & Najafi, B. (2010). Diabetic Foot Biomechanics and Gait Dysfunction. *Journal of Diabetes Science and Technology, 1*(4), 833-845.

Wu, S. C., Crews, R. T., & Armstrong, D. G. (2005). The pivotal role of offloading in the management of neuropathic foot ulceration. *Curr Diab Rep, 5*(6), 423-429.

Wu, S. C., Driver, V. R., Wrobel, J. S., & Armstrong, D. G. (2007). Foot ulcers in the diabetic patient, prevention and treatment. *Vascular Health and Risk Management, 3*(1), 65-76.

Wu, S. C., Jensen, J. L., Weber, A. K., Robinson, D. E., & Armstrong, D. G. (2008). Use of pressure offloading devices in diabetic foot ulcers: do we practice what we preach? *Diabetes Care, 31*(11), 2118-2119.

Yavuz, M. (2014). Plantar shear stress distributions in diabetic patients with and without neuropathy. *Clin Biomech (Bristol, Avon), 29*(2), 223-229.

Yavuz, M., Botek, G., & Davis, B. L. (2007a). Plantar shear stress distributions: comparing actual and predicted frictional forces at the foot-ground interface. *J Biomech, 40*(13), 3045-3049.

Yavuz, M., Erdemir, A., Botek, G., Hirschman, G. B., Bardsley, L., & Davis, B. L. (2007b). Peak plantar pressure and shear locations: relevance to diabetic patients. *Diabetes Care, 30*(10), 2643-2645.

Yavuz, M., Tajaddini, A., Botek, G., & Davis, B. L. (2008). Temporal characteristics of plantar shear distribution: relevance to diabetic patients. *J Biomech, 41*(3), 556-559.

Yavuzer, G., Yetkin, I., Toruner, F., Koca, N., & Bolukbas, N. (2006). Gait deviations of patients with diabetes mellitus: looking beyond peripheral neuropathy. *Europa Medicophysica, 42*(2), 127-133.

Yentes, J. M., Sayles, H., Meza, J., Mannino, D. M., Rennard, S. I., & Stergiou, N. (2011). Walking abnormalities are associated with COPD: An investigation of the NHANES III dataset. *Respiratory Medicine, 105*(1), 80-87.

Yi, L. C., Sartor, C. D., Souza, F. T., & Sacco, I. C. N. (2016). Intralimb Coordination Patterns in Absent, Mild, and Severe Stages of Diabetic Neuropathy: Looking Beyond Kinematic Analysis of Gait Cycle. *PLoS ONE, 11*(1), e0147300.

Zammit, G. V., Menz, H. B., & Munteanu, S. E. (2010). Reliability of the TekScan MatScan(R) system for the measurement of plantar forces and pressures during barefoot level walking in healthy adults. *J Foot Ankle Res, 18*(3), epub.

Zhang, P., Lu, J., Jing, Y., Tang, S., Zhu, D., & Bi, Y. (2017). Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. *Annals of medicine, 49*(2), 106-116.

Żuk, M., & Pezowicz, C. (2015). Kinematic Analysis of a Six-Degrees-of-Freedom Model Based on ISB Recommendation: A Repeatability Analysis and Comparison with Conventional Gait Model. *Applied Bionics and Biomechanics, 55*(1), 369–376.

Appendices

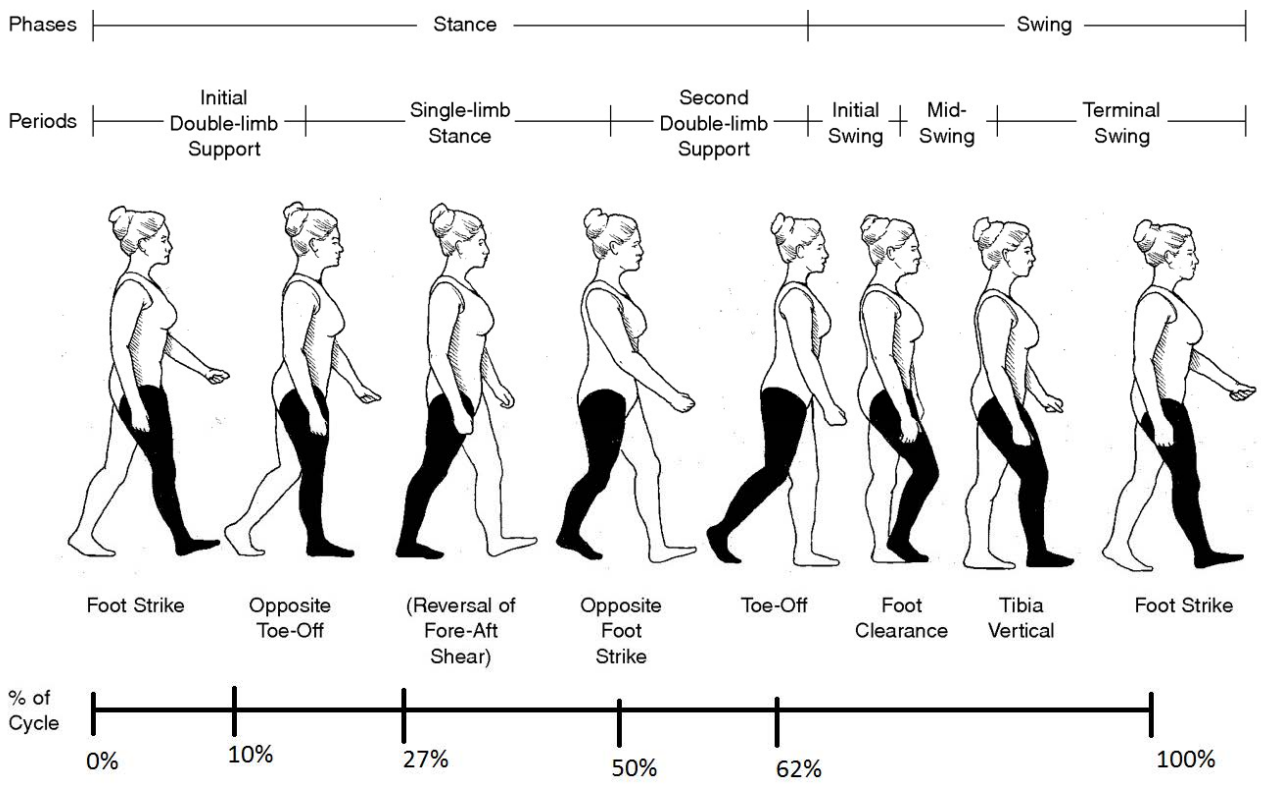
Appendix A: Detailed description of biomechanical outcomes

The gait cycle

The ability to walk upright is a unique characteristic of man which influences the features of human locomotion, also known as gait. The gait cycle (GC) is a time-interval or sequence of motion occurring from initial contact of one limb (foot strike) to the subsequent contact of the same limb (DeLisa, 1998). Appendix Figure 1 represents a right limb-gait cycle. This cycle is then further divided into periods and phases. The phases incorporate stance; during which the limb is in contact with the ground, and swing; during which the limb is in the air (Perry & Burnfield, 2010). The stance phase begins when the foot strikes the ground and ends with toe-off (Lim et al., 2007). Stance is subdivided into three intervals; firstly, initial double limb support, secondly, second/terminal double limb support (when both limbs are in contact with the ground) and single-limb stance (when one foot is in contact with the ground) (Perry & Burnfield, 2010). It can also be thought of as initial double limb support (when weight is being transferred from contralateral to ipsilateral limb, and terminal support (when weight is being transferred from ipsilateral to the contralateral limb) (Kirtley, 2006). Consequently, the series of events that follow from the initial foot strike are; opposite limb toe-off, heel lift (also known as reversal of fore-aft shear), opposite foot strike, toe-off, foot clearance, tibia vertical and then the subsequent foot strike (Lim et al., 2007). Swing phase begins when the stance phase ends with toe-off and incorporates initial swing, mid swing and terminal swing, prior to second limb contact (Perry & Burnfield, 2010).

The periods in the gait cycle are; initial double limb support, single limb stance, second double limb support, initial swing, mid-swing and terminal swing (Lim et al., 2007). The generic normal distributions of the GC percentages for each of the phases are 60% for stance and 40% for swing phase (DeLisa, 1998; Kirtley, 2006; Lim et al., 2007; Perry & Burnfield, 2010). In the average population, it has been postulated that toe-off occurs at 62% of the GC, with double stance representing 12% of the gait cycle (Kirtley, 2006; Lim et al., 2007). The precise durations of the GC intervals however vary with individual

walking velocity (Kirtley, 2006; Perry & Burnfield, 2010). Although the relative duration of both stance and swing reduces with increase speed of walking, walking faster lengthens the single stance period and shortens the double stance period (Kirtley, 2006; Perry & Burnfield, 2010). Thus, the analysis of the characteristics of the GC enables temporal-spatial gait characteristics to be examined.



Appendix Figure 1 The Gait Cycle (Lim et al., 2007)

Temporal-spatial parameters (TSPs)

Together, speed of walking, cadence and stride length constitutes temporal-spatial parameters (TSPs) (Kirtley, 2006; J. Whittle, 2012). Since gait is repetitive in nature, TSPs provide valuable analytical information for quantifying the timing of critical events in the GC (DeLisa, 1998). As the GC incorporates the sequence of events from the contact of a single limb to its subsequent contact, it has also been identified by the term 'stride' and therefore two steps constitute a stride (Kirtley, 2006; Perry & Burnfield, 2010; J. Whittle, 2012). The number of steps per minute is termed 'cadence' and since there are two steps in every stride, 'steps per minute' can be converted to 'strides per second' and 'stride-time' can also be calculated in the same way (Kirtley, 2006). By calculating the measurements over a defined walking distance, 'average walking velocity' and 'stride length' also can be defined. Therefore, walking speed is a result of cadence and stride length (Kirtley, 2006). It is anticipated that as a person gets weaker, has painful joints, or reduces balance, walking speed will decrease and less time will be spent in single limb support on the affected side (Lim et al., 2007). The speed of walking is determined using the equation below;

$$\text{Walking speed} = \frac{(\text{cadence} \times \text{stride length})}{120}$$

The key TSPs include;
Cadence – number of steps walked per minute;
Speed of walking; average walking speed (m/s);
Stride length- longitudinal length between two consecutive contacts of the same limb;
Stance time- amount of time (s) or (% gait cycle) spent in stance phase;
Double support time- amount of time spent in double support phase (when both limbs are in contact with the ground);
Single support time- amount of time (% gait cycle) spent in single limb support (both left and right limbs);
Swing time- amount of time spent in the swing phase of gait.

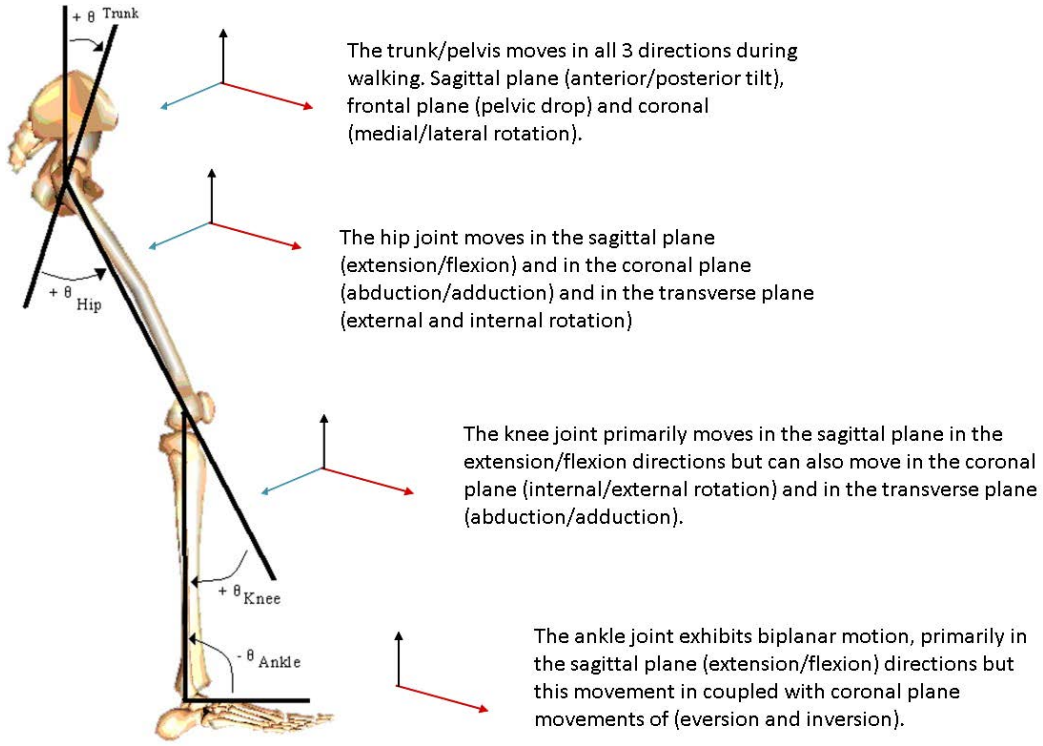
Therefore, TSPs provide critical information regarding the vital signs of gait and can be used to screen individuals for risk of falls, as a performance measure, to grade level of disability, to measure effects of interventions and to 'normalize' other gait characteristics, especially in order to compare results between participants of divergent walking speeds (Kirtley, 2006; J. Whittle, 2012). These gait characteristics include kinematics, kinetics and dynamic electromyography measurements.

Joint angular kinematics

The term 'joint angular kinematics' or simply 'kinematics' refers to the description of gait which encompasses angles, positions (displacements), velocities and accelerations of the body segments, and joints (Kirtley, 2006). Movements in the human body can occur in three possible axes; sagittal, coronal (frontal) and transverse. The sagittal plane lies vertically and divides the body into right and left parts, the frontal plane lies vertically but divides the body into anterior and posterior parts and the transverse plane lies horizontally and divides the body into superior and inferior parts (Perry & Burnfield, 2010). A fixed-body joint-coordinate system may be determined for a body segment (assumed to be rigid) that has at least three non-colinear markers attached to it; i.e. the pelvis coordinate system is constructed from three-dimensional location vectors of three non-colinear pelvis anatomical locations (Davis, Ounpuu, Tyburski, & Gage, 1991). As the relative position of the body segment vectors do not change in length or move on the body segment, but changes orientation in space as the body segment rotates, this is how the rotation of the body is tracked during the GC (DeLisa, 1998; Kadaba, Ramakrishnan, & Wootten, 1990). Human body segments are thus modeled as rigid bodies and the relative rotation is assumed to take place about a fixed point in the proximal segment, which is considered the center of the joint.

In order to investigate the moments and torques acting on a joint, this joint center must first be defined (DeLisa, 1998; Kadaba et al., 1990). It is possible to define a kinematic joint center using an instantaneous center of rotation for sagittal plane analysis or an instantaneous helical axis for general three-dimensional analysis using complex

mathematical modeling (DeLisa, 1998). The relative orientation of one body segment to another defines the joint angles (see Appendix Figure 2). For example, the orientation of the tibia to the femur defines the three clinical angles of the knee; flexion/extension, abduction/adduction, and internal/external rotation of the tibia relative to the femur, which are based on Euler angles (DeLisa, 1998). When a curve is plotted of the relative movement, i.e. knee flexion/extension, the slope of this curve is the angular velocity component in flexion and extension (DeLisa, 1998). Hence, we can use this approach to investigate the movements occurring at the three lower limb joints during a GC; hip, knee and ankle.

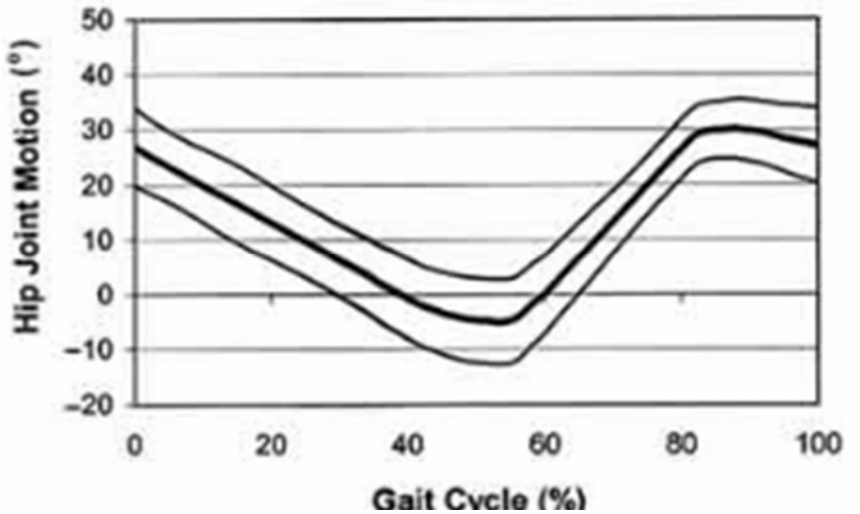


Appendix Figure 2 Joint angle conventions and directions of movement of the pelvis/trunk, hip, knee and ankle joints in various planes of movement (Crowther, Spinks, Leicht, Quigley, & Golledge, 2007).

Joint angular kinematics of the hip

During standard gait, hip motion takes place in all three anatomical planes, yet more pronounced in the sagittal plane (Nordin & Frankel, 2001). The hip moves through two arcs of movement during a normal stride in the sagittal plane; extension during stance and flexion in swing (see Appendix Figure 3). The normal arc of motion ranges from 40 degrees (deg) to 48 deg (Perry & Burnfield, 2010). However, some consider maximum hip extension as zero degrees and maximum hip flexion as 40 deg (Perry & Burnfield, 2010). The thigh is flexed 20 deg at Initial Contact (IC) and loses flexion during loading of the limb to the ground. The hip then extends and reaches neutral (0 deg) at 27% GC. The hip then extends further until it reaches a peak of 20 deg as the contralateral limb contacts the ground at 50% GC (Perry & Burnfield, 2010).

In the coronal plane, the hip moves through a small arc of adduction and abduction. This action begins at the onset of stance phase. At IC, the hip is approximately neutral (i.e. zero deg) due to the anatomical angle between the femur and the tibia (Perry & Burnfield, 2010). As body mass loads onto the limb, hip adduction increases to 10 deg, as a result of the contralateral pelvic drop and displacement of the femur. During single limb support, adduction diminishes and by pre-swing (56% GC), the hip regains its neutral position. In the transverse plane, during each stride each limb moves through an arc of internal rotation followed by a similar arc of external rotation. Total hip rotation averages 15 deg (Perry & Burnfield, 2010).



Appendix Figure 3 Sagittal plane hip motion (thigh relative to pelvis) during gait. Mean and SD (Perry & Burnfield, 2010).

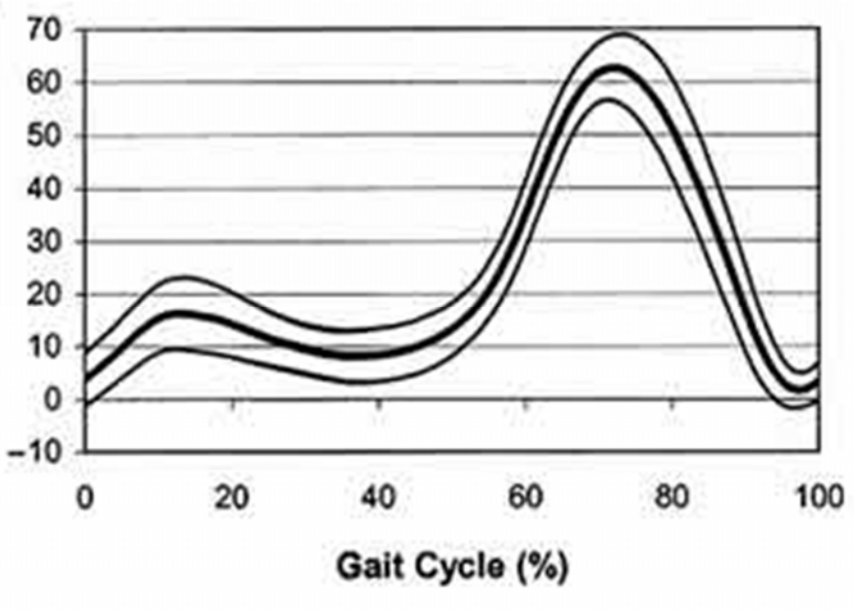
Joint angular kinematics of the knee

Thee knee joint undergoes much for complex movements compared to the hip. However, similar to the hip, the knee is also a sagittal plane dominant joint with small arcs of coronal and transverse plane mobility. Knee flexion and extension in the sagittal plane (see Appendix Figure 4) are used for limb progression in stance phase, and limb clearance and advancement during swing phase. Normal knee motion during walking represents greater and lesser degrees of flexion with in the full range zero to 60 deg. During each stride, the knee experiences two waves of flexion. The smaller first wave peaks at 20 deg flexion at the transition between just after initial contact to midstance and contributes to controlled shock absorption at the knee. The latter, larger wave contributes towards 60 deg knee flexion during initial swing and assists with limb clearance.

The rate of change of a joint angle is different from the joint angle i.e. the relative position/displacement of the joint. For example, the knee could be in a flexed position, whilst it is undergoing extension (Nordin & Frankel, 2001). Hence at IC, the knee is

extended with a mean posture of five degrees extension (Perry & Burnfield, 2010). Following limb contact, the knee rapidly flexes and at forefoot contact (12% GC) the knee remains at 20 deg flexion where the joint is said to be under maximum weight bearing load.

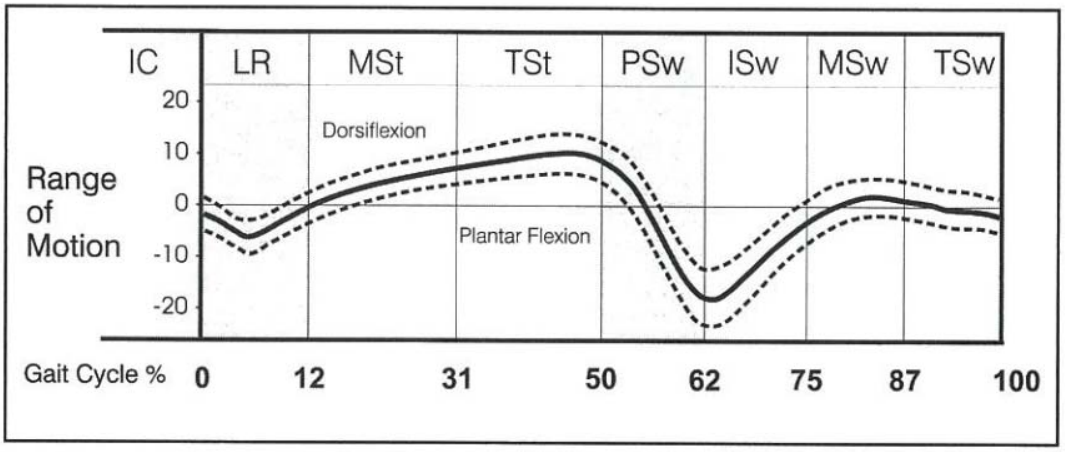
In the transverse plane, the magnitude and direction of rotation at the knee changes across gait phases. At IC the tibia is in slight external rotation relative to the femur (also known as the knee locking mechanism). Following this, the internal rotation of the tibia followed by the femur leads to internal knee rotation. By the end of the foot to floor contact, the tibia has moved through four to eight degrees of internal rotation relative to the femur. At single limb stance, the tibia externally rotates at a faster rate than the femur and again this relative external rotation locks the knee when knee extension is required for stability. In the coronal plane, the knee moves into both abduction and adduction within each GC. The knee is abducted during stance phase (up to 4 deg) and is adducted with swing phase (up to 2 deg) and terminal swing marks the transition to knee adduction.



Appendix Figure 4 Sagittal plane knee motion during gait. Mean and SD (Perry & Burnfield, 2010).

Joint angular kinematics of the ankle

While the dominant movements at the ankle are dorsiflexion and plantarflexion, the ankle joint is capable of bi-planar movement (see Appendix Figure 5). Therefore, any plantarflexion movement is accompanied by inversion and similarly any dorsiflexion movement is supplemented by eversion. During each GC, the ankle joint travels through four arcs of motion and alternates between plantarflexion and dorsiflexion. The first three arcs of motion occur during stance (plantarflexion, dorsiflexion then plantarflexion) which is critical for limb progression and shock absorption. The fourth arc occurs during swing phase and contributes to foot clearance (dorsiflexion). The entire range of ankle joint motion during each stride averages 25 deg (see Appendix Figure 5).



Appendix Figure 5 Sagittal plane ankle motion during gait. Mean and SD (Perry & Burnfield, 2010).

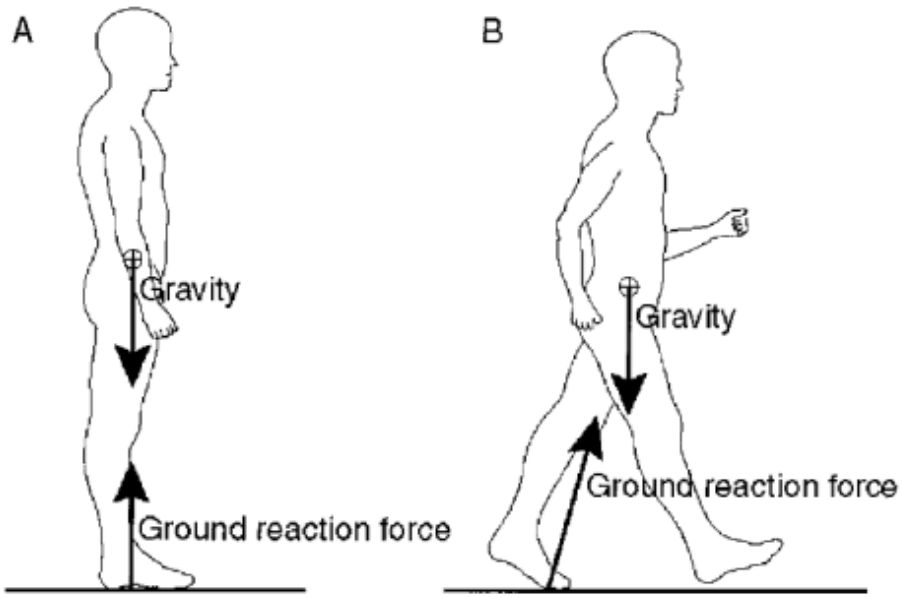
Kinetics during the gait cycle

Whilst kinematic analysis offers vast amount information regarding the relative movements and positions of the lower limb joints, it does not illustrate the forces and moments which are crucial to understanding the intricacies of human locomotion during the GC. Kinetics involves both static and dynamic analysis of the forces and moments

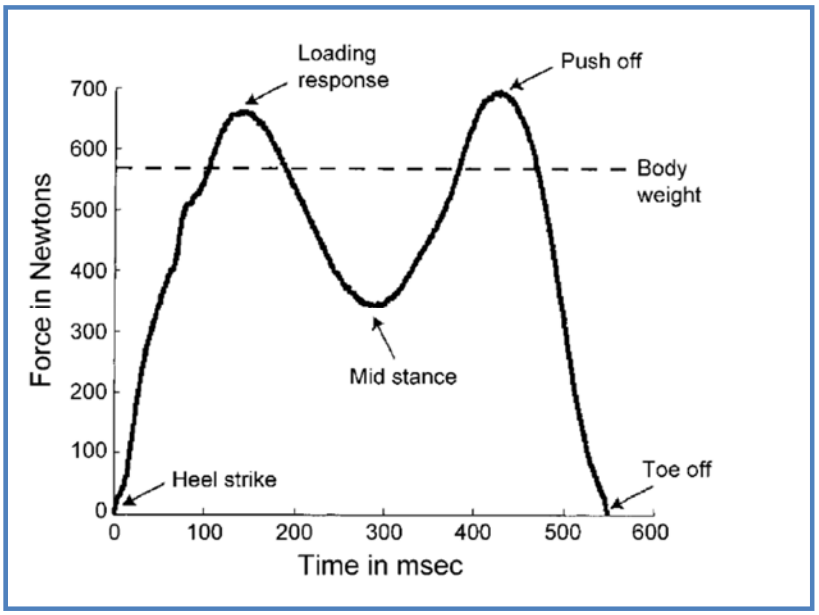
acting on a joint (Nordin & Frankel, 2001). Force platform systems are commonly used to measure the forces acting on the foot against the ground and the forces applied to the ground by the foot (Ma, 2010). Kinetic analysis therefore enables one to determine the magnitude of moments and forces on a joint produced by body mass, muscle action, soft tissue resistance and externally applied loads in a static or dynamic situation.

The ground reaction force

During static standing (also known as quite standing) the ground produces a reaction force equal and opposite to a person's body mass, a consequence of Newton's third law known as the Ground Reaction Force (GRF). This force is an average of all the forces or pressures under the feet and falls approximately five centimeters anterior to the ankle joint, also known as the Center of Pressure (CoP) (Kirtley, 2006). It also has three vector components; vertical, anterior-posterior and medial-lateral. Appendix Figure 6 demonstrates the forces that act on the body whilst stationary (A) and then during movement (B). The GRF is therefore the force applied by the standing surface to the plantar foot. This is counteracted by the force of gravity, having a downward displacement of opposing force. Thus, in quite standing, the GRF is equal and opposite to the body mass and gravity pulls at the body's Center of Mass (CoM) which is opposed by the GRF of similar magnitude, according to Newton's third law. However, during walking, the GRF changes with the GC, and the vertical component of the GRF resembles an 'M' shaped curve (see Appendix Figure 7) and provides for progression of limb movement (Van Deursen, 2004).

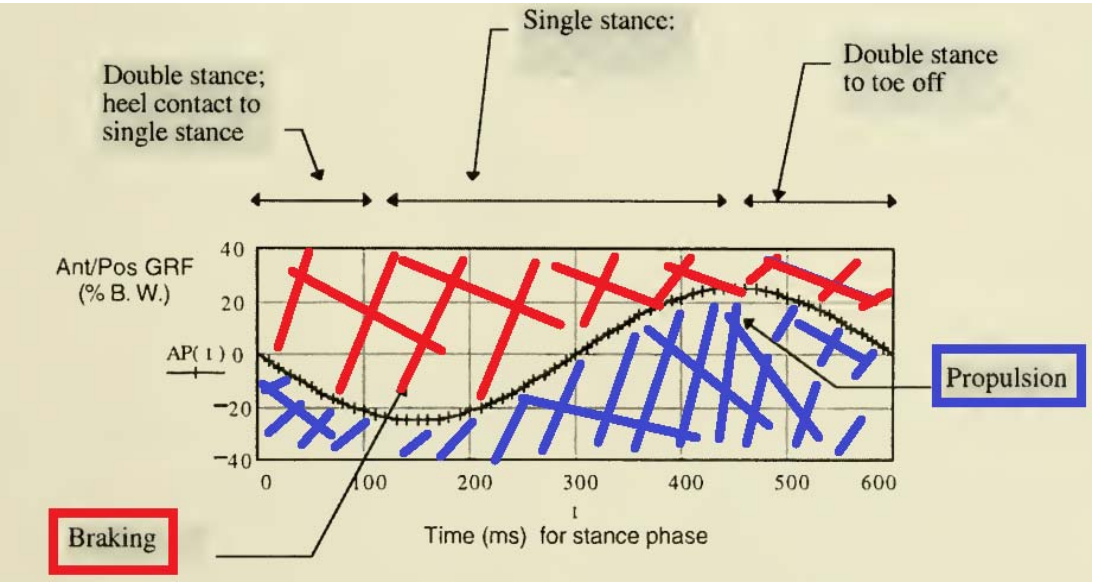


Appendix Figure 6 Force vectors for the Ground Reaction Force (GRF) during standing and walking. (Van Deursen, 2004).



Appendix Figure 7 Vertical (F_V) component of the GRF during gait (Van Deursen, 2004).

During initial double support, the force quickly rises as weight is transferred from the contralateral limb (represented by the first 100ms of the stance phase (see Appendix Figure 7), the force rises above resting body mass in early stance and falls below resting body mass during midstance (Kirtley, 2006). The two peaks in the curve represents IC and toe-off (TO) events of the gait cycle (Van Deursen, 2004).

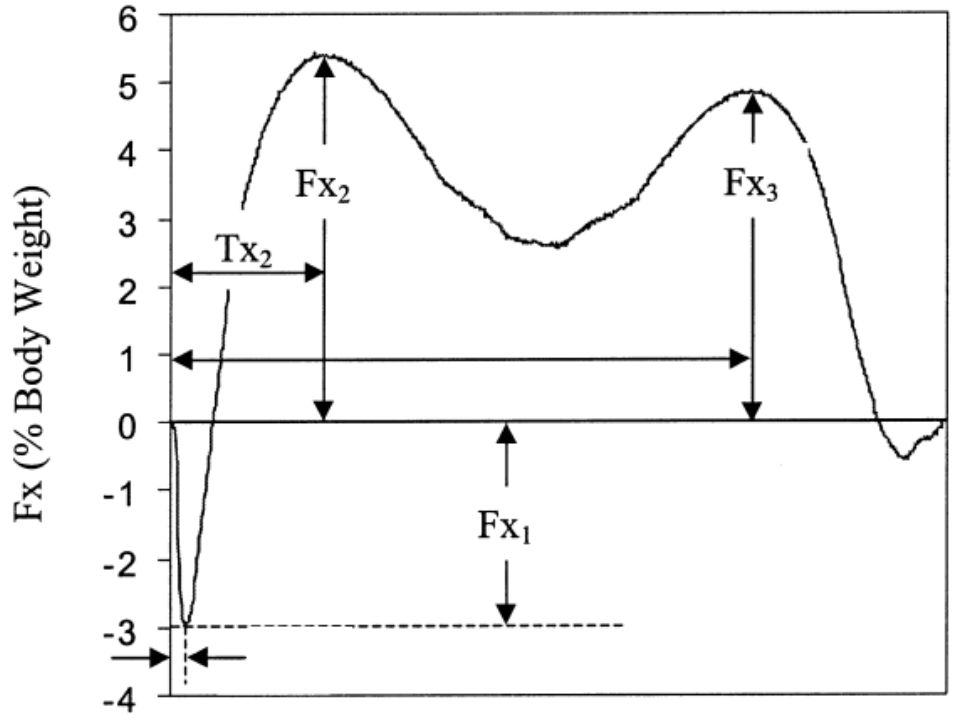


Appendix Figure 8 Anterior-Posterior (F_{AP}) component of the GRF during gait (DeLisa, 1998).

The anterior–posterior (AP) GRF is a braking force up the mid-stance period, followed by propulsion and usually represents a sine curve with an amplitude of 25% body mass (see Appendix Figure 8). The area under the sine curve represents the braking impulse, or the time integral of the force and this should be approximately equal to the propulsion impulse for balanced gait left to right. This is true due to the fact that the total impulse in the AP direction for a GC should be zero as the impulse is equal to the momentum in the forward direction. Appendix Figure 8 represents the AP GRF, where the blue area represents the propulsion component under the curve and the red area the braking component.

Lastly, the Medial-Lateral (ML) component of the GRF is of lower magnitude in most situations and relates to balance during the GC (see Appendix Figure 9). This component

acts in the medial direction with a magnitude of approximately 10% body mass or less and then acts laterally during the stance phase to maintain balance (DeLisa, 1998).



Appendix Figure 9 Medial-Lateral (F_{ML}) component of the GRF during gait (Wearing, Urry, & Smeathers, 2000).

Therefore, GRF characteristics during gait can be an important descriptor of pathological gait (Kirtley, 2006; Perry & Burnfield, 2010; M. W. Whittle, 1996; Winiarski & Rutkowska-Kucharska, 2009). GRF can be measured using force plates which consist of a top plate (mounted level with the surrounding floor) separated from a bottom frame by force transducers near each corner (DeLisa, 1998).

The role of the foot during the gait cycle

The human foot is an intricate and complex multi-articular mechanical structure consisting of 26 bones,33 joints and more than 100 soft tissue structures (muscles, tendons, ligaments), with an extremely important role in the biomechanical function of the lower extremity (Lorimer, French, O'Donnell, Burrow, & Wall, 2006; Nordin & Frankel, 2001). The foot provides support and balance during standing and stabilizes the body during the GC. During single-limb stance phase (between heel strike and toe off) the foot has to adapt to a changing pattern of loading as the center of mass of the body moves anteriorly (De Souza, 2007). Thus normal lower-extremity function during the GC requires normal foot function and vice versa (Kirby, 2000). The foot must also be relatively compliant to cope with uneven ground, while maintaining its functional integrity (Nordin & Frankel, 2001). The important mechanical structures of the foot include;

4) The bony skeleton, which together with the ligaments and arches provide relative rigidity and the essential lever arm mechanism required to maintain balance during standing and facilitate propulsion;

5) The joints which confer flexibility;

6) The muscles and tendons which control foot movement;

7) And the skin which facilitates extensibility and dynamic changes to forces acting on the internal structures of the foot. (De Souza, 2007)

The sole of the foot is uniquely responsible for transmitting forces from the ground to the body and so the distribution of pressure over the surface is of great interest and provides insight into pathological deviations in the structures of the foot, which increases mechanical pressure on the foot whilst weight-bearing during the GC.

Plantar pressure assessment

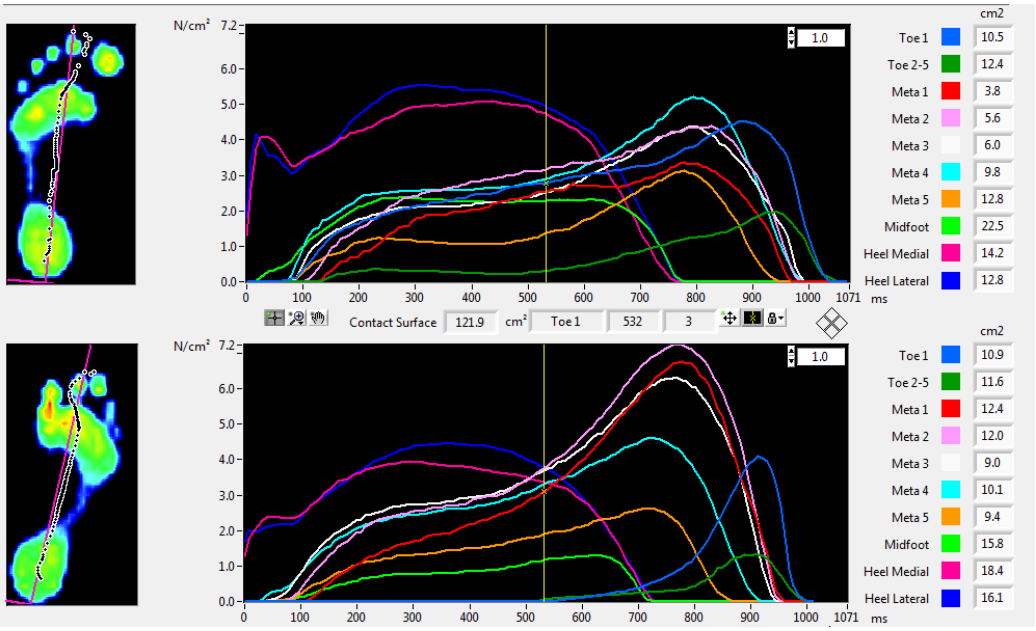
As mentioned above, feet provide the primary surface of interaction with the environment during locomotion in humans (Kirtley, 2006; Razak, Zayegh, Begg, & Wahab, 2012). As body mass acts on the foot during the GC, compressive force is imposed on the plantar tissues of the foot. Relating this force to the area of contact defines the plantar pressure (Perry & Burnfield, 2010).

Therefore, plantar pressure is defined as force per unit area. Force, when measured using a force platform is the net result of the three components of the ground reaction or resultant force acting on the foot. The three components of the ground reaction force are in the AP, ML and vertical directions, as mentioned above (Orlin & McPoil, 2000). When assessing plantar pressure, a discrete sensor or matrixes of multiple sensors are used to measure the vertical forces acting on each sensor while the foot is in contact with the supporting surface (Kirtley, 2006). The magnitude of pressure is then determined by dividing the measured force by the known area of the sensors while the foot was in contact with the supporting surface (Kirtley, 2006; Orlin & McPoil, 2000; Perry & Burnfield, 2010; Razak et al., 2012). The two most widely used measures of plantar pressure are the peak plantar pressure (MPP), which measures the mean peak pressure at a given site and the pressure time integral (PTI) the amount of time over which this pressure is applied (Melai et al., 2011; Waaijman & Bus, 2012).

Two distinct types of pressure measurement exist; in-shoe plantar pressure assessment and platform plantar pressure assessment (Perry & Burnfield, 2010; Razak et al., 2012). The in-shoe plantar pressure method provides comprehension as to how offloading modalities and footwear may be impacting on the change in plantar pressure of individuals during the GC and hence influence the design of footwear and offloading devices (Erdemir et al., 2005; Mueller, 1999; Raspovic, Newcombe, Lloyd, & Dalton, 2000). The platform methods provide insight on plantar pressures related to disease processes which can be documented and compared across-groups without the influence of footwear or walking devices. However, as data cannot be extrapolated from one collection method to the other, the

general recommendation is to use one standardised approach and not a combination of the two methods listed above (Chevalier, Hodgins, & Chockalingam, 2010).

However, both methods of plantar pressure measurement have a common limitation. In general, the sensors used for pressure measurement are incapable of measuring the AP and the ML components of the force component which requires specialized sensors and modified platforms (Orlin & McPoil, 2000). This is a limitation in measuring the 'shear-pressure' components which is often implicated in diabetes foot ulceration. Appendix Figure 10 represents a diagram of a typical plantar pressure measurement using the platform method and the corresponding plantar pressure values obtained from vertical pressure measurement. Regional plantar pressures are also influenced by the cadence and speed of walking, as speed and cadence increases, the contact time decreases yet the PTI and MPP increases (Segal et al., 2004; Zhu, Wertsch, Harris, & Alba, 1995). However, studies reporting this finding are usually in-shoe pressure measurement studies and therefore the effect of footwear also has to be taken into account. Despite these known limitations, plantar pressure assessment during the GC provides important information regarding the high-stress plantar locations in people with diabetes mellitus which are often the sites at risk of ulceration (Armstrong, Peters, Athanasiou, & Lavery, 1998; Boulton et al., 1983; Lavery, Armstrong, Wunderlich, Tredwell, & Boulton, 2003; Sauseng, Kästenbauer, Sokol, & Irsigler, 1999; Stess, Jensen, & Mirmiran, 1997).



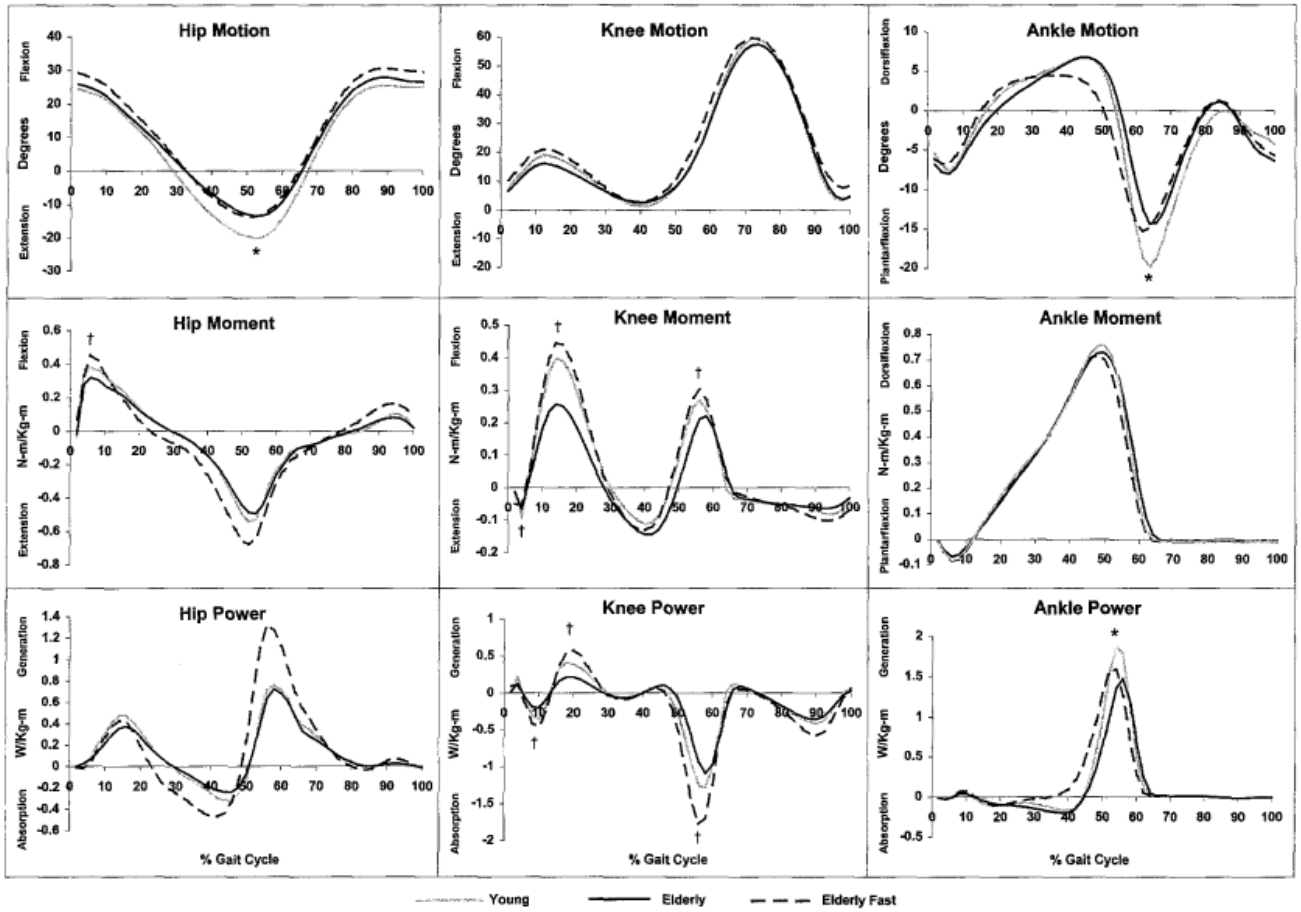
Appendix Figure 10 Dynamic barefoot plantar pressure assessment.

Normative gait, gait variability and changes in gait due to ageing

Gait analysis is the systematic measurement, description and assessment of the quantities and characteristics listed above, thought to characterize human locomotion (Davis et al., 1991; J. Whittle, 2012). However, it must be emphasized that with all gait variables, natural biological variability exists when measurements of gait are repeated several times and hence variation can occur within the same subject (intra-subject variation) or in a group of subjects (inter-subject variation) (Kirtley, 2006). The variation may also originate from the methods used in the analysis such as the instructions given to participants, the environment, length of the walkway and number of investigators involved in measurement (Kirtley, 2006). However, the amount of variation is usually less when measurements are taken at the same time (intra-session) versus a different time of day or different day (intra-session variation) (Kirtley, 2006). Although variation in gait was deemed to be of no use in the past and potentially representative of 'random noise', recent investigation has indicated that this variability may demonstrate fractal behavior which is representative of falls risk

and gait deviations due to pathology (Hausdorff, 2005; Kang & Dingwell, 2008; Kirtley, 2006; Perry & Burnfield, 2010). Thus, variability can also arrive from natural temporal dynamics of neuromotor control, pathologies of the neurological or musculoskeletal systems and the effects of ageing (Callisaya et al., 2011; Chau, Young, & Redekop, 2005). Recent findings demonstrate that gait variability is likewise observed in those with neuropathic pain, originating from the long-term effects of DPN (Lalli et al., 2013).

It is also anticipated that with the effects of ageing manifesting as decline in neuromuscular control, reductions muscle co-ordination and joint ranges of motion, that changes in the kinematic, kinetic, EMG and plantar pressure characteristics are also expected. A number of studies comparing kinematic variables between elderly and young adult subjects have reported subtle age associated reductions in total joint kinematics, particularly at the hip and ankle (Judge, Davis, & Ounpuu, 1996; Kerrigan et al., 1998; Lim et al., 2007). It is highly regarded that walking speed, which is a good baseline indicator of overall walking performance, declines with advanced age (Kirtley, 2006; Murray, Drought, & Kory, 1964; Perry & Burnfield, 2010; Samson et al., 2001; Schimpl et al., 2011). Appendix Figure 11 demonstrates sagittal plane mobility characteristics of elderly and young participants.



Appendix Figure 11 Sagittal plane joint kinematics, moments and powers for the hip, knee and ankle in young subjects, and healthy older subjects at normal and increased walking speed (Kerrigan, Todd, Della Croce, Lipsitz, & Collins, 1998).

In this particular study (Appendix Figure 11) comprising of 31 healthy young participants (aged 28.5, 4.5) and 31 healthy older participants (aged 72.7, 5.5 S.D) the characteristics which were significantly different between the young and elderly groups at both comfortable and fast walking speeds were peak hip extension, peak ankle plantar flexion, peak ankle power generation, and anterior pelvic tilt (Kerrigan et al., 1998). Hip extension is repeatedly reported to be reduced in the gait of elderly persons, associated with an overall increase in anterior pelvic tilt (Perry & Burnfield, 2010; J. Whittle, 2012). Hip flexion contracture was also found to produce a shorter contralateral step-length and reduced hip extension and the increased anterior pelvic tilt was associated with a compensatory attempt to maintain stability (Judge et al., 1996; Kerrigan et al., 1998). Alternatively, this increased pelvic tilt may also be caused by kyphosis and lumbar lordosis, with secondary effects on hip extension (Perry & Burnfield, 2010). Increased levels of hamstring activity in the older adults were also present during loading and mid-stance at faster walking speeds, which may contribute to the increased hip extensor power during early-stance commonly observed in older adult gait (Schmitz, Silder, Heiderscheit, Mahoney, & Thelen, 2009).

A reduction in both peak ankle plantar flexion and ankle power generation at slow and fast walking speeds in the elderly has prompted researchers towards the possibility of restricted ankle joint plantar flexion range of motion in the elderly (Cofré, Lythgo, Morgan, & Galea, 2011; DeVita & Hortobagyi, 2000; Kerrigan et al., 1998). Reduced ankle plantarflexion range perhaps represents a strategy to preserve balance during walking, in order to maintain greater foot-floor contact and broaden the base of support; therefore this may be a plausible cause of reduced power generation (Kerrigan et al., 1998). Strength in the quadriceps muscles of the knee and the extension range of motion of the knee were not significantly different between healthy and elderly participants as elderly participants were able to recruit more strength in the knee extensor muscles during fast walking. Although other findings have demonstrated that less knee extension is increased by elderly participants during walking (DeVita & Hortobagyi, 2000). Therefore, a limiting factor can also be gender and this can play a vital role in gait variations (Murray et al., 1964; Murray, Kory, & Sepic, 1970; Samson et al., 2001).

It has been postulated that age, gender and body height account for up to 34-51% of within subject gait variability (Senden, Meijer, Heyligers, Savelberg, & Grimm, 2012). While reductions in joint motion during gait is age related in men, only knee flexion at IC was found to be influenced by age in women (Chao, Laughman, Schneider, & Stauffer, 1983). However, these results were associated with the different types of footwear utilized by women. There were statistically significant differences in the total range of motion in the sagittal and frontal planes between men and women, highlighting the anatomical differences which inherently influences the range of motion (Chao et al., 1983). Furthermore, increasing age in in women was related to deviation in GRF whereas the knee joint motion was affected in men. Although this was a seminal study in this area, the instrumentation used in this study limits the comprehensiveness of the findings and applicability to current methods of gait analysis.

Although there are age and gender related changes in gait, the gait differences between a group of diseased and non-diseased participants can provide vital information about how movement patterns may be affected by pathological processes and how these may be contributing to further decline in physical function and limitations in walking ability. For example, it is possible to compare differences between a group of diseased (pathological) participants against a group of healthy controls to investigate functional limitations and differences during the GC which may be a result of the associated pathology, considering the anticipated variances from gender, BMI and age.

Therefore, using this type of comparison, the gait characteristics and plantar pressures of people with DPN and DFUs can be compared with the gait characteristics and plantar pressures of people with diabetes mellitus without DFUs and in comparison, to healthy controls.

References relating to Appendix A

1. Armstrong, D. G., Peters, E. J., Athanasiou, K. A., & Lavery, L. A. (1998). Is there a critical level of plantar foot pressure to identify patients at risk for neuropathic foot ulceration? *Journal of Foot & Ankle Surgery, 37*(4), 303-307.

2. Boulton, A. J., Hardisty, C. A., Betts, R. P., Franks, C. I., Worth, R. C., Ward, J. D., & Duckworth, T. (1983). Dynamic foot pressure and other studies as diagnostic and management aids in diabetic neuropathy. *Diabetes Care, 6*(1), 26-33.

3. Callisaya, M. L., Blizzard, L., Schmidt, M. D., Martin, K. L., McGinley, J. L., Sanders, L. M., & Srikanth, V. K. (2011). Gait, gait variability and the risk of multiple incident falls in older people: a population-based study. *Age and ageing, 40*(4), 481-487.

4. Chao, E. Y., Laughman, R. K., Schneider, E., & Stauffer, R. N. (1983). Normative data of knee joint motion and ground reaction forces in adult level walking. *J Biomech, 16*(3), 219-233.

5. Chau, T., Young, S., & Redekop, S. (2005). Managing variability in the summary and comparison of gait data. *J Neuroeng Rehabil, 2*, 22.

6. Chevalier, T. L., Hodgins, H., & Chockalingam, N. (2010). Plantar pressure measurements using an in-shoe system and a pressure platform: A comparison. *Gait Posture, 31*(3), 397-399.

7. Cofré, L. E., Lythgo, N., Morgan, D., & Galea, M. P. (2011). Aging modifies joint power and work when gait speeds are matched. *Gait Posture, 33*(3), 484-489.

8. Crowther, R., G, Spinks, W., L, Leicht, A., S, Quigley, F., & Golledge, J. (2007). Relationship between temporal-spatial gait parameters, gait kinematics, walking performance, exercise capacity, and physical activity level in peripheral arterial disease. *Journal of Vascular Surgery, 45*(6), 1172-1178.

9. Davis, R., B, Ounpuu, S., Tyburski, D., & Gage, J., R. (1991). *A gait analysis data collection and reduction technique* (Vol. 10): Human movement science.

10. De Souza, V. A. (2007). Design of insoles using image base analysis. *Thesis*.

11. DeLisa, J. A. (1998). *Gait Analysis in the Science of Rehabilitation*. Rehabilitation research and development service, Baltimore.: U.S. Department of Veterans Affairs, Veterans Health Administration, Rehabilitation Research and Development Service.

12. DeVita, P., & Hortobagyi, T. (2000). Age causes a redistribution of joint torques and powers during gait. *Journal of Applied Physiology, 88*(5), 1804-1811.

13. Erdemir, A., Saucerman, J. J., Lemmon, D., Loppnow, B., Turso, B., Ulbrecht, J. S., & Cavanagh, P. R. (2005). Local plantar pressure relief in therapeutic footwear: design guidelines from finite element models. *J Biomech, 38*(9), 1798-1806.

14. Hausdorff, J. M. (2005). Gait variability: methods, modeling and meaning. *J Neuroeng Rehabil, 2*, 19.

15. Judge, J. O., Davis, R. B., 3rd, & Ounpuu, S. (1996). Step length reductions in advanced age: the role of ankle and hip kinetics. *Journals of Gerontology Series A-Biological Sciences & Medical Sciences, 51*(6), M303-312.

16. Kadaba, M. P., Ramakrishnan, H. K., & Wootten, M. E. (1990). Measurement of lower extremity kinematics during level walking. *Journal of Orthopaedic Research, 8*(3), 383-392.

17. Kang, H. G., & Dingwell, J. B. (2008). Separating the effects of age and walking speed on gait variability. *Gait Posture, 27*(4), 572-577.

18. Kerrigan, D. C., Todd, M. K., Della Croce, U., Lipsitz, L. A., & Collins, J. J. (1998). Biomechanical gait alterations independent of speed in the healthy elderly: Evidence for specific limiting impairments. *Arch Phys Med Rehabil, 79*(3), 317-322.

19. Kirby, K. A. (2000). Biomechanics of the normal and abnormal foot. *Journal of the American Podiatric Medical Association, 90*(1), 30-34.

20. Kirtley, C. (2006). *Clinical Gait Analysis Theory and Practice* (R. Edwards Ed. First ed. Vol. 1). New York, USA: Elsevier.

21. Lalli, P., Chan, A., Garven, A., Midha, N., Chan, C., Brady, S., . . . Toth, C. (2013). Increased gait variability in diabetes mellitus patients with neuropathic pain. *Journal of Diabetes & its Complications, 27*(3), 248-254.

22. Lavery, L. A., Armstrong, D. G., Wunderlich, R. P., Tredwell, J., & Boulton, A. J. M. (2003). Predictive value of foot pressure assessment as part of a population-based diabetes disease management program. *Diabetes Care, 26*(4), 1069-1073.

23. Lim, M. R., Huang, R. C., Wu, A., Girardi, F. P., & Cammisa, F. P., Jr. (2007). Evaluation of the elderly patient with an abnormal gait. *Journal of the American Academy of Orthopaedic Surgeons, 15*(2), 107-117.

24. Lorimer, D. L., French, G., O'Donnell, M., Burrow, J., & Wall, B. (2006). *Neale's Disorders of the Foot* (Seventh ed.). Scotland: Churchill Livingstone.

25. Ma, W. (2010). *Instrumentation of Gait Analysis ,.* (Master of Science Electronic), Kungliga Tekniska Högskolan (KTH), Stockholm, Sweden.

26. Melai, T., T.H, I. J., Schaper, N. C., de Lange, T. L. H., Willems, P. J. B., Meijer, K., Savelberg, H. H. C. M. (2011). Calculation of plantar pressure time integral, an alternative approach. *Gait and Posture, 34*(3), 379-383.

27. Mueller, M. J. (1999). Application of plantar pressure assessment in footwear and insert design. *Journal of Orthopaedic & Sports Physical Therapy, 29*(12), 747-755.

28. Murray, M. P., Drought, A. B., & Kory, R. C. (1964). Walking patterns of normal men. *Journal of Bone & Joint Surgery - American Volume, 46*, 335-360.

29. Murray, M. P., Kory, R. C., & Sepic, S. B. (1970). Walking patterns of normal women. *Arch Phys Med Rehabil, 51*(11), 637-650.

30. Nordin, M., & Frankel, V. (2001). *Basic Biomechanics of the Musculoskeletal System* (Third edition ed. Vol. 1). Baltimore, USA: Lippincott Williams and Wilkins.

31. Orlin, M. N., & McPoil, T. G. (2000). Plantar Pressure Assessment. *Phys Ther, 80*(4), 399-409.

32. Perry, J., & Burnfield, J. M. (2010). *Gait Analysis Normal and Pathological Function* (Second Edition ed. Vol. 1). New Jersey, USA: SLACK Incorporated.

33. Raspovic, A., Newcombe, L., Lloyd, J., & Dalton, E. (2000). Effect of customized insoles on vertical plantar pressures in sites of previous neuropathic ulceration in the diabetic foot. *Foot, 10*(3), 133-138.

34. Razak, A. H., Zayegh, A., Begg, R. K., & Wahab, Y. (2012). Foot plantar pressure measurement system: a review. *Sensors (Basel), 12*(7), 9884-9912.

35. Samson, M. M., Crowe, A., de Vreede, P. L., Dessens, J. A., Duursma, S. A., & Verhaar, H. J. (2001). Differences in gait parameters at a preferred walking speed in healthy subjects due to age, height and body mass. *Aging (Milano), 13*(1), 16-21.

36. Sauseng, S., Kästenbauer, T., Sokol, G., & Irsigler, K. (1999). Estimation of risk for plantar foot ulceration in diabetic patients with neuropathy. *Diabetes, Nutrition and Metabolism - Clinical and Experimental, 12*(3), 189-193.

37. Schimpl, M., Moore, C., Lederer, C., Neuhaus, A., Sambrook, J., Danesh, J., . . . Daumer, M. (2011). Association between Walking Speed and Age in Healthy, Free-Living Individuals Using Mobile Accelerometry—A Cross-Sectional Study. *PLoS ONE, 6*(8), e23299.

38. Schmitz, A., Silder, A., Heiderscheit, B., Mahoney, J., & Thelen, D. G. (2009). Differences in lower-extremity muscular activation during walking between healthy older and young adults. *J Electromyogr Kinesiol, 19*(6), 1085-1091.

39. Segal, A., Rohr, E., Orendurff, M., Shofer, J., O'Brien, M., & Sangeorzan, B. (2004). The effect of walking speed on peak plantar pressure. *Foot & Ankle International, 25*(12), 926-933.

40. Senden, R., Meijer, K., Heyligers, I. C., Savelberg, H. H. C. M., & Grimm, B. (2012). Importance of correcting for individual differences in the clinical diagnosis of gait disorders. *Physiotherapy, 98*(4), 320-324.

41. Stess, R. M., Jensen, S. R., & Mirmiran, R. (1997). The role of dynamic plantar pressures in diabetic foot ulcers. *Diabetes Care, 20*(5), 855-858.

42. Van Deursen, R. (2004). Mechanical Loading and Off-Loading of the Plantar Surface of the Diabetic Foot. *Clinical Infectious Diseases, 39*, 87-91.

43. Waaijman, R., & Bus, S. A. (2012). The interdependency of peak pressure and pressure-time integral in pressure studies on diabetic footwear: no need to report both parameters. *Gait Posture, 35*(1), 1-5.

44. Wearing, S. C., Urry, S. R., & Smeathers, J. E. (2000). The effect of visual targeting on ground reaction force and temporospatial parameters of gait. *Clinical Biomechanics, 15*(8), 583-591.

45. Whittle, J. (2012). *Whittle's Gait Analysis* (Fifth Edition ed. Vol. One). China: Elsevier.

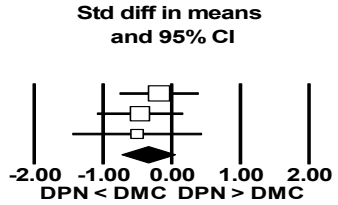
46. Whittle, M. W. (1996). Clinical gait analysis: A review. *Human Movement Science, 15*(3), 369-387.

47. Winiarski, S., & Rutkowska-Kucharska, A. (2009). Estimated ground reaction force in normal and pathological gait. *Acta Bioeng Biomech, 11*(1), 53-60.

48. Zhu, H., Wertsch, J. J., Harris, G. F., & Alba, H. M. (1995). Walking cadence effect on plantar pressures. *Arch Phys Med Rehabil, 76*(11), 1000-1005.

Appendix B Forest plots from Chapter 2

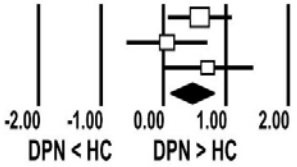
Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Sawacha 2009a	-0.184	0.294	0.086	-0.760	0.393	-0.625	0.532
Sawacha 2012b	-0.461	0.320	0.103	-1.089	0.167	-1.440	0.150
Savelberg 2010	-0.503	0.482	0.232	-1.447	0.441	-1.045	0.296
	-0.343	0.198	0.039	-0.730	0.044	-1.736	0.083



Meta Analysis

Appendix Figure 12 DPN versus DMC Stride length Forest Plot.

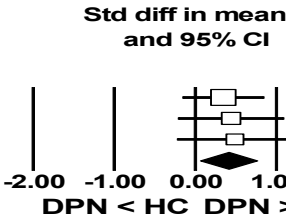
Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Melai 2011	0.581	0.260	0.068	0.072	1.090	2.235	0.025
Bacarin 2009	0.058	0.330	0.109	-0.589	0.704	0.175	0.861
Sacco 2009	0.708	0.370	0.137	-0.018	1.434	1.911	0.056
	0.455	0.186	0.035	0.090	0.821	2.443	0.015



Meta Analysis

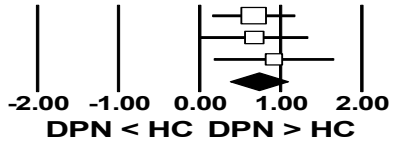
Appendix Figure 13 DPN versus HC; Peak plantar pressure Rearfoot Forest Plot.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Melai 2011	0.347	0.258	0.066	-0.158	0.852	1.346	0.178
Bacarin 2009	0.440	0.334	0.111	-0.214	1.095	1.319	0.187
Sacco 2009	0.489	0.365	0.133	-0.226	1.204	1.341	0.180
Meta Analysis	0.407	0.178	0.032	0.058	0.756	2.288	0.022



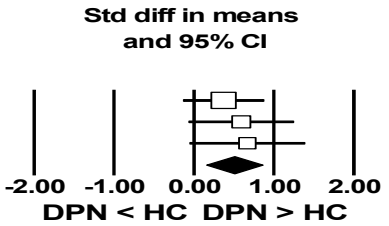
Appendix Figure 14 DPN versus HC; Pressure time integral Rearfoot Forest Plot.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Melai 2011	0.668	0.261	0.068	0.157	1.180	2.561	0.010
Bacarin 2009	0.677	0.339	0.115	0.012	1.342	1.997	0.046
Sacco 2009	0.919	0.378	0.143	0.178	1.659	2.431	0.015
Meta Analysis	0.729	0.181	0.033	0.373	1.084	4.015	0.000



Appendix Figure 15 DPN versus HC; Peak plantar Pressure Midfoot Forest Plot.

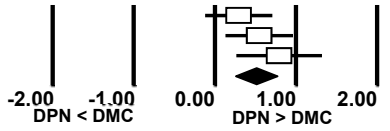
Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Melai 2011	0.370	0.258	0.067	-0.136	0.875	1.435	0.151
Bacarin 2009	0.592	0.337	0.114	-0.068	1.253	1.757	0.079
Sacco 2009	0.668	0.369	0.136	-0.056	1.392	1.809	0.070
	0.503	0.179	0.032	0.152	0.854	2.808	0.005



Meta Analysis

Appendix Figure 16 DPN versus HC; Pressure time integral Midfoot Forest Plot.

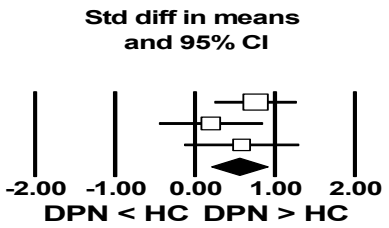
Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Melai 2011	0.293	0.209	0.044	-0.118	0.703	1.398	0.162
Guldemond 2008	0.547	0.212	0.045	0.132	0.961	2.584	0.010
Caselli 2002	0.793	0.268	0.072	0.269	1.318	2.964	0.003
	0.510	0.138	0.019	0.240	0.780	3.707	0.000



Meta Analysis

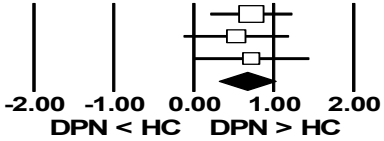
Appendix Figure 17 DPN versus DMC; Peak Plantar Pressure Forefoot Forest Plot.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Melai 2011	0.758	0.262	0.069	0.243	1.272	2.888	0.004
Bacarin 2009	0.199	0.331	0.109	-0.449	0.847	0.603	0.547
Sacco 2009	0.583	0.367	0.135	-0.137	1.302	1.588	0.112
Meta Analysis	0.552	0.179	0.032	0.200	0.903	3.077	0.002



Appendix Figure 18 DPN versus HC; Peak Plantar Pressure Forefoot Forest Plot.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Melai 2011	0.721	0.262	0.069	0.208	1.234	2.756	0.006
Bacarin 2009	0.532	0.336	0.113	-0.125	1.190	1.586	0.113
Sacco 2009	0.719	0.371	0.138	-0.008	1.446	1.939	0.053
Meta Analysis	0.666	0.180	0.033	0.313	1.020	3.694	0.000



Appendix Figure 19 DPN versus HC; Pressure time integral Forefoot Forest Plot.

Appendix C: Supporting files, Figures and Tables for Chapter 3

MOOSE Checklist[2]

From: Stroup DF, Berlin JA, Morton SC, et al (2000) Meta-analysis of observational studies in epidemiology: A proposal for reporting. JAMA 283:2008–2012. doi:10.1001/jama.283.15.2008.

Appendix Table 1 MOOSE Checklist[2]

Reporting of background should include	Reported on page	Comments
Problem definition	4	
Hypothesis statement	4	
Description of study outcome(s)	4	
Type of exposure or intervention used		Does not apply
Type of study designs used	5	
Study population	4	
Reporting of search strategy should include		
Qualifications of searchers (e.g. librarians and investigators)	2	
Search strategy, including time period used in the synthesis and key words	5	
Effort to include all available studies, including contact with authors	7	
Databases and registries searched	5	
Search software used, name and version, including special features used (e.g. explosion)	5	
Use of hand searching (e.g. reference lists of obtained articles)	5	

[2] These page references apply to the original manuscript

List of citations located and those excluded, including justification		Figure 2
Method of addressing articles published in languages other than English	5	
Method of handling abstracts and unpublished studies	5	
Description of any contact with authors	7	
Reporting of methods should include		
Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested		
Rationale for the selection and coding of data (e.g. sound clinical principles or convenience)		Does not apply
Documentation of how data were classified and coded (e.g. multiple raters, blinding and interrater reliability)	5	
Assessment of confounding (e.g. comparability of cases and controls in studies where appropriate)		Does not apply
Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	5 and 6	
Assessment of heterogeneity	8-9	
Description of statistical methods (e.g. complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	7	
Provision of appropriate tables and graphics		See figures and tables
Reporting of results should include		

Graphic summarizing individual study estimates and overall estimate		See Forest Plots
Table giving descriptive information for each study included		See Table 1
Results of sensitivity testing (e.g. subgroup analysis)	9	See Meta-analysis Table (Table 2)
Indication of statistical uncertainty of findings		
Reporting of discussion should include		
Quantitative assessment of bias (e.g. publication bias)	11	
Justification for exclusion (e.g. exclusion of non-English language citations)	5-6	
Assessment of quality of included studies	8-9	See Quality of Studies Table
Reporting of conclusions should include		
Consideration of alternative explanations for observed results	11	
Generalization of the conclusions (i.e. appropriate for the data presented and within the domain of the literature review)	11-12	
Guidelines for future research	12	
Disclosure of funding source	12	

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title page
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Intro
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	N/A
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Data extraction and synthesis
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Study selection
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Search strategy
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supp Fig 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Study selection
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Data extraction and synthesis
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Data extraction and synthesis
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Search strategy and quality assessment
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Statistical methods
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Statistical methods

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Statistical methods
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Statistical methods
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Search results
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	N/A
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	N/A
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Primary and secondary outcome measures
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Supp Table 1
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Sensitivity and subgroup analyses
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Discussion
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Risk of bias in included studies
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Discussion
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Funding statement

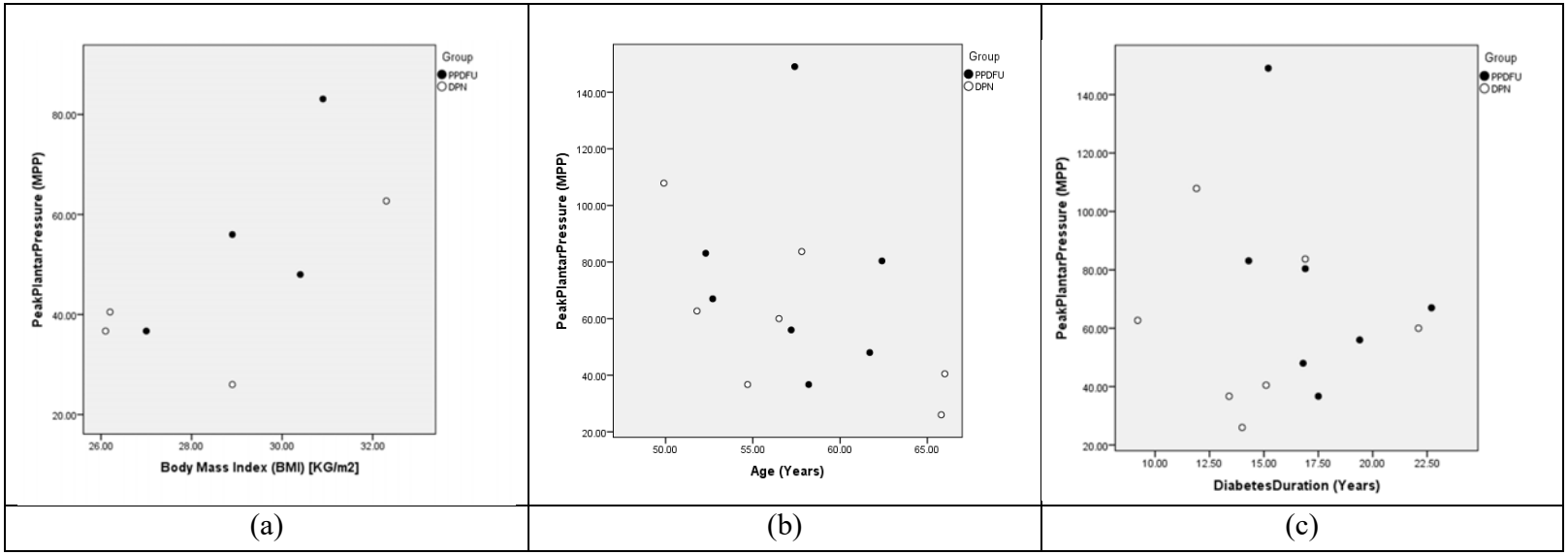
From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

Appendix Table 2 Plantar pressure distribution

Study	Rear foot		Mid foot		Fore foot		Overall Plantar Pressure	
Group	PPDFU	DPN	PPDFU	DPN	PPDFU	DPN	PPDFU	DPN
Bacarin 2009								
MPP (N/cm^2)	34.2 (11.9)	34.2 (7.6)	29.0 (15.1)	20.5 (11.8)	36.7 (8.6)	36.7 (8.9)	36.7 (8.6)	36.7 (8.9)
PTI (Ns/Cm^2)	10.2 (3.7)	9.4 (2.9)	6.8 (3.6)	4.3 (0.9)	12.5 (3.3)	11.9 (3.1)	12.9 (3.3)	11.9 (3.1)
Cavanagh[a] 1991								
MPP (N/cm^2)							80.4 (34.2)	83.7 (35.8)
Sauseng[b] 1999								
MPP (N/cm^2)			10.5 (11.1)	20.5 (19.3)	56.0 (48.2)	26.0 (12.6)	56.0 (48.2)	26.0(12.6)
PTI (Ns/Cm^2)			3.8 (3.5)	8.1 (8.0)	23.2 (16.6)	9.4 (4.7)	23.2 (16.6)	9.4 (4.7)
Armstrong 1998								
MPP (N/cm^2)					83.1 (24.7)	62.7 (21.4)	83.1 (24.7)	62.7 (21.4)
Boulton 1983[a]								
MPP (N/cm^2)							149.06 (67.7)	107.87 (47.6)
Rich [c] 2000								
MPP (N/cm2)	34.0 (18.0)	31.0 (15.0)			82.0 (43.0)	66 (28.0)	82.0 (43.0)	66.0 (28.0)
Brash 1996								
MPP (N/cm2)					67.0 (20.0)	60.0 (13.0)	67.0 (20.0)	60.0 (13.0)
Stess 1997[a, d]								
MPP (N/cm2)					48.0 (12.6)	40.5 (10.5)	48.0 (12.6)	40.5 (10.5)
PTI (Ns/Cm2)					32.0 (14.0)	23 (7.5)	32.0 (14.0)	23 (7.5)

Legend: Reported foot plantar pressures and pressure time integrals normalised to body mass (mean) and (SD).
Where multiple results were reported, the highest value in the PPDFU group was used with the corresponding value in the control group. For overall peak pressure, the highest reported MPP and PTI was used, irrespective of location.
[a] This study did not report S D therefore the S D were estimated, the values in brackets indicate estimated S D (please see manuscript for details of how these were approximated).
[b] This study did not report Mean and s.d and in place (IQR) was reported.
[c] This study reported findings as number of feet instead of patients.
[d] This study reported absolute values for MPP but not for PTI, therefore the PTI values were estimated from graphs provided.



(a) (b) (c)

Legend: Scatterplots displaying potential variables influencing the differences in plantar pressure between PPDFU and DPN, at the aggregate level. These included diabetes duration (years), body mass index (BMI) and age.

Appendix Figure 20 Scatterplots for potential variables causing differences in plantar pressure

Appendix D: Case report form used in prospective studies

Case Report Form

Protocol Title:	**Full Title-** **Plantar pressure and gait characteristics of Type 2 diabetic/ plantar ulcer/non-ulcer patients and correlations of ulceration to histopathological and haematological findings.** **Short title-** **Diabetes and Foot Ulceration Study**
Participant Initials:	____ _____
Unique identification number	___ ___ ___ ___ ___ ___ ___
Date of Birth:	___ ___/___ ___ ___/___ ___ ___ ___
Site:	The Townsville Hospital- Diabetes Clinic, Department of Endocrinology & James Cook University Movement Analysis Laboratory
Scheduled date of first Gait analysis investigation	___ ___/___ ___ ___/___ ___ ___ ___
Participant Alerts: (Allergies or specific requirements)	
Date Completed:	___ ___/___ ___ ___/___ ___ ___ ___

Queensland Government
Queensland Health

JAMES COOK UNIVERSITY
AUSTRALIA

Case Report Form (CRF) Completion Instructions

Please ensure the headers are completed on each page

- Participant Initials should be recorded as a three-letter sequence of the participant's initials (First, Middle, Last). If the participant does not have a middle name use a dash (-)
- Patient identification-number for the study.

Complete all pages in a medium to heavy point black ink pen ONLY

Ensure all figures are written inside the designated space, try not to touch the boundaries of this space to maximise accuracy

All text and explanatory comments should be brief and written within the space provided

To answer multiple choice questions place a cross (X) inside the designated space

DO NOT use liquid paper or permanently remove or cover an error. To make a correction, drawn a single line through the original value and write the new value as close as possible to the original space. Initial and date the correction

Do not fold the forms.

If there are any questions please contact the principal study investigator:

Mr. Malindu (Mal) Fernando
Podiatrist and Cohort Doctoral Candidate
James Cook University, Faculty of Medicine and Molecular Sciences.
Telephone: +61 7 47813144
Fax: + 61 7 47813179
Email- malindu.fernando@my.jcu.edu.au

Visit 1 – Baseline

Eligibility Criteria

Inclusion Criteria

*All criterion listed below must be answered **YES** for the participant to be considered eligible*

1	Has the participant been clinically diagnosed with Type II diabetes mellitus or receiving medication for this?	Yes No
2	Is the participant able to walk unassisted, utilising two feet?	Yes No
3	Has the participant agreed to comply with all study procedures and instructions?	Yes No

Exclusion Criteria

*All criterion listed below must be answered **NO** for the participant to be considered eligible*

1	Does the participant have type I diabetes	Yes No
2	Is the participant an amputee, either BKA or have any foot amputation?	Yes No
3	Does the participant have a known vascular reconstruction or orthopaedic surgical procedure scheduled or planned within the next 12 months?	Yes No
4	Has the participant previously had foot and ankle surgery?	Yes No
5	Does the participant have a bleeding disorder?	Yes No
6	Is the participant under 18 years of age?	Yes No
7	Does the participant require ambulatory assistance?	Yes No
8	Is the participant pregnant?	Yes No

Note- If the patient has a present plantar foot ulcer, allocate patient to case group and if not, allocate patient to control group.

Is the participant a healthy control? **Yes** **No**

Visit 1

Informed Consent

PICF version	Patient Has consented to sections **A B C**
Date on which Informed Consent was obtained (dd/mm/yyyy)	/ /

Demographics

Date of birth / / (dd/mm/yyyy)	
Gender	Male Female
Ethnicity Caucasian Asian African Aboriginal/ Torres strait islander Other _____	
Aboriginal or Torres strait Islander?	Yes No

Smoking status

Never smoked	
Ex-smoker (has not smoked in the last month)*	Current smoker*
*If an ex- or current smoker please answer the following	
Number of years smoking	
Average number of cigarettes per day	

Medical History

Please note that the definition of these conditions is based on a history of diagnosis or treatment

Hypertension	Yes No
Dyslipidemia	Yes No
Stroke/ TIA – Date _____	Yes No

Peripheral Arterial Disease	Yes No
Cancer or other neoplastic syndrome	Yes No
Ischemic Heart Disease	Yes No
Cardiac or vascular surgery-	Yes No
Congestive Heart Failure	Yes No
Chronic Pulmonary Disease	Yes No
Chronic Liver Disease	Yes No
Chronic Renal Impairment	Yes No
DVT- Deep vein thrombosis	Yes No
Charcot's neuroarthropathy/ Charcot foot	Yes No
Visual impairment from Macular degeneration or Glaucoma/ other	Yes No

Diabetes and ulcer history

Year and month Diagnosed- Duration_____years and _____ months.	Uses insulin as a part of diabetes management? Yes No
Positive Family History Yes No	HAB1C-
Have you had a foot ulcer in the past? Yes No	Has this ulcer been plantar in location? Yes No
Is the present ulcer in the same location where you had the last ulcer? Yes No	Have you had more than one previous ulcer in the past? Yes No

Physical Activity History

1. How many hours a week would you be standing? (work, home, leisure) _____hrs	2. How many hours a week are you on your feet and walking? _____hrs
3. Do you do regular exercise that includes walking? Yes No	4. Do you get pain in your feet, legs or buttock areas that keep you from walking regularly? Yes No

3A. If yes how many hours a week walking is this? _____ hrs.	5. How many hours a week are you off your feet (resting, sleeping, sitting _____ hrs.

Medication	**Use**	**Daily Dose**

1. Sports 2. Dress 3.Diabetic special shoe 4.Custom made shoe 5.Thongs 6.None

On average per week how many days do you use this shoe?

Days out of seven? **/7**

Do you wear orthoses? **Yes No** **Custom Off-the shelf**

Physical Measurements

Height: cm	Body mass: kg
BMI: _____	
Waist circumference _____cm	
Hip circumference _____cm	

Visual test (Diabetic retinopathy)

Has the patient seen an optometrist in the last 12 months? Yes No

Optometrist Details-

Does the patient need optometrist referral?

Visual acuity score-

Podiatric Examination- To be conducted using a goniometer for values for ROM

Examination	**Right**	**Left**
Ankle Joint ROM (deg)		
STJ ROM (deg)		

1st MTPJ ROM (deg)		
HAV- Hallux Abducto Valgus Deformity Stage Manchester Scale		
Foot type	Pes Planus Pes Cavus Regular Arch Contour	Pes Planus Pes Cavus Regular Arch Contour
Muscle strength DF/PF	1 2 3 4 5	1 2 3 4 5
Muscle Strength Inv/Evr	1 2 3 4 5	1 2 3 4 5
Muscle Strength Abduction/ Adduction	1 2 3 4 5	1 2 3 4 5
Lesser toe deformities (Claw toe, Hammer Toe, Mallet toe)	Claw Toe Hammer Toe Mallet Toe	Claw Toe Hammer Toe Mallet Toe
Plantar hyperkeratosis locations	Plantar first metatarsal head Plantar PMA 1-3 Plantar Apex of toes Plantar Calcaneal area Medial Hallux Plantar 5th metatarsal head Plantar PMA 4-5 Plantar Cuboid	Plantar first metatarsal head Plantar PMA 1-3 Plantar Apex of toes Plantar Calcaneal area Medial Hallux Plantar 5th metatarsal head Plantar PMA 4-5 Plantar Cuboid
Lunge test	<35 Degrees 35 Degrees >35 Degrees	<35 Degrees 35 Degrees >35 Degrees
MSRT	1 2 3 4 5	1 2 3 4 5
Jack's test	Positive Negative	Positive Negative

Vascular and Neurological examination

Investigation	Right	Left
Foot Pulses		
Posterior Tibialis	Present/Normal Reduced Absent	Present/Normal Reduced Absent
Dorsalis Pedis	Present/Normal Reduced Absent	Present/Normal Reduced Absent
Anterior tibialis	Present/Normal Reduced Absent	Present/Normal Reduced Absent
ABI		
Brachial	1	1
Posterior Tibialis	2	2

Dorsalis Pedis Anterior Tibial	3 4 **ABI =**	3 4 **ABI =**
Toe perfusion pressure (Hallux Pressure)	1 2 3 **Mean=**	1 2 3 **Mean=**
Monofilament test Site specification (/10)	1. Plantar Hallux 2. Plantar metatarsal 2 3. Plantar metatarsal 3 4. Plantar metatarsal 4 5. Plantar metatarsal 5 6. Plantar Arch- (Navicular) 7. Plantar 2nd toe apex 8. Plantar 5th toe apex 9. Dorsal Hallux 10. Plantar Medial Tubercle	1. Plantar Hallux 2. Plantar metatarsal 2 3. Plantar metatarsal 3 4. Plantar metatarsal 4 5. Plantar metatarsal 5 6. Plantar Arch- (Navicular) 7. Plantar 2nd toe apex 8. Plantar 5th toe apex 9. Dorsal Hallux 10. Plantar Medial Tubercle
Neurothesiometer Reading at which vibration felt at tip of hallux.	>25 V 25 V < 25 V	>25 V 25 V < 25 V

Does the patient have a present plantar foot ulcer?

Yes No

(If yes, please fill out planimetry and ulcer assessment table below and also get consent for biopsy)

Planimetry and Ulcer assessment

Investigation	**Ulcer 1**
Ulcer Location	
Estimated duration of ulcer In weeks	
Planimetric depth cm3	
Planimetry Length cm Width cm Area cm	 Length _____cm Width_____ cm Area_____cm

Wound exudate/ discharge present and type of exudate	Mild Serous Moderate Purulent High Haemoserous
UTWCS Grading	
Wound Bed	Necrotic Granulating Epithelializing Sloughy Pale Hypergranulating Bone
Wound Edge	Regular Irregular Undermined Rolling
Sinus Formation Yes No	Yes No mm_____
Is the ulcer infected? -Conduct Wound Swab for presence of pathological microbes.	Yes No
Type of organism causing infection (As per pathology test)	
Type of ulcer (Neuropathic, Neuroischemic)	Neuropathic Neuroischemic
Surrounding Skin	Macerated Hyperkeratotic Indurated Normal/healthy Fragile Erythematous Oedematous Anhidrotic
Orthoses Used?	Yes No
Offloading shoe or boot-walker used?	Yes No
Type of Off-loading Device	TCC Foot Orthoses AFO Padding No offloading

How often is the ulcer seen by a health professional in a month? (X 3)	
How often is the ulcer dressing changed per month? (x 3)	

Is a photograph of the ulcer taken? Yes No

Blood Collection – Pathology

Refer patient to SNP pathology for blood collection.

Blood collection form given to patient?	Yes No
Date of Collection	/ / (dd/mm/yyyy)
Haemoglobin g/L	LD U/L
WCC . 10^9/L	Cholesterol . mmol/L
Platelets 10^9/L	Triglyceride . mmol/L
Fibrinogen . g/L	HDL . mmol/L
Sodium mmol/L	LDL . mmol/L
Potassium . mmol/L	CRP mg/L
Urea mmol/L	Chloride mmol/L
Creatinine µmol/L	Bicarbonate mmol/L
eGFR mL/min/1.73m^2	Calcium . mmol/L
Fasting Glucose . µmol/L	Phosphorus . mmol/L
HbA1C _____%	Total protein g/L
CRP _____mg/L	Hematocrit . mg/dL
ESR _____mm/hr	Neuts . 10^9/L
Parathyroid Hormone _____pg/ml	Lymphs . 10^9/L
N-(carboxymethyl) lysine (CML) _____	Monos . 10^9/L

Non-CML advanced glycation end products (AGEs) _____	Eos . 10^9/L
Pentosidine _____	Baso . 10^9/L
Homocystine	

Vascular Biology Unit Blood Sample Storage Form

Blood Collection –For VBU Biomarker Staff to complete

Has blood been collected for study analysis? (Check with SNP and Ratnesh)	Yes No
Date of Collection	/ / (dd/mm/yyyy)
2 x SST	Yes No
2 x EDTA	Yes No
1 x Sodium Citrate	Yes No

Sample Storage

Have study bloods been processed and stored according to the lab manual?		Yes No *If NO, record a Protocol Deviation	
Date of Processing		/ / (dd/mm/yyyy)	
	Number of Samples:	Have samples been stored at the Protocol defined temperature?	Location of Samples
Serum (from SST tubes)	(Store at -80°C)	Yes No *If NO, record a Protocol Deviation	Box # Position #
EDTA Plasma (Purple top EDTA tubes)	(Store at -80°C)	Yes No *If NO, record a Protocol Deviation	Box # Position #
EDTA RBC Pellet (Remaining pellet from EDTA tubes with plasma removed)	(Store at -80°C)	Yes No *If NO, record a Protocol Deviation	Box # Position #

Citrate Plasma (Blue top Citrate tubes)	(Store at -80°C)	Yes No* *If NO, record a Protocol Deviation	Box # Position #

Patient has completed blood test	Yes No
Patient fulfils the criteria for the study?	Yes No
Patient is going to be placed in the group	Case Control
Patient has filled out a quality of life questionnaire/s	Yes No
Patient has been advised about the gait assessment date and time and location of the gait lab	Yes No
Patient has been advised of the date of next visit and advised to come after fasting for this, as if having a blood test	Yes No

Patient Contact information for follow-up visits

Name
Unique Identification Number -
Telephone number
Address
Email
Gait Assessment date and time -
Next estimated visit date (3 months) -

Prior to Commencing Gait assessment
Claudication Questionnaire Checked? Yes No
ABI is within normal range for participation? Yes No
Need vascular review? Yes No
Needs ulcer dressing? Yes No

Visit 1 – Part 2: Gait, Pressure and Biomechanical Assessment. Site- James Cook University, Gait Laboratory.

Physical Measurements

ASIS Breadth cm	Mass: kg
Left Leg length cm	Height cm
Left Knee diameter cm	
Left malleolus width cm	
Right Leg length cm	
Right Knee diameter cm	
Right malleolus width cm	

Testing protocol

Measure up: ❑

EMG prep: ❑

MVC: ❑

Reflective markers prep ❑

Static capture ❑

10 walking trails ❑

Treadmill walking 4 minutes ❑

(Capture only 2:00 onwards)s
Self-Selected Walking Speed _____km/hr.

Plantar pressure capture (3 walks) ❑

FINISHED

Visit 2
At 3 Months

Initial assessment

1	Is the participant in the case or control group?	Case Control
2	Does the participant have a present plantar foot ulcer?	Yes No
3	Does the participant have a newly formed plantar ulcer?	Yes No
3A	Has a plantar ulcer healed in this duration?	**Yes No**
4	Does the participant give consent for biopsy of the ulcer/ulcers?	Yes No
5	Has the participant's diabetes medication or dose changed since last visit? Please note changes: 1. 2. 3. 4. 5.	Yes No
6	Has the participant increased/decreased treatment frequency for the ulcer?	Yes No
	If yes, how so?	Increased Decreased
7	Has the patient commenced any new treatment for a disease process or as a part of a health measure? I.e.- Dialysis, HRT, Warfarin treatment, Radiation therapy	Yes No
	Please state _____	
8	Has the patient commenced or is awaiting any surgical procedure?	Yes No
	Please state _____	

9	Has the patient increased or decreased weight-bearing activity levels in the last 3 months?	Yes No
	Please state new number of hours per week, on weight-bearing activities? _____hrs Please state the new number of hours per week weight-bearing but not doing any activity (i.e. standing) _____hrs Please state the number of hours completely not weight bearing (Sitting) _____hrs	
Total	Total hours in three months standing (weight bearing) _____ hrs Total hours in three months walking (weight bearing) _____hrs Total hours in three months non-weight bearing _____hrs	

Physical Measurements

Body mass: kg	Waist circumference _____cm
BMI: _____	Hip circumference _____cm

Does the patient have a present plantar foot ulcer?

Yes No

If yes, please fill out planimetry and ulcer assessment table below.

Planimetry and Ulcer assessment

Investigation	**Ulcer 1**	**Ulcer 2**	**Ulcer 3**
Ulcer Location			

Estimated duration of ulcer In weeks			
Planimetric depth cm3			
Planimetry Length cm Width cm Area cm	Length _____cm Width_____ cm Area_____cm	Length _____cm Width_____ cm Area_____cm	Length _____cm Width_____ cm Area_____cm
Wound exudate/ discharge present and type of exudate	Mild Serous Moderate Purulent High Haemoserous	Mild Serous Moderate Purulent High Haemoserous	Mild Serous Moderate Purulent High Haemoserous
UTWCS Grading			
Wound Bed	Necrotic Granulating Epithelializing Sloughy Pale Hypergranulating Bone	Necrotic Granulating Epithelializing Sloughy Pale Hypergranulating Bone	Necrotic Granulating Epithelializing Sloughy Pale Hypergranulating Bone
Wound Edge	Regular Irregular Undermined Rolling	Regular Irregular Undermined Rolling	Regular Irregular Undermined Rolling
Sinus Formation Yes No	Yes No mm_____	Yes No mm _____	Yes No mm _____
Is the ulcer infected? -Conduct Wound Swab for presence of pathological microbes.	Yes No	Yes No	Yes No
Type of organism causing infection (As per pathology test)			
Type of ulcer (Neuropathic, Neuroischemic)	Neuropathic Neuroischemic	Neuropathic Neuroischemic	Neuropathic Neuroischemic

Surrounding Skin	Macerated Hyperkeratotic Indurated Normal/healthy Fragile Erythematous Oedematous Anhidrotic	Macerated Hyperkeratotic Indurated Normal/healthy Fragile Erythematous Oedematous Anhidrotic	Macerated Hyperkeratotic Indurated Normal/healthy Fragile Erythematous Oedematous Anhidrotic
Orthoses Used?	Yes No	Yes No	Yes No
Offloading shoe or boot-walker used?	Yes No	Yes No	Yes No
Type of Off-loading Device	TCC Foot Orthoses AFO Padding No offloading	TCC Foot Orthoses AFO Padding No offloading	TCC Foot Orthoses AFO Padding No offloading
How often is the ulcer seen by a health professional in a month? (X 3)			
How often is the ulcer dressing changed per month? (x 3)			

Is a photograph of the ulcer taken? Yes No

Footwear Assessment

What type of shoe do you currently use most of the time?

1. Sports 2. Dress 3.Diabetic special shoe 4.Custom made shoe 5.Thongs 6.None

On average per week how many days do you use this shoe?

Days out of seven? **/7**

Do you wear orthoses? **Yes No** **Custom Off-the shelf**

Blood Collection – Pathology

Refer patient to SNP pathology for blood collection.

Blood collection form given to patient?	Yes No
Date of Collection	/ / (dd/mm/yyyy)
Haemoglobin g/L	LD U/L

WCC . 10^9/L	Cholesterol . mmol/L
Platelets 10^9/L	Triglyceride . mmol/L
Fibrinogen . g/L	HDL . mmol/L
Sodium mmol/L	LDL . mmol/L
Potassium . mmol/L	CRP mg/L
Urea mmol/L	Chloride mmol/L
Creatinine µmol/L	Bicarbonate mmol/L
eGFR mL/min/1.73m^2	Calcium . mmol/L
Fasting Glucose . µmol/L	Phosphorus . mmol/L
HAB1C _____%	Total protein g/L
CRP _____mg/L	Hematocrit . mg/dL
ESR _____mm/hr	Neuts . 10^9/L
Parathyroid Hormone _____pg/ml	Lymphs . 10^9/L
N-(carboxymethyl) lysine (CML) _____	Monos . 10^9/L
Non-CML advanced glycation end products (AGEs) _____	Eos . 10^9/L
Pentosidine _____	Baso . 10^9/L
Homocystine	

Vascular Biology Unit Blood Sample Storage Form

Blood Collection –For VBU Biomarker Staff to complete

Has blood been collected for study analysis? (Check with SNP and Ratnesh)	Yes No

Date of Collection	/ / (dd/mm/yyyy)
2 x SST	Yes No
2 x EDTA	Yes No
1 x Sodium Citrate	Yes No

Sample Storage

Have study bloods been processed and stored according to the lab manual?		Yes No* *If NO, record a Protocol Deviation	
Date of Processing		/ / (dd/mm/yyyy)	
	Number of Samples:	Have samples been stored at the Protocol defined temperature?	Location of Samples
Serum (from SST tubes)	(Store at -80°C)	Yes No* *If NO, record a Protocol Deviation	Box # Position #
EDTA Plasma (Purple top EDTA tubes)	(Store at -80°C)	Yes No* *If NO, record a Protocol Deviation	Box # Position #
EDTA RBC Pellet (Remaining pellet from EDTA tubes with plasma removed)	(Store at -80°C)	Yes No* *If NO, record a Protocol Deviation	Box # Position #
Citrate Plasma (Blue top Citrate tubes)	(Store at -80°C)	Yes No* *If NO, record a Protocol Deviation	Box # Position #

Part 2: Gait, Pressure and Biomechanical Assessment.
Site- James Cook University, Gait Laboratory.

Physical Measurements

ASIS Breadth cm	Mass: kg
Left Leg length cm	Height cm
Left Knee diameter cm	
Left malleolus width cm	

Right Leg length	cm
Right Knee diameter	cm
Right malleolus width	cm

Testing protocol

Measure up: ❑

EMG prep: ❑

MVC: ❑

Reflective markers prep ❑

Static capture ❑

10 walking trails ❑

Treadmill walking 4 minutes ❑

(Capture only 2:00 onwards)s
Self-Selected Walking Speed _____km/hr.

Plantar pressure capture (3 walks) ❑

FINISHED

Summary of Findings

Is the initial ulcer still present?	**YES**	**NO**
Has the ulcer size increased?	**YES**	**NO**
Has the ulcer size decreased?	**YES**	**NO**
Is there formation of a new ulcer?	**YES**	**NO**
Has the ulcer completely healed?	**YES**	**NO**
Is there a decrease in glycaemic control?	**YES**	**NO**
Is there a decrease in renal function?	**YES**	**NO**
Has the patient commenced dialysis?	**YES**	**NO**

Is there increased AGE formation present?	**YES**	**NO**
Are there differences in the ROM of joints?	**YES**	**NO**
Is there deterioration of vascular function?	**YES**	**NO**
Is there a deterioration of neurological function?	**YES**	**NO**
Has there been a reduction in treatment frequency?	**YES**	**NO**
Has there been an increase in treatment frequency?	**YES**	**NO**
Was a biopsy conducted?	**YES**	**NO**
Is there infection present?	**YES**	**NO**
Has haematological status deteriorated critically? I.e.- Lipid profile, WCC, CRP, ESR	**YES**	**NO**
Is the participant still suitable for the study?	**YES**	**NO**
Next visit date provided?	**YES**	**NO**

If the participant is no longer suitable for the study, please send a letter of thanks for being involved in the study, otherwise provide date and time of next consultation.

Date and time of next consultation _____

Visit 3
At 6 Months

Initial assessment

1	Is the participant in the case or control group?	Case Control
2	Does the participant have a present plantar foot ulcer?	Yes No
3	Does the participant have a newly formed plantar ulcer?	Yes No
3A	Has a plantar ulcer healed in this duration?	**Yes No**
4	Does the participant give consent for biopsy of the ulcer/ulcers?	Yes No

5	Has the participant's diabetes medication or dose changed since last visit? Please note changes: 1. 2. 3. 4. 5.	Yes No
6	Has the participant increased/decreased treatment frequency for the ulcer?	Yes No
	If yes, how so?	Increased Decreased
7	Has the patient commenced any new treatment for a disease process or as a part of a health measure? I.e.- Dialysis, HRT, Warfarin treatment, Radiation therapy	Yes No
	Please state _____	
8	Has the patient commenced or is awaiting any surgical procedure?	Yes No
	Please state _____	
9	Has the patient increased or decreased weight-bearing activity levels in the last 3 months?	Yes No
	Please state new number of hours per week, on weight-bearing activities? _____hrs Please state the new number of hours per week weight-bearing but not doing any activity (ie standing) _____hrs Please state the number of hours completely not weight bearing (Sitting) _____hrs	
Total	Total hours in three months standing (weight bearing) _____ hrs Total hours in three months walking (weight bearing) _____hrs Total hours in three months non-weight bearing	

	_____hrs

Physical Measurements

Body mass: kg	Waist circumference _____cm
BMI: _____	Hip circumference _____cm

Does the patient have a present plantar foot ulcer?

Yes No

If yes, please fill out planimetry and ulcer assessment table below.

Planimetry and Ulcer assessment

Investigation	**Ulcer 1**	**Ulcer 2**	**Ulcer 3**
Ulcer Location			
Estimated duration of ulcer In weeks			
Planimetric depth cm3			
Planimetry Length cm Width cm Area cm	Length _____cm Width_____ cm Area_____cm	Length _____cm Width_____ cm Area_____cm	Length _____cm Width_____ cm Area_____cm
Wound exudate/ discharge present and type of exudate	Mild Serous Moderate Purulent High Haemoserous	Mild Serous Moderate Purulent High Haemoserous	Mild Serous Moderate Purulent High Haemoserous
UTWCS Grading			

Wound Bed	Necrotic Granulating Epithelializing Sloughy Pale Hypergranulating Bone	Necrotic Granulating Epithelializing Sloughy Pale Hypergranulating Bone	Necrotic Granulating Epithelializing Sloughy Pale Hypergranulating Bone
Wound Edge	Regular Irregular Undermined Rolling	Regular Irregular Undermined Rolling	Regular Irregular Undermined Rolling
Sinus Formation Yes No	Yes No mm_____	Yes No mm _____	Yes No mm _____
Is the ulcer infected? -Conduct Wound Swab for presence of pathological microbes.	Yes No	Yes No	Yes No
Type of organism causing infection (As per pathology test)			
Type of ulcer (Neuropathic, Neuroischemic)	Neuropathic Neuroischemic	Neuropathic Neuroischemic	Neuropathic Neuroischemic
Surrounding Skin	Macerated Hyperkeratotic Indurated Normal/healthy Fragile Erythematous Oedematous Anhidrotic	Macerated Hyperkeratotic Indurated Normal/healthy Fragile Erythematous Oedematous Anhidrotic	Macerated Hyperkeratotic Indurated Normal/healthy Fragile Erythematous Oedematous Anhidrotic
Orthoses Used?	Yes No	Yes No	Yes No
Offloading shoe or boot-walker used?	Yes No	Yes No	Yes No
Type of Off-loading Device	TCC Foot Orthoses AFO Padding No offloading	TCC Foot Orthoses AFO Padding No offloading	TCC Foot Orthoses AFO Padding No offloading

How often is the ulcer seen by a health professional in a month? (X 3)			
How often is the ulcer dressing changed per month? (x 3)			

Footwear Assessment

What type of shoe do you currently use most of the time?

1. Sports 2. Dress 3.Diabetic special shoe 4.Custom made shoe 5.Thongs 6.None

On average per week how many days do you use this shoe?

Days out of seven? **/7**

Do you wear orthoses? **Yes No Custom Off-the shelf**

Blood Collection – Pathology

Refer patient to SNP pathology for blood collection.

Blood collection form given to patient?	Yes No
Date of Collection	/ / (dd/mm/yyyy)
Haemoglobin g/L	LD U/L
WCC . 10^9/L	Cholesterol . mmol/L
Platelets 10^9/L	Triglyceride . mmol/L
Fibrinogen . g/L	HDL . mmol/L
Sodium mmol/L	LDL . mmol/L
Potassium . mmol/L	CRP mg/L
Urea mmol/L	Chloride mmol/L
Creatinine µmol/L	Bicarbonate mmol/L
eGFR mL/min/1.73m^2	Calcium . mmol/L
Fasting Glucose . µmol/L	Phosphorus . mmol/L

HAB1C _____%	Total protein g/L
CRP _____mg/L	Hematocrit . mg/dL
ESR _____mm/hr	Neuts . 10^9/L
Parathyroid Hormone _____pg/ml	Lymphs . 10^9/L
N-(carboxymethyl) lysine (CML) _____	Monos . 10^9/L
Non-CML advanced glycation end products (AGEs) _____	Eos . 10^9/L
Pentosidine _____	Baso . 10^9/L
Homocystine	

Vascular Biology Unit Blood Sample Storage Form

Blood Collection –For VBU Biomarker Staff to complete

Has blood been collected for study analysis? (Check with SNP and Ratnesh)	Yes No
Date of Collection	/ / (dd/mm/yyyy)
2 x SST	Yes No
2 x EDTA	Yes No
1 x Sodium Citrate	Yes No

Sample Storage

Have study bloods been processed and stored according to the lab manual?		Yes No* *If NO, record a Protocol Deviation	
Date of Processing		/ / (dd/mm/yyyy)	
	Number of Samples:	Have samples been stored at the Protocol defined temperature?	Location of Samples

Serum (from SST tubes)	(Store at -80°C)	Yes No* *If NO, record a Protocol Deviation	Box # Position #
EDTA Plasma (Purple top EDTA tubes)	(Store at -80°C)	Yes No* *If NO, record a Protocol Deviation	Box # Position #
EDTA RBC Pellet (Remaining pellet from EDTA tubes with plasma removed)	(Store at -80°C)	Yes No* *If NO, record a Protocol Deviation	Box # Position #
Citrate Plasma (Blue top Citrate tubes)	(Store at -80°C)	Yes No* *If NO, record a Protocol Deviation	Box # Position #

Part 2: Gait, Pressure and Biomechanical Assessment
Site- James Cook University, Gait Laboratory.

Physical Measurements

ASIS Breadth cm	Mass: kg
Left Leg length cm	Height cm
Left Knee diameter cm	
Left malleolus width cm	
Right Leg length cm	
Right Knee diameter cm	
Right malleolus width cm	

Testing protocol

Measure up: ❑

EMG prep: ❑

MVC: ❑

Reflective markers prep ❑

Static capture ❑

10 walking trails ❑

Treadmill walking 4 minutes ❑

(Capture only 2:00 onwards)s
Self-Selected Walking Speed _____km/hr.

Plantar pressure capture (3 walks) ❑

FINISHED

Summary of Findings

Is the initial ulcer still present?	**YES**	**NO**
Has the ulcer size increased?	**YES**	**NO**
Has the ulcer size decreased?	**YES**	**NO**
Is there formation of a new ulcer?	**YES**	**NO**
Has the ulcer completely healed?	**YES**	**NO**
Is there a decrease in glycaemic control?	**YES**	**NO**
Is there a decrease in renal function?	**YES**	**NO**
Has the patient commenced dialysis?	**YES**	**NO**
Is there increased AGE formation present?	**YES**	**NO**
Are there differences in the ROM of joints?	**YES**	**NO**
Is there deterioration of vascular function?	**YES**	**NO**
Is there a deterioration of neurological function?	**YES**	**NO**
Has there been a reduction in treatment frequency?	**YES**	**NO**
Has there been an increase in treatment frequency?	**YES**	**NO**
Was a biopsy conducted?	**YES**	**NO**
Is there infection present?	**YES**	**NO**
Has haematological status deteriorated critically? I.e.- Lipid profile, WCC, CRP, ESR	**YES**	**NO**

End of Study:

Did the participant complete all aspects of the study?

Yes	No
According to the protocol	Participant withdrew*
With protocol deviations or violations (ensure these are recorded on the protocol deviation page)	Lost to follow-up
	Participant deceased (ensure an SAE has been recorded & reported)
	Patient declined further involvement*
	Other*
*Comment:	

Protocol Deviations:

Deviation Number	CRF Page Number	Deviation	Recorded by
1			
2			

End of CRF:

Data collection completed by (Name)	
Signature	
Date completed	/ /
Principle Investigator (Name):	
Signature	
Date	/ /

Appendix E: Ethics approvals and site-specific approvals for studies

This administrative form
has been removed

This administrative form
has been removed

This administrative form
has been removed

This administrative form
has been removed

Appendix F: Supporting files, extended results and Tables for Chapter 5

Supplementary File 1: Results

Participant characteristics

There were three males and one female in the DFU group; two males and three females in the DMC group; and two males and three females in the HC group (see Table 5.1). The median age [IQR] was 56.5 [47.0-71.3] in the DFU group; 58.0 [52.0-64.0] in the DMC group and 64.0 [52.0-72.5] in the HC group. There was no statistical difference in age, gender, foot arch-type, diabetes duration, height or body mass between groups (p>0.05). The hip circumference and waist circumference however differed between groups. The DFU group had a larger hip and waist circumference compared to the other two groups (see Table 5.1).

The reproducibility of identifying anatomical landmarks

Table 5.2 displays the mean [95% CI], minimum and maximum (absolute) differences in identifying all anatomical landmarks between observers for the entire study population. Almost all mean differences and 95% CIs were within the pre-defined 7mm level of agreement, except the 95% CI for the right head of the fibula which was between 0 and 7 mm. The maximum group difference was greater than the pre-defined 7mm for five of the 16 landmarks assessed, namely the left tibial tuberosity (15 mm), the left ASIS (10 mm), the right tibial tuberosity (10 mm), the right head of fibula (35 mm) and the right ASIS (12 mm).

The reproducibility of assessing leg dimensions

The group CCC [95% CI] values for limb and joint assessments for measurement 1 vs. 2; measurement 2 vs. 3 and measurement 1 vs. 3 are shown in Table 5.3. The CCCs between the three measurements ranged between 0.919 [95% CI 0.766-0.972] to 0.982 [95% CI 0.949-0.994] for left leg length and likely contained an outlier (see Figure 5.1). Removing the outlier from one participant increased the lowest CCC value to 0.976 [95% CI 0.928-0.992]. The range of CCCs for other leg dimensions were 0.944 [95% CI 0.841-0.981] to 0.997 [95% CI 0.990-0.999] for the left leg and between 0.957 [95% CI 0.876-0.985] to 0.980 [95% CI 0.943-0.993] for the right leg.

The reproducibility of processing gait measurements

The mean CVs for the repeated processing of gait data were all considerably below the acceptable 10% (see Table 5.4). The highest CVs were noted for double support-time in both left and right limbs (1.3% and 1.9% respectively). All other CV values were below 1% (Table 5.4).

The reproducibility of plantar pressure measurements

The calculated mean CVs for plantar pressures are presented in Table 5.5. The right foot demonstrated a smaller range of CVs compared to the left foot. Contact area demonstrated the highest reproducibility over five days for both feet (CVs <30% in 19/20 locations) and was followed by mpp (CVs <30% in 17/20 locations) and pti (CVs <30% in 14/20 locations. CVs for msp demonstrated the lowest level of reproducibility overall. In the left foot, only 3/10 CVs for msp were <30% compared to 8/10 CVs for the right foot (see

Table 5.5). The highest CVs for mpp and pti and ca measurements were at toe 1 for the left foot and toes 2-5 for the right foot. The highest CVs for msp were at metatarsal 5 in the left foot (41.2%) and toes 2-5 in the right foot (32.7%).

Influence of group on the reproducibility of measurements

We found no clear association between participant group and the reproducibility of assessing anatomical locations and leg dimensions (see Appendix Tables 3-6). Large CVs in assessing double support time were observed within all three groups (see Appendix Table 5). The CVs for plantar pressure measurements appeared to vary little between groups and were generally low for all groups for msp and pti (see Appendix Table 6). Out of all the plantar pressure outcomes, the msp measurements had the higher CVs in all groups. The CVs for mpp measurements were lower than the pti and msp measurements in all groups. The DFU group had 59/80 CVs which were <30%, the DMC group had 68/80 CVs which were <30% and the HC group had 58/80 CVs which were <30% (see Appendix Table 6). The left foot measurements for mpp, pti, ca and msp had much higher CVs compared to the right foot irrespective of group (see Appendix Table 6).

Supplementary File 2: Supplementary Tables with group specific data and anthropometric and clinical measurement reproducibility assessments

Appendix Table 3 Assessment of reproducibility by sub-groups for anatomical measurements.

Variable	DFU (n=4)	DMC (n=5)	HC (n=5)
Left Limb			
Tibial tuberosity	4	4	4
Head of fibula	0	4	4
Lateral malleolus	4	4	3
Medial malleolus	5	4	4
Lateral shin	4	0	5
Central posterior calcaneus	3	3	4
Head of second metatarsal	3	3	3
ASIS*	10	10	3
Right limb			
Tibial tuberosity	5	6	0
Head of fibula	4	3	35
Lateral malleolus	6	3	
Medial malleolus	5	4	4
Lateral shin	3	2	2
Central posterior calcaneus	4	3	6
Head of second metatarsal	3	0	3
ASIS	5	12	0

Legend: The numerical value indicates the maximum difference in (mm) for measurements between two assessors by group. Difference relates to the difference between examiner 1 (MF) and examiner 2 (RC). For purposes of clinical significance a 7mm margin of error was acceptable for this analysis. *ASIS = anterior superior iliac spine. A zero indicates measurements for which differences did not exist between the two assessors.

Appendix Table 4 Assessment of reproducibility by sub-groups for limb measurements.

Variable	CCC measurement 1 v 2 [95% CI]	CCC measurement 1 v 3 [95% CI]	CCC measurement 2 v 3 [95% CI]
DFU group (n=4)			
Left leg length	0.992[0.947-0.998]	0.987[0.887-0.998]	0.998[0.970-0.999]
Left knee diameter	0.951[0.554-0.995]	0.941[0.551-0.993]	0.991[0.906-0.999]
Left ankle diameter	0.961[0.762-0.994]	0.938[0.906-0.960]	0.960[0.791-0.993]
Right leg length	0.997[0.965-0.999]	0.992[0.901-0.999]	0.995[0.952-0.999]
Right knee diameter	0.972[0.789-0.996]	0.982[0.820-0.998]	0.964[0.610-0.997]
Right ankle diameter	0.831[0.108-0.986]	0.961[0.532-0.997]	0.920[0.419-0.991]
ASIS distance	*0.999 [0.999-0.999]*	0.999[0.999-0.999]	0.999[0.999-0.999]
Mass	0.999[0.995-0.999]	0.999[0.996-999]	0.999[0.995-0.999]
Height	0.999[0.998-0.999]	0.999[0.996-0.999]	0.999[0.999-0.999]
DMC group (n=5)			
Left leg length	0.920[0.474-0.990]	0.704[0.030-0.945]	0.774[0.252-0.960]
Left knee diameter	0.997[0.986-0.999]	0.996[0.967-0.999]	0.997[0.987-0.999]
Left ankle diameter	0.927[0.609-0.988]	0.944[0.692-0.991]	0.995[0.956-0.999]
Right leg length	0.979[0.909-0.995]	0.915[0.632-0.982]	0.929[0.564-0.990]
Right knee diameter	0.965[0.866-0.991]	0.957[0.783-0.992]	0.992[0.929-0.999]
Right ankle diameter	0.992[0.982-0.996]	0.989[0.921-0.998]	0.989[0.927-0.998]
ASIS* distance	0.988[0.899-0.998]	0.993[0.941-0.999]	0.997[0.977-0.999]
Mass	0.998[0.998-0.999]	0.999[0.997-0.999]	0.999[0.998-0.999]
Height	0.999[0.999-0.999]	0.999[0.998-0.999]	0.999[0.997-0.999]
HC group (n=5)			
Left leg length	0.997[0.982-0.999]	0.992[0.933-0.999]	0.997[0.976-0.999]
Left knee diameter	0.644[0.212-0.940]	0.657[0.220-0.946]	0.996[0.982-0.999]
Left ankle diameter	0.978[0.939-0.992]	0.855[0.329-0.976]	0.899[0.374-0.987]
Right leg length	0.996[0.974-0.999]	0.987[0.899-0.998]	0.973[0.810-0.996]
Right knee diameter	0.996[0.981-0.999]	0.995[0.964-0.999]	0.973[0.810-0.996]
Right ankle diameter	0.936[0.599-0.991]	0.758[0.07-0.968]	0.812[0.05-0.976]
ASIS distance	0.994[0.939-0.999]	0.993[0.942-0.999]	0.996[0.982-0.999]
Mass	0.999[0.999-0.999]	0.999[0.999-0.999]	0.999[0.998-0.999]
Height	0.998[0.988-0.999]	0.999[0.998-0.999]	0.998[0.989-0.999]

Legend: Numerical figures indicate CCC measurements and two-sided 95% confidence intervals. Concordance Correlation Coefficients (CCC) were calculated using http://www.niwa.co.nz/node/104318/concordance. Two sided 95% confidence intervals are presented with the CCC value for each measurement. The strength of agreement was considered as: Almost perfect >0.90; Substantial >0.8-0.9; Moderate 0.65-0.8; and Poor <0.65. *ASIS = anterior superior iliac spine.

Appendix Table 5 Coefficients of variation (CV) for repeated processing of gait trials.

Variable	DFU group (n=4) CV (%)	DMC group (n=5) CV (%)	HC group (n=5) CV (%)
Left limb			
Cadence	0.1	0.2	0.0
Walking speed	0.7	0.3	0.6
Stride time	0.1	0.2	0.2
Step time	0.9	0.3	0.3
Opposite foot off	0.3	0.5	1.1
Opposite foot contact	0.1	0.2	0.0
Foot off time	0.1	0.1	0.1
Single support time	1.5	0.4	0.8
Double support time	1.8	0.6	1.7
Stride length	0.6	0.2	0.5
Step length	1.1	0.2	0.7
Right limb			
Cadence	0.0	0.2	0.2
Walking speed	0.6	0.3	0.8
Stride time	0.6	0.2	0.2
Step time	1.1	0.2	0.4
Opposite foot off	0.2	1.1	1.0
Opposite foot contact	0.1	0.2	0.0
Foot off time	0.0	0.2	0.2
Single support time	1.6	0.4	0.6
Double support time	1.6	2.1	1.9
Stride length	0.5	0.6	0.7
Step length	1.2	1.0	0.6

Legend: Figures represent coefficients of variation (CV) reported as percentages (%).Cadence refers to number of steps per minute. The CV measurements reported as 0.0 contained CVs which were below 0.001.

Appendix Table 6 Coefficients of variation (CV) for plantar pressure measurements by group for left and right foot.

Variable	DFU group (n=4) CV (%)				DMC group (n=5) CV (%)				HC group (n=5) CV (%)			
	MPP	*PTI*	*CA*	*MSP*	*MPP*	*PTI*	*CA*	*MSP*	*MPP*	*PTI*	*CA*	*MSP*
Right foot												
Toe1	21.5	30.4	12.0	27.3	18.9	23.3	12.0	29.5	32.5	39.7	29.3	39.2
Toes 2-5	31.4	38.0	22.8	23.7	25.2	44.1	27.2	35.9	22.2	48.9	34.3	36.6
Metatarsal 1	30.9	30.4	24.4	27.5	23.4	24.4	11.6	27.9	24.4	24.9	14.3	30.5
Metatarsal 2	29.8	29.9	24.0	22.0	14.4	17.1	14.0	23.5	20.4	17.9	7.5	27.1
Metatarsal 3	25.6	29.4	22.4	18.7	12.4	14.3	12.8	19.4	18.3	15.6	6.8	23.3
Metatarsal 4	25.3	29.1	18.1	25.2	16.2	17.6	8.3	15.4	17.7	17.9	6.4	21.4
Metatarsal 5	27.4	32.1	17.6	25.8	18.3	18.9	9.8	19.7	16.8	19.5	9.3	19.8
Midfoot	13.2	18.2	8.1	18.0	14.3	17.6	5.6	27.3	13.5	15.9	9.2	18.3
Medial Heel	15.6	35.1	15.6	19.2	16.9	19.7	5.1	22.9	24.6	20.5	4.3	36.0
Lateral Heel	21.0	37.2	16.3	21.3	20.1	24.3	4.5	32.3	17.1	16.1	4.6	24.3
Left foot												
Toe1	26.0	38.0	38.9	27.4	30.5	36.9	17.2	34.8	38.5	48.3	25.0	34.9
Toes 2-5	31.5	38.0	36.8	38.9	29.1	37.6	26.4	38.5	33.7	40.5	32.5	41.3
Metatarsal 1	29.5	38.9	15.1	32.7	26.1	29.5	12.7	36.5	28.6	36.9	20.7	35.5
Metatarsal 2	14.3	13.9	7.5	23.7	21.9	22.9	7.7	28.4	29.6	27.5	17.8	39.5
Metatarsal 3	16.7	17.8	6.6	31.5	23.3	23.2	6.7	30.7	23.4	24.3	14.5	29.6
Metatarsal 4	23.7	27.1	6.5	38.6	24.3	25.8	7.1	27.4	30.0	33.4	16.3	40.6
Metatarsal 5	28.8	38.0	18.6	40.2	29.2	31.0	16.9	36.6	35.0	38.4	23.8	46.5
Midfoot	28.3	36.3	13.9	28.5	22.1	23.9	6.2	25.1	16.2	18.7	28.4	28.8
Medial Heel	23.6	28.8	4.0	23.8	16.4	21.3	4.3	22.6	18.4	20.5	12.6	26.6
Lateral Heel	20.5	24.0	5.1	15.3	19.2	16.2	4.9	25.5	24.4	26.1	13.4	32.9

Legend: Figures represent the average coefficients of variation (CV) reported as percentages (%).Maximum sensor pressure in a plantar anatomical location (msp), mean peak pressure in a plantar anatomical location (mpp), pressure time integral (pti) and contact area (ca). CV= Coefficient of variation reported as a percentage for each group based on the average CV of the group from five selected walks per individual over five days.

Appendix Table 7 Concordance Correlation Statistics for Continuous variables [clinical and anthropometric].

Variable	CCC measurement 1 v 2 [95% CI]	CCC measurement 1 v 3 [95% CI]	CCC measurement 2 v 3 [95% CI]
Left brachial systolic BP	*0.917 [0.746-0.974]*	0.798 [0.533-0.920]	0.829 [0.654-0.920]
Left brachial diastolic BP	0.840 [0.564-0.947]	0.719 [0.375-0.889]	0.786 [0.440-0.928]
Left dorsalis pedis systolic BP	0.924 [0.811-0.971]	0.909 [0.730-0.971	0.925 [0.864-0.984]
Left posterior tibialis systolic BP	0.937 [0.739-0.986]	0.924 [0.681-0.984]	0.975 [0.885-0.995]
Left ABPI	0.862 [0.615-0.955]	0.753 [0.365-0.918]	0.873 [0.651-0.962]
Right brachial systolic BP	0.943 [0.828-0.982]	0.863 [0.651-0.951]	0.849 [0.660-0.936]
Right brachial diastolic BP	*0.836 [0.698-0.914]*	0.957 [0.871-0.986]	0.841 [0.645-0.933]
Right dorsalis pedis systolic BP	0.979 [0.931-0.994]	0.987 [0.964-0.996]	0.984 [0.962-0.993]
Right posterior tibialis systolic BP	0.942 0.759-0.987]	0.969 [0.874-0.992]	0.985 [0.931-0.997]
Right ABPI	0.738 [0.317-0.916]	0.801 [0.480-0.933]	0.842 [0.587-0.945]
Left 1st MTPJ RoM	0.987 [0.960-0.996]	0.974 [0.925-0.991]	0.983 [0.951-0.994]
Left monofilament score	0.995 [0.984-0.998]	0.989 [0.970-0.996]	0.995 [0.987-0.998]
Left leg length	0.982 [0.949-0.994]	0.919 [0.776-0.972]	0.933 [0.822-0.975]
Left knee diameter	0.965 [0.895-0.988]	0.962 [0.891-0.987]	0.997 [0.990-0.999]
Left ankle diameter	0.961 [0.892-0.985]	0.944 [0.841-0.981]	0.971 [0.910-0.991]

Right 1st MTPJ RoM	0.977 [0.933-0.992]	0.951 [0.859-0.983]	0.961 [0.895-0.986]
Right monofilament score	0.994 [0.986-0.997]	N/A	0.994 [0.987-0.997]
Right leg length	0.994 [0.983-0.998]	0.972 [0.917-0.991]	0.977 [0.931-0.992]
Right knee diameter	0.986 [0.958-0.996]	0.984 [0.955-0.994]	0.980 [0.943-0.993]
Right ankle diameter	0.967 [0.902-0.989]	0.959 [0.878-0.986]	0.957 [0.876-0.985]
ASIS distance	0.994 [0.981-0.998]	0.995 [0.984-0.998]	0.997 [0.992-0.999]
Mass	0.999 [0.999-0.999]	0.999 [0.999-0.999]	0.999 [0.999-0.999]
Height	0.999 [0.999-0.999]	0.999 [0.999-0.999]	0.999 [0.999-0.999]
% Body Fat	0.999 [0.999-0.999]	0.981 [0.940-0.993]	0.983 [0.948-0.994]
Hip circumference	0.999 [0.996-0.999]	0.999 [0.996-0.999]	0.999 [0.998-0.999]
Waist circumference	0.999 [0.998-0.999]	0.998 [0.995-0.999]	0.999 [0.996-0.999]

Legend: Concordance Correlation Coefficients (CCC) were calculated using http://www.niwa.co.nz/node/104318/concordanceresults.
Two sided 95% confidence intervals are presented with the CCC value for each measurement
ABPI refers to the ankle brachial pressure index, calculated as the highest pedal systolic measurement divided by the highest brachial systolic pressure. The strength of agreement was considered as; Almost perfect >0.90, Substantial >0.8-0.9, Moderate 0.65-0.8 and Poor <0.65. N/A indicates that CCC was unable to be computed

Appendix G: Extended results and Tables for Chapter 6[3]

Supplementary File 1:

[3] Some Tables have been resized to fit the pages enclosed.

Appendix Table 8 Mean differences in kinematics (joint angles) and odds ratios by group.

Joint angles	Mean difference DFU vs. DMC [95% CI of difference]	OR [95% CI]	Mean difference DFU vs. HC [95% CI of difference]	OR [95% CI]	Mean difference DMC vs. HC [95% CI of difference]	OR [95% CI]
Sagittal plane						
Initial strike						
Knee flexion angle (º)	-2.1 [-5.1;-1.0]		-4.2 [-7.3;1.1]	0.8 [0.7;0.9]^	-2.1 [-4.3;0.0]	
Anterior pelvic tilt (º)	2.9 [-1.6;7.5]		6.8 [2.1;11.5]	1.1 [1.0;1.2]^	3.9 [0.6;7.2]	1.1 [1.0;1.2]^
Toe-off						
Anterior pelvic tilt (º)	3.2 [-1.3;7.7]		6.2 [1.6;10.9]	1.1 [1.0;1.2]^	3.0 [-0.2;6.3]	
Gait cycle characteristics						
Maximum ankle plantarflexion (º)	3.3 [0.1;6.6]	1.1 [1.0;1.2]^	4.8 [1.5;8.2]	1.2 [1.1;1.4]^	1.5 [-0.9;3.8]	
Maximum ankle dorsiflexion (º)	2.1 [-0.9;5.1]		-1.2 [-4.2;-1.9]		-3.3 [-5.4;-1.1]	0.9 [0.8;0.9]^
Total ankle ROM (º)	-0.2 [-3.5;3.1]		-5.3 [-8.7;-1.9]	0.8 [0.7;0.9]^	-5.1 [-7.5;-2.7]	0.8 [0.8;0.9]^
Maximum knee flexion (º)	-6.5 [-11.6;-1.4]	0.9 [0.9;1.0]^	-9.6 [-14.8;-4.4]	0.9 [0.8;1.0]^	-3.1 [-6.8;0.6]	
Minimum anterior pelvic tilt (º)	2.3 [-2.0;6.5]		6.4 [2.0;10.7]	1.1 [1.0;1.2]^	4.1[1.1;7.2]	1.1 [1.0;1.2]^

Maximum anterior pelvic tilt (º)	2.4[-1.6;6.4]		6.1 [2.0;10.2]	1.1 [1.0;1.2]^	3.7 [0.8; 6.5]	1.1 [7.0;1.2]^
Frontal plane						
Toe-off						
Pelvic obliquity (º)	1.6 [0.2;3.0]	1.4 [1.1;1.8]^	2.2 [0.8;3.7]	1.4 [1.1;1.7]^	0.6 [-0.4;1.6]	
Gait cycle characteristics						
Maximum ankle valgus (º)	2.0 [0.0;4.0]	1.1 [0.9;1.3]	3.7 [1.7;5.7]	1.3 [1.1;1.6]^	1.7[0.2;3.1]	1.2 [1.0;1.3]^
Maximum knee varus (º)	5.4 [-1.8;12.5]		10.0 [2.7;17.3]	1.1 [1.0;1.1]^	4.6 [-0.5;9.8]	
Minimum Pelvic obliquity (º)	2.2 [0.6;3.9]	1.6 [1.2;2.2]^	3.1 [1.4;4.8]	1.5 [1.2;2.0]^	0.9 [-0.3;2.0]	
Total pelvic ROM (º)	-1.2 [-2.9;-0.5]		-3.7- [-5.4;1.9]	0.6 [0.5;0.8]^	-2.4- [-3.7;1.2]	0.7 [0.6;0.9]^
Transverse plane						
Toe-off						
Foot progression angle (º)	-8.5 [-17.6;-0.7]		-13.6 [-23.0;-4.3]	0.9 [0.9;1.0]^	-5.2 [-11.8;1.4]	

Legend: All mean differences are in degrees (^{0}). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. ROM= Range of motion, CI= Confidence Interval, OR= Odds Ratio. Odds Ratios were only computed from binary logistic regression analyses for variables which remained significantly different on Sidak's post hoc comparisons. Odds Ratios were calculated for the DFU group compared to control groups and for the DMC group compared to the HC group. ^= Binary logistic regression analysis was significant at $p<0.05$ after adjusting for age, gender and BMI. Sagittal plane movements represent dorsiflexion and plantarflexion of the ankle joint and extension and flexion of the hip and knee and anterior and posterior pelvis tilt; frontal plane movements represent abduction and adduction of the ankle, knee and hip and left lateral and right lateral rotation of the pelvis ; transverse plane movements represent inversion and eversion of the ankle, medial and lateral rotation of the knee and internal and external rotation of the hip and transverse rotations of the pelvis. All angles are reported for initial strike, toe-off and during stride.

Appendix Table 9 Mean differences in kinetics (forces) and odds ratios by group.

Ground reaction forces (Newtons N)	Mean difference DFU vs. DMC [95% CI of difference]	OR [95% CI]	Mean difference DFU vs. HC [95% CI of difference]	OR [95% CI]	Mean difference DMC vs. HC [95% CI of difference]	OR [95% CI]
Anterior-posterior ground reaction force F_{AP}						
Min	-15.2 [-27.5;-2.8]	1.0 [1.0;1.1]^	-28.5 [-41.2;-15.8]	0.9 [0.9;1.0]^	-13.4 [-22.3;-4.5]	1.0 [0.9;1.0]^
Max	4.3 [-3.1;11.7]		11.9 [4.3;19.5]	1.1 [1.0;1.2]^	7.6 [2.3;12.9]	1.1 [1.0;1.1]^
Range	24.9 [8.4;41.3]	1.0 [1.0;1.1]^	41.9 [25.0;58.8]	1.0 [1.0;1.1]^	17.1 [5.2;28.9]	1.0 1.0;1.0]^
Total	-3.0 [-10.2;4.2]		-9.4 [-16.8;-2.0]	0.9 [0.9;1.0]^	-6.5 [-11.7;-1.3]	0.9 [0.9;1.0]^
Medial-lateral ground reaction force F_{ML}						
Total	-0.8 [-4.7;3.1]		-4.3 [-8.3;-0.3]	0.9 [0.8;1.0]^	-3.5 [-6.3;-0.7]	0.9 [0.9;1.0]^
Vertical ground reaction force F_V						
Final	0.6 [0.1;1.1]	2.2 [1.0;5.0]	0.2 [-0.3;0.7]		-0.4 [-0.8;0.0]	0.4 [0.2;0.8]^

Max	104.5 [-7.0;215.9]		216.7 [102.2;331.1]	1.0 [1.0;1.0]^	112.2 [31.8;192.7]	1.0 [1.0;1.0]^
Range	112.6 [-1.9;227.2]		222.9 [105.3;340.5]	1.0 [1.0;1.0]^	110.3 [27.6;93.0]	1.0 [1.0;1.0]^
Total	89.3 [6.61;71.9]	1.0 [1.0;1.0]^	186.3 [101.4;271.2]	1.0 [1.0;1.0]^	97.0 [37.3;156.7]	1.0 [1.0;1.0]^

Legend: All mean differences are in Newtons (N). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. CI= Confidence Interval, OR= Odds Ratio. Odds Ratios were only computed from binary logistic regression analyses for variables which remained significantly different on Sidak's post hoc comparisons. Odds Ratios were calculated for the DFU group compared to control groups and for the DMC group compared to the HC group. ^= Binary logistic regression analysis was significant at $p<0.05$ after adjusting for age, gender and BMI. The ground reaction forces in the anterior-posterior, medial-lateral and vertical directions are reported. Initial force indicates the force at initial contact of the ground, final force indicates the force before the limb leaves the ground, the minimal and maximum forces represent the minimal and maximal recorded values during astride and the range and total indicates the range and total force during a stride.

Appendix Table 10 Mean differences in Temporal-spatial parameters (TSPs) and odds ratios by group.

Variable	Mean difference DFU vs. DMC [95% CI of difference]	OR [95% CI]	Mean difference DFU vs. HC [95% CI of difference]	OR [95% CI]	Mean difference DMC vs. HC [95% CI of difference]	OR [95% CI]
Cadence (steps/min)	-4.2 [-9.6;1.3]	0.9 [0.9;1.0]	-8.2 [-13.7;-2.6]	0.9 [0.8;1.0]^	-4.0 [-7.9;-1.0]	0.9 [0.9;1.0]
Walking speed (m/s)	-0.1[-0.2;0.0]	0.2 [0.0;0.5]^	-0.2 [-0.3;-0.1]	0.0 [0.0;0.1]^	-0.1[-0.2;0.0]	0.3 [0.0;0.3]^
Opposite foot-off-time (s)	0.4 [-0.8;1.7]	1.1 [0.9;1.4]	1.9 [0.7;3.2]	1.8 [1.3;2.7]^	1.5 [0.6;2.4]	1.5 [1.2;1.9]^
Stride length (m)	-0.1 [-0.2;0.0]	0.3 [0.0;0.8]^	-0.2 [-0.3;-0.1]	0.0 [0.0;0.2]^	-0.1 [-0.2;0.0]	0.0 [0.0;0.8]^
Step length (m)	-0.1 [-0.1;0.0]	0.0 [0.0;0.2]^	-0.1 [-0.1;0.0]^	0.0 [0.0;0.0]^	0.0 [-0.1;0.0]	0.0 [0.0;0.6]^

Legend: All mean differences are in units reported in the table. DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. CI= Confidence Interval, OR= Odds Ratio. Odds Ratios were only computed from binary logistic regression analyses for variables which remained significantly different on Sidak's post hoc comparisons. Odds Ratios were calculated for the DFU group compared to control groups and for the DMC group compared to the HC group. ^= Binary logistic regression analysis was significant at $p<0.05$ after adjusting for age, gender and BMI.

Appendix Table 11 Kinematic outcomes that remained significantly different after Sidak's correction.

Joint angles	DFU (n=21)	DMC (n=69)	HC (n=56)	Corrected p-value	Mean difference DFU vs. DMC [95% CI of difference]	Mean difference DFU vs. HC [95% CI of difference]	Mean difference DMC vs. HC [95% CI of difference]
Sagittal plane							
Initial strike							
Knee flexion angle (º)	2.4 (6.4)[b]	4.5 (4.4)	6.6 (5.1)	0.042	-2.1 [-5.1;1.0]	-4.2 [-7.3;-1.1]	-2.1 [-4.3;0.0]
Anterior pelvic tilt (º)	18.4 (9.5)[b]	15.4 (6.2)[b]	11.6 (8.3)	0.016	2.9 [-1.6-7.5]	6.8 [2.1;11.5]	3.9 [0.6;7.2]
Toe-off							
Anterior pelvic tilt (º)	17.6 (8.2)[b]	14.4 (6.4)	11.3 (8.4)	0.042	3.2 [-1.3;7.7]	6.2 [1.6;10.9]	3.0 [-0.2;6.3]
Gait cycle characteristics							
Maximum ankle plantarflexion (º)	11.8 (4.7)[ab]	15.1 (5.2)	16.6 (5.9)	0.042	3.3 [0.1;6.6]	4.8 [1.5;8.2]	1.5 [-0.9;3.8]
Maximum ankle dorsiflexion (º)	14.2 (5.3)	12.1 (4.3)[b]	15.4 (5.5)	0.016	2.1 [-0.9;5.1]	-1.2 [-4.2;1.9]	-3.3 [-5.4;-1.1]
Total ankle ROM (º)	26.6 (5.0)[b]	26.8 (5.1)[b]	31.9 (6.2)	0.002	-0.2 [-3.5;3.1]	-5.3 [-8.7;-1.9]	-5.1- [-7.5;2.7]
Maximum knee flexion (º)	45.6 (10.3)[ab]	52.2 (8.2)	55.3 (7.9)	0.002	-6.5 [-11.6;-1.4]	-9.6 [-14.8;-4.4]	-3.1 [-6.8;0.6]
Minimum anterior pelvic tilt (º)	16.4 (8.5)[b]	14.1 (5.0)[b]	10.0 (8.4)	0.002	2.3 [-2.0;6.5]	6.4 [2.0;10.7]	4.1[1.1;7.2]

Maximum anterior pelvic tilt (º)	19.7 (8.1)[b]	17.3 (5.4)[b]	13.6 (7.4)	0.002	2.4[-1.6;6.4]	6.1 [2.0;10.2]	3.7 [0.8; 6.5]
Frontal plane							
Toe-off							
Pelvic obliquity (º)	-0.8	2.4 (1.6)	3.0 (2.6)	0.017	1.6 [0.2;3.0]	2.2 [0.8;3.7]	0.6 [-0.4;1.6]
Gait cycle characteristics							
Maximum ankle valgus (º)	0.7 (3.7)[ab]	-1.3 (2.0)[b]	-3.0 (4.3)	0.002	2.0 [0.0;4.0]	3.7[1.7;5.7]	1.7[0.2;3.1]
Maximum knee varus (º)	4.5 (3.6)[b]	3.8 (2.7)	3.6 (4.1)	0.048	5.4 [-1.8;12.5]	10.0 [2.7;17.3]	4.6 [-0.5;9.8]
Minimum Pelvic obliquity (º)	1.0 (2.5)[ab]	3.2 (2.0)	4.0 (3.5)	0.002	2.2 [0.6;3.9]	3.1 [1.4;4.8]	0.9 [-0.3;2.0]
Total pelvic ROM (º)	4.9 (2.3)[b]	6.1 (1.6)[b]	8.5 (3.9)	0.002	-1.2 [-2.9;0.5]	-3.7 [-5.4;-1.9]	-2.4 [-3.7;-1.2]
Transverse plane							
Toe-off							
Foot progression angle (º)	-11.3 (18.8)[b]	-2.8 (14.5)	2.4 (14.5)	0.040	-8.5 [-17.6;0.7]	-13.6 [-23.0;-4.3]	-5.2 [-11.8;1.4]

Legend: All data are in degrees (º). Group data represents mean (standard deviation). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. CI= Confidence Interval. ROM= Range of motion. Corrected p-values indicate p-values obtained after Holm correction rounded to 3decimal places. [a]= $p<0.05$ vs. DMC on Sidak's post hoc test, [b]=$p<0.05$ vs. HC on Sidak's post hoc test. Sagittal plane movements represent dorsiflexion and plantarflexion of the ankle joint and extension and flexion of the hip and knee and anterior and posterior pelvis tilt; frontal plane movements represent abduction and adduction of the ankle, knee and hip and left lateral and right lateral rotation of the pelvis ; transverse plane movements represent inversion and eversion of the ankle, medial and lateral rotation of the knee and internal and external rotation of the hip and transverse rotations of the pelvis. All angles are reported for initial strike, toe-off and during stride.

Appendix Table 12 Kinetic (force) characteristics that remained significantly different after Sidak's correction.

Ground reaction forces (Newtons N)	DFU (n=21)	DMC (n=69)	HC (n=56)	Corrected p-value	Mean difference DFU vs. DMC [95% CI of difference]	Mean difference DFU vs. HC [95% CI of difference]	Mean difference DMC vs. HC [95% CI of difference]
Anterior-posterior ground reaction force F_{AP}							
Min	77.3 (31.9)[ab]	62.1 (17.6)[b]	48.7 (18.5)	0.001	-15.2 [-27.5;-2.8]	-28.5 [-41.2;-15.8]	-13.4 [-22.3;-4.5]
Max	33.0 (18.1)[b]	28.7 (12.4)[b]	21.1 (9.0)	0.001	4.3 [-3.1;11.7]	11.9 [4.3;19.5]	7.6 [2.3;12.9]
Range	114.4 (43.7)[ab]	89.5 (23.1)[b]	72.5 (24.2)	0.001	24.9 [8.4;41.3]	41.9 [25.0;58.8]	17.1 [5.2;28.9]
Total	35.8 (17.8)[b]	32.8 (10.8)[b]	26.3 (10.5)	0.024	-3.0 [-10.2;4.2]	-9.4 [-16.8;-2.0]	-6.5 [-11.7;-1.3]
Medial-lateral ground reaction force F_{ML}							
Total	0.1 (6.3)[b]	0.9 (6.2))[b]	4.4 (6.7)	0.044	-0.8 [-4.7;3.1]	-4.3 [-8.3;-0.3]	-3.5 [-6.3;-0.7]
Vertical ground reaction force F_V							
Final	25.1 (1.2)[a]	24.5 (0.9)[b]	24.9 (0.5)	0.044	0.6 [0.1;1.1]	0.2 [-0.3;0.7]	-0.4 [-0.8;0.0]^

Max	1019.4 (241.2)[b]	914.9 (154.7)[b]	802.7 (195.9)	0.001	104.5 [-7.0;215.9]	216.7 [102.2;331.1]	112.2 [31.8;192.7]
Range	995.1 (241.2)[b]	882.5 (163.3)[b]	772.2 (199.9)	0.001	112.6 [-1.9;227.2]	222.9 [105.3;340.5]	110.3 [27.6;193.0]
Total	736.4 (174.4)[ab]	647.1 (112.4)[b]	550.1 (149.4)	0.001	89.3 [6.61;71.9]	186.3 [101.4;271.2]	97.0 [37.3;156.7]

Legend: All data are reported in Newtons (N). Group data represents mean (standard deviation). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. Corrected p-values indicate p-values obtained after Holm correction rounded to 3 decimal places. CI=Confidence Interval. [a]= $p<0.05$ vs. DMC on Sidak's post hoc test, [b]=$p<0.05$ vs. HC on Sidak's post hoc test. The ground reaction forces in the anterior-posterior, medial-lateral and vertical directions are reported. Initial force indicates the force at initial contact of the ground, final force indicates the force before the limb leaves the ground, the minimal and maximum forces represent the minimal and maximal recorded values during astride and the range and total indicates the range and total force during a stride.

Appendix Table 13 Temporal-spatial parameters (TSPs) that remained significantly different after Sidak's correction.

Variable	DFU (n=21)	DMC (n=69)	HC (n=56)	Corrected p-value	Mean difference DFU vs. DMC [95% CI of difference]	Mean difference DFU vs. HC [95% CI of difference]	Mean difference DMC vs. HC [95% CI of difference]
Cadence (steps/min)	105.7 (7.4)[b]	109.9 (10.2)[b]	113.9 (7.8)	0.007	-4.2 [-9.6;1.3]	-8.2 [-13.7;-2.6]	-4.0 [-7.9;1.0]
Walking speed (m/s)	0.9 (0.2)[ab]	1.1 (0.2)	1.2 (0.2)	0.001	-0.1[-0.2;0.0]	-0.2 [-0.3;-0.1]	-0.1 [-0.2;0.0]
Opposite foot-off-time (s)	12.3 (2.2)[b]	11.9 (2.3)[b]	10.4 (1.5)	0.001	0.4 [-0.8;1.7]	1.9 [0.7;3.2]	1.5 [0.6;2.4]
Stride length (m)	1.1 (0.2)[b]	1.1 (0.2)[b]	1.3 (0.1)	0.001	-0.1 [-0.2;0.0]	-0.2 [-0.3;-0.1]	-0.1 [-0.2;0.0]
Step length (m)	0.5 (0.1)[ab]	0.6 (0.1)[b]	0.6 (0.1)	0.001	-0.1 [-0.1;0.0]	-0.1 [-0.1;0.0]	0.0 [-0.1;0.0]

Legend: All data values are in the units provided. Group data represents mean (standard deviation). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. CI=Confidence interval. Corrected p-values indicate p-values obtained after Holm correction rounded to 3decimal places. [a]= $p<0.05$ vs. DMC on Sidak's post hoc test, [b]=$p<0.05$ vs. HC on Sidak's post hoc test. *= Binary logistic regression analysis was significant at $p<0.05$ after adjusting for age, gender and BMI. ^= Binary logistic regression analysis was significant at $p<0.05$ but BMI was also significantly different between groups $p<0.05$. ~= Binary logistic regression was significant at $p<0.05$ for BMI after adjustment. Cohen's d scores and Binary logistic regression analyses were only calculated for variables which were significant on the Sidak's post-hoc test.

Appendix Table 14 All joint angles (kinematics) outcome variables investigated by group.

Joint angles		DFU (n=21)			DMC (n=69)			HC (n=56)			ANOVA p-value			Corrected p-value		
		Sagittal	Frontal	Transverse	Sagittal	Frontal	Transverse	Sagittal	Frontal	Transverse	Sagittal	Frontal	Transverse	Sagittal	Frontal	Transverse
Initial strike																
	Ankle (º)	-6.0 (5.4)	0.2 (4.9)	-4.4 (15.4)	-5.6 (3.5)	-0.5 (2.6)	2.2 (12.7)	-5.2 (3.5)	-0.8 (3.4)	4.1 (15.1)	0.677	0.481	0.063	0.960	1.000	0.732
	Knee (º)	2.4 (6.4)[b]	-0.8 (9.6)	-36.5 (16.5)	4.5 (4.4)	0.5 (5.7)	-32.1 (14.3)	6.6 (5.1)	0.6 (5.8)	-33.0 (11.9)	0.003	0.665	0.445	0.042	1.000	1.000
	Hip (º)	37.8 (9.2)	-0.3 (7.7)	24.6 (13.8)[b]	38.2 (8.1)	1.2 (4.8)	18.9 (15.5)	34.9 (9.5)	0.0 (3.4)	15.5 (12.7)	0.093	0.242	0.044	0.558	1.000	0.704
	Pelvis (º)	18.4 (9.5)[b*]	1.7 (1.8)[a*b]	2.0 (4.9)	15.4 (6.2)	0.5 (1.4)	2.4 (2.8)	11.6 (8.3)	0.6 (1.5)	3.3 (3.8)	0.001	0.004	0.247	0.016	0.060	1.000
Toe-off																
	Ankle (º)	-5.8 (7.3)[b]	2.6 (3.8)[a*b*]	-11.3 (18.8)[b*]	-10.0 (6.5)	0.6 (2.6)	-2.8 (14.5)	-10.6 (8.2)	0.4 (3.8)	2.4 (14.5)	0.033	0.021	0.002	0.330	0.294	0.040
	Knee (º)	24.8 (11.3)[b]	20.0 (11.6)	-21.0 (14.7)	28.3 (7.7)	14.3 (11.8)	-18.6 (12.8)	30.5 (9.6)	13.6 (9.5)	-22.4 (11.0)	0.043	0.064	0.236	0.387	0.640	1.000
	Hip (º)	10.0 (11.3)	-1.9 (6.9)	25.9 (18.1)	7.2 (9.8)	-2.8 (5.5)	22.5 (14.7)	5.6 (8.1)	-3.9 (4.5)	19.4 (11.9)	0.192	0.298	0.180	0.960	1.000	1.000
	Pelvis (º)	17.6 (8.2)[b*]	-0.8 (3.3)[ab*]	-2.9 (6.7)	14.4 (6.4)	-2.4 (1.6)	-3.4 (2.3)	11.3 (8.4)	-3.0 (2.6)	-2.7 (3.6)	0.003	0.001	0.538	0.042	0.017	1.000
Gait cycle																
	Ankle (º) min	-11.8 (4.7)[ab*]	0.7 (3.7)[ab*]	-19.2 (16.0)	-15.1 (5.2)	-1.3 (2.0)	-15.4 (11.0)	-16.6 (5.9)	-3.0 (4.3)	-11.5 (14.7)	0.003	<0.001	0.061	0.042	0.002	0.732
	Ankle (º) max	14.2 (5.3)	4.5 (3.6)	3.6 (18.7)[b]	12.1 (4.3)	3.8 (2.7)	9.1 (11.2)	15.4 (5.5)	3.6 (4.1)	12.3 (14.6)	0.001	0.547	0.049	0.016	1.000	0.704
	Ankle (º) range	26.6 (5.0)[b*]	4.5 (2.3)[b]	22.2 (9.1)	26.8 (5.1)	5.4 (1.6)	23.6 (4.6)	31.9 (6.2)	6.2 (3.4)	22.8 (8.1)	<0.001	0.030	0.650	0.002	0.390	1.000

Knee (º) min	-2.2 (5.7)	-2.7 (4.9)	-39.2 (18.3)	0.4 (5.1)	-2.0 (3.9)	-38.6 (12.0)	1.3 (6.3)	-1.3 (5.1)	-39.1 (12.3)	0.061	0.426	0.962	0.960	1.000	1.000
Knee (º) max	45.6 (10.3)[ab*]	34.0 (13.9)[b*]	-16.0 (15.3)[a]	52.2 (8.2)	28.6 (10.8)	-8.2 (9.8)	55.3 (7.9)	24.0 (12.2)	-11.8 (12.1)	<0.001	0.003	0.020	0.002	0.048	0.360
Knee (º) range	46.3 (8.8)[b]	33.5 (15.7) [b]	27.0 (11.8)	51.1 (9.0)	29.2 (9.7)	29.5 (7.9)	52.9 (8.2)	24.5 (12.5)	25.7 (9.5)	0.013	0.08	0.067	0.143	0.064	0.732
Hip (º) min	2.0 (11.5)	-3.5 (6.4)[b]	19.3 (18.8)	0.6 (8.4)	-5.6 (3.4)	15.9 (13.3)	-0.6 (8.2)	-6.1 (3.6)	11.2 (12.9)	0.468	0.045	0.046	0.440	0.540	0.704
Hip (º) max	41.3 (9.5)	8.0 (5.7)	34.5 (13.6)[b]	41.1 (7.9)	6.2 (2.9)	29.7 (11.7)	38.8 (8.6)	5.9 (3.0)	25.8 (12.2)	0.241	0.051	0.018	0.960	0.561	0.342
Hip (º) range	38.1 (6.3)[a]	11.3 (4.2)	14.4 (9.8)	41.3 (5.0)	11.7 (2.1)	13.9 (4.8)	40.7 (5.4)	12.0 (3.4)	13.6 (5.1)	0.055	0.661	0.860	0.440	1.000	1.000
Pelvis (º) min	16.4 (8.5)[b*]	-1.0 (2.5)[a*b*]	-2.1 (4.8)[a]	14.1 (5.0)	-3.2 (2.0)	-4.1 (2.6)	10.0 (8.4)	-4.0 (3.5)	-3.7 (2.7)	<0.001	<0.001	0.032	0.002	0.002	0.544
Pelvis (º) max	19.7 (8.1)[b*]	2.9 (3.3)	4.2 (2.9)	17.3 (5.4)	3.3 (1.9)	4.4 (2.5)	13.6 (7.4)	4.2 (3.2)	5.3 (2.7)	<0.001	0.065	0.138	0.002	0.640	1.000
Pelvis (º) range	4.0 (4.0)	4.9 (2.3)[b*]	6.9 (3.7)[b]	3.2 (0.7)	6.1 (1.6)	8.6 (2.1)	3.5 (1.3)	8.5 (3.9)	8.8 (3.9)	0.204	<0.001	0.049	0.960	0.002	0.704

Legend: All data are in degrees (^{0}). All data represents mean and standard deviation (SD). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. ANOVA p-value indicates the uncorrected p-value from one way ANOVA. The corrected ANOVA p-value indicates p-values after performing the Holm step-wise correction for multiple testing. A significance of $p < 0.05$ was used throughout. Sagittal plane movements represent dorsiflexion and plantarflexion of the ankle joint and extension and flexion of the hip and knee and anterior and posterior pelvis tilt; frontal plane movements represent abduction and adduction of the ankle, knee and hip and left lateral and right lateral rotation of the pelvis ; transverse plane movements represent inversion and eversion of the ankle, medial and lateral rotation of the knee and internal and external rotation of the hip and transverse rotations of the pelvis. All angles are reported for initial strike, toe-off and during stride.

Appendix Table 15 All kinetic (force) characteristics investigated by group.

Ground reaction forces (Newtons N)	DFU (n=21)	DMC (n=69)	HC (n=56)	ANOVA p-value	Corrected p-value
(X-axis) Anterior-posterior ground reaction force F_{AP}					
Initial	4.5 (2.3)[ab]	3.1 (2.3)	3.0 (1.8)	0.012	0.108
Final	-1.7 (2.6)	-1.7 (1.9)	-1.1 (2.5)	0.298	1.000
Min	-77.3 (31.9)[ab*]	-62.1 (17.6)	-48.7 (18.5)	<0.001	0.001
Max	33.0 (18.1)[b*]	28.7 (12.4)	21.1 (9.0)	<0.001	0.001
Range	114.4 (43.7)[a*b*]	89.5 (23.1)	72.5 (24.2)	<0.001	0.001
Total	-35.8 (17.8)[b]	-32.8 (10.8)	-26.3 (10.5)	0.002	0.024
(Y-axis) Medial-lateral ground reaction force F_{ML}					
Initial	1.3 (3.5)	0.3 (2.7)	0.7 (1.9)	0.287	1.000
Final	0.5 (4.1)	-1.4 (2.4)	-0.7 (3.7)	0.051	0.357
Min	-113.5 (35.4)	-123.1 (30.9)	-106.9 (29.6)	0.016	0.128
Max	117.8 (43.3)	127.7 (35.4)	118.2 (31.9)	0.265	1.000
Range	231.3 (72.2)	249.3 (62.8)	230.8 (52.6)	0.194	1.000
Total	0.1 (6.3)[b]	0.9 (6.2)	4.4 (6.7)	0.004	0.044
(Z-axis) Vertical ground reaction force F_V					
Initial	26.6 (6.3)	30.8 (35.0)	28.9 (17.3)	0.801	1.000
Final	25.1 (1.2)[a]	24.5 (0.9)	24.9 (0.5)	0.004	0.044
Min	24.2 (0.3)	24.1 (0.9)	27.5 (19.9)	0.266	1.000
Max	1019.4 (241.2)[b*]	914.9 (154.7)	802.7 (195.9)	<0.001	0.001
Range	995.1 (241.2)[b*]	882.5 (163.3)	772.2 (199.9)	<0.001	0.001
Total	736.4 (174.4)[ab*]	647.1 (112.4)	550.1 (149.4)	<0.001	0.001

Legend: All data are in Newtons (N). All data represents mean and standard deviation (SD). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. ANOVA p-value indicates the uncorrected p-value from one way ANOVA. The corrected ANOVA p-value indicates p-values after performing the Holm step-wise correction for multiple testing. A significance of $p < 0.05$ was used throughout. All values are in Newtons (N). The ground reaction forces in the anterior-posterior, medial-lateral and vertical directions are reported. Initial force indicates the force at initial contact of the ground, final force indicates the force before the limb leaves the ground, the minimal and maximum forces represent the minimal and maximal recorded values during astride and the range and total indicates the range and total force during a stride.

Appendix Table 16 All Temporal-spatial parameters (TSPs) investigated by group.

Variable	DFU (n=21)	DMC (n=69)	HC (n=56)	ANOVA p-value	Corrected p-value
Cadence (steps/min)	105.7 (7.4)	109.9 (10.2)	113.9 (7.8)	0.001	0.007
Walking speed (m/s)	0.9 (0.2)	1.1 (0.2)	1.2 (0.2)	<0.001	0.001
Stride time (s)	1.1 (0.1)	1.1 (0.1)	1.2 (0.2)	0.053	0.212
Step time (s)	0.6 (0.0)	0.6 (0.1)	0.5 (0.1)	0.028	0.140
Opposite foot-off-time (s)	12.3 (2.2)	11.9 (2.3)	10.4 (1.5)	<0.001	0.001
Opposite foot-contact-time (s)	49.9 (1.8)	50.1 (2.6)	50.7 (3.8)	0.379	1.000
Foot-off-time (s)	62.2 (3.1)	61.8 (2.5)	60.6 (2.4)	0.019	0.114
Single-support time (s)	0.4 (0.1)	0.4 (0.0)	0.4 (0.0)	0.613	1.000
Double-support time (s)	0.3 (0.1)	0.3 (0.1)	0.3 (0.6)	0.667	1.000
Stride length (m)	1.1 (0.2)	1.1 (0.2)	1.3 (0.1)	<0.001	0.0011
Step length (m)	0.5 (0.1)	0.6 (0.1)	0.6 (0.1)	<0.001	0.0011

Legend: All data are in the units provided. All data represents mean and standard deviation (SD). DFU= diabetic foot ulcer group, DMC= diabetes mellitus control group without foot ulcers, HC= healthy control group. ANOVA p-value indicates the uncorrected p-value from one way ANOVA. The corrected ANOVA p-value indicates p-values after performing the Holm step-wise correction for multiple testing. A significance of $p < 0.05$ was used throughout.

Appendix H: Extended results and Tables for Chapter 7[4]

Appendix Table 17 Mean peak pressure and pressure time integral characteristics of the cohort by group.

	DFU Group (n=21)	DMC Group (n=69)	HC Group (n=56)	p value	Corrected p-value
Plantar Pressure (mpp)) (N/cm²)					
Toe 1/ Hallux	3.6 [2.5-8.2]	4.0 [3.1-5.0]	3.5 [2.9-4.8]	0.347	0.347
Toes 2-5	3.0 [2.4-5.6]	2.5 [1.9-3.1]	2.1 [1.8-2.7]	0.001	0.007
Metatarsal 1	5.7 [4.5-8.5]	5.5 [4.3-6.4]	4.6 [3.8-5.4]	0.002	0.008
Metatarsal 2	6.8 [4.9-9.2]	7.0 [6.0-7.9]	5.7 [4.8-7.1]	0.003	0.009
Metatarsal 3	6.6 [4.7-9.9]	7.2 [5.9-8.3]	5.8 [4.4-7.3]	0.004	0.009
Metatarsal 4	5.7 [4.1-7.6]	6.1 [5.2-7.1]	4.8 [4.0-5.7]	<0.001	<0.001
Metatarsal 5	4.7 [3.4-6.2]	4.9 [3.9-5.8]	3.6 [2.8-4.7]	<0.001	<0.001
Mid Foot	3.8 [3.1-6.5]	3.0 [2.5-3.7]	2.2 [1.8-2.9]	<0.001	<0.001
Medial Heel	5.8 [4.5-9.2]	6.1 [5.3-6.8]	5.2 [4.4-6.0]	0.001	0.007
Lateral Heel	6.3 [4.8-9.6]	6.1 [5.5-7.1]	5.1 [4.2-6.6]	0.001	0.007
Pressure Time Integral (pti) (Ns/cm²)					
Toe 1/ Hallux	0.9 [0.5-1.9]	1.0 [0.7-1.2]	0.8 [0.6-1.1]	0.231	0.231
Toes 2-5	0.9 [0.6-1.4]	0.6 [0.5-0.8]	0.5 [0.3-0.6]	0.0001	0.001
Metatarsal 1	2.3 [1.5-2.9]	1.8 [1.4-2.4]	1.4 [1.1-1.7]	0.0001	0.001
Metatarsal 2	2.7 [1.7-3.7]	2.5 [2.0-2.9]	1.9 [1.5-.4]	0.0001	0.001

[4] Some Tables have been resized to fit the pages enclosed.

Metatarsal 3	2.9 [1.7-3.9]	2.6 [2.0-3.1]	1.5 [1.6-2.7]	0.002	0.004
Metatarsal 4	2.3 [2.0-3.3]	2.3 [2.0-2.8]	1.7 [1.4-2.2]	0.0001	0.001
Metatarsal 5	1.9 [1.3-2.7]	1.8 [1.4-2.1]	1.2 [0.9-1.7]	0.0001	0.001
Mid Foot	1.7 [1.2-2.5]	1.0 [0.8-1.3]	0.6 [0.4-0.9]	0.0001	0.001
Medial Heel	2.3 [1.5-3.6]	2.0 [1.6-2.3]	1.5 [1.2-1.9]	0.0001	0.001
Lateral Heel	2.2 [1.5-3.6]	2.0 [1.6-2.3]	1.5 [1.1-1.9]	0.0001	0.001

Legend: Data displays median and interquartile range [IQR] values based on the ulcerated and non-ulcerated feet of the DFU group compared to the average values in the DMC and HC groups. The Kruskal-Wallis test was used for all between group comparisons with a significance of $p<0.05$ and these are the main p-values reported. The corrected p-value indicates p-values after performing the Holm step-wise correction for multiple testing. A significance of $p <0.05$ was used throughout.

Appendix Table 18 Contact area and maximum sensor pressure characteristics of the cohort by group.

	DFU Group (n=21)	DMC Group (n=69)	HC Group (n=56)	p value	Corrected p-value
Max Sensor Pressure (msp) (N/cm²)					
Toe 1/ Hallux	17.9 [7.4-30.7]	14.5 [10.0-18.5]	13.7 [10.4-18.4]	0.696	0.696
Toes 2-5	11.7 [8.3-23.9]	8.4 [6.8-11.3]	8.0 [6.3-9.8]	0.001	0.009
Metatarsal 1	19.5 [10.8-27.5]	13.2 [10.8-17.4]	12.5 [9.5-16.3]	0.015	0.120
Metatarsal 2	18.0 [12.7-21.4]	17.4 [14.2-21.2]	15.7 [12.5-19.0]	0.195	0.588
Metatarsal 3	17.0 [12.3-24.3]	16.7 [13.3-20.6]	15.1 [12.4-18.6]	0.165	0.588
Metatarsal 4	14.1 [10.6-15.9]	12.9 [10.7-16.6]	12.0 [9.3-15.0]	0.147	0.588
Metatarsal 5	11.7 [8.1-15.8]	11.7 [9.0-15.1]	8.8 [6.9-12.8]	0.016	0.120
Mid Foot	12.4 [9.4-19.4]	9.3 [7.7-11.1]	6.7 [5.6-8.7]	0.0001	0.001
Medial Heel	17.1 [11.9-26.7]	15.4 [13.2-18.5]	14.4 [11.8-17.0]	0.076	0.380
Lateral Heel	16.2 [11.4-28.2]	15.9 [13.5-19.3]	13.8 [10.8-17.4]	0.021	0.126
Contact Area (ca) (cm²)					
Toe 1/ Hallux	11.0 [8.4-13.3]	10.9 [9.3-12.3]	11.3 [9.5-12.4] 3.3	0.694	0.694
Toes 2-5	10.1 [8.0-11.7]	8.4 [6.4-10.2]	8.6 [6.5-10.1]	0.044	0.132
Metatarsal 1	12.8 [10.2-15.2]	11.1 [10.0-12.2]	10.1 [8.6-10.6]	0.0001	0.001
Metatarsal 2	10.0 [8.7-11.9]	9.7 [8.7-10.4]	9.0 [8.4-9.6]	0.002	0.014

Metatarsal 3	8.9 [7.5-9.7]	8.1 [7.5-8.9]	7.9 [7.2-8.4]	0.018	0.072
Metatarsal 4	9.5 [8.5-10.3]	8.6 [8.0-9.5]	8.5 [8.0-9.1]	0.014	0.070
Metatarsal 5	8.5 [6.6-10.6]	9.4 [8.2-10.6]	9.0 [7.8-9.7]	0.081	0.165
Mid Foot	37.4 [28.1-42.4]	26.9 [22.0-32.4]	21.7 [13.9-25.9]	0.0001	0.001
Medial Heel	17.4 [15.5-18.8]	16.1 [14.2-17.5]	15.1 [13.6-15.7]	0.0001	0.001
Lateral Heel	14.9 [13.8-15.7]	14.0 [12.4-15.5]	13.5 [12.2-14.1]	0.003	0.018

Legend: Data displays median and interquartile range [IQR] values based on the ulcerated and non-ulcerated feet of the DFU group compared to the average values in the DMC and HC groups. The Kruskal-Wallis test was used for all between group comparisons with a significance of $p<0.05$ and these are the main p-values reported. The corrected p-value indicates p-values after performing the Holm step-wise correction for multiple testing. A significance of $p <0.05$ was used throughout.

Appendix Table 19 Post-hoc test results and median differences by group.

	DFU Group (n=21)	DMC Group (n=69)	HC Group (n=56)	Corrected p-value	Median difference DFU vs. DMC	Median difference DFU vs. HC
Plantar Pressure (mpp) (N/cm²)						
Toes 2-5	3.0 [3.1][ab]	2.5 [1.1]	2.1 [0.9]	0.007	-0.8 [-1.5 to -0.9]	-1.0 [-1.9 to -0.5]
Metatarsal 1	5.7 [4.0][b]	5.5 [2.1]	4.6 [1.6]	0.008	-	-1.5 [-2.7 to -0.5]
Metatarsal 2	6.8 [4.3]	7.0 [1.9] .0.0	5.7 [2.3]	0.009	-	-
Metatarsal 3	6.6 [5.2]	7.2 [2.5]	5.8 [2.8]	0.009	-	-
Metatarsal 4	5.7 [3.5]	6.1 [1.9	4.8 [1.7]	<0.001	-	-
Metatarsal 5	4.7 [2.8][b]	4.9 [1.9]	3.6 [1.8]	<0.001	-	-1.1 [-2.0 to -0.1]
Mid Foot	3.8 [3.4][ab]	3.0 [1.1]	2.2 [1.1]	<0.001	-0.9 [-2.0 to -0.3]	-1.7 [-3.0 to -1.1]
Medial Heel	5.8 [4.7][b]	6.1 [1.4]	5.2 [1.6]	0.007	-	-1.0 [-2.3 to -0.8]
Lateral Heel	6.3 [4.8][b]	6.1 [1.6]	5.1 [2.3]	0.007	-	-1.3 [-2.7 to -0.2]
Max Sensor Pressure (msp) (N/cm²)						
Toes 2-5	11.7 [15.7][ab]	8.4 [4.5]	8.0 [3.5]	0.009	3.8 [1.1 to 8.6]	-4.6 [-9.5 to -1.9]
Mid Foot	12.4 [10.0][ab]	9.3 [3.5]	6.7 [3.1]	0.001	3.8 [1.4 to 6.8]	-5.8 [-8.8 to -3.4]
Pressure Time Integral (pti) (Ns/cm²)						
Toes 2-5	0.9 [0.9][ab]	0.6 [0.3]	0.5 [0.3]	0.001	0.3 [0.1 to -0.6]	-0.4 [-0.8 to -0.2]
Metatarsal 1	2.3 [1.4][b]	1.8 [0.9]	1.4 [0.6]	0.001	-	-0.8 [-1.2 to -0.1]
Metatarsal 2	2.7 [2.0][b]	2.5 [10]	1.9 [0.9]	0.001	-	-0.7 [-1.3 to -0.1]
Metatarsal 3	2.9 [2.2][b]	2.6 [1.0]	1.5 [2.0]	0.004	-	-0.8 [-1.5 to -0.1)

Metatarsal 4	2.3 [1.4][b]	2.3 [0.8]	1.7 [0.8]	0.001	-	-0.7 [-1.1 to -0.2]
Metatarsal 5	1.9 [1.4][b]	1.8 [07]	1.2 [0.8]	0.001	-	-0.7 [-1.1 to -0.3]
Mid Foot	1.7 [1.4][ab]	1.0 [0.5]	0.6 [0.4]	0.001	0.6 [0.3 to 1.0]	-1.0 [-1.3 to -0.7]
Medial Heel	2.3 [2.1][b]	2.0 [0.7]	1.5 [0.6]	0.001	-	-0.8 [-1.4 to -0.2]
Lateral Heel	2.2 [2.1][b]	2.0 [0.7]	1.5 [0.8]	0.001	-	-0.7 [-1.4 to -0.2]
Contact Area (ca) (cm²)						
Metatarsal 1	12.8 [5.0][ab]	11.1 [2.3]	10.1 [2.0]	0.001	-2.0 [-3.4 to -0.4]	-3.0 [-5.0 to -1.6]
Metatarsal 2	10.0 [3.2][b]	9.7 [1.8]	9.0 [1.3]	0.014	-	-1.2 [-2.4 to -0.3]
Mid Foot	37.4 [14.3][ab]	26.9 [10.4]	21.7 [12.4]	0.001	-8.4 [-13.5 to -3.7]	-15.1 [20.0 to -9.7]
Medial Heel	17.4 [3.3][b]	16.1 [3.3]	15.1 [2.1]	0.001	-	-2.2 [-3.3 to-1.2]
Lateral Heel	14.9 [1.9][b]	14.0 [3.1]	13.5 [1.9]	0.018	-	-1.6 [-2.5 to -0.7]

Legend: Data displays median and interquartile range [IQR] values based on the ulcerated and non-ulcerated feet of the DFU group compared to the reported maximum values in the DMC and HC groups. The Kruskal-Wallis test was used for all between group comparisons with a significance of $p<0.05$ and these are the main p-values reported. The Man Whitney U test was used for Posthoc comparisons for all significant outcomes. [a]= $p<0.05$ when compared to the DMC group in post-hoc analyses [b] =$p<0.05$ compared to the HC group in post hoc analyses. - = not statistically significant on Posthoc analyses and therefore median differences were not reported. Hodges-Lehmann estimates of the median difference and 95% confidence intervals are reported.

Appendix Table 20 Post-hoc test results and odds ratios from binary logistic regression.

	Median difference DFU vs. DMC [95% CI of difference]	OR [95% CI]	Median difference DFU vs. HC [95% CI of difference]	OR [95% CI]
Plantar Pressure (mpp)) (N/cm²)				
Toes 2-5	-0.8 [-1.5-(-)0.9]	2.2 [1.3-3.8]^	-1.0 [-1.9 to -0.5]	4.9 [1.3 to 18.0]^
Metatarsal 1	-		-1.5 [-2.7 to -0.5]	1.9 [1.1 to 3.4]^
Metatarsal 5	-		-1.1 [-2.0 to -0.1]	1.3 [0.9 to 2.0]
Mid Foot	-0.9 [-2.0-(-)0.3]	2.0 [1.3-3.2]^	-1.7 [-3.0 to -1.1]	2.5 [1.2 to 5.2]^
Medial Heel	-		-1.0 [-2.3 to -0.8]	1.6 [0.9 to 2.5]
Lateral Heel	-		-1.3 [-2.7 to -0.2]	1.6 [1.0 to 2.4]
Max Sensor Pressure (msp) (N/cm²)				
Toes 2-5	3.8 [1.1-8.6]	1.2 [1.1-1.4]^	-4.6 [-9.5 to -1.9]	1.5 [1.0 to 2.2]^
Mid Foot	3.8 [1.4-6.8]	1.3 [1.1-1.5]	-5.8 [-8.8 to -3.4]	1.3 [1.1 to 1.6]^
Pressure Time Integral (pti) (Ns/cm²)				
Toes 2-5	0.3 [0.1-0.6]	21.9 [4.0-120.0]^	-0.4 [-0.8 to -0.2]	19.2 [1.9 to 199.0]^
Metatarsal 1	-		-0.8 [-1.2 to -0.1]	2.3 [0.8 to 7.0]
Metatarsal 2	-		-0.7 [-1.3 to -0.1]	1.5 [0.8 to 2.8]
Metatarsal 3	-		-0.8 [-1.5 to -0.1)	1.5 [0.8 to 2.9]
Metatarsal 4	-		-0.7 [-1.1 to -0.2]	1.6 [0.8 to 3.3]

Metatarsal 5	-		-0.7 [-1.1 to -0.3]	2.0 [0.8 to 5.0]
Mid Foot	0.6 [0.3-1.0]	5.1 [1.8-14.6]^	-1.0 [-1.3 to -0.7]	5.9[1.5 to 23.8]^
Medial Heel	-		-0.8 [-1.4 to -0.2]	2.2 [1.0 to 5.0]
Lateral Heel	-		-0.7 [-1.4 to -0.2]	2.2 [1.0 to 5.0]
Contact Area (ca) (cm^2)				
Metatarsal 1	-2.0 [-3.4-(-)0.4]	1.3 [1.1-1.7]^	-3.0 [-5.0 to -1.6]	1.4 [1.0 to 1.9]^
Metatarsal 2	-		-1.2 [-2.4 to -0.3]	1.7 [1.1 to 2.8]^
Mid Foot	-8.4 [-13.5-(-)3.7]	1.1 [1.0-1.2]^	-15.1 [20.0 to -9.7]	1.2 [1.0 to 1.2]^
Medial Heel	-		-2.2 [-3.3 to -1.2]	1.8 [1.1 to 2.9]^
Lateral Heel	-		-1.6 [-2.5 to -0.7]	1.6 [1.0 to 2.4]

Legend: Data displays median and interquartile range [IQR] values based on the ulcerated and non-ulcerated feet of the DFU group compared to the reported maximum values in the DMC and HC groups. The Kruskal-Wallis test was used for all between group comparisons with a significance of $p<0.05$ and these are the main p-values reported. The Man Whitney U test was used for between two group comparisons for all significant outcomes. CI= Confidence Interval, OR= Odds Ratio, - = negative value. Odds Ratios were only computed from binary logistic regression analyses for variables which remained significantly different on post hoc comparisons. ^= Binary logistic regression analysis was significant at $p<0.05$ after adjusting for age, sex and BMI.

Appendix Table 21 Post-hoc test results and odds ratios from binary logistic regression and Cohen's d scores.

	Median difference DFU vs. DMC [95% CI of difference]	OR [95% CI]	Cohen's d	Median difference DFU vs. HC [95% CI of difference]	OR [95% CI]	Cohen's d
Plantar Pressure (mpp) (N/cm²)						
Toes 2-5	-0.8 [-1.5-(-)0.9]	2.2 [1.3-3.8]^	0.21	-1.0 [-1.9 to -0.5]	4.9 [1.3 to 18.0]^	0.40
Metatarsal 1	-			-1.5 [-2.7 to -0.5]	1.9 [1.1 to 3.4]^	0.36
Metatarsal 5	-			-1.1 [-2.0 to -0.1]	1.3 [0.9 to 2.0]	
Mid Foot	-0.9 [-2.0-(-)0.3]	2.0 [1.3-3.2]^	0.32	-1.7 [-3.0 to -1.1]	2.5 [1.2 to 5.2]^	0.63
Medial Heel	-			-1.0 [-2.3 to -0.8]	1.6 [0.9 to 2.5]	
Lateral Heel	-			-1.3 [-2.7 to -0.2]	1.6 [1.0 to 2.4]	
Max Sensor Pressure (msp) (N/cm²)						
Toes 2-5	3.8 [1.1-8.6]	1.2 [1.1-1.4]^	0.29	-4.6 [-9.5 to -1.9]	1.5 [1.0 to 2.2]^	0.33
Mid Foot	3.8 [1.4-6.8]	1.3 [1.1-1.5]		-5.8 [-8.8 to -3.4]	1.3 [1.1 to 1.6]^	0.80
Pressure Time Integral (pti) (Ns/cm²)						
Toes 2-5	0.3 [0.1-0.6]	21.9 [4.0-120.0]^	0.45	-0.4 [-0.8 to -0.2]	19.2 [1.9 to 199.0]^	0.60
Metatarsal 1	-			-0.8 [-1.2 to -0.1]	2.3 [0.8 to 7.]	
Metatarsal 2	-			-0.7 [-1.3 to -0.1]	1.5 [0.8 to 2.8]	
Metatarsal 3	-			-0.8 [-1.5 to -0.1)	1.5 [0.8 to 2.9]	
Metatarsal 4	-			-0.7 [-1.1 to -0.2]	1.6 [0.8 to 3.3]	
Metatarsal 5	-			-0.7 [-1.1 to -0.3]	2.0 [0.8 to 5.0]	
Mid Foot	0.6 [0.3-1.0]	5.1 [1.8-14.6]^	0.67	-1.0 [-1.3 to -0.7]	5.9[1.5 to 23.8]^	1.07
Medial Heel	-			-0.8 [-1.4 to -0.2]	2.2 [1.0 to 5.0]	
Lateral Heel	-			-0.7 [-1.4 to -0.2]	2.2 [1.0 to -5.0]	
Contact Area (ca) (cm²)						
Metatarsal 1	-2.0 [-3.4-(-)0.4]	1.3 [1.1-1.7]^	0.44	-3.0 [-5.0 to -1.6]	1.4 [1.0 to 1.9]^	0.70
Metatarsal 2	-			-1.2 [-2.4 to -0.3]	1.7 [1.1 to 2.8]^	0.41

Mid Foot	-8.4 [-13.5-(-)3.7]	1.1 [1.0-1.2]^	0.84	-15.1 [20.0 to -9.7]	1.2 [1.0 to 1.2]^	1.17
Medial Heel	-			-2.2 [-3.3 to -1.2]	1.8 [1.1 to 2.9]^	0.83
Lateral Heel	-			-1.6 [-2.5 to -0.7]	1.6 [1.0 to 2.4]	

Legend: Data displays median and interquartile range [IQR] values based on the ulcerated and non-ulcerated feet of the DFU group compared to the reported maximum values in the DMC and HC groups. The Kruskal-Wallis test was used for all between group comparisons with a significance of $p<0.05$ and these are the main p-values reported. The Man Whitney U test was used for between two group comparisons for all significant outcomes. CI= Confidence Interval, OR= Odds Ratio, - = negative value. Odds Ratios were only computed from binary logistic regression analyses for variables which remained significantly different on post hoc comparisons. ^= Binary logistic regression analysis was significant at $p<0.05$ after adjusting for age, sex and BMI. Cohen's d scores were calculated for the DFU group (DFU group- control).

Appendix Table 22 Paired analysis of mean peak pressure and pressure time integral characteristics of cases.

	DFU Group (n=21)		*p value*	*Corrected p-value*
	Ulcerated feet (n=21)	*Non-ulcerated feet (n=21)*		
Plantar Pressure (mpp) (N/cm²)				
Toe 1/ Hallux	3.6 [2.5-8.2]	5.0 [2.1-5.6]	*0.465*	*1.00*
Toes 2-5	3.0 [2.4-5.6]	3.1 [2.1-4.5]	*0.455*	*1.00*
Metatarsal 1	5.7 [4.5-8.5]	5.8 [4.4-7.0]	*0.232*	*1.00*
Metatarsal 2	6.8 [4.9-9.2]	6.6 [4.4-8.0]	*0.433*	*1.00*
Metatarsal 3	6.6 [4.7-9.9]	6.1 [4.7-6.8]	*0.191*	*1.00*
Metatarsal 4	5.7 [4.1-7.6]	5.0 [4.0-5.8]	*0.073*	*0.73*
Metatarsal 5	4.7 [3.4-6.2]	4.2 [2.6-5.6]	*0.126*	*1.00*
Mid Feet	3.8 [3.1-6.5]	3.2 [2.3-4.9]	*0.114*	*1.00*
Medial Heel	5.8 [4.5-9.2]	6.1 [4.0-7.1]	*0.159*	*1.00*
Lateral Heel	6.3 [4.8-9.6]	5.8 [4.0-4.6]	*0.279*	*1.00*
Pressure Time Integral (pti) (Ns/cm²)				
Toe 1/ Hallux	0.9 [0.5-1.9]	1.1 [0.7-1.6]	*0.794*	*1.00*
Toes 2-5	0.9 [0.6-1.4]	1.0 [0.6-1.1]	*0.601*	*1.00*
Metatarsal 1	2.3 [1.5-2.9]	2.1 [1.3-2.4]	*0.224*	*1.00*
Metatarsal 2	2.7 [1.7-3.7]	2.3 [1.7-3.2]	*0.217*	*1.00*

Metatarsal 3	2.9 [1.7-3.9]	2.5 [1.5-3.2]	*0.110*	*0.77*
Metatarsal 4	2.3 [2.0-3.3]	2.1 [1.1-3.0]	*0.058*	*0.46*
Metatarsal 5	1.9 [1.3-2.7]	1.6 [0.8-2.3]	*0.017*	*0.17*
Mid Feet	1.7 [1.2-2.5]	1.3 [0.8-1.8]	*0.030*	*0.27*
Medial Heel	2.3 [1.5-3.6]	2.3 [1.5-3.1]	*0.467*	*1.00*
Lateral Heel	2.2 [1.5-3.6]	2.3 [1.4-3.1]	*0.296*	*1.00*

Legend: Data displays median and interquartile range [IQR] values based on the ulcerated and non-ulcerated feet of the DFU group. The Wilcoxon Signed Rank test was used to compare differences between the ulcerated and non-ulcerated feet of cases. The corrected p-value indicates p-values after performing the Holm step-wise correction for multiple testing. A significance of $p < 0.05$ was used throughout.

Appendix Table 23 Paired analysis of estimated vertical ground reaction force, contact area and maximum sensor pressure characteristics of cases.

	DFU Group (n=21)		*p value*	*Corrected p-value*
	Ulcerated feet (n=21)	*Non-ulcerated feet (n=21)*		
Max Sensor Pressure (msp) (N/cm²)				
Toe 1/ Hallux	17.9 [7.4-30.7]	16.49 [7.8-24.7]	*0.414*	*1.00*
Toes 2-5	11.7 [8.3-23.9]	10.9 [8.5-16.0]	*0.498*	*1.00*
Metatarsal 1	19.5 [10.8-27.5]	12.9 [10.7-16.5]	*0.050*	*0.05**
Metatarsal 2	18.0 [12.7-21.4]	16.2 [11.3-21.2]	*0.639*	*1.00*
Metatarsal 3	17.0 [12.3-24.3]	14.1 [9.9-21.3]	*0.205*	*1.00*
Metatarsal 4	14.1 [10.6-15.9]	10.8 [7.4-16.1]	*0.205*	*1.00*
Metatarsal 5	11.7 [8.1-15.8]	8.4 [5.5-13.8]	*0.068*	*0.54*
Mid Feet	12.4 [9.4-19.4]	9.9 [6.3-13.8]	*0.058*	*0.52*
Medial Heel	17.1 [11.9-26.7]	15.6 [8.7-22.1]	*0.159*	*1.00*
Lateral Heel	16.2 [11.4-28.2]	15.2 [8.8-19.5]	*0.181*	*1.00*
Contact Area (ca) (cm²)				
Toe 1/ Hallux	11.0 [8.4-13.3]	11.0 [8.3-13.2]	*0.205*	*1.00*
Toes 2-5	10.1 [8.0-11.7]	7.4 [6.0-11.6]	*0.099*	*0.89*
Metatarsal 1	12.8 [10.2-15.2]	9.4 [7.4-14.1]	*0.144*	*1.00*
Metatarsal 2	10.0 [8.7-11.9]	9.3 [7.8-11.4]	*0.187*	*1.00*

Metatarsal 3	8.9 [7.5-9.7]	8.3 [6.5-9.6]	*0.106*	*0.89*
Metatarsal 4	9.5 [8.5-10.3]	9.1 [6.8-10.4]	*0.076*	*0.76*
Metatarsal 5	8.5 [6.6-10.6]	8.6 [7.4-10.2]	*0.986*	*1.00*
Mid Feet	37.4 [28.1-42.4]	30.1 [25.5-38.0]	*0.232*	*1.00*
Medial Heel	17.4 [15.5-18.8]	16.3 [13.3-18.7]	*0.375*	*1.00*
Lateral Heel	14.9 [13.8-15.7]	14.3 [10.8-17.2]	*0.330*	*1.00*

Legend: Data displays median and interquartile range [IQR] values based on the ulcerated and non-ulcerated feet of the DFU group. The Wilcoxon Signed Rank test was used to compare differences between the ulcerated and non-ulcerated feet of cases. The corrected p-value indicates p-values after performing the Holm step-wise correction for multiple testing. A significance of $p < 0.05$ was used throughout.

Appendix I: Extended results and statistical outcomes for Chapter 8

This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed

This raw data has been removed

This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed

This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed


This raw data has been removed

This raw data has been removed


This raw data has been removed


This raw data has been removed

This raw data has been removed

This raw data has been removed


This raw data has been removed

This raw data has been removed

This raw data has been removed


This raw data has been removed

This raw data has been removed

This raw data has been removed

This raw data has been removed


This raw data has been removed

Appendix J: Copyright and Permissions

For Figure 1.1

For Figure 1.2

For Plate 1.1