BUILDING THE REPERTOIRE OF NEUROMUSCULOSKELETAL MODELLING IN CEREBRAL PALSY… ONE STEP AT A TIME

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Written patient consent has been received and archived for the research involving patient data reported in this thesis.

The work described in this thesis was funded by the Royal Australasian College of Physicians AFRM Ispen Open Research Fellowship.

This thesis contains published work and/or work prepared for publication, some of which has been co-authored.

PhD Candidate Signature (Caroline Alexander)
DECLARATION FOR THESIS CONTAINING PUBLISHED WORK AND WORK PREPARED FOR PUBLICATION

This thesis contains published work and/or work prepared for publication, some of which has been co-authored. The work involved in designing the studies described was performed primarily by Caroline Alexander (candidate). The thesis outline and experimental design was planned and developed by the candidate, in consultation with Senior Lecturer Siobhan Reid, Senior Lecturer Cyril J Donnelly, Professor Catherine Elliott, and Ms Katherine Stannage (the candidate’s academic and clinical supervisors), along with Doctor Jane Valentine (Head of Paediatric Rehabilitation at Princess Margaret Hospital for Children, from where participants were recruited).

The candidate was responsible for all recruitment, participant management, data collection and analysis. The candidate drafted the original thesis chapters as well as papers arising from this thesis that have been published or prepared for future publication. Senior Lecturer Siobhan Reid, Senior Lecturer Cyril J Donnelly, Professor Catherine Elliott, Ms Katherine Stannage and Doctor Jane Valentine provided guidance on data collection, data analysis and all drafts associated with the thesis until the examinable version was finalised.

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Accepted for publication in ‘Journal of Biomechanics’, January 2017

### LOCATION IN THESIS: Chapter Three

### STUDENT CONTRIBUTION TO WORK: The candidate was responsible for method development, data analysis and manuscript preparation.

**CO-AUTHORS:** Lum, I., Reid, S, Clarke, E., Stannage, K., El-Sallam Abd, A., Herbert, R.D., Donnelly, C.J.

### DETAILS OF THE WORK: Children with cerebral palsy have larger in-vivo and linearly scaled Achilles tendon moment arms than typically developing children.

Submitted for publication in ‘Journal of Biomechanics’, August 2017

### LOCATION IN THESIS: Chapter Four

### STUDENT CONTRIBUTION TO WORK: The candidate was responsible for all recruitment, participant management, data collection and analysis, and manuscript preparation.

**CO-AUTHORS:** Reid, S., Stannage, K., Elliott, C., Valentine, J., Dwyer, B., Donnelly, C.J.
### DETAILS OF THE WORK: An Open Source Vector Field Statistic Template for Clinical Gait Reporting

In preparation for publication in ‘Gait and Posture’

**LOCATION IN THESIS:** Chapter Seven

**STUDENT CONTRIBUTION TO WORK:** The candidate was responsible for the development of the template, including the output content and presentation, data collection and analysis and manuscript preparation.

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**LOCATION IN THESIS:** Chapter Five

**STUDENT CONTRIBUTION TO WORK:** The candidate was responsible for all recruitment, participant management, data collection and analysis, and manuscript preparation.

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Abstract

Cerebral palsy (CP) is the most common childhood physical disability treated in paediatric rehabilitation programs [1]. The impairments associated with the disorder have significant effects on all components of functioning, particularly gait [2]. This is particularly true when considering the plantarflexor muscle group at the ankle, which is frequently implicated among children with CP [2] and is a critical muscle group in regulating gait function [3]. As there is no known cure for CP, treatments aim to optimise growth and development of function. Conventional 3D gait analysis (3DGA) is commonly used to objectively assess gait function, enabling individualised clinical care plans [2, 4]. Neuromusculoskeletal modelling is a sophisticated tool that, in combination with 3DGA, has the potential to provide further insight into the mechanisms underlying pathological gait, thereby optimising outcomes for individual patients [5]. Current models, however, do not accurately reflect the complexity and variability of musculoskeletal impairments seen among children with CP [5, 6].

The overarching aim of this thesis is to assess differences in musculoskeletal geometry and muscle tendon structure at the ankle in a way that both builds on the repertoire of neuromusculoskeletal models for children with CP, and directly informs current clinical practice. The second aim of this research is to develop a statistical framework specifically for clinically relevant analysis of the complex biomechanical outputs from neuromusculoskeletal modelling that could also be utilised directly in current clinical 3DGA practices.

This doctorate is written as a series of research papers, each building on the knowledge base required to develop neuromusculoskeletal models appropriate for children with CP. Following a review of the literature, chapter three validates a novel technique for assessing 3D Achilles tendon moment arm (ATMA) using common clinical Magnetic Resonance Imaging (MRI) scans. Using a previously validated dynamic MRI technique for comparison, this chapter demonstrates that 3D ATMA can be accurately estimated from a single, static MRI scan at a fixed joint angle. This method overcomes many of the barriers to assessing in-vivo 3D ATMA in children with CP, which to date has not been assessed in any published research. The ability to take subject specific 3D ATMA measurements in clinical settings will enhance both the specificity of neuromusculoskeletal models for clinical decision making, as well as the understanding of the mechanisms behind functional pathologies at the ankle.

Once the validity of this 3D ATMA measurement technique was established, the method was applied, in chapter four, to compare 3D ATMA in children with CP with typically developing (TD) children, as well as adults. Children with CP were found to have larger ATMA compared to their TD counterparts, possibly due to alterations in bony structure leading to altered joint axes of rotation. The findings are contrary to what was hypothesised, and may reveal important
mechanisms relating to the functional pathology of the ankle. These results suggest the plantarflexor muscle function may be more impaired than is currently believed, as the advantage of an increased ATMA may mitigate the muscular impairments when assessed by joint kinetics. The secondary aim of this chapter is to assess the validity of using linear scaling of an existing generic neuromusculoskeletal model among children with CP. As hypothesised, the results indicated that linear scaling of existing models will result in statistically and clinically significant differences in ATMA. This work demonstrates that linear scaling will result in overestimation of muscle force profiles contained within the plantarflexor group. This is not trivial, as it may lead to incorrect assumptions around muscle function, and have unintended negative impacts on clinical intervention decision making.

Chapter five addresses the potential implications of a common treatment, Botulinum Toxin Type A (BoNT-A) on muscle morphology parameters. Again utilising MRI for measurement, this chapter is concerned with changes in muscle volume of both the BoNT-A targeted, and the synergistic muscles of the lower limb. As hypothesised, BoNT-A treatment had significant effects on muscle volume that are dependent on the time of assessment relative to treatment. This chapter is the first research to track changes in muscle morphology from the first injection, including alterations in synergistic muscles, suggesting that functional benefits of BoNT-A are significant, although consideration should be given to the timing of repeat injections relative to muscle volume recovery. The chapter demonstrates the variable nature of the disorder, and the significant impact interventions may have on musculoskeletal parameters during child development. This highlights the importance of developing neuromusculoskeletal models that are highly subject specific to accurately represent the individual patient, thus allowing optimal intervention and prescription.

Shifting the focus from the inputs of neuromusculoskeletal model to data outputs, chapter six explores a number of statistical methods that may be used to overcome many of the challenges faced by the analysis of complex time varying biomechanical data. The chapter aims to identify a tool that is statistically robust and appropriate, is computationally effective to run, and provides outputs that retain clinical meaningfulness. After first identifying the challenges and requirements of a statistical tool, four methods are considered for their ability to meet these requirements: Bootstrap Confidence Bands, Principal Component Analysis, Functional Data Analysis and Vector Field Statistics. Through this investigation, Vector Field Statistics (the application of Statistical Parametric Mapping (SPM)) was identified as the methodology that best meets the scope required by a statistical tool for use in a clinical setting, with evidence provided to support this claim.

Finally, chapter seven presents a case study demonstrating the use of vector field statistics in clinical gait reporting. The report template was developed for kinematic outputs from standard
3DGA, to allow for immediate application within current clinical practice. This chapter also presents, for the first time, effect magnitude statistics, derived from the SPM statistic, to objectively quantify the overall differences observed in each parameter. The case study is that of a 12 year old boy with spastic diplegia, functioning at a Gross Motor Functional Classification Scale level III, who underwent 3DGA prior to BoNT-A treatment. The results reveal statistically significant changes in kinematic traces that supported the clinical interpretation of the case. The SPM analysis on the 2D vectors, combined with the effect magnitudes, also provides more accurate, objective assessment of knee function on the left and right sides, despite concerns that poor anatomical coordinate system definitions resulted in a varus/valgus artefact. The use of vector field statistics, in addition to analysis on the individual components, as highlighted in this case study, has significant potential to overcome procedural limitations of assessing gait pathology using 3DGA. It is a viable clinical tool and highly applicable within clinical research settings.

This research contributes to the understanding of musculoskeletal geometry among children with CP, and the impact of interventions on the alterations of muscle-tendon properties over time. Significant differences in musculoskeletal structure and geometry are apparent between children with CP and TD controls, while interventions such as BoNT-A may also contribute to alterations in musculoskeletal structure. The results of this research, along with contributing to the knowledge base required to develop appropriate neuromusculoskeletal models for children with CP, have direct application and significance to current clinical care. Furthermore, this thesis presents novel methods developed for direct application into current clinical practices, and may directly impact on clinical decision making and ultimately patient outcomes.

REFERENCES

I have been so fortunate to be supported by the most incredible people and groups throughout this journey. Firstly, I must thank those who have supported me financially: the Australian Government Research Training Program (RTP) Scholarship; the University of Western Australia Safety Net Top-Up Scholarship, and the Ernest and Evelyn Havill Shacklock Scholarship. Without this support, none of this would have been possible.

To my supervisors:

Throughout this journey I have been mentored and guided by an incredible team of academic and clinical supervisors. People who care deeply about the children we work with and the integrity of research. People who valued and respected me as a person as much as a student. I will forever be indebted to each and every one of them.

Si – Six years ago I walked in to your office, as a young undergraduate visiting from New Zealand, and we spoke about the possibility of me coming to UWA for one year as your honours student. Today, I sit in your office, my swollen feet up on a chair, as we flip between discussing the final adjustments on my PhD thesis, and you being on call to look after Agnes when I go into labour. Never could I have imagined, all those years ago, what a pivotal role you would play in my life, both academic and otherwise. Thank you for helping shape this PhD into what it is today, for being a constant source of knowledge and wisdom, reason and sanity.

Jon – I am so fortunate you came on board as a supervisor in those early days. Thank you for encouraging and inspiring me to look outside the box when thinking about the analysis of gait pathology, and for sharing the knowledge and expertise that allowed me to do that. It is through you that I have been fortunate enough to collaborate with some incredible individuals and I am incredibly grateful for those opportunities.

Cath – Like Si, you have been on this journey with me every step of the way, and I couldn’t have asked for anything better. Thank you for guiding me through the crazy world of research, from ethics applications, to the first chaotic data collection (sorry I set all the alarms off!), to managing patients and their participation. Thank you for sitting me down half way through the proposal process to ask what I wanted out of this PhD, and then ensuring my PhD was constructed in a way that helped me achieve those research and career goals.

Kate – It is an incredible thing, to be mentored and supervised by someone who you respect and admire on so many levels. Thank you for your constant support over the last five years, for encouraging me to pursue my academic, career and personal goals, and for the insight and passion you brought to every meeting. Your input into the creation of this thesis, from guiding the research
development, to editing work (whether it be removing two commas, or suggesting a complete overhaul) has been invaluable.

Jane – Thank you for welcoming me in to the research team at Paediatric Rehabilitation all those years ago and ensuring no stone was left unturned as I navigated my way through conducting and reporting research in a clinical setting ever since. Despite your insanely hectic and demanding schedule, you always found the time for reading through stacks of patient files looking for illusive clinic notes with me, identifying eligible patients, and sending me the latest references of interest, and for that I am truly grateful.

To the clinical teams:

None of this would have been possible without the incredible teams I have worked with at PMH. The Department of Paediatric Rehabilitation and the Department of Diagnostic Imaging at Princess Margaret Hospital. Thank you for your support and guidance in this research, from protocol development, to patient recruitment and management, to data collection. Your passion and dedication to the field and the families we work with is a constant source of inspiration.

To those that this research is for:

The children and their families, thank you for the value you placed in this work. Thank you for giving up your weekends, for your enthusiasm at being involved in these studies, and for your patience and understanding when things didn’t run so smoothly. To participate in research requires some level of sacrifice, whether it be driving hours to attend assessments, or missing out on a birthday party, but you always showed up with a smile, and for that I will always be grateful.

To my village:

They say it takes a village to raise a child. It certainly has taken a village to finish a PhD.

My fellow PhD students, the PMH Peer Support group and the members of The 1.55 Office. Thank you for always being there to celebrate, to commiserate and to procrastinate. A PhD can sometimes feel like a lonely battle, but I was so lucky to have each and every one of you battling alongside me. Thank you especially to Claire, Jess and Kirsty. Each of you have helped make this thesis what it is, in very different, but equally important ways. I could not have done it without you.

To my cousin Carl, thank you for proof reading this thesis in its entirety, from the other side of the globe, when you had much bigger and better things to be doing. Thank you for your constant enthusiasm and for teaching me how to use spell check properly.

Mum and Dad. Even from across the ditch, your support of me and this crazy endeavour is relentless. Thank you for never doubting me, for never holding it against me that I extended my
“one year trip” indefinitely, for printing off countless pages to mark up with your red pen, and for constantly being two of my biggest fans. While you may not be next door, I know without a shadow of doubt you will be here at a moment’s notice when I need you. Thank you for showing me what an incredible, supportive, loving parent is. I can only hope I can live up to the bar you both have set.

Agnes and bump – Agnes, bubub, thank you for being my sidekick earth-side over the last 14 months. You won’t ever remember how the first year of your life was filled with hanging out on the office floor of whoever I was meeting with that day, or bouncing on my knee as I wrote or ran code, but I will never forget it, and will always be grateful that you not only tolerated every minute of it, you gave me a reason to smile all along the way. Bump, my sidekick womb-side for the last 8 months: thank you for giving me the deadline to finish, and for not making me as ill as your sister did so that I could meet that deadline. I cannot wait to meet you little one. To both of you: thank you for the privilege of being your mum. I love you both more than you will ever know.

And most importantly, to Adam – Words. Despite finding 60,000 of them for this thesis, I can’t find the right ones to put here. Without your constant support, reassurance and reality checks, I would not have made it here in one piece. I will never forget the sacrifices you have made for me to do this PhD, from the day you agreed to move to Perth until the day it finished, and the dining table was reinstated for eating, not my late night typing. Thank you for never letting me drown in my research, for being my rock, my biggest advocate and my constant reminder of what is truly important in life. Thank you for being the most incredible best friend, husband and father. I’m so lucky to be on this journey with you. Forever.
Publications and Abstracts

The following is a list of publications and abstracts to which the candidate has contributed during the course of her candidature, arising both directly and indirectly from this thesis. Published manuscripts and all abstracts are included in the Appendices section of this thesis. Note, some publications and abstracts were made under the candidate’s maiden name of Caroline F Davis.

PEER REVIEWED PUBLICATIONS


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List of Abbreviations

0D – Zero-dimensional
1D – One-dimensional
2D – Two-dimensional
3D – Three-dimensional
3DGA – Three-dimensional gait analysis
6MWT - Six Minute Walk Test
aCSA – Anatomical cross sectional area
ATMA – Achilles tendon moment arm
BoNT-A – Botulinum Toxin Type A
CI – Confidence intervals
CNS – Central Nervous System
CP – Cerebral Palsy
CSA – Cross sectional area
CT – Computed tomography
EM – Effect magnitude
EMG – Electromyography
FoV – Field of View
fPC – Functional principal components
GDI – Gait Deviation Index
GLM – General Linear Model
GMFCS – Gross Motor Function Classification System
GPS – Gait Profile Score
GRF – Ground reaction force
HJC – Hip joint centre

ICC – Intra-class correlation coefficient

ICF – International Classification of Functioning, Disability and Health

LoA – Limits of Agreement

MAP – Movement Analysis Profile

MRI – Magnetic Resonance Imaging

MV – Muscle volume

PCA – Principal Component Analysis

pCSA – Physiological cross sectional area

PMH – Princess Margaret Hospital for Children

PNS – Peripheral Nervous System

RE – Relative effect

RFT – Random Field Theory

SDR – Selective Dorsal Rhizotomy

SPM – Statistical Parametric Mapping

TD – Typically developing

TUG – Timed “Up and Go”

WGH – Winters, Gage and Hicks
1.1 INTRODUCTION

Cerebral Palsy (CP) is a lifelong disorder of movement and posture caused by a lesion to the developing brain [1]. In Australia, the incidence of CP has been estimated to be 1.4 - 2.1 cases in every 1,000 live births [2], and clinically it makes up the largest diagnostic group treated in paediatric rehabilitation. The economic burden of CP is not insignificant, with research suggesting the lifetime cost (incorporating healthcare, productivity and social costs) to be between AUD$1,200,000 and AUD$1,300,000 [3, 4]. The manifestation of CP has considerable variability within the population, and is a result of both the extent and location of the underlying brain lesion, as well as the timing of when that lesion was acquired relative to brain development. As such, CP is considered an “umbrella term”, which covers a large array of specific disorders. It is, therefore, an inherently complex and variable disorder with many potential permutations and combinations of features.

It is accepted, however, that growth and development of the musculoskeletal system in the presence of abnormal neural activity leads to the development of secondary musculoskeletal abnormalities. There have been well documented musculoskeletal alterations from the level of the muscle fibre up to the gross muscle function level. In children with spastic CP, alterations in muscle fibre type, and selective fibre atrophy [5-7], increased intramuscular collagen content [5], reduced muscle volume and cross sectional area [8], fixed shortening of the muscle-tendon unit [9], increased muscle tendon lengths [10, 11], reduced strength [12, 13] and increased involuntary activation [14, 15] have all been identified. Musculoskeletal parameters such as these have direct and significant implications for a range of properties including contractile strength and velocity, range of motion, functional strength and ultimately gait characteristics [16]. Indeed, musculoskeletal abnormalities are found to be correlated with gross motor function in this population [5, 8, 17]. Along with the various types and degrees of abnormalities, the site of the abnormalities also play a role. In children with CP, the gastrocnemius, which acts as a plantarflexor about the ankle, is the most commonly affected muscle group [18]. This muscle plays a crucial role in gait mechanics [19], and is therefore of primary concern for this population.

Although the brain lesion associated with CP is non-progressive, the musculoskeletal pathologies and their functional consequences are progressive [1, 18], and deterioration is gait expected without intervention [20]. Maintenance or improvement of gait is, logically, a primary goal in the clinical management of CP; thus musculoskeletal abnormalities that impact upon gait are frequently the target for intervention [18]. To facilitate this, three-dimensional gait analysis (3DGA) is employed to understand the specific gait pathology of each individual. Reflective of
conventional clinical gait analysis, the term 3DGA is used within this thesis to refer to the assessment of joint kinematics and kinetics during walking gait.

The uptake of 3DGA has led to significant improvements in surgical outcomes [21, 22], however is limited in its ability to reveal the causal mechanisms behind the gait pathology. Neuromusculoskeletal modelling is a sophisticated tool that takes 3DGA data, in combination with various subject specific musculoskeletal parameters, to estimate variables such as muscle activation and muscle forces [23]. An enhanced understanding of the underlying causes of the outward gait pathology would be of undeniable benefit to surgical decision making. Furthermore, it can be used to simulate alterations, such as surgical interventions, and may therefore facilitate more accurate predictions of surgical outcomes, and adjunct rehabilitation to maximise or optimise function outcomes.

1.1.1 STATEMENT OF THE PROBLEM

Despite the obvious potential for neuromusculoskeletal modelling within clinical CP settings, there has been limited uptake. This is due to many factors including the time and financial costs of research and validation of models in clinical settings, and because current models not accurately represent children with CP [24]. As mentioned above, CP affects the musculoskeletal system at many different levels, from the fibre level to the musculoskeletal geometry [18]. For a model to be valid, and therefore useful, the specificity of the model must align with the individual being assessed. Current models, however, are typically based on a small sample of healthy adults’ cadavers [25], which are unlikely to reflect myriad musculoskeletal pathologies present in children with CP. Furthermore, current methods for obtaining musculoskeletal parameters, such as musculoskeletal geometries, may not be feasible for children with CP, or practical in clinical settings, which together have impeded the wide scale uptake within standard clinical 3DGA and modelling procedures.

Along with the known fact that neuromusculoskeletal models in general do not reflect a child with CP, the analysis of generated outputs, such as muscle force estimates over the gait cycle, is also lacking. This is not unique to neuromusculoskeletal modelling however, as the same can be said for the analysis of conventional 3DGA data. Within research settings, simple statistical methods are often employed [26] that do not accurately reflect the complexity of biomechanical data [27], while clinical settings use subjective methods for identifying differences [28]. Clinicians and researchers spend time, money and effort to obtain high fidelity, time varying data from 3DGA and neuromusculoskeletal modelling. However, the methodologies employed for the analysis of the data is neither objective nor robust against potential biases, introducing risk for errors. This potentially impacts on the success of 3DGA and neuromusculoskeletal modelling in achieving the goal it is being applied to, and may result in suboptimal clinical management decisions being made.
1.1.2 STUDY AIMS

The overarching aim of this thesis was to assess differences in musculoskeletal geometry and muscle tendon structure in a way that both builds the repertoire of knowledge informing neuromusculoskeletal models for children with CP, and directly informs current clinical practice.

The second aim of this research was to develop an appropriate statistical method for the handling of the complex time varying biomechanical outputs from neuromusculoskeletal modelling that could also be utilised directly in current clinical 3DGA practices.

1.2 THESIS OUTLINE

This introduction briefly establishes the context for this thesis, communicating the research problem and the approach taken to address this problem. The majority of this thesis is presented as a series of individual papers, born out of two research studies. The first study, from which chapters three and four are developed, is a cross sectional study assessing differences in musculoskeletal geometry. Specifically assessing differences in Achilles tendon moment arm (ATMA), chapter three demonstrates in healthy controls the development and validation of a novel assessment technique using Magnetic Resonance Imaging (MRI) scan protocols that are achievable and practical among children with CP. Chapter four then utilises this technique to compare ATMA among children with CP, typically developing (TD) children, and healthy adults. In-vivo moment arms are also directly compared to estimates from a generic scaled neuromusculoskeletal model to assess validity and specificity of common scaling methods. The second study, from which data for chapters five and seven are derived, is a longitudinal study assessing the morphological and functional implications of a common treatment intervention, Botulinum toxin type A (BoNT-A). Chapter five addresses alteration in muscle morphology, while chapter seven presents a new statistical method, vector field analysis, for the exploratory analysis of differences in gait function compared to normative populations. Each chapter stands alone, with full references provided at the conclusion of each, and makes significant contributions to both the future development of neuromusculoskeletal models for children with CP, as well as current clinical practice. The thesis is concluded with a synthesis of results and a discussion.

The University of Western Australia supports the submission of PhD theses that comprise a series of papers prepared for publication. This structure has been adopted by the candidate in the submission of this thesis. As such, while the theoretical linking between the studies (i.e. chapters in this thesis) should be made clear for the examiner, each study must be stand-alone in content. Consequently, theses adopting a series of papers approach sometimes result in repetition of methodology from study to study. Please note that, where possible, reference to previous papers has been undertaken, however at times the examiner may find some repeated methodology redundant in the course of reading.
1.2.1 CHAPTER TWO
LITERATURE REVIEW

The literature review builds further on the thesis context established in the introduction through a comprehensive review of the current published research. Starting with a brief introduction of CP, the literature review focuses on the assessment of gait for children with CP, and the potential benefit of neuromusculoskeletal modelling for enhancing our understanding of gait mechanics. The elements of a neuromusculoskeletal model are detailed, along with a brief synopsis on the myriad ways CP may affect the specificity of various model elements. Following this, the ways in which neuromusculoskeletal models for children with CP may be improved are explored. Finally, the handling of data outputs from neuromusculoskeletal models is addressed through exploration of statistical approaches to understand the complexity of 3DGA data.

1.2.2 CHAPTER THREE
A SIMPLE BUT RELIABLE METHOD FOR MEASURING 3D MOMENT ARM GEOMETRY FROM A SINGLE, STATIC MAGNETIC RESONANCE SCAN

The aims of this chapter are to:

- Develop a simple and reliable participant specific \textit{in-vivo} three-dimensional (3D) ATMA method from a static clinical MRI scan (static ankle position, and a single scan sequence).
- Validate the simple static method with a previously validated 3D dynamic method.

It is hypothesised that:

- The novel method will be reliable at estimating \textit{in-vivo} 3D ATMA from a single clinical MRI scan in healthy adults and TD children.
- The method will be valid compared to a previously validated dynamic 3D method.

1.2.3 CHAPTER FOUR
CHILDREN WITH CEREBRAL PALSY HAVE LARGER \textit{IN-VIVO} AND LINEARLY SCALED ACHILLES TENDON MOMENT ARMS THAN TYPICALLY DEVELOPING CHILDREN.

The aims of this chapter are to:

- Compare \textit{in-vivo} 3D ATMA estimates between adults, TD children and children with CP.
- Compare 3D ATMA derived from an established musculoskeletal model linearly scaled to tibia length with participant specific \textit{in-vivo} 3D ATMA for adults, TD children, and children with CP.

It is hypothesised that:
• Adults will possess the largest 3D ATMA estimates, while children with CP will have the smallest.
• No differences in 3D ATMA estimates will be observed between methods for the adult population, however significant differences will be found between methods for both paediatric groups.

1.2.4 CHAPTER FIVE
MUSCLE VOLUME ALTERATIONS IN CHILDREN WITH CEREBRAL PALSY FOLLOWING THE FIRST BOTULINUM TOXIN TREATMENT: A 6 MONTH PROSPECTIVE COHORT STUDY.

The aim of this chapter is to:

• Assess muscle volume change, using MRI, of the injected and synergistic muscles, following the first exposure to clinically given BoNT-A in children with CP.

It is hypothesised that:

• The injected gastrocnemius muscle will show atrophy at each time point, with the greatest atrophy expected at 3 months post injection.
• Hypertrophy will be found in the synergistic muscles of the soleus and medial hamstring group at each time point.

1.2.5 CHAPTER SIX
STATISTICAL METHODOLOGIES FOR ANALYSIS OF TIME VARYING WAVEFORMS IN CLINICAL GAIT ANALYSIS

This chapter steps through the rationale behind the selection of vector field statistics for the statistical analysis of 3DGA data. It outlines the other potential options considered, and their merits and limitations, with specific reference to use in a clinical setting. This chapter acts as an extended methodology detailing the theoretical basis of vector field statistics.

1.2.6 CHAPTER SEVEN
VECTOR FIELD STATISTICS FOR CLINICAL GAIT REPORTING: A CASE STUDY

The aim of this chapter is to:

• Present a case study demonstrating the application of a vector field statistics template for a full lower limb kinematic 3DGA data set in a clinical setting.

It is hypothesised that:

• Vector field statistics will provide objective support to subjective interpretation of kinematic variables.
Vector field statistics will provide additional information, of clinical relevance, beyond that obtained by scalar field analysis.

1.2.7 CHAPTER EIGHT

SYNTHESIS OF RESULTS AND CONCLUSIONS

The final chapter aims to provide an overall synthesis of the results presented in the thesis by integrating the major findings of each chapter. It also provides an overall conclusion of the research in its entirety. Finally, this chapter outlines the clinical applicability and significance of the research contained in this thesis, and suggestions for future research directions.

1.3 LIMITATIONS AND DELIMITATIONS

1.3.1 LIMITATIONS

As with many disorders, CP manifests differently in each patient. The individual experience of CP, including the pathology itself and the interventions used, results in limitations when comparing group data, which are the reality of working with a clinical population such as this. Typically, a highly variable sample may be overcome by a large sample size. However, this was not possible within the constraints of this research where it relates to BoNT-A treatments. Over the two-year time frame of participant recruitment, a total of 22 patients attending the Rehabilitation Mobility Service at Princess Margaret Hospital met the inclusion criteria, with 13 consenting to and attending the baseline appointment. The primary reason for the low rate of recruitment is that BoNT-A is best practice care in Australia, and as a result, the majority of children are not toxin naïve at age 4 years. Further drop out, missing data, and exclusions on clinical grounds were apparent following the baseline assessments. The resultant small sample size is a limitation to this research.

While conventional 3DGA includes both joint kinematic and kinetic analysis, the application of vector field statistics was developed for kinematic outputs only. To achieve stable variance and appropriate statistical power, 15 – 30 trials are required for analysis of 3D vectors. This was not practical or achievable within the constraints of the data collection protocol employed, which took place in a clinical 3DGA laboratory with two in-ground force plates. Rates of successful foot strike on a force plate for young children, or children with reduced stride lengths, are often low. As a result very large numbers of trials are required to obtain sufficient successful trials to run full kinetic analysis, which was not feasible for young children or those who fatigue quickly within this study. While this research has not explicitly developed templates for the use of vector field statistics on joint kinetics, the templates developed could be easily extended to include joint kinetics for future applications.

Finally, the exclusion of strength assessments is a significant limitation to this research. The first five chapters of this thesis are concerned with musculoskeletal parameters about the ankle that
are likely to impact upon plantarflexor strength and function. Strength assessments would allow more specific conclusions about the operating characteristics of a muscle in relation to altered 3D ATMA and muscle morphology, and the functional implications of altered operating characteristics. This relationship may contribute to how such parameters may be included in a CP specific neuromusculoskeletal model. This omission was not taken lightly, however the lack of a reliable and valid way to assess isolated plantarflexor strength in children with CP led to the decision to exclude a direct strength measurement.

1.3.2 DELIMITATIONS
As these studies involved assessments for which a high level of understanding and compliance was required (MRI and 3DGA), the inclusion criterion for age was 4 years and older. In Western Australia, BoNT-A is prescribed to children with CP, where clinically indicated, from the age of 2 years. In addition to limiting the sample size attainable, this criterion also altered the characteristics of the sample in terms of clinical presentation. Those patients presenting late for first BoNT-A treatment likely do so due to delayed diagnosis. This may be a result of a mild presentation, a complex presentation that was initially misdiagnosed, or lack of early access to care due to physical, geographical or social circumstances. Regardless, a delayed clinical management plan is likely to result in increased incidence of fixed contractures or bony deformities. The response to BoNT-A exposure, therefore, cannot be generalised to younger children receiving first BoNT-A treatment.

Due to local constraints, acquisition of dynamic MRI scans, or static scans at multiple controlled joint angles, were not possible for children with CP. As such, validation of the 3D ATMA measurement technique was conducted on a pre-existing dataset from healthy adults. This also limited validity assessments of model-estimated ATMA at a standardised joint angle for children with CP.

1.4 SIGNIFICANCE OF THE STUDY
As reflected in the aims, this thesis has two major avenues for significant impact on current best clinical practice. Firstly, the research aims to inform the development of neuromusculoskeletal modelling practices that are appropriate for children with CP. This development is a massive undertaking, one that cannot be tackled by one thesis alone, or even one research group, but rather the collaboration of the entire international research community. However, the research contained in this thesis will contribute to the knowledge base that will see development take place through exploration of the variations in the morphology of the plantarflexor, and the geometry of the plantarflexor moment arms among children with CP.

Secondly, as is ethically responsible, each chapter in this thesis has direct application to current clinical practice. Assessment of musculoskeletal geometry among children with CP compared to TD children will allow clinicians to make informed decisions regarding surgical procedures that
may directly or indirectly impact on that geometry. Enhanced understanding of the effect of interventions on muscle-tendon structure, and the timeline for that effect, will contribute important information around the timing of subsequent interventions. Finally, the development of statistical methods to appropriately handle complex time varying biomechanical data can be used directly for the generation of 3DGA reports. Improved analysis of 3DGA data will directly benefit the planning and assessment of interventions, and ultimately patient outcomes. It is anticipated the results of this research will be disseminated widely to clinical staff responsible for the clinical management of children with CP.

1.5 REFERENCES

2.1 CEREBRAL PALSY

Cerebral palsy (CP) is a common neurodevelopmental disorder making up the largest childhood physical disability treated in paediatric rehabilitation programs. The term CP describes:

“a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain” [1].

The Australian CP register has reported the first ever drop in the incidence of CP, decreasing to 1.4-2.1 cases in every 1,000 live births [2], from 2.0-2.5 cases in every 1,000 live births in 2006 [3]. This declining prevalence in Australia is reflective of that found in recent research on European rates of incidence [4]. The lifetime economic costs of CP are significant, with economic reports suggesting the lifetime cost, including healthcare, productivity and social costs, to be between AUD$1,200,000 and AUD$1,300,000 [5, 6].

As an umbrella term, CP describes a large array of motor disorders that are frequently classified in three ways. Firstly, the neural subtype of CP is described. Neural subtype refers to the specific movement disorder present, with the most common subtypes being spastic CP and dyskinetic CP. Of the two, spasticity (a velocity dependent increase in the stretch reflex [7]) occurs in 72-91% of patients with CP [2, 3], and is more common than dyskinesia (sustained or intermittent repetitive muscular co-contractions [2, 8]). The second means for classifying children with CP is by topographical, or limb involvement. Unilateral CP, which includes monoplegia or hemiplegia, sees impairments predominantly on one side of the body. In contrast, bilateral CP, which includes diplegia, triplegia and quadriplegia, sees impairments on both sides of the body. Of these, hemiplegia (involvement of the upper limb and lower limb on the same side of the body) is the most common, with 21-40% of children with CP falling into this group [2, 3]. Diplegia (both lower limbs involved), is the next most common, with 13-36% of children being classified as diplegic [2, 3]. Finally, the functional capacity of the child is described. This is most typically done using the Gross Motor Function Classification System (GMFCS) to describe mobility [9] and the Manual Ability Classification System to describe upper limb function [10]. Both systems have five levels, with level I describing the highest functioning children, to level V describing children with the least level of independent function. Within the GMFCS, children classified as levels I-III display some level of ambulation, but walking aids may be required in some contexts [9]. While independent ambulation may be achievable over various distances or terrains, some level of gait impairment exists even in the most highly functioning individuals [11]. The 2016
Australian Cerebral Palsy Register has found that 71.3% of individuals with CP are classified as GMFCS level I-III, with 35.3% of all individuals functioning at GMFCS level I [2].

Although the brain lesion is non-progressive, the disorders of movement and posture are not static [1, 8], adding further complexity to the description of CP. Functional mobility declines with age, with longitudinal studies showing that mobility peaks at seven years of age, then declines through adolescence for children functioning at GMFCS level III [12]. Furthermore, older children with CP are found to take fewer daily steps than younger children with CP [13]. When assessing gait function specifically, a study conducted by Bell and colleagues on the natural progression of gait in children with CP aged 8 ±2 years showed the rate of surgical interventions recommended increased from 50% to 92.9% of participants over the 4 ±2 year time frame [14]. These findings suggest a deterioration in gait over time when surgical intervention was not employed. Such findings are not insignificant as research has found a strong relationship between gait function and parent- and child- reported quality of life [15]. Graham and colleagues eloquently stated in their recent primer on CP:

“Helping [young people and their families] to achieve optimal outcomes and the best possible participation in every aspect of community life must continue to be the prime goal while researchers work on more long term solutions [cures]” [8].

The International Classification of Functioning, Disability and Health (ICF), is a classification of health and health related domains [16]. The ICF provides a comprehensive framework for the assessment of health outcomes that incorporates the interlinked elements of ‘health condition’, ‘body structures and function’, ‘activity’, ‘participation’, ‘environmental factors’, and ‘personal factors’ to ensure this overarching goal of optimal participation in community life can be achieved. The maintenance or improvement of gait function, falling within the ‘body structure and function’ element of the ICF, is undoubtedly an important part of this goal and it should come as no surprise, therefore, that it is frequently the focus of interventions.

2.2 GAIT PATHOLOGY

The prevalence of specific gait abnormalities in children with CP varies depending on age, GMFCS level, and the distribution of limb involvement [11, 17]. Recent research has found significant differences in gait outcomes of children with CP compared to typically developing children (TD), with deviations evident even when gait patterns are described as “no or minor differences” by clinical standards [18]. To help make sense of the vast array of gait abnormalities seen, and in turn treatment options, a number of classic descriptive classifications, or gait patterns, have been proposed.
The most widely utilised classification for gait patterns in children with hemiplegia was put forward by Winters, Gage and Hicks (WGH) in 1987, from a sample of 46 patients who attended the Newington Children’s Hospital Kinesiology Laboratory [19]. Originally comprised of 4 patterns, Rodda and colleagues extended the classification to include two subtypes within Type 2 (True Equinus), to reflect the subtle but distinct differences in knee flexion angle (Figure 2.1) [20]. Although specific analysis has not been conducted on the prevalence of the gait patterns with regards to age, analysis has been conducted in relation to GMFCS. It was found that those children classified as WGH Type 1 and 2 were also generally classified as GMFCS level I. In contrast, a larger proportion of children classified as GMFCS level II were described as having WGH Type 4 [21]. While no participants in this group were classified as WGH Type 3, a study by Rethlefsen and colleagues (2017) found excessive knee flexion (evident in WGH Type 3 and 4) increased in prevalence with GMFCS level and age [17]. It was also noted by Rodda and colleagues, the order of the gait patterns reflects differences in muscle involvement, starting most distal with Group 1, and ending most proximal with Group 4 [20].

**Common Gait Patterns: Spastic Hemiplegia**

![Figure 2.1 Sagittal gait patterns for patients with spastic hemiplegia, adapted from Rodda et al (2001) [20]. Dashed lines indicated the subdivision of Winter’s original Type 2 pattern [19] proposed by Rodda and colleagues (2001)](image)

Along with typical gait patterns for children with hemiplegia, common sagittal gait patterns were also proposed for children with diplegia. Work by Rodda and colleagues (2004) classified five sagittal gait patterns, based on kinematic data from 174 children with CP who attended the Hugh Williamson Gait Laboratory at Melbourne’s Royal Children’s Hospital (Figure 2.2) [22]. As with the hemiplegia gait patterns, the order reflects differences in muscle involvement, starting most distal with Group I (True Equinus), and ending with Group IV (Crouch Gait) [note group V is Asymmetrical Gait, resulting in any combination of groups I-IV] [22]. As with specific gait
abnormalities, the prevalence of sagittal gait patterns also changes with age as well as previous interventions, and can occur in conjunction with frontal and transverse abnormalities [22].

Clinical gait patterns such as these can be clinically useful, however they drastically simplify complex biomechanical parameters and interactions, with multiple specific conditions potentially resulting in the same pattern presentation [8]. Therefore, while “typical” management strategies can be suggested for each gait pattern [20], interventions should not be prescribed on gait pattern type alone, but should reflect the result of full biomechanical, physical and clinical assessment of each patient throughout their life. While incomplete, gait patterns do offer a snapshot of the variability of gait pathology within children with CP.

Figure 2.2 Sagittal gait patterns for patients with spastic diplegia, adapted from Rodda et al (2004) [22]

2.3 INTERVENTIONS

Reflective of the broad range and scope of gait pathology that exists under the umbrella term “CP”, interventions targeting these gait abnormalities range from conservative to invasive, and can be broadly categorised as therapeutic (including, but not limited to physical therapy, occupational therapy, casting and splinting), pharmacological, or surgical (including orthopaedic and neurosurgical procedures). The type and dose of each are determined by the age, classification, assessments and goals of each individual child and family, as well as access to services. In an attempt to categorise interventions with regard to their effectiveness and the level of evidence supporting their use, Novak and colleagues [23] conducted a comprehensive analysis of systematic reviews of interventions for children with CP. Interventions were classified according to a traffic light system, with a “green light” indicating strong evidence for
effectiveness, a “yellow light” indicating low quality evidence and/or low levels of effectiveness, while a “red light” indicated strong evidence of ineffectiveness/unfavourable outcomes. Of the interventions targeting functional mobility (walking, gait parameters, function or gross motor function), the majority received a “yellow light”, suggesting it should “probably be used” as an intervention. Of the therapeutic interventions, those awarded a “yellow light” for functional mobility outcomes were: assistive technology; biofeedback; early intervention; electrical stimulation; goal directed training; hippotherapy; hydrotherapy; orthotics; stretching; and whole body vibration. Two more therapeutic interventions, massage and therasuits, also received “yellow lights”, with conflicting evidence supporting their use. Baclofen, a generalised antispasticity medication administered intrathecally, was the only pharmacological intervention targeting gait to receive a “yellow light”. Orthopaedic surgeries, and single event, multi-level surgeries both received “yellow lights” based on the evidence supporting their effectiveness at improving functional mobility. The authors noted the intervention “orthopaedic surgeries” includes many different specific surgical interventions, and insufficient evidence exists to support one technique as superior. Along with this, the age of intervention significantly impacts on the long term outcomes.

While the vast majority of interventions fell into the “yellow light” category, most did so because of insufficient evidence, or low quality evidence. There is difficulty in conducting research with high level evidence, such as randomised control trials, when a treatment is considered to be gold standard, as is the case with orthopaedic surgery. Some interventions receiving a “yellow light” may have excellent outcomes, but the evidence is difficult to collect in an ethically appropriate manner. Three interventions had strong evidence supporting their effectiveness in targeting functional mobility, resulting in a “green light” grading. These interventions were casting (therapeutic), botulinum toxin type A (BoNT-A) (pharmacological) and Selective Dorsal Rhizotomy (SDR) (neurological surgery). For SDR, while the quality of the evidence was considered low, the effectiveness on gait kinematics was high enough to be given the “green light”, however for other domains (activity and participation) SDR was not awarded a “green light”. For casting and BoNT-A, both also had low to moderate evidence for their use, but effectiveness was moderate to high, when used in combination with other therapies (BoNT-A and physiotherapy respectively). This highlights the complexity of treating a child with CP, where the individual patient, their goals and the treatments and their interactions must all be considered carefully to maximise the benefit and long term outcomes.

The systematic review by Novak and colleagues [23] was a bold attempt to make sense of the huge array of interventions available for children with CP. If nothing else, it highlighted the importance of quality evidence, and of accurately measuring the effectiveness of an intervention to improve specific outcomes with large, generalisable samples. However, as previously mentioned, the variability of the population, both between children and within children over time, is extremely
high, and treatment plans must be developed on a case by case basis [24]. Successfully matching the right intervention with the right child involves a complete understanding of the child itself and the underlying causes of the functional presentation, and how the specific intervention will impact on that specific case.

2.4 **THREE-DIMENSIONAL GAIT ANALYSIS**

In terms of functional mobility, a large leap forward in the selection of specific interventions for individual patients was made with the uptake of three-dimensional gait analysis (3DGA) for children with CP. A systematic review, conducted in 2011 by Wren and colleagues, found consistent evidence for the efficacy (both in terms of technical and diagnostic accuracy) of 3DGA for children with CP [25]. Furthermore, there is strong evidence to show 3DGA significantly influences treatment plans. A randomised control trial assessed the surgical procedures performed when a 3DGA report was made available to the surgeon (after the initial treatment plan was developed, but before the surgery was carried out), compared to when the 3DGA report was not made available [26]. It found when a procedure was initially planned and then recommended by 3DGA, the procedure went ahead 91% of the time when the report had been seen, compared to only 70% of the time when the report was not seen. This demonstrated 3DGA can be used as support for an initial treatment plan. The study also assessed how frequently surgical decisions were changed when the treatment plan and the 3DGA report were not in agreement. When a procedure was initially planned and was not recommended by 3DGA, the decision was changed 48% of the time when the report was available, compared to just 27% when the report was not available. When a procedure was not initially planned, and was recommended by 3DGA, the decision was changed 12% of the time when the report was available, compared to 7% when the report was not available. These results indicate that surgeons, when they have 3DGA reports available, change the treatment plan more often to agree with the 3DGA report, than when the report is not available [26].

As well as aiding in pre-intervention decision making, 3DGA is has an important role in the assessment of outcomes. There is consistent evidence, from cohort and case control studies, to show 3DGA used as part of surgical decision making, leads to improved gait and functional mobility outcomes [25]. Importantly, function is found to deteriorate when surgical recommendations by 3DGA are not carried out [25]. Post-operative 3DGA has the dual benefit of informing further treatments for the individual patient, as well as acting as an educational tool by quantifying the outcomes, positive or negative, for that intervention. This education can take the form of informal learning within a surgical department [25], or formal documentation through published research.

Significant advances in patient outcomes have been made by the uptake of 3DGA into clinical decision making, however approximately 23% of cases have negative outcomes, even when
While there are many factors that influence surgical outcomes, and relate in complex ways, enhancements in 3DGA techniques may improve such statistics. While 3DGA has strong technical and diagnostic efficacy, it only assesses the resultant movement patterns of the child. It provides a far greater level of accuracy in the assessment of the complex biomechanical system than simple observation alone, however cannot reveal the underlying causal mechanisms for the disordered movement. 3DGA employs a simple model that converts 3D marker trajectories into meaningful joint angles (kinematics). By combining 3D marker trajectories with ground reaction forces (GRF), and approximations of inertial properties of the body segments using a process called “inverse dynamics”, joint moments (kinetics), are able to be determined [29]. Even in healthy, typical gait, the complex 3D linkage that make up the human body means the roles of muscles in producing movement are difficult to discern from assessment of joint kinetics and muscle activation profiles (recorded by surface or fine wire electromyography (EMG)) alone [30].

2.5 NEUROMUSCULOSKELETAL MODELLING

Neuromusculoskeletal modelling takes a traditional 3DGA model further, by employing a series of approximations and algorithms to represent the underlying muscle function. In 2017, Sartori, Fernandez and colleagues summarised the exciting potential of neuromusculoskeletal modelling for children with CP by stating

“the translation of neuromusculoskeletal modelling pipelines to the clinical level is a promising avenue for deriving a new class of biomarkers that directly correlate to a patient’s impairment and subsequent recovery” (pg. 16) [31].

At its highest level, neuromusculoskeletal modelling can be used to simulate changes in muscle activation patterns to investigate the effect on gait kinematics. For example, the team at Stanford University’s Neuromuscular Biomechanics Laboratory conducted a series of simulations using neuromusculoskeletal modelling [30, 32, 33] in an effort to understand the mechanisms behind the use of the surgical procedure of rectus femoris transfer in the treatment of “stiff-knee gait”. Through systematically altering the modelled forces in the lower limb muscles, the simulations revealed the potential mechanisms behind why patients treated for crouch gait using a hamstring or gastrocnemius lengthening may develop stiff-knee gait due to the reduced capacity to generate knee flexion force [33]. While the theory behind the rectus femoris transfer surgery (changing the function of the rectus femoris from a knee extensor to a knee flexor) appears sound, in practice the surgery is not always successful. It is conjectured that scarring may be responsible for the rectus femoris not functioning effectively as a knee flexor following surgical transfer [34], a suggestion supported by neuromusculoskeletal modelling simulations [32].
2.5.1 ELEMENTS OF A NEUROMUSCULOSKELETAL MODEL

Generation of movement in the human body is incredibly complex, and therefore any neuromusculoskeletal model designed to represent the active and passive tissue of the human body requires the same complexity to ensure the results attained are valid and applicable to the population being assessed. Hicks and colleagues offered a concise overview of the elements of neuromusculoskeletal modelling (Figure 2.3) in their paper which established guidelines for assessing model validity [35]. Starting with the initial neural command, each model element feeds into the subsequent element, ultimately resulting in the final movement. Modelling can incorporate all the elements shown in Figure 2.3, or a subset of these. For example, traditional 3DGA is limited to assessment of “multibody dynamics” which is achieved through assessment of “movement” (3D marker trajectories) and “contact” (GRF). As previously mentioned, this simple model, while it has greatly enhanced our understanding of pathological gait and treatment outcomes, it is not able to explore causal mechanisms underlying the abnormal movements. To do so, we must continue to build layers of complexity into the model by considering the higher level elements.

Figure 2.3 Elements of neuromusculoskeletal modelling, adapted from Hicks et al. (2015) [35]

2.5.1.1 Musculoskeletal geometry

The next layer of complexity that can be included in neuromusculoskeletal modelling, as described by Hicks and colleagues (2015) is “musculoskeletal geometry”. In the context of neuromusculoskeletal modelling, musculoskeletal geometry can be broadly categorised into three components: body, joint and muscle-tendon units [35]. The body properties are concerned with the segmental inertial properties, such as mass and mass distribution, which will impact on how the segment acts under the influence of different forces. Each segment is considered a rigid body. For example the thigh segment, made up of the bone, muscle, connective tissue, adipose tissue and skin, is considered to be fixed in length, mass, volume and mass distribution. Joint definitions include both the type of joint (from simple to incredibly complex), the shape and properties of the articular surfaces, as well as the properties of the passive structures surrounding the joint. As with
most aspects of neuromusculoskeletal modelling, the passive structures can be modelled simply
as a restriction to the range of motion available, or if the study requires, a complex model of the
properties and geometry of ligaments, cartilage, joint capsules, menisci and surrounding muscle
structures that all play a role in regulating the motion of the joint. Within the element of
musculoskeletal geometry, the muscle path (the attachments, insertions and pathway constraints
such as via points and wrapping surfaces), is used to accurately represent the muscles of interest
(models can include varying numbers of muscles, depending on the research question). Combined, the body, joint and muscle-tendon properties all impact the muscle moment arm, which is responsible for mapping muscular forces to joint moments.

As always, there are inherent limitations and assumptions associated with every element of a
model. It is generally accepted that many factors within the musculoskeletal geometry are
simplified and sometimes unknowingly ignored within neuromusculoskeletal models. The ability
for segments to deform due to internal forces such as muscular contraction, or external forces
such as compression from other segments at the end range of motion, is not accounted for through
the rigid body assumption [36]. Muscles are modelled as a single line with each muscle attaching
to an individual tendon, however in the biological system it is known that multiple muscles attach
to a single tendon, such as with the triceps surae [31, 35]. Assumptions and limitations are simply
a fact of modelling, as we attempt to represent a complex biological system using a series of
algorithms in a way that is computationally feasible [35]. Awareness of these limitations and
assumptions, and the influences these have on the specific research question, allows researchers
to select the most appropriate model, as well as interpret corresponding results according to
known constraints. In doing so, every effort should be made to reduce the limitations of the
selected model and thereby enhance its validity. The main recommendations for enhancing the
validity of neuromusculoskeletal models with respect to musculoskeletal geometry is to utilise
high quality medical imaging, such as magnetic resonance imaging (MRI) or computed
tomography (CT) for subject specific measurements, as evident in Table 2.1.

<table>
<thead>
<tr>
<th>Component</th>
<th>Minimum complexity</th>
<th>Maximum complexity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodies</td>
<td>Accurate marker based segment lengths</td>
<td>Subject specific, MRI or CT based models</td>
</tr>
<tr>
<td>Joints</td>
<td>Use of functional joint centres Restriction model motion to known degrees of freedom</td>
<td>Developing new models based on high resolution imaging</td>
</tr>
</tbody>
</table>

Table 2.1 Recommendations for optimising validity of musculoskeletal geometry in neuromusculoskeletal models, as described by Hicks and colleagues (2015) [35]
### Muscle tendon units

| Use of subject specific moment arm data to calibrate generic model | Subject specific muscle path geometry from MR and CT images |

#### 2.5.1.2 Muscle-tendon dynamics

After musculoskeletal modelling, the next layer of complexity in neuromusculoskeletal modelling, as described by Hicks and colleagues, is the element “muscle-tendon dynamics” [35]. The most common computational model of muscle-tendon dynamics is the Hill-type model [37], which incorporates contractile fibres, elastic elements (both series and parallel) and activation dynamics. Based on this model, there are five main inputs required: i) maximum isometric force (which is calculated using the Physiological Cross Sectional Area (pCSA) and the specific tension of the muscle); ii) fibre pennation angle; iii) the force-length-velocity relationship; iv) timing parameters for activation dynamics (electromechanical delay); and v) the stiffness and slack length of the tendon. Deeper into the complexity of the biological system, the accessibility or ability to measure these parameters becomes increasingly difficult. Additionally, at this level, most measurements are not feasible in-vivo in human subjects, which is important for model specificity. As a result, architectural properties typically come from cadaveric research, while dynamic parameters come from animal studies [38]. Additional information is, however, available using more sophisticated non-invasive imaging, such as MRI or CT, to determine pCSA [39] and muscle volume [40], laser diffraction to measure sarcomere length [41] and ultrasound imaging to measure fascicle length [42].

As with musculoskeletal geometry, the assumptions and limitations associated with muscle-tendon dynamics should be a) acknowledged, and b) minimised where possible. Within this element of neuromusculoskeletal modelling, given the difficulties in measuring properties in-vivo, minimising assumptions is primarily achieved by ensuring the population on which the model was developed is as similar as possible to the population on which the model will be implemented. A large number of current models have been developed using a historical dataset from a small number of elderly cadaveric subjects [43]. This is problematic in at least two ways: i) older persons have significantly different muscle architectural properties to young persons [44] and ii) advances in measurement techniques have shown measurement uncertainties among the historical measurement techniques used to obtain these said measures [45]. Hicks and colleagues recommended for the greatest validity of neuromusculoskeletal models, new models should be created using comprehensive assessment of muscle architecture among in-vivo and cadaveric subjects using the most up to date, sophisticated imaging techniques [35]. Where the research question does not require the level of detail from a full muscle-tendon model, simplifications are possible. For slow, small movements, ignoring the elastic properties of the muscle and tendon, as well as omitting the force-length-velocity relationship can provide a simpler model, but this must
be used with caution as even in normal walking, muscles can operate in a large range of length and velocity, in which case, the simpler model may not be appropriate [35]. Finally, the Hill-type model of muscle is not a complete representation of all muscle-tendon dynamics. The omission of muscle mass, neuromuscular and physiological fatigue, fibre type profiling and more detailed tendon properties should be taken into account when any research question is being asked.

2.5.1.3 Neural control

The final, and arguably most challenging element within neuromusculoskeletal modelling, as described by Hicks and colleagues (2015) is neural control. Neural control within a biological system is the result of descending commands from the central nervous system (CNS), and their complex interaction with peripheral nervous system (PNS) feedback loops, including reflexes and sensory feedback. There are two ways in which neural control can be modelled: tracking control or predictive simulation. As the name suggests, tracking control involves solving the muscle redundancy problem to calculate individual muscle moments from net joint moments within known movements. Predictive simulation involves optimising control signals to find trajectory, moments and forces that achieve the goals of a task (e.g. minimise energy cost of walking), although the precise movements required to achieve that goal are unknown. Both types of modelling employ an effort based cost function, which aims to achieve the given or desired motion with the smallest energy cost.

The major barrier to valid modelling of neural command is that the very nature of neural control in a biological system is still unknown. Broad questions remain over the role of many factors with motor control that must be answered before they can be accurately incorporated into a neuromusculoskeletal model. These include, but are not limited to: reflexes and feedback loops, muscle synergies, central pattern generators, and internal cost functions. Where neural control is an important factor in the research question, and therefore required in the neuromusculoskeletal model, Hicks and colleagues recommended to compare simulated or predicted muscle activation timing with experimental EMG data (issues with normalisation and measurement error mean analysis of signal intensity is not recommended). At the very least, one should always ensure the simulated motion is indeed realistic for humanlike motion [35].

2.5.2 Barriers to application to Cerebral Palsy

The elements of neuromusculoskeletal modelling are, logically, reflective of the pathway to generate movement within the biological system. The neural command descends to the muscle, regulated by peripheral feedback loops, where the muscle-tendon dynamics influence the force produced. The musculoskeletal geometry then maps that force to joint moments, which combined with external forces, result in movement. It should come as no surprise that this pathway is also reflective of the development of movement pathology from the initial brain disturbance that we see in children with CP. The most widely accepted model for the development of musculoskeletal
pathology by Graham and Selber (2003), tracks changes originating in the CNS, resulting in alterations in the PNS, and subsequently the musculoskeletal system [46] with early musculoskeletal deformities representing changes in muscle-tendon dynamics, while late musculoskeletal deformities effect musculoskeletal geometry [8, 47, 48].

The extent to which CP will affect the elements of neuromusculoskeletal modelling are complex, and while a comprehensive systematic review is beyond the scope of this thesis, a brief summary has been presented in *Error! Reference source not found.*. As shown, each and every element of a full neuromusculoskeletal model is implicated when considering a child with CP. As mentioned previously, questions remain unanswered with regards to neural control in healthy adult populations, particularly around internal cost functions, which are amplified in pathological patients, for whom co-contractions, pain and sensory deficits may all play a role. In a similar way, multiple abnormalities seen in CP contribute to a single factor within muscle-tendon dynamics: the length-velocity-tension relationship. Children with CP are found to have longer resting sarcomere lengths [63, 66, 70], which would suggest they function within a different range on the length-force curve, though this is questioned by some research [95], with studies demonstrating children with CP are operating within the optimal point of the length-force curve [96]. Logically, given spasticity is, by definition, a velocity dependent increase in the stretch reflex, it has been found to result in alterations in the velocity-force curve [97].

The research however, is not always conclusive, with conflicting evidence existing around the presence, direction and magnitude of abnormalities in even some of the more simple to measure parameters. For example, while early studies show a decrease in pennation angles [74], some studies report no alterations [71, 98], while others suggest an increase [73]. Similarly, variability is also found in fascicle length measurements, with some studies reporting a decrease in children with CP relative to TD controls [71, 72, 99, 100], while others report no differences [98, 101]. The observed inconsistency in study results reflects, in part, the need for further research with increased standardisation of measurement techniques, but may also be a reflection of the heterogeneity of the CP population. There is a significant range of neural and musculoskeletal pathology within the CP group. At one end of the spectrum there is the young child who has mild spastic hemiplegia and functions at a GMFCS level I, while at the other end there is the older child who has significant spasticity and dystonia affecting all four limbs and trunk musculature, functioning at GMFCS level V. Along with this, variability exists within each patient, with musculoskeletal pathology changing as the musculoskeletal system develops and adapts in the presence of abnormal neural activity [8, 46], and as a result of intervention.
Table 2.2 The effect of CP on components of the neuromusculoskeletal system considered in modelling

<table>
<thead>
<tr>
<th>Neuromusculoskeletal Modelling Element</th>
<th>Component</th>
<th>Feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural Command</td>
<td>CNS Command</td>
<td>Brain lesions [8, 49]</td>
</tr>
<tr>
<td></td>
<td>PNS reflexes and feedback loops</td>
<td>Spasticity [7, 8, 50, 51]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dyskinesia [49, 52] (54-56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proprioception deficits [53]</td>
</tr>
<tr>
<td></td>
<td>Muscle synergies</td>
<td>Co-contractions [51, 54, 55]</td>
</tr>
<tr>
<td>Muscle-tendon Dynamics</td>
<td>Architectural Parameters</td>
<td>Reduced PCSA [47, 56, 57]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced Muscle-tendon slack length [58]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced tendon thickness [59]</td>
</tr>
<tr>
<td></td>
<td>Dynamic Parameters</td>
<td>Increased muscle stiffness [60-65]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shift in operating range on length-force relationship [66]</td>
</tr>
<tr>
<td></td>
<td>Un-modelled parameters</td>
<td>Fibre type distribution [67-69]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced fibre diameter [56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased sarcomere length [63, 66, 70]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced fascicle length [71-73]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Altered pennation angle [73, 74]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Altered transcriptional profiling [75, 76]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced satellite cell count [77]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased intramuscular fat [78]</td>
</tr>
<tr>
<td>Musculoskeletal Geometry</td>
<td>Bone</td>
<td>Torsion of long bone [46, 79-81]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foot deformities [82, 83]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced bone strength [84-86]</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>Muscle contracture [8, 80, 87, 88]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased tendon length [59, 73]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Altered attachment [89]</td>
</tr>
<tr>
<td></td>
<td>Joint</td>
<td>Joint degeneration [90, 91]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Joint instability [92, 93]</td>
</tr>
<tr>
<td></td>
<td>Body geometry</td>
<td>Altered moment arm [94]</td>
</tr>
</tbody>
</table>
2.5.2.1 Role of interventions

The interventions we use to manage the symptoms of CP add another layer of complexity to the situation. Many interventions are known to alter musculoskeletal parameters as a direct and desired outcome. One such example is the altered muscle-tendon path as a result of the surgical procedure of a rectus femoris transfer [34]. Interventions such as surgical procedures typically have clear, pre-defined influences on musculoskeletal parameters. However, other interventions, such as pharmacological interventions, may result in more complex and indirect effects.

Focal spasticity treatment using BoNT-A injections is a prime example of an intervention that has a complex effect on the musculoskeletal system that is not yet well understood. Acting by pre-synaptically blocking acetylcholine release at the neuromuscular junction, BoNT-A has a temporary neurochemical effect that lasts 12-16 weeks [102]. Clinical gains from BoNT-A, however, last much longer, with 80% of children treated showing improvements in spasticity six to twelve months after a single injection [103]. As evidence based, best practice care, there is consistent evidence for BoNT-A efficacy in improving spasticity [46, 103, 104], gross motor function [105], ambulation [106] and quality of life [107]. Indeed, as previously mentioned, BoNT-A is one of the few treatments to be graded a “green light” in Novak and colleagues comprehensive review on interventions for children with CP, based on the high level of evidence for favourable outcomes [23].

Alongside the positive clinical and functional outcomes, research suggests BoNT-A also effects musculoskeletal properties. Altered muscle histology, specifically altered fibre type distributions, are found to be significantly correlated with the number of injections received [108]. Animal models also show BoNT-A is associated with alterations in the amount of contractile material in the injected muscle that are maintained up to six months following BoNT-A exposure [109]. The effect of BoNT-A on the musculoskeletal system is not limited to muscle histology. Research demonstrates gross muscle atrophy in response to BoNT-A exposure in children with CP [110-112], while muscle-tendon unit length is found to increase in the short term [113, 114], although reduce with long term exposure [115]. Furthermore, animal models suggest the ratio of muscle belly length to tendon length is maintained with BoNT-A, while there is a relative shortening of the muscle belly when not exposed to BoNT-A [116].

The effects of interventions such as BoNT-A on musculoskeletal pathology are complex not only due to the range of parameters affected, but also due to the variability introduced by factors. Factors may include dosage, timing [112], and other treatment variables such as specific injection site [111], as well as interactions with other interventions including strength training [117].

2.5.3 DEVELOPMENT OF APPROPRIATE MODELS

While the primary recommendation in neuromusculoskeletal modelling is to ensure a close match between the target population and the population upon which the model was developed [35], it is
clear that in the case of CP, currently available models (typically based on healthy, older adults) are not reflective of this unique pathological population. As such there is a need for the development of CP specific neuromusculoskeletal models within the field of biomechanics. It is possible, given the variability both within the population and within the individuals, including the impact of interventions, that a generic “CP model” will never be appropriate and that subject specific models will always be required. Where does one go from here? The most logical place to start is the first layer of complexity that can be added into the model as described by Hicks and colleagues: musculoskeletal geometry [35]. Musculoskeletal geometry is the gateway through which the neural command, and subsequent muscle-tendon dynamics, are modulated to convert muscular force into meaningful joint moments. Importantly, most components of musculoskeletal geometry can be measured accurately and non-invasively, meaning subject specificity can be achieved where existing models currently fail.

2.5.3.1 Subject specific models

The optimal solution for creating subject specific models for CP or any population, would be to create a fully subject specific model derived from medical imaging, complete with subject specific bone geometry and muscle tendon paths. This has been demonstrated by Arnold and colleagues (2000), who found MRI-derived models estimations of muscle lengths and moment arms were within 10% of true experimental values of 3 adult cadaveric specimens [118]. The development of CT or MRI-derived models is the same; they use reconstructions of high definition images to acquire detail(s) of gross bony geometry and muscular attachments and paths. Similar methods have also been used among TD children [119]. These methods however, still rely on the scaling of existing rigid models. While the scaling is highly subject specific and accurate, it does not take in to account the potential variability in bone shape that may be expected in children with CP [1, 120]. A continuum model, such as finite element modelling, involves accurate subject specific bone models, where the model bone shape is manipulated to match the 3D mesh created from the MRI reconstruction [121, 122]. As pointed out by Sartori, Fernandez and colleagues (2017), methods such as finite element modelling are prone to error and are not feasible or practical in clinical settings [31]. Furthermore, the experience of this research team in the collection of MRI images among young children with CP has highlighted the need to carefully balance the scan time (which will influence the quality of the images attained) with the child’s ability to comply with the scan protocol. A 45 minute scan, would provide exceptional images of bone shape and muscle attachments, however, is simply not achievable for young children, limiting the potential sample population. Current research is developing workflows for generating accurate 3D bony geometry and shapes from limited data sets, with the aim of reducing errors and computational costs [123]. This work provides an exciting potential future for the field of neuromusculoskeletal modelling, though much work remains until these technologies are streamlined into clinical settings where
these models have the largest potential impact on the health related quality of life among CP populations.

2.5.3.2 Ensuring model validity

While work continues in creating a feasible workflow for generating the optimal, subject specific neuromusculoskeletal model for children with CP, there are intermediate goals between current models, and their ultimate end goal. At a minimum, Hicks and colleagues recommendations for ensuring validity of musculoskeletal geometry within neuromusculoskeletal models include: (1) accurate marker based or careful manual measurements of segment lengths; (2) the use of functional joint centres [and axes]; (3) restricting model joint motions to known degrees of freedom; and (4) using subject specific moment arm data to calibrate models [35].

The recommendation by Hicks and colleagues (31) for the use of subject specific segment lengths to scale an existing neuromusculoskeletal model, as opposed to a standard linear protocol, allows inherent variability between homo- and heterogeneous populations to be accounted for. Research by Rezgui and colleagues (2013), has shown that while generic segment scaling may be appropriate among healthy adult populations, when applied to TD children, and children with CP, subject specific scaling methods are imperative for kinematic estimates to match in-vivo measures [124], a finding replicated by other research groups [89, 125, 126]. The restriction of model joint motions to known degrees of freedom, is based on the understanding of the joint structure and the movement the joint and surrounding tissue allows. Combined with the knowledge that most human movement, such as walking and running, occurs primarily in the sagittal plane, eliminating degrees of freedom from model joints allows a simpler computation approach, allowing for more stable estimates of model dynamics [35]. However, there is research to suggest variables such as co-contraction are highly sensitive to the simplification of joint mobility from three degrees of freedom to one or two degrees [127] and therefore simplification of the model may not be appropriate, depending on the research question being asked.

The second recommendation by Hicks and colleagues [35] is concerned with the use of functional joint centre [and axis], which may not be applicable among CP populations. Research has shown that functional joint centres [and axes] calibration techniques are capable of estimating joint centre locations and axis orientations with high accuracy in both mechanical models [128, 129] and healthy adults [130], and demonstrate high levels of repeatability [131]. Pilot research from clinicians at Princess Margaret Hospital and University of Western Australia among very young children, and children with limited range of motion available at the hip, indicate functional calibration trials are difficult to comply with, and can lead to questionable results if used [132]. Research conducted at the Hugh Williamson Gait Laboratory at Melbourne’s Royal Children’s Hospital has demonstrated functional calibration trials, in some circumstances, may actually be inappropriate for individuals with impaired ranges of motion. Sangeux and colleagues (2014)
compared hip joint centre (HJC) location derived from high resolution medical imaging (true location), with functional calibrations, and common regression equations among healthy participants in three conditions: (1) comfortable range of movement; (2) reduced range of movement; and (3) with assistance from a support person to move the leg. As expected, for those with a good range of motion available at the hip, functional calibrations provided a HJC closest to the true location (on average 1.1cm from the true HJC). However, when joint range of motion was impaired, or assistance was required to complete the task, the Harrington regression [133] gave the most accurate result, with a mean difference of 1.7cm from the true HJC, compared to errors of up to 3.7cm for some functional techniques [134]. As is the case with most aspects of neuromusculoskeletal modelling, there is no “one-size fits all” approach, and care needs to be taken to ensure the technique being used is the most appropriate for the participant of interest.

The final recommendation by Hicks and colleagues [35] is to calibrate musculoskeletal models using subject specific moment arms. While moment arms have been used to assess the validity of model scaling among adults [118], this has not been assessed in children with CP. Accurate model scaling relies on the ability of anthropometric variables (such as segment lengths) to predict the measurement variability of moment arms among heterogeneous adult or paediatric populations. Research on these relationships is inconsistent, and may depend on the specific moment arm being estimated and/or the population of interest. Knee extensor moment arms have been predicted by anthropometric variables, such as tibia length and leg length, in TD children [135], but not adults [135, 136]. No studies have assessed the ability of anthropometric variables to predict the Achilles tendon moment arm (ATMA) in adults, despite the critical role of the plantarflexors in modulating appropriate and efficient walking and running gait dynamics. In TD children, results have been contradictory, with one study showing a weak relationship between anthropometry and ATMA [137], while another study shows a moderate to strong relationship [94].

The ability of anthropometric variables to predict moment arms in children with CP is of importance when considering neuromusculoskeletal models are typically scaled for this population. Children with CP frequently demonstrate abnormalities in parameters which in healthy adults are known to influence the moment arm geometry, such as altered muscle cross sectional areas [138-141] or bony deformities that may influence a joint’s axis of rotation, which is an underappreciated, but key determinant of 3D moment arm estimations [142, 143]. To date, only one study has been conducted to quantify ATMA in children with CP. Kalkman and colleagues (2017), measured ATMA using a 2D tendon excursion method, reporting that children with CP had significantly smaller (15% or 7mm) ATMA compared to TD children. Among children with CP, anthropometric variables were found to predict up to 81% of the variability in ATMA, with leg length predicting the greatest variability at 81%, and body mass predicting the least variability at 66% [94]. While this may be a positive indication for model scaling for children
with CP, future research is needed to directly compare subject specific 3D moment arms and linearly scaled estimates from neuromusculoskeletal models.

2.5.3.3 Medical imaging

In addition to musculoskeletal geometry, medical imaging can be used to acquire highly accurate measurements on parameters such as muscle volume, muscle cross sectional area and muscle belly length. Again, the non-invasive nature of these measurements is critical to their use for creating subject specific musculoskeletal models. Accurate measures of muscle belly length is important as it will impact muscle-tendon dynamics, by shifting where the muscle is functioning within the length-force relationship [144]. However, it is the force generating capacity as a function of the sarcomere length that is of primary interest [35]. This is of particular importance in children with CP as research demonstrates increased resting sarcomere length in this population [63, 66, 70]. Without relating to sarcomere length, and therefore to its capacity to produce force, muscle belly length in itself has little value within musculoskeletal models, aside from its potential use as a scaling factor [145]. The relationship between muscle belly length and sarcomere length depends on the fibre arrangement (fusiform vs pennate), and therefore differs with muscle architecture making it a difficult parameter to scale without advanced medical imaging.

Physiological cross sectional area is known to contribute to the maximum isometric force, an important input parameter into the Hill-type muscle model [35, 37]. Anatomical cross sectional area (aCSA), which is the area perpendicular to the muscle belly length, can be determined from standard musculoskeletal MRI scans alone. However, aCSA can be significantly different from pCSA, and by varying degrees for different muscles, depending on the muscle architecture, or the pennation angle of the muscle fibres, therefore should not be used as a substitute [146]. While correlation exist between pCSA and muscle force [147, 148], and is sensitive to alterations in pCSA [149-151], muscle force estimates are most sensitive to other parameters such as tendon slack length [152, 153]. Calculation of pCSA requires muscle volume, and fibre length, and can be assessed using a number of techniques such as: 1) diffusion-tensor MRI scans [154], from which fibre lengths and pennation angles can be measured; 2) standard MRI scans (to measure muscle volume) in combination with a) ultrasound imaging [155]; or b) cadaveric data [156], to acquire the relevant fibre parameters.

As previously described, research suggests that fibre length and pennation angles are similar between children with CP and TD children [71, 98, 101]. There is growing evidence to show that fibre lengths may not be a good parameter for comparison between the two groups, as it may mask the underlying, but critical, differences in sarcomere lengths [157]. If this is correct, then the measurement of subject specific fibre length and angle characteristics may be redundant. Muscle volume takes into account both cross sectional area and muscle length, and when combined with muscle moment arm estimates, contributes to the maximum isometric joint
moment capacity of a muscle [119]. Indeed, research demonstrates that muscle volume among healthy adults is strongly related to its torque generating capacity [158-160]. The use of muscle volume as a muscle-tendon dynamic parameter may be an appropriate substitute for pCSA in modelling, when used in combination with fibre lengths and pennation angles from scaled models, and has been used in both adults and children [145]. The correlation between muscle volume and maximum isometric torque is found to be weaker in children with CP [161] than adults. While this may be a function of recruitment deficiencies, not muscle morphology, the use of muscle volume as a substitute for pCSA is not without limitation or assumption. While research has suggested muscle force estimates during modelling simulations have been found to be relatively insensitive to perturbations in peak isometric force [145, 150, 152, 153], during dynamic movements such as gait it is isokinetic force production that is of greater potential significance than isometric force production. It is therefore interesting to note that in children with CP, while muscle volume is a weak predictor of isometric force, it is a stronger predictor of isokinetic force and overall work [161]. With further developments into the determinants of maximum torque generating potential in children with CP (including, but not limited to the potential role of moment arm geometry, altered length-force relationship due to increased sarcomere length, altered muscle activation dynamics and altered neural control), the use of muscle volume as an input parameter may be a viable option for children with CP.

2.6 ANALYSIS OF OUTPUTS

Research continues to develop subject specific inputs parameters for neuromusculoskeletal models among children with CP. As this work continues, the application of subject specific modelling in clinical practice will lead to improved data quality for analysis. However, regardless of the quality of the inputs, the handling and interpretation of the data derived is critical to its utility within both clinical or research settings. The quality of the recommendations made is directly related to the quality of the final data presented to the clinician for final decision making.

2.6.1 GAIT INDICES

Within clinical settings, statistical analysis is rarely, if ever, used in isolation to map differences in gait kinematics or kinetics to normative data bands, or changes over time. Instead, clinicians use subjective identification of gait “features”, such as “increased internal rotation of the hip throughout the gait cycle”, “reduced plantarflexion at foot off”, or “reduced knee flexion range during swing”. For quantification of differences, gait indices are frequently used. Gait indices, such as the Gait Deviation Index (GDI) [162], or the Gait Profile Score and Movement Analysis Profile (GPS/MAP) [163], collapse multiple time varying gait waveforms into either a single score, or a series of scores to reflect the variation from what would be considered a normative gait waveform. The GDI achieves this by assessing the scaled distance between kinematics of the participant and normative data on 15 key features, while the GPS does not rely on scaling or restrict analysis to 15 features. Within the GPS, the MAP is created by quantifying the variability
of a subject’s kinematics compared to normative data independently for each of the 9 kinematic variables of interest: Pelvis Tilt, Obliquity, Rotation; Hip Flexion, Abduction, Rotation; Knee Flexion; Ankle Flexion; and Foot progression. The MAP thus provides further detail compared to the GDI, allowing clinicians to assess gait variability from the normative band by joint and plane, although valuable temporal information is still lost.

In the original publication demonstrating the utility of GDI as an index of gait pathology by Schwartz and colleagues (2008) [162] the complexity of gait data was highlighted. The motion of a joint at a single time point is inherently linked to other time points, other planes of motion as well as other joints. This highlights the interdependent nature of the time varying gait data. Schwartz and colleagues go on to state:

“it is clear, therefore, that some method for dealing with this complexity and interdependence is necessary to gain an overall sense of gait pathology”

[162].

Gait indices have aimed to capture this complexity through statistical reduction of gait data to an easily digestible number that represents gait abnormality. The fundamental assumption in gait indices is a high score (caused by large deviations from the normative sample) represents low quality gait, while a low score indicates high quality gait. While research supports the use of gait indices as sensitive [164] and repeatable [165-167] measures, with clinical usefulness [164, 168-171], the limitations of such approaches are not insignificant. Whilst the recent gait indices (GDI and GPS) aim to capture differences over the entire gait cycle as opposed to relying on predetermined discrete time points, as older gait indices have done [172], they do not allow identification of where, within a specific variable curve, the differences were generated. Furthermore, the interdependence, or coupling, of motions are not modelled or not assessed quantitatively.

2.6.2 STATISTICAL METHODS
The need for a simple method for quantitatively representing time varying gait deviations for use in clinical practice is clear, as the goal of gait analysis is the determination of highly individual treatment plans by teams of experienced clinicians and surgeons. In research settings, however, the goal is objective, hypothesis driven analysis of outcomes. Current standards for the analysis of 3DGA data in a research setting involve the use of discrete comparisons at a priori defined time points. For example, it is common to compare knee flexion angle at mid-stance, or ankle dorsiflexion angle at foot contact using simple parametric (student t-tests or one-way ANOVAs) [173, 174] or non-parametric tests (Mann-Whitney U or Wilcoxon signed-rank tests) [175, 176]. The particular discrete variables, or features, analysed are selected a priori based on perceived clinical importance and expected change. Given the subjectivity of this, the variables selected within the literature vary extensively, as no one variable, or even set of variables, can
appropriately represent the time varying waveform. A literature review conducted by Nieuwenhuys and colleagues (2016) [177] identified 26 articles reporting BoNT-A treatment outcomes in children with CP using 3DGA. On average, papers presented data on five features (range 2-49), with a total of 53 unique kinematic features and 33 kinetic features assessed across the 26 papers. The most commonly presented kinematic feature was maximum ankle dorsiflexion during stance, which was reported in 23 papers. Across the 26 papers, 12 features were only reported once, another 12 only reported twice and 11 were deemed too ambiguous to reproduce [177]. Along with the potential for Type II (false negative) errors [177], recent studies have found the use of discrete statistical comparisons on time varying data, like gait kinematics, is likely to result in Type I (false positive) errors [178].

The limitations of selecting discrete variables to represent the waveform have not gone unnoticed, with analysis of time varying, interdependent data a noted concern within 3DGA research. While gait indices collapse the entire gait cycle into a single variable, discrete analysis pick a single variable from within the entire gait cycle. Multiple methods have been put forward to address this issue within clinical gait analysis, such as Bootstrap intervals [179], functional limits of agreement [180], analysis of variance in combination with confidence interval bands [181], curve registration [182] and continuous wave radar [183, 184], with limited success. This is evident in their sparse uptake within clinical and research settings. To date, the most prominent statistical technique used in clinical research is Principal Component Analysis (PCA).

2.6.2.1 Principal Component Analysis

The aim of PCA is to reduce a large number of components to a smaller set, which represents the variance of the dataset as a whole. It produces a unique set of components for each dataset, in theory a smaller, representative number of components will facilitate an easier interpretation of the larger dataset [185]. A PCA works on the understanding that within continuous data, a single data point is related to points either side (e.g. knee flexion angle at 60% of the gait cycle is related to knee flexion at 59% of gait cycle, as well as ankle flexion at 61% of the gait cycle etc.). It assumes linear relationships between these data points, transforming them into unique orthogonal (i.e. independent and uncorrelated) components. The subset of components that account for a predetermined amount of variance are labelled “principal components”. Each principal component is made up of an unspecified number of dependent variables. Thus, the success of a PCA depends greatly on the ability to interpret the results, which requires the subjective, meaningful labelling of principal components. For example, in a study by Olney and colleagues (1998) [186], only three out of four principal components identified were able to be given meaningful labels. [186]. This highlights a significance weakness in PCA: the information generated, while statistically significant, may lose its physical meaning and therefore its clinical relevance [185] as clinicians are faced with the task of correctly interpreting these complex outputs.
One of the strengths of PCA, however, is the retention of temporal information. Deluzio and colleagues (1997) [187] demonstrated this potential in a case study comparing a patient with knee osteoarthritis pre and post operatively with healthy controls to identify differences in knee flexion throughout the gait cycle. Each principal component explained high levels of variability at differing points in the gait cycle. Combined with the patient’s individual principal component scores, the authors were able to determine when significant differences were occurring. In this instance, the patient demonstrated a low PC1 score pre-operatively, which was related to variability in the stance phase. Thus, significant differences in knee flexion/extension could be attributed here. The ease of interpretation in this case, however, is reliant on the smaller number of principal components and the clear separation of the effect on variability throughout the gait cycle. A greater number of principal components, or disjointed areas of their effects on variability, would mean attribution of differences to particular points in the gait cycle is not feasible [187].

In an attempt to take into account the interactions between components, PCA has also been applied to entire 3DGA datasets. Federolf and colleagues (2013) [188] used PCA to assess differences in gait kinematics and kinetics in patients with osteoarthritis compared to healthy controls. A total of 12,432 0D vector components (made up of 36 markers and GRF, in 3D space, over 112 time points) were analysed with the resultant principal vector component accounting for 26.2% of the variability in gait between osteoarthritis patients and controls. The interpretation of the results was conducted by the subjective examination of stick figures of osteoarthritis patients and controls at various intervals of time, with the authors selecting 13 perceived differences. No statistical analyses were used in the selection of these 13 variables, nor were they correlated in any way to the principal vector component identified. As with PCA for single waveforms, PCA for complete 3DGA datasets is let down by the difficulty in interpretation. Moran and colleagues (2014), go so far as to suggest that temporal information is in fact, lost by the expansion of PCA to include the full dataset [189], negating the technique’s greatest strength. For uptake into a clinical setting, an analysis tool is required that is clear, complete and offers an intuitive, quick and simple interpretation.

2.6.2.2 Vector field statistics

Vector field statistics, or the application of statistical parametric mapping (SPM) to nD vector fields, was initially developed for the analysis of spatially normalised signals. This gave rise to the development of functional MRI analysis, allowing researchers to analyse time varying changes in brain images. Later, Pataky and colleagues (2010) applied this technique to time normalised signals within the biomechanics literature, which allows for the time varying vector and scalar analysis of biomechanical data [190]. They have demonstrated its appropriateness in analysing variables of varying dimensions, from 1D (such as time varying knee kinematics), to 2D (such as contact pressure) and 3D (such as bone strain). Analysis can be conducted on a vector field (such as 9D lower limb kinematics), or scalar field (such as knee flexion, knee abduction
and knee rotation) [190]. Analysis of nD vectors allows us to analyse the time varying data in the medium in which they are collected. Pataky and colleagues stress the importance of implementing the various levels of analysis in a hierarchical manner, starting with vector field analysis, then progressing to scalar field analysis if statistical significance is found. They liken this to the accepted procedure for first running an ANOVA, before following up with post-hoc t-tests. The justification for this is the removal of potential bias and source of errors [191].

This approach means vector field analysis is able to overcome two common sources of bias in traditional statistical analyses of biomechanical data: 1) ‘Regional Focus Bias’: where analysis of any variables or time points other than the specific ones addressed in the directed hypothesis will result in bias (refer to Pataky, 2013 Appendix A[191]); and 2) ‘Intercomponent Covariation Bias’: where ignoring the inter-relatedness between time points and joints will result in bias [191]. These sources of bias are not insignificant and can result in Type I and Type II statistical errors, which would result in error with the clinical interpretation of the data, and may impact on decision making and patient outcomes when used in clinical settings. The risk of error was demonstrated by Pataky and colleagues (2013) by re-analysing publically available historical datasets using SPM [191]. To demonstrate the use of SPM on kinematic variables, 3D knee rotation data from 8 participants performing a side shuffle, and a v-cut manoeuvre was used. Looking at the kinematic traces, two variables were identified as the most affected by the experiment and were extracted for analysis: 1) maximum flexion at ≈50% stance; and 2) abduction at 0% stance. Scalar paired t-tests showed both to be significantly different (knee flexion at ≈50% stance t=3.093, p=0.018 and abduction at 0% stance t=3.948 p=0.006). However, vector field analysis revealed differences in the knee kinematic vector at 1%, 10%, 20%, 30-35% and 95-100% of stance. This highlights both Type I and Type II errors resulting from scalar analysis: scalar results were not supported by SPM (Type I error) and differences identified by SPM were not found in scalar analysis (Type II error). The vector field analysis was followed by a scalar field analysis, demonstrating that differences could be attributed to increased flexion (p=0.015) and increased external rotation (p=0.004) at 30-35% and 95-100%. That scalar field analysis was unable to identify all the differences identified by vector field analysis highlights the potential bias associated with not using SPM in the appropriate hierarchical manner [191].

While in some respects, vector field statistics is working toward the same goal as statistical techniques such as PCA, however by taking into account the time varying and interrelated nature of biomechanical variables, it overcomes many of the limitations PCA still grapples with. Therein, the statistical maps produced by SPM not only explicitly reveal the temporal characteristics of the statistical differences, but are directly related to specific experimental variables, meaning the functional interpretation of the statistical difference is inherent. These characteristics lend themselves to clinical application as despite the high level of mathematical complexity and computational costs, the intuitive presentation and interpretation of the data is streamlined within
freely available pipelines for data processing (processing takes merely seconds to complete [190]), therefore reduce computational barriers and make it a clinically viable tool.

There has been a relatively swift uptake of vector field statistics within the biomechanics community, with SPM being utilised in pedobarography [192-194] kinematics and kinetics [195-202], musculoskeletal modelling validation [203, 204], variability assessments [205, 206] and multi-muscle EMG [207] in a variety of research settings. However, it has only been applied to a limited number of clinical scenarios, such as chronic ankle instability [201, 202, 208] and anterior cruciate ligament rupture [209].

The application of SPM in a research setting to 3DGA data of CP populations has recently been explored by Nieuwenhuys and colleagues (2016) [177]. The authors conducted a literature review of the impact of multi-level BoNT-A intervention on 3DGA parameters, identifying 53 kinematic features had been used in previous research to describe changes. Of these, 42 were clearly defined and able to be replicated in their study. The experimental outcome portion of the paper compared these 42 scalar variables to SPM analyses of the pelvis (in 3 dimensions), hip (in 3 dimensions), sagittal knee, sagittal ankle, and foot progression. Retrospective data of 53 patients with CP, including patients with diplegia and hemiplegia, and classified as GMFCS levels I, II or III, were used. Participants had full 3DGA prior to, and on average 58 days post multi-level BoNT-A and casting. At the pelvis and hip, no changes were identified by SPM vector field analysis, which was consistent with no changes in any of the 7 and 15 scalar variables assessed respectively. At the knee, although SPM scalar field analysis identified changes at 41-59% and 86% of the gait cycle, traditional scalar analysis showed no changes in any of the 7 variables assessed, indicating Type II error resulting from a traditional statistical approach. While some scalar variables do address pre-swing phase (which SPM found to show differences (41-59%)), they are evidently insufficient to reflect the true underlying difference. This highlights that even when appropriate scalar variables are selected, traditional statistical approaches may still fail to be effective. At the ankle, SPM scalar field analysis identified changes at 0-2% and 22-100% of the gait cycle. Traditional scalar analysis showed changes in 11 out of 12 variables assessed. The authors suggest the significant changes at the ankle are expected, given the frequent targeting of the gastrocnemius, and its characteristic changes of plantarflexion angle throughout the gait cycle, and thus contribute to the consistency of the results seen. Finally, no changes were identified in foot progression angle through SPM scalar field analysis, although the only scalar variable assessed, mean foot progression during stance, was significantly different. The authors suggest this may be the result of the strict Bonferroni correction used in the SPM analysis in an attempt to account for the co-variance between the joints.

The authors conclude that traditional scalar analyses are acceptable so long as a clear, definite hypothesis is constructed a priori and that the statistical approach used is adequate to assess that
hypothesis [177]. This is consistent with Pataky and colleagues identification of Regional Focus Bias and the errors this can result in [191]. In contrast, where the existence, direction, and/or magnitude of changes is difficult to predict and therefore hypothesise, vector field statistics can overcome this bias. While some cases and treatments may be relatively predictable, the incredible variability and complexity of CP means this is not always the case, with positive results not guaranteed even when 3DGA is used [27, 28]. While vector field statistics is indeed a clinically viable tool, with established validity for the statistical analysis of biomechanical data, further research and work is required to establish user-friendly templates and guidelines for their clinical application, as well as assess their validity and usefulness in a clinical setting.

One proposed application to facilitate the application of SPM within clinical practice is the development of algorithms for the automatic detection of gait patterns. An international consensus study, conducted by Nieuwenhuys and colleagues (2016) identified 49 gait patterns found in children with CP [210]. Comprised of patterns in three degrees of freedom of the pelvis (n=14), three degrees of freedom of the hip (n=10), sagittal knee patterns during stance (n=7) and swing (n=6), sagittal ankle patterns during stance (n=5) and swing (n=4) and foot progression angle (n=3), the gait patterns aimed to make sense of the gait variability in a quantitative way. The gait patterns were identified based on the subjective assessment of a panel of international experts, with reference to supporting literature. Nieuwenhuys and colleagues (2017) used SPM to assess if gait patterns identified were a true reflection of the gait data underlying them, as well as confirm the individual patterns were mutually exclusive. The results generally support the gait patterns, with SPM finding statistical differences to confirm every pattern change identified. Forty-seven out of the 49 patterns were also found to be mutually exclusive. The identification of some differences, using SPM analysis, that were not described in the original gait patterns has led the authors to offer recommendations for the improvement of the originally defined gait patterns [18].

Overall the results of the study suggest SPM can be used to develop algorithms to automatically detect gait patterns. Automation of this kind has many benefits within a clinical setting. Firstly, it reduces the time involved in generating a 3DGA report, thus reducing computational costs and facilitating widespread use. Secondly, and potentially more importantly, it reduces the reliance on human accuracy, and therefore reliance on an individual’s experience in interpreting 3DGA data. The authors state the future vision for such a tool is to understand how gait patterns respond to treatments, and use this knowledge in clinical decision making [18]. However, algorithms such as these still rely on discrete categorisation of gait, albeit into 49 unique patterns over nine degrees of freedom. Currently, there is no suggestion of how information about magnitude may be incorporated into such results. The simplification of the complex 3DGA data by using such algorithms, while appealing, may compromise the success of this future vision.
Vector field statistics’ application to clinical gait analysis need not be restricted to kinematic traces. As has been demonstrated in other research settings, it can be successfully applied to many other time-varying experimental variables that are of interest within clinical CP gait analysis, such as ground reaction forces[200], kinetics [198, 199], EMG [207] and gait variability [205, 206] and is appropriate for variables estimated from modelling procedures, such as muscle forces and lengths. Not only are such variables time-varying, but also demonstrate interdependence, making them prime candidates for vector field statistical analysis. Demonstrating this potential, SPM has been used to assess changes in experimental fascicle lengths of the gastrocnemius over the gait cycle in children with CP [211]. While the uptake of vector field statistics in research settings shows considerable appetite for a statistical tool that can appropriately handle the complexity of biomechanical data, the appropriate application of SPM in clinical practice requires further work, namely in the development of user-friendly templates and guidelines for clinical interpretation.

2.7 SUMMARY

The international research community has some way to go to build an appropriate neuromusculoskeletal model for children with CP. Substantial research is required in many areas of modelling, including elemental inputs, model validation and tools for the appropriate analysis of the outputs. It is critical, however, that while this research is conducted with the greater aim in mind, to ensure the results have application to current clinical settings. Researchers have moral and ethical responsibilities to respect the commitment participants make to research, and ensure the research aims are meaningful to the greater goal, and wherever possible, to current understandings and therefore clinical practice. Indeed, the exploration of the individual components of neuromusculoskeletal modelling can be multifaceted in their application. Research driven “purely” by clinical goals, can and should be used to inform neuromusculoskeletal modelling where possible, just as neuromusculoskeletal modelling research can, and should where possible, have direct clinical meaning. Through multifaceted goals clinical research can have the greatest impact on improving the lives of children with CP, which is the overriding goal of all clinical and research endeavours.

2.8 REFERENCES


Chapter 3 A simple but reliable method for measuring 3D moment arm geometry from a single, static magnetic resonance scan

This manuscript was accepted for publication into Journal of Biomechanics in January 2017.

The most simple neuromusculoskeletal model relies on the linear scaling of a generic model to basic anthropometric variables, such as segment lengths. Musculoskeletal geometry parameters, such as moment arms are estimates derived from this linear scaling. Current techniques for acquiring subject specific moment arms are costly and may not be feasible for children with impaired range of motion about the joint of interest. Chapter three in this thesis validates a novel method for measuring ATMA from a single, static MRI scan that is achievable for all children with CP who can comply with a standard clinical MRI protocol. This validation was performed on a sample of healthy adults who also participated in a full dynamic scan protocol that has been previously published as a valid method for measuring ATMA.

The published version of this paper is supplied in this thesis as Appendix A – Publications.
3.1 ABSTRACT

Current methods for measuring in-vivo 3D muscle-tendon moment arms generally rely on the acquisition of magnetic resonance imaging (MRI) scans at multiple joint angles. However, for patients with musculoskeletal pathologies such as fixed contractures, moving a joint through its full range of motion is not always feasible. The purpose of this research was to develop a simple, but reliable in-vivo 3D Achilles tendon moment arm (ATMA) technique from a single static MRI scan. To accomplish this, for nine healthy adults (five males, four females), the geometry of a cylinder was fit to the 3D form of the talus dome, which was used to estimate the talocrural flexion/extension axis, and a fifth-order polynomial fit to the line of action of the Achilles tendon. The single static scan in-vivo 3D ATMA estimates were compared to estimates obtained from the same subjects at the same ankle joint angles using a previously validated 3D dynamic MRI based in-vivo ATMA measurement technique. The ATMA estimates from the single scan in-vivo 3D method (52.5 mm ± 5.6) were in excellent agreement (ICC = 0.912) to the validated in-vivo 3D method (51.5 mm ± 5.1). These data show reliable in-vivo 3D ATMA can be obtained from a single MRI scan for healthy adult populations. The single scan, in-vivo 3D ATMA technique provides researchers with a simple, but reliable method for obtaining subject-specific ATMAS for musculoskeletal modelling purposes.
3.2 INTRODUCTION

Subject-specific musculoskeletal models offer the ability to estimate an individual’s muscle mechanics and dynamics, allowing enhanced understanding of healthy and pathological movement. This information may be beneficial for the development and implementation of patient-specific treatment plans. Subject-specific muscle mechanics are estimated using many model parameters, including three-dimensional (3D) moment arms. When unknown, these parameters are estimated by linearly scaling the model to subject-specific anthropometric measurements (e.g. segment lengths). Reliance on musculoskeletal model scaling comes into question when considering pathological and/or paediatric populations (particularly those with known skeletal malalignment and deformities), for whom standard assumptions around model scaling may be more likely to be violated [1-3]. As such, much research has been focused on the development of reliable and valid methods for obtaining subject-specific muscle parameters like 3D musculotendon moment arms.

There are currently many in-vivo methods available in the literature for the measurement of musculotendon moment arms, such as the Achilles tendon moment arm (ATMA). Surface measurements, while appealing in their simplicity, do not have the validity and reliability associated with medical imaging techniques such as ultrasound [2, 4-6] and magnetic resonance imaging (MRI) [4, 6-9]. For most measures of the ATMA, the axis of rotation must be defined, which is complex, as there are no surface landmarks to define its anatomical coordinate system. Therefore, most in-vivo methods utilise the motion of the foot as placed through its dorsi-/plantar-flexion range, imaged through dynamic scans, or multiple static scans, to estimate the axis of rotation. While this is achievable for most populations, such procedures may not be feasible in pathological populations with muscle contractures and/or bone contractures/deformities. To bridge this gap in the literature, a subject-specific method for calculating 3D ATMA from a single joint posture is required.

The objective of this technical note was to develop a simple, accurate and repeatable (i.e. reliable) participant specific in-vivo 3D ATMA method from a single MRI scan (static ankle position, and a single scan sequence). The second objective was to validate the ATMA method with a previously validated 3D finite helical axis method.

3.3 METHOD

From a previously published dataset of 10 healthy adults [10], nine participants (five men, four women) aged between 22 and 48 years were used in this investigation. The participant data not used was excluded as the dataset was corrupted while in data storage. Written, informed, consent was provided by all participants, and methods were approved by the Human Research Ethics committee of the University of New South Wales.
Participants lay prone in a 3T MRI scanner (Phillips, Achieva, Netherlands) with the thighs and hips supported in a neutral positioning. The knees were held in a flexed position between 5° and 10° degrees. A flexible surface coil was wrapped around the ankle.

High-resolution anatomical scans in the sagittal plane of the right ankle were used to create the \textit{in-vivo} 3D ATMA\textregistered s for each participant. The high-resolution scan used the following parameters: 3D T1- weighted FSE; 90° flip angle; 320 x 320 matrix; 160mm x 160mm FOV; 355.76/16.68ms TR/TE; and 1mm slice thickness. The scans used for validation purposed were a series of low-resolution dynamic sagittal scans of the right ankle. Parameters used in the low resolution scans can be found in Clarke et al., (2015) [10].

The high resolution static scans were processed using a digitisation tablet (Intuos2, Wacom Technology Corp., Vancouver, WA, USA) and Mimics software package (version 16.0, Materialise, Leuven, Belgium) (\textbf{Figure 3.1A}). The low-resolution scans were processed as per methods described by Clarke et al., (2015) [10].

The Achilles tendon (AT) was manually defined using seven digitised points on the scan slice closest to the midline of the joint (\textbf{Figure 3.1A}). The first point was placed at the most proximal point of bony insertion, and the fifth point placed at the most distal appearance of muscle, with the second, third and fourth points distributed between. The sixth and seventh points were placed immediately proximal to the musculotendinous junction (See \textbf{Figure 3.1B} lateral view and \textbf{Figure 3.1C}).

Although the ankle joint is complex, made up of multiple joints with multiple degrees of freedom, previous research has shown the majority of plantar-/dorsi-flexion motion occurs at the talocrural joint [11]), and the orientation of its plantar-/dorsi- flexion axis of the talocrural joint is fairly consistent [12]. This model, therefore assumes the joint axis to be the talocrural plantar-/dorsi- flexion axis. To estimate the location of this axis, the talus was manually segmented from the high resolution MRI scan and reconstructed as a 3D form. To this 3D form, a cylinder was manually fitted to the talar dome. The orientation and radius of the cylinder was manually adjusted to subjectively ‘best-fit’ the curvature of each individual’s talus dome. The plantar-/dorsi-flexion axis of the talocrural was defined as the polar axis of the cylinder (\textbf{Figure 3.1B}). A manual approach was chose to fit the cylinder after much pilot testing, which showed a least squares approach was not able to appropriately and repeatedly fit a cylinder to the irregular convex shape of the talar dome.

All seven digitised points of the AT as well as the cylinder parameters were exported into Matlab (R2015a, The Mathwords, Massachusetts, U.S.A.) to calculate each participant’s \textit{in-vivo} 3D ATMA (\textbf{Figure 3.1C}). In Matlab, the seven digitised points of the AT were fit with a fifth-order polynomial. The ATMA was calculated as the shortest Euclidean distance between the polar axis
Figure 3.1 A) Sample slice showing manual digitisation of talus and location of point markers. B) 3D reconstructed talus (green) with manually fit cylinder. Calcaneus (yellow) and tibia and fibula (pink) are presented for display purposes only. C) Graphical representation on moment arm calculation, where the joint axis is shown in green, the Achilles tendon, modelled as a polynomial curve is shown in red, and the 3D ATMA in dark blue.
of the cylinder and the polynomial defining the orientation of the AT (Matlab script can be found in Appendix F – 3D ATMA Code).

The in-vivo 3D ATMA estimates from the proposed model were compared with validated 3D ATMA estimates [10]. Briefly, the validated 3D ATMA estimates contain a series of ATMA measures at discrete joint angles over each participant’s ankle range of motion. The discrete talo-tibial joint angle for each of these scans were estimated by taking the vector dot product between the long axis of the tibia and the talus body, which were defined using bony anatomical landmarks visible within both the high and low resolution scans. The anatomical landmarks used to define the tibia vector were (a) the midpoint between the anterior and posterior surface of the tibia at the level of the fibular notch and (b) the midpoint between the anterior and posterior surface of the tibia at the most superior portion of the image plane. The anatomical landmarks used to define the talus body vector were (c) the anterior superior ridge of the talus head and (d) the most posterior aspect of the posterior process for the talus body. The validated ATMA measure from the low resolution scan that matched talo-tibial joint angle of the high resolution scan were used for further analysis.

The foot-tibia angles from Clarke et al. (2015) [10] were obtained from the same scans as the talo-tibial estimates were obtained. The foot-tibia angles from Clarke et al. (2015) [10] were used for comparison to the literature.

The intra-rater measurement agreement and repeatability of the talo-tibial joint angle estimates and in-vivo 3D ATMA estimates were assessed using: (i) a two-way, mixed model intra-class correlation coefficient (ICC) for absolute agreement, (ii) Bland-Altman plots and (iii) limits of agreement (LoA) (95% confidence intervals (CI)). The accuracy of the in-vivo 3D ATMA estimates were compared to the validated 3D ATMA estimates [10] using: (i) a two tailed, paired student t-test, (ii) a two way, mixed model ICC for absolute agreement, (iii) Bland-Altman plots and (iv) limits of agreement (LoA) (95% confidence intervals (CI)). Lastly, the mean was plotted in-vivo 3D ATMA estimates against previously literature estimating in-vivo 3D ATMAs.

For all ICC tests, post hoc achieved power (1 - β) were calculated with an alpha of 0.05. For the ICC estimates; <0.4 indicates poor agreement, 0.4–0.75 indicates good agreement, and >0.75 indicates excellent agreement [13].

Briefly, Bland-Altman or difference plots were used as they are purposely designed for the comparison of two measurements or measurement systems. Cartesian coordinates of a given sample S with values of S1 and S2 can be determined by either Equation 1 or Equation 2. Limits of agreement are defined using the variance of the observed measurement difference (i.e., 1σ, 2σ, 3σ etc.) [14]. Bland-Altman plots are typically used to evaluate mean bias between measurement
systems, as well as the expected interval or range measurement of agreement, which must be set *a priori* based on clinical or practical necessity.

**Equation 1**

\[ S(x, y) = \left( \frac{S_1 + S_2}{2}, (S_1 - S_2) \right) \]

**Equation 2**

\[ S(x, y) = (S_1, (S_1 - S_2)) \]

Lastly, the *in-vivo* 3D ATMA estimates were only validated to the 3D ATMA estimates published by Clarke et al. (2015) [10] for two reasons. First, the dataset was made available to the research group, which allowed for direct comparisons. Second, this was the only dataset which could be directly mapped to the operationally defined talo-tibial joint angles estimates.

### 3.4 RESULTS

Intra-rater repeatability of the talus-tibial angle measurement was very high with an ICC of 0.939 (p<0.001; 1 – β = 99.9%). The mean measurement error was -0.63°, with a LoA of -6.08° to +4.82°.

The current ATMA method showed excellent intra-rater repeatability with an ICC of 0.996 (p<0.001; 1 – β = 99.9%). The mean measurement error for intra-rater repeatability was -0.1mm, with a LoA of -1.1mm to +0.9mm (Figure 3.2). This method also possess excellent inter- and intra-tester repeatability with an ICC ≥ 0.985 (p<0.001; 1 – β = 99.9%) when used to estimate the 3D *in-vivo* ATMA among a larger sample of 15 typically developing children. These data and experimental procedures can be found within Appendix G - Achilles tendon moment arm repeatability among typically developing children.

The current ATMA method was in excellent agreement with the validated 3D ATMA method, with an ICC of 0.912 (p < 0.001; 1 - β = 99.9%). Additionally, when comparing the validated 3D ATMA method, there was no significant difference in talo-tibial joint angles (p = 0.23) at which the ATMA were compared. Descriptive statistics demonstrated a mean difference of 3.0 ± 6.9°.

There was no difference (p=0.21) in ATMA length when assessed using the current method compared to the validated 3D ATMA method. The mean ATMAs calculated by the current method and validated 3D ATMA method were 52.5mm (±5.6mm) and 51.5mm (±5.1mm) respectively. Descriptive statistics showed a mean ATMA difference of 1.0mm (±2.2mm) with a LoA of -3.2mm to +5.2mm (Figure 3.3). The mean and maximum measurement error of 1.0mm and 4.6mm, equates to approximately 1.9% and 8.9% of the mean ATMA (as measured by the
validated 3D ATMA method) respectively. To compare to the existing literature, the mean foot-tibia angle measured during testing was estimated. The current method showed \textit{in-vivo} 3D ATMA estimates to existing literature as shown in Figure 3.4.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{A) Bland Altman plot to assess the intra-rater repeatability of the current method shows a very small mean measurement error and tight limits of agreement B) Comparison of two blinded measurements of moment arm using the current method shows very high repeatability}
\end{figure}

\textbf{Figure 3.2} A) Bland Altman plot to assess the intra-rater repeatability of the current method shows a very small mean measurement error and tight limits of agreement B) Comparison of two blinded measurements of moment arm using the current method shows very high repeatability
**Figure 3.3** A) Bland Altman plot comparing the current method for moment arm and the validated 3D method ATMA demonstrating a small mean measurement error and tight limits of agreement. B) Comparison of the measured moment arm using the current method and the validated 3D ATMA method for each of the 9 participants.
Figure 3.4 Comparison of ATMA estimates from MRI based models in the literature. Data from Fath et al., Hashizume et al., Rugg et al and Maganaris et al are mean moment arms measured using a 2D centre of rotation method. Data from Sheehan et al, Hashizume et al, and Clarke et al are mean moment arms measured using 3D models.

3.5 DISCUSSION

The current method is a reliable way to calculate in-vivo 3D ATMA from a single, static MRI scan, exhibiting excellent repeatability and accuracy. The intra-tester measurement error of the current ATMA method (error 1.0mm ± 2.2mm) is less than that reported by previous literature which compared the validated 3D ATMA estimates to physical measurement (error 2.9mm ± 2.1mm) [10]. The maximum inter-model measurement error observed in the current study was 8.9%, which is smaller than the maximum measurement error observed from a validated in-vivo 3D ATMA method when compared against physical measurements (13-22%) [10]. What these results and previous literature show is that MRI based in-vivo 3D ATMA models appear repeatable with typical errors of 1.5-1.6mm [15].

As shown in Figure 3.4, the in-vivo 3D ATMA measured using the current method displays reassuring similarity with 3D ATMA measurements among healthy adults published previously the literature [4, 6-10]. The exception for this is the data published by Hashizume et al. (2012) which shows smaller ATMA compared to both 2D centre of rotation estimates, and other 3D estimates [7]. It is also worth noting the mean and variance of error of the in-vivo 3D ATMA
method presented in the manuscript is within the accepted error margins presented previously in the literature [4, 6-10].

One limitation of the study was the measurement of talo-tibial joint angle to make direct comparisons between the high and low resolution scans for validation purposes. Foot-tibia joint angles were not measured externally during scanning and the base of the foot was not visible in the static scans, which is why joint kinematics were estimated from the bony geometry of the talus body. The talo-tibial angle was deemed appropriate for comparison purposes as it could be measured objectively and repeatedly (ICC = 0.939; 1 − β = 99.9%) from both static and dynamic scan sets. Though a practical method to obtain ankle joint kinematics for comparison purposes, this method was limited as the motion of the talus during the dynamic scan was prone to perspective error, meaning the bony body, at times was observed to move in and out of the scan plane. Future applications of this technique would benefit from the use of MRI compatible motion capture markers to directly calculate foot-tibia angles. External kinematic markers would also allow researchers to place a given musculoskeletal model into the correct posture during model scaling to ensure its ATMA matches the participant’s in-vivo estimates with the highest fidelity possible.

An inherent limitation of this current method is that it is restricted to the measurement of ATMA at one posture. ATMA length is known to change with change in joint kinematics, and while this pattern of variability is frequently modelled as predictable in musculoskeletal calculations and simulations [16] it may be highly subject-specific even among healthy adult populations [10]. While in a single static posture, it is also difficult to standardise musculotendon force estimates, which may add additional errors to ATMA estimates. While these limitations are acknowledged, it is important to keep in mind the population for whom this method has been developed: individuals with pathologies which prevent or impede on their ability to move joints through full or partial ranges of motion. In such populations, these limitations are mitigated as the method currently provides the only feasible method for reliable 3D assessment of ATMA, and/or given the reduced range of motion available, less extrapolation is required to estimate ATMA at the end range of motion for the joint, thereby reducing potential errors.

While the development and validation of the current method was performed using high resolution scans, the nature of the technique (where a simple geometric shape is being fitted to a full bone, without reliance on precise identification of specific landmarks) suggests lower resolutions scans, might produce 3D ATMA measurements of nearly comparable high quality. Furthermore, the scan plane is not required to be aligned with the sagittal plane of the ankle, as the model is a true 3D model, using 3D forms and coordinates.

The current method has many advantages as a model to measure in-vivo 3D ATMA. To the authors’ knowledge, this is the first method that provides validated measures of 3D ATMA from
a single, static MRI scan. It may be a feasible option for measuring subject specific, 3D ATMA in clinical and research settings for subjects with limited ankle range of motion, offering a pragmatic solution for implementing subject specific measurements of ATMA into musculoskeletal modelling purposes.

**ETHICAL APPROVAL**

Ethical approval was attained for this project from the University of NSW (09179) and the University of Sydney (12192).

**ACKNOWLEDGMENTS**

The authors would like to thank David Lloyd, Chris Carty, Jane Valentine and Catherine Elliott for useful discussions and support.

EC and RH are supported by NHMRC research fellowships. The scans for this study were collected as a pilot project, funded by a grant from the Sydney Medical School, University of Sydney.

**CONFLICTS OF INTEREST**

The authors have no conflicts of interest to declare.

3.6 **REFERENCES**

5. Manal K, Cowder JD, Buchanan TS. (2013). Subject-specific measures of Achilles tendon moment arm using ultrasound and video-based motion capture. *Physiological Reports, 1*(6), e00139.


**Chapter 4** Children with cerebral palsy have larger *in-vivo* and linearly scaled Achilles tendon moment arms than typically developing children.

This manuscript was submitted for publication into *Journal of Biomechanics* in August 2017 and is currently under review.

Chapter three validated a simple MRI-derived ATMA protocol that is feasible for children with CP to complete, regardless of level of contracture at the ankle. This chapter uses this validated protocol to compare ATMA in children with CP, TD children and healthy adults. To investigate the appropriateness of linear scaling of a generic model for each of the populations, in-vivo ATMA were compared to scaled estimates from a commonly used neuromusculoskeletal model. This study highlights the importance of developing neuromusculoskeletal models that are truly reflective of the unique musculoskeletal geometry seen in children with CP.
4.1 Abstract
The effectiveness of the plantarflexor muscle group to generate desired plantarflexion moments is modulated by the geometry of the Achilles tendon moment arm (ATMA). Children with cerebral palsy (CP) frequently have reduced plantarflexor function, which is attributed to impaired muscle structure and function, however little attention has been paid to the potential contribution of ATMA geometry. Our use of musculoskeletal modelling for the simulation of gait and understanding of gait mechanics, rely on accuracy of ATMA estimates. This study aimed to compare 3D in-vivo estimates of ATMA of adults, children with CP and typically developing (TD) children, as well as compare 3D in-vivo estimates to linearly scaled musculoskeletal model estimates. MRI scans for eight children with CP, 11 TD children and nine healthy adults were used to estimate in-vivo 3D ATMA using a validated method. A lower limb musculoskeletal model was linearly scaled to individual tibia length to provide a scaled ATMA estimate. Normalised in-vivo 3D ATMA for children with CP was 17.2% ± 2.0 tibia length, which was significantly larger than for TD children (15.2% ± 1.2, p=0.013) and adults (12.5% ± 0.8, p<0.001). Scaled ATMA estimates from musculoskeletal models significantly underestimated in-vivo estimates for all groups, by up to 34.7%. The results demonstrate that children with CP have larger normalised 3D ATMA compared to their TD counterparts, which may have implications in understanding reduced plantarflexor function and the efficacy of surgical interventions that have a primary aim of modifying the musculoskeletal geometry of this muscle group.
4.1 INTRODUCTION

The Achilles tendon and the plantarflexor muscle group play a crucial role in the functioning and regulation of gait mechanics. The impact of this is particularly evident when assessing the gait of children with cerebral palsy (CP). This neurodevelopmental movement disorder is related to a number of skeletal muscle-tendon unit impairments, with the plantarflexors being commonly affected. Impairments include spasticity, reduced selective motor control, reduced muscle volume and cross sectional area, and altered muscle histology [1]. It is well known that children with CP have reduced plantarflexor strength (i.e. joint moment estimates) [2, 3], when compared to typically developing (TD) children. While the aforementioned impairments are contributing factors [1, 4], our understanding of strength impairments among this population is incomplete. One contributing mechanism may be differences in three dimensional (3D) Achilles tendon moment arm (ATMA) geometry.

The moment arm of any musculotendon unit is understood as the “effectiveness” of skeletal muscle to generate a moment of force about an axis of rotation [5], and thereby actuating the desired moment about a given joint to generate movement. The moment of force produced by a musculotendon unit is a product of the linear muscular force, and the 3D moment arm (the perpendicular distance between the forces line of action and the axis of rotation). Many musculotendon and bony geometry factors, which are frequently altered in children with CP, have been found to influence moment arm geometry among healthy adult populations. Training studies have shown that muscle hypertrophy increases the cross sectional area of the muscle belly, which in turn increases moment arm length [6-9]. Children with CP have smaller plantarflexor muscle volumes and cross sectional areas than TD children [10], so it is reasonable to expect that children with CP would have smaller ATMA. Another factor influencing the size of the ATMA is the location and orientation of the plantarflexion/dorsiflexion axis of rotation which is typically assumed to be the talocruatal flexion/extension axis [11, 12]. The orientation and location of this axis is dictated by the bony geometry of the foot [11, 12], and further modulated by muscle activation and loading [8, 9]. Children with CP commonly demonstrate bony deformities at the foot [13-15], as well as abnormal muscle tone and activation [1]. To the authors’ knowledge, only one study has assessed ATMA geometry among this population, finding normalised 2D ATMA of children with CP to be smaller than their TD peers [16]. These results suggest that ambulatory paediatric CP populations also possess the mechanical disadvantage of short ATMA. Further research into AMTA geometry in 3D will allow for better understanding of mechanical disadvantages, and facilitate more precise modelling, leading to improved clinical treatment plans for pathological populations.

Internal muscle forces can be estimated when moment arms are known (scaled estimates or measured in-vivo) and when net joint moments can be calculated via inverse dynamics during gait or dynamometric assessments [17, 18]. Estimation of internal muscle forces, obtained through
musculoskeletal modelling facilitate detailed exploration of muscle mechanics underpinning pathological movement patterns typically observed among CP populations [19, 20]. While this computation method is powerful, it is not a common measurement approach within most clinical research settings [11, 19, 21]. Along with factors such as computational time and costs, one of the primary reasons for this is the sensitivity and specificity of the modelling parameters used to represent the clinical populations they are meant to measure. When in-vivo estimates of a participant’s 3D moment arms are not available, it is common for researchers to linearly scale these musculoskeletal modelling parameters to accessible anthropometric information such as segment lengths. Most of the regression equations available in the literature are derived from small homogenous cadaveric populations [22] meaning their suitability among pathological and/or paediatric populations is questionable [11, 19]. Gastrocnemius muscle function has been found to be highly sensitive to changes in muscle-tendon architectural properties, including ATMA [23], highlighting the importance of sensitive and participant specific musculoskeletal models. It is imperative to establish more accurate methods to correctly prepare and scale musculoskeletal models that are representative of paediatric and/or pathological populations to increase the specificity of the mechanical information derived.

The primary aim of this study is to compare in-vivo 3D ATMA estimates between adults, TD children and children with CP. It is hypothesised that adults will possess the largest 3D ATMA estimates, while children with CP will have the smallest. The secondary aim of this study is to compare 3D ATMA derived from an established musculoskeletal model linearly scaled to tibia length with participant specific in-vivo 3D ATMA for adults, TD children, and children with CP. It is hypothesised no differences in 3D ATMA estimates will be observed between methods for the adult population, however significant differences will be found between methods for both paediatric groups.

4.2 METHODS

Eight children with spastic CP, aged 9.7 years (±2.6), and a convenience sample of 11 TD children, aged 8.7 years (±2.3), participated. Children with CP were classified as Gross Motor Function Classification System level I (n=4) and II (n=4), with diplegia (n=4) and hemiplegia (n=4). An existing dataset of 9 healthy adults was used [24]. Full participant characteristics can be found in Error! Reference source not found.. Informed consent was obtained from adults and parents of paediatric participants.

Participants had an MRI scan of the dominant (TD and adults) or most affected (CP) ankle at a comfortable resting angle. For the paediatric groups, a 1.5T whole body MRI unit (Siemens Medical Solutions, Erlangen, Germany) was used, employing a T1-weighted spin echo sequence; 256 x 256 matrix; 160mm x 160mm FOV; 3mm slice thickness; and 3.3mm slice gap. A mean of 18.2 slices were acquired, with a range of 16 – 20 slices. Adult scans were conducted in a 3T
Table 4.1 Participant demographics, tibia length and MRI derived joint angles **p<0.01 compared to other groups, *p<0.05 compared to other groups

<table>
<thead>
<tr>
<th></th>
<th>Gender</th>
<th>Age (years)</th>
<th>Tibia Length (mm)</th>
<th>MRI derived joint angle (°)</th>
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<td>n</td>
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<td>Adult</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>29.3**</td>
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<tr>
<td>TD</td>
<td>11</td>
<td>5</td>
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<td>8.7</td>
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<tr>
<td>CP</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>9.7</td>
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whole body MRI unit (Philips Healthcare, Achieva, Netherlands). Adult scan parameters can be found at Clarke et al [24].

Scans were processed using Mimics software (version 16.0, Materialise, Leuven, Belgium). The Achilles tendon was defined using seven manually digitised points. The talus was manually segmented and reconstructed, with a cylinder fitted to the reconstructed form. The bipolar axis of the cylinder was taken to represent the flexion/extension axis of the talocrural joint [25]. 3D coordinates of the digitised Achilles tendon and the cylinder’s bipolar axis were output and processed through a custom Matlab code to calculate in-vivo 3D ATMA. This method has been validated among adult populations [26] and possesses established repeatability among TD children [27]. Ankle joint angles measured from the MRI scans were operationally defined as the angle between the long axis of the tibia and the plantar surface of the hindfoot.

Tibia length for the paediatric groups were measured from lower leg coronal MRI. Tibial length was defined as the most superior lateral aspect of the tibial plateau, to the most inferior lateral aspect of the fibular notch. Coronal lower leg scans were not available for the adult sample, so Grieves Reference Length was used as a surrogate to estimate tibial length [28].

Musculoskeletal modelling was conducted in OpenSim (version 3.3, SimTK, Stanford, CA, USA) [20], using a lower limb model described by Arnold et al., [22]. All rigid bodies and musculoskeletal properties were manually scaled to tibial lengths. Following model scaling, the scaled moment arms were output at the same ankle joint angles measured from the MRI scans, as shown in Figure 4.1 (blue closed circle). For the purposes of this comparison, the soleus moment arm was selected to represent the ATMA as it is mono-articular, thereby unaffected by knee positioning.

The soleus insertion onto the calcaneus body in OpenSim was adjusted in the anterior/posterior plane until the modelled moment arm was equal to the in-vivo ATMA at the experimentally measured joint angle (Figure 4.1, red open circle). This allowed ATMA to be estimated over the model’s plantar/dorsiflexion range of motion [5]. To compare ATMA across all participants, a predicted ATMA was taken at 20° plantarflexion, and normalised to tibia length. A joint angle of 20° plantarflexion was selected as it was similar to the mean joint angle measured, and an angle most children with CP can achieve despite presence of spasticity or contracture.

Participant age and tibia length, as well as MRI derived joint angle estimates were compared using a one-way ANOVA. To compare the absolute and normalised predicted ATMA at 20° plantarflexion between the three groups, a one-way ANOVA was used. To compare in-vivo and linearly scaled 3D ATMA estimates between the three groups, a SPANOVA was used. All ANOVA’s were followed up with post-hoc analysis conducted using Tukey’s HSD. If a significant main effect for condition was found from the SPANOVA, two-tailed t-tests were
conducted. All statistical analyses were performed in SPSS (version 23.0, IBM Analytics, Armonk, NY, USA), with an alpha of 0.05.

**Figure 4.1** Example output for one participant, showing the modelled ATMA derived from a linearly scaled musculoskeletal model (blue) and the subject-specific modelled ATMA derived from the *in-vivo* ATMA measured from MRI scans at the subject specific joint angles, as represented by a dotted line. The dashed line represents 20° of plantarflexion. The ATMA used for comparisons are indicated by circle. An open circle represents an *in-vivo* measurement, while a closed circle represents a modelled measurement.

### 4.3 Results

There were significant main effects in age and tibia length (*p*<0.001). Post-hoc analysis showed no significant differences in age (*p*=0.911) or tibia length (*p*=0.994) between TD and CP, while adults were significantly different than both paediatric groups for both variables (*p*<0.001). A significant main effect in MRI derived joint angles was found (*p*=0.001). Post-hoc analysis showed CP had significantly larger joint angles compared to both TD (*p*=0.003) and adult (*p*=0.003), but no significant difference was found between TD and adults (*p*=0.986). A significant main effect was found (*p*<0.001) when normalised to tibia length; mean predicted ATMAs were 12.5%±0.8 for adults, 15.2%±1.2 for TD and 17.2%±2.0 for CP (Figure 4.2). The post-hoc analysis showed normalised ATMA at 20° plantarflexion were smaller among adults.
compared to TD ($p<0.001$), and CP ($p<0.001$). CP was found to have significantly larger normalised ATMA at $20^\circ$ plantarflexion, compared to TD ($p = 0.013$)

![Box plot showing normalised predicted ATMA for each group at 20° plantarflexion. **p<0.01](image)

**Figure 4.2** Box plot showing normalised predicted ATMA for each group at $20^\circ$ plantarflexion, **p<0.01**

When comparing the *in-vivo* and scaled methods for estimating ATMA, a main effect for group was observed ($p<0.001$), as was a main effect for method ($p<0.001$). The interaction of these two factors was also significant ($p<0.001$) (**Figure 4.3**). *Post-hoc* analysis showed the effect of ATMA method (*in-vivo* or scaled) was significantly different among adults compared to CP and TD ($p<0.001$), while the effect of method was not different between CP and TD ($p=0.705$).

*Post-hoc* testing revealed that *in-vivo* 3D ATMAs were significantly larger than the linearly scaled 3D ATMA at the same MRI derived joint angles for all groups ($p<0.001$). The mean difference was -5.8mm for adults, -11.0mm for TD and -15.7mm for CP. This corresponds to a 10.7%, 26.2%, and 34.7% underestimation of *in-vivo* measurements for adults, TD and CP respectively.
Figure 4.3 Relationship between in-vivo and scaled ATMA for each group **within group t test of p<0.01, ^^^ condition effect of p<0.01

4.4 DISCUSSION

The primary aim of this study was to compare 3D ATMA estimates for adults, children with CP and their TD counterparts. It was hypothesised that adults would have the largest ATMA, and children with CP would have smaller 3D ATMA than TD children, contributing to the documented reduced ‘plantarflexor strength’ (as measured by joint moments) [2, 3]. This hypothesis has been rejected with adults showing no difference to children with CP, who in turn demonstrate larger normalised 3D ATMA compared to their TD counterparts, of 9% or 4.3mm. The secondary aim of this study, was to compare 3D ATMA derived from an established musculoskeletal model linearly scaled to tibia length with participant specific in-vivo 3D ATMA. As hypothesised, linear scaling of an adult musculoskeletal model did not demonstrate participant specificity for TD children or children with CP, with scaling underestimating in-vivo 3D ATMA estimates by 27.68% and 35.82% respectively. While scaled ATMA for adults was also significantly smaller than in-vivo ATMA, the effect of the ATMA method (in-vivo vs scaled) was significantly less than in either paediatric group and is comparable to the error range accepted in the literature of 10% [29].

Literature suggests a peak plantarflexion moment of 0.86-1.11Nm/kgBW for children with CP during walking gait [2, 30-33]. Based on a 4.3mm longer ATMA in children with CP, the peak internal muscle force for a 30kg child with CP would be 62N (10%) less than an appropriately matched TD child producing the same joint moment. Given the observed deficiencies in muscle structure among children with CP [10, 34], a larger ATMA may in fact act as a mechanical compensation for impaired muscle function, allowing children to maintain function. More research is required to understand gait mechanics and the role of ATMA among pathological
paediatric populations as this may this impact surgical treatments directly manipulate the 3D ATMA geometry.

Underestimations of ATMA as a result of linear scaling in musculoskeletal modelling will have significant implications on internal muscle force estimates. For a 30kg child with CP, these underestimates would result in a 345N (52.83%) overestimation of internal plantarflexor muscle force, assuming a peak plantarflexion moment of 0.985Nm/kgBW [2, 30-33]. For a TD child of the same mass and plantarflexion moment, internal muscle forces would be overestimated by 259N or 36.21%. Linear scaling of adult musculoskeletal models, with the intention of estimating internal muscle forces are not appropriate for paediatric populations.

This study relies on the assumption that the musculoskeletal model correctly modelled the 3D ATMA of a paediatric CP population through their full joint range of motion. This relationship is based on an established algorithm for computing ATMA at each angle of interest [5]. A complete explanation of this algorithm is beyond the scope of this discussion, but simply, the algorithm involves three individual calculations. First, the determination of the coupling matrix, which relates the generalised speeds to the joint angle of interest, satisfying all velocity-level constraints. Then, unit tension is “applied” to the muscle of interest and mapped to body spatial forces. This calculation assumes the tension is uniform within the muscle tendon unit, and there is a linear relationship between this tension and the transmission of force. These body spatial forces are then mapped to generalised forces. This calculation combines information about forces (applied and inertial), velocities and positions, using a number of different constraint matrices. The final algorithm uses this coupling matrix, the unit tension and the generalised force to compute the instantaneous ATMA at that joint angle. This calculation relies on numerous assumptions around muscle properties, many of which are known to be altered in children with CP[1]. While it was not feasible to measure ATMA at multiple joint angles within this study, the results suggest endeavouring to do so would be a worthwhile pursuit, and allow for a better understanding of the relationship between ATMA and joint angle over the range of motion for this population.

The findings of this study are in direct contrast to previously presented work by Kalkman et al. (2017) who found children with CP possessed smaller normalised 2D ATMAs when compared to their TD peers [16]. When comparing the presented 3D ATMA estimates from the TD population (15.2%), they were in agreement with the published 2D ATMA estimates (16%). However, for the paediatric CP population in this study, normalised 3D ATMA were notably larger (17.2%) than the 2D estimates reported by Kalkman et al (14%). The observed differences in ATMA for the paediatric CP population may in part be to the computational method used to estimate ATMAs. The 2D tendon excursion method used has been shown to result in measurement errors up to 40% among healthy adult populations [35]. Three factors have been shown to contribute to these measurement errors: 1) the flexion/extension axis is not orthogonal
to the sagittal plane; 2) the sagittal plane selected for analysis; and 3) visual identification of anatomical landmarks used in the measurement. While the second and third proposed source of error would uniformly affect all participants regardless of pathology, the orientation of the flexion-extension axis, given the deformities frequently seen among children with CP, would represent a significant source of error for this population, and may explain the differences found between previous research and results presented here. Further research should be conducted to directly compare reliable robust 3D methodologies, such as that employed in this study [26], and 2D methods among children with CP.

While this study is, to the authors’ knowledge, the first to compare linear scaling to *in-vivo* ATMA measurements among children with CP, previous research has compared linear scaling to subject specific MRI musculoskeletal models for this population. Correa et al. (2011) found a linearly scaled model resulted in a 2.4% (±3.9%) overestimation of ATMA compared to MRI derived musculoskeletal model (an individualised model based on subject specific bone geometry and muscle insertions) for four children with CP [11]. This suggests ATMA estimated from the MRI based musculoskeletal model would not correlate with the *in-vivo* measurements, however important differences between the studies must be considered. The current study directly measured *in-vivo* ATMA using a static, comfortable joint angle, without voluntary muscle activation or external force applied. The previous research compared the AMTA obtained from modelling single leg stance phase during gait. Given the computational expense of a full MRI derived lower limb model, simple *in-vivo* measurements hold strong appeal, and further research comparing the *in-vivo* measurements at MRI derived models would be beneficial.

While the mechanisms for altered ATMA cannot be determined from the current study, potential mechanisms are worth considering. Two factors that influence the ATMA are the tendon line of action, and the joint axis orientation and location. Research suggests a muscle with a smaller cross sectional area would have smaller 3D ATMA [6-9], due to a shift in the tendon line of action associated with reduced muscle bulk. Indeed, the smaller muscles of children with CP [10, 34] was put forward as an explanation for the smaller 2D ATMA found by Kalkman et al [16]. However, this finding was not upheld in 3D ATMA measurements, suggesting proximal muscle properties are not contributing to the increased 3D ATMA via the tendon line of action. Abnormal forces acting on the calcaneus, including reduced external forces due to lack of heel strike at foot contact [36-38], and altered muscular forces due to spasticity, impaired neural activation, contracture and co-contraction, can result in an deformity of the bone [13, 14, 39], and may impact on tendon insertion and therefore line of action. Similarly, bony abnormalities of the foot may also impact on the second critical factor in ATMA length: the location of the joint axis. Pes planovalgus, a common foot deformity in children with CP, sees the lateral column of the foot shorten both functionally and structurally relative to the medial column [14], and may result in rotation or translation of the talocrural axis. Indeed, the contrasting findings between the 3D
ATMA results of this study, and the previously reported 2D ATMA results of children with CP [16] provide support for the importance of joint axis as potential mechanism for altered ATMA geometry. Further research into the mechanisms behind altered ATMA, as well as the impact of various surgical interventions targeting the foot and ankle may have, is required.

This study presents two critical findings. Firstly, children with CP were found to have significantly larger normalised 3D ATMA when compared to TD children. This has implications for the understanding of muscle function and torque generation at the ankle. Secondly, as hypothesised, linearly scaled musculoskeletal models cannot replicate in-vivo measured ATMA in TD children or children with CP. Future research examining the relationship between ATMA and joint angle for these populations is warranted for developing appropriate musculoskeletal models, facilitating their use in a clinical setting.

ETHICS

This study was approved by the Princess Margaret Hospital (#2013085), University of Western Australia (#RA/4/1/6780), University of New South Wales (#09179) and University of Sydney (#12192) Human Research Ethics Committees.

ACKNOWLEDGMENTS

The authors would like to thank the Cerebral Palsy Mobility Services and Diagnostic Imaging teams at Princess Margaret Hospital, the PMH Foundation, as well as the children and their families who participated in this research.

The authors would like to thank Elizabeth Clarke and Robert Herbert for the use of their adult population data and their meaningful contributions to the development of this study.

CA is supported by the Australian Post-graduate Award, the UWA Safety-net top-up and the Ernest and Evelyn Havill Shacklock top-up scholarships for her PhD candidature; SR is supported by the BrightSpark Foundation and the Pay-it-Forward Foundation.

4.5 REFERENCES


Chapter 5 Muscle volume alterations in children with cerebral palsy following the first botulinum toxin treatment: A 6 month prospective cohort study.

This manuscript was submitted for publication in *Developmental Medicine and Child Neurology* in September 2017.

The manuscript was accepted for publication June 2018.

FOREWORD

Chapters three and four in this thesis established and utilised a method for assessing musculoskeletal geometry, specifically the ATMA. The findings highlighted the limitation of scaling a generic model to anthropometric variables, as children with CP demonstrate significant differences to TD children. Alterations in musculoskeletal parameters in children with CP, when compared to TD children, however, may not be consistent within the population, or over time. A large number of interventions used in the treatment of children with CP have the potential to impact upon muscle-tendon dynamic parameters. Accurate understanding of the way in which such interventions do so is critical to developing neuromusculoskeletal models that accurately represent children with CP. BoNT-A widely used for the treatment of focal spasticity in children with CP as a part of evidence based best practice care. While it has an excellent safety profile, and strong, consistent evidence for functional and clinical gains, there is some concern of the potential atrophic effects on the targeted muscle. This has been documented in children with CP who have received multiple BoNT-A injections previously, however animal models suggest the first exposure to BoNT-A may have the greatest impact on muscle morphology. Chapter five investigates the effect of the first BoNT-A exposure on muscle volume in toxin naïve children with CP. This is the first study to document the initial impact of BoNT-A treatment on the targeted and surrounding muscles, with changes tracked over a six-month period of time. This information confirms the impact of interventions on muscle-tendon dynamic parameters in not only the targeted muscle, but also surrounding musculature, highlighting the importance of accounting for treatment history in developing subject specific neuromusculoskeletal models. Clinically, this chapter implicates future research, and may guide treatment plans for the use of BoNT-A in children with CP.
5.1 ABSTRACT

AIM

This study aimed to track muscle volume (MV) alterations for six months in children with cerebral palsy (CP) following the first exposure to botulinum toxin (BoNT-A), a commonly used focal spasticity treatment.

METHODS

Eleven ambulant children (eight male, three female) with spastic CP, aged 8.8 years (±3.1) participated. Participants received injections to the affected gastrocnemius. MV of the gastrocnemius, soleus and hamstrings was measured using Magnetic Resonance Imaging. MV was normalised to bone length, and changes analysed relative to baseline. Assessments were conducted one week prior to, and four, 13 and 25 weeks post BoNT-A treatment.

RESULTS

All children demonstrated positive clinical and functional gains. MV of the injected gastrocnemius was found to be significantly reduced at four weeks (-0.15ml/mm; 95%CI -0.27, -0.05), 13 weeks (-0.24ml/mm; 95%CI -0.37, -0.12) and 25 weeks (-0.22ml/mm; 95%CI -0.34, -0.10) compared to baseline. Significant increases in normalised soleus MV were identified at each follow up, while hamstrings showed significant increase at four weeks only.

INTERPRETATION

Absolute and normalised MV of the injected muscle reduces following first BoNT-A exposure, and does not return to baseline volume by 25 weeks. Hypertrophy is seen in the soleus up to 25 weeks, but not in the hamstrings.
5.1 Introduction

Botulinum toxin type-A (BoNT-A) is a widely used treatment for focal spasticity [1, 2], a common motor impairment seen in children with cerebral palsy (CP) [3], with a well-documented safety profile [4], and an array of positive clinical and functional outcomes [1]. With muscle volume (MV) in children with CP reduced compared to their typically developing (TD) counterparts [5, 6], research has focused on the potential for BoNT-A exposure to further reduce MV. Data from animal and healthy adult human studies have both found muscle atrophy in response to BoNT-A exposure [7-10]. Muscle atrophy (reduction in absolute MV) may significantly impact the muscle’s functional ability.

Recent research has provided evidence to support the hypothesis that BoNT-A exposure among children with CP is associated with impaired muscle growth in the short term. BoNT-A exposure results in atrophy of between 4.47% and 20.5% of injected muscle, between five and 12 weeks post injection, depending on the muscle assessed and the measurement technique used [11-13]. While the pharmacological effect of BoNT-A washes out by approximately 12 weeks [14], it is important to understand the long term impact beyond this period. Studies have identified in children with CP, injected psoas atrophy of 13.8% at six months [11], and injected gastrocnemius hypertrophy of 13% at 12 months [15], though the authors noted this rate of growth was reduced compared to TD children.

While atrophy of the injected muscle is consistently found in the first six months following exposure, there is no clear consensus on the expected magnitude of this reduction. This variation may stem from multiple factors, including: measurement techniques; normalisation; muscles assessed; ambulation status; BoNT-A dosage; and previous BoNT-A exposure (which has been found to be an important factor in predicting atrophy in animal models [8, 9]). While some of these factors were well described in the aforementioned studies, others were not, clouding interpretation.

For a greater understanding of the global impact of the targeted intervention, assessment of the synergistic and antagonistic muscles is required. This has been shown to be true in animal models, with significant alterations in non-injected muscle still apparent six months post injection [9]. To the authors’ knowledge, only one study has done this in children with CP, whom were serial BoNT-A receivers, reporting a significant increase of soleus volume at five weeks post gastrocnemius injection [13]. The authors suggest this is evidence of compensatory hypertrophy, in order to maintain function of the plantarflexor group. The bi-articular gastrocnemius acts as a both a plantarflexor and a knee flexor [16]. To date, no research has investigated the possibility of compensatory hypertrophy of the hamstrings to maintain knee flexion function. Such assessment of the associated muscles is important in contributing to the understanding of the functional implications of muscle growth alterations.
The objective of this study is to assess MV change, using MRI, of the injected and synergistic muscles, following the first exposure to clinically given BoNT-A in children with CP. MV changes were tracked over a six month period, with assessments at four, 13 and 25 weeks, relative to baseline (pre BoNT-A) volume. It is hypothesised the injected gastrocnemius muscle will show atrophy at each time point, with the greatest atrophy expected at 13 weeks post injection, while hypertrophy will be found in the synergistic muscles of the soleus and medial hamstring group at each time point.

5.2 METHOD
This is an observational prospective cohort design. BoNT-A for focal spasticity management in children with CP is now best practice care [2], therefore case controlled, or randomised control trials are not ethically appropriate. Baseline assessments were conducted prior to BoNT-A exposure, with follow up assessments conducted at four, 13 and 25 weeks post injection. Time points correspond to peak neurological effect [14], period of clinical usefulness [2] and recommended consideration for repeat injection [17]. Approval was granted by Princess Margaret Hospital (PMH) (2013085) and University of Western Australia (RA/4/1/6780) ethics committees.

Participants were recruited from the CP Mobility Service, PMH, Perth, Western Australia from July 2013 until August 2015, with data collection occurring until September 2015.

Children were eligible for this study by meeting the following criteria: (i) diagnosis of spastic CP, (ii) aged between four and 13 years, (iii) toxin naïve at time of enrolment, (iv) no previous lower limb surgery, (v) clinical management plan involved lower limb BoNT-A. Children were excluded from this study if the following criteria were met: (i) failure to comply with MRI protocol, (ii) additional interventions, beyond standard physical therapy, during the data collection period.

In total, 22 patients were identified as eligible and invited to participate. Informed consent was provided for 13 participants, and 11 participants (eight males, three females) completed baseline and at least one follow up assessment. Participants were aged between five and 13 years (8y 10m ± 3y 1m), and were classified as Gross Motor Function Classification System level I (n=7) or II (n=4). Seven participants were classified with diplegia, four with hemiplegia. All participants had spasticity, as measured by the Modified Tardieu Scale [18] of the gastrocnemius with a mean dynamic muscle length of 18.3° (± 10.8°) plantarflexion, and a mean static muscle length of 4.8° (± 5.4°) dorsiflexion. The average height of the participants was 131.2cm (± 11.53cm), and average weight was 31.8kg (± 6.3kg).

Participants received ultrasound guided injections of BoNT-A (Botox®; Allergan, Irvine, CA, USA) to the targeted muscle, with a total dose of between 60 and 340 units of BoNT-A total
(1.6U/kg to 12U/kg), using 1.2ml dilution (1.47ml ± 0.51ml). All participants received BoNT-A to the affected gastrocnemius (18 legs injected, 1.4-4.8U/kg). Other muscles injected were the medial hamstrings (6 legs, 1.4U/kg); tibialis posterior (2 legs, 1U/kg); and rectus femoris (2 legs, 0.6U/kg).

Figure 5.1 Participant attrition and sample size at each follow up assessment. FES, functional electrical stimulation.

Timing for baseline assessment was 1.17 week (±1.10) prior to their first BoNT-A. Follow up assessments were conducted at 4.3 weeks (±1.1) (Ax1), 13.1 weeks (±1.2) (Ax2) and 24.7 weeks (±1.9) (Ax3). Seven participants completed all follow ups, while four completed between one and two follow ups (Figure 5.1). All participant data were used in the analysis assessing lower leg MV, and functional results, with sample sizes outlined in Figure 5.1. For the hamstring MV analysis, only those who did not have upper leg injections were assessed: 12 legs were assessed at baseline, nine legs at Ax1, nine legs at Ax2 and 11 legs at Ax3.

Clinical outcomes, including technical response at the six week clinical follow up, further interventions and adverse events, were noted. A technical response was taken as a reduction in the dynamic component of muscle length (the difference between R1 and R2) detected on the
Modified Tardieu Scale [18] of more than 5°. To assess functional mobility capacity, the 6-minute walk test (6MWT) [19] and Timed Up and Go (TUG) [20] assessments were used.

A 1.5T whole-body magnetic resonance imaging (MRI) unit (Magnetom Sonato Maestro Class, Siemens Medical solutions, Erlangen, Germany) was used to acquire axial scans from the iliac crest to the ankle malleoli, using a T1 weighted spin-echo sequence. Participants lay prone with their hips and knees in a neutral position, with ankles in a relaxed posture. Positioning was maintained passively.

Images were collected using a repetition time of 572ms and an echo time of 13ms. Slices were 5mm thick, with an average slice increment of 10.2mm (10.0-12.5mm). Scan matrix size and field of view (FoV) were optimised for each participant, with an average lower leg matrix of 256.0 x 124.5mm and FoV of 333.1mm, and an average thigh matrix of 256.0 x 129.0mm and FoV of 343.4mm. The mean number of slices for the lower leg was 35.2 (27-43 slices), and for the thigh was 37.9 (27-50 slices).

MR images were processed using Mimics® (version 16.0, Materialise, Leuven, Belgium). Muscles were manually traced by a single, blinded assessor (CA). Bilateral MV assessments were made for participants with diplegia, while only the affected side was assessed for participants with hemiplegia. Muscle segmented and used for analysis were the gastrocnemius (medial and lateral heads), soleus, tibialis anterior and hamstrings (biceps femoris, semitendinosus and semimembranosus). MV was calculated as the product of the number of voxels in each reconstruction and the voxel size. MV were analysed both as absolute volumes, and normalised volumes, to account for skeletal growth over the study period. For this, lower leg MV were normalised to tibia length, and hamstring volume was normalised to femur length [13].

The repeatability of this method has been previously reported, with Intraclass Correlation Coefficient (ICC) for inter- and intra-rater reliability being greater than 0.94 and 0.92, respectively [21]. For the single assessor in this study (CA), intra-rater repeatability was assessed on a random selection of five participants. For these participants 10 muscles were assessed and found to possess excellent repeatability (ICC = 0.998), and a mean measurement difference of 1ml.

Statistical analysis was conducted in Stata (StataCorp LLC, Texas, USA) to compare each follow up assessment to baseline measurements. For all functional and MV data, mixed model analysis were used to account for repeated measures over time and missing data points, as well as correlation between left and right limbs for children with diplegia. Predicted means are presented, with 95% confidence intervals. Statistical significance was accepted at \( \alpha = 0.05 \).
5.3 RESULTS

5.3.1 PARTICIPANTS
At the six week clinical follow up, mean dynamic muscle length of the plantarflexors was 5.8° (±7.1°) plantarflexion, and a mean static muscle length of 6.5° (±4.6°) dorsiflexion. All participants showed a technical response to BoNT-A, with the mean difference between R1 and R2 measurements of the gastrocnemius reducing from 23.1° (±9.5°) to 12.2° (±6.6°). No adverse events were reported. Nine participants went on to receive further BoNT-A at an average of 26.7 weeks (±2.0). Two participants were deemed to not require further intervention at their 26 week clinical follow up, and had not received any further intervention by 52 week follow-up. Significant improvements in 6MWT were identified at 13 and 25 weeks, while significant improvements in TUG were identified at all time points following BoNT-A exposure (Error! Reference source not found.).

5.3.2 MUSCLE VOLUME
Absolute changes in MV are presented in Error! Reference source not found.. The injected gastrocnemius showed significant atrophy at four, 13 and 25 weeks post injection, equating to a 5.9%, 9.4% and 6.8% reduction in MV respectively. The soleus showed significant hypertrophy at four, 13 and 25 weeks of 6.0%, 7.7% and 10.8% respectively. The total plantarflexor group showed significant hypertrophy at 25 weeks post injection of 5.1%. No changes were found in the tibialis anterior. Of those who received gastrocnemius injections only, the hamstrings showed significant hypertrophy at four (2.1%), 13 (5.7%) and 25 (7.0%) weeks.

Normalised MV’s are presented in Error! Reference source not found.. Gastrocnemius showed significant reductions of 6.7%, 10.7% and 9.8% at four, 13 and 25 weeks respectively. Soleus showed significant increase in normalised MV of 5.4%, 6.1% and 7.4% at four, 13 and 25 weeks respectively. No changes were identified in the total plantarflexor volume, or the tibialis anterior. Of those who received gastrocnemius injections only, the hamstring group showed significant increase in normalised MV at the four week follow up only of 5.5%.

5.4 DISCUSSION
The results support the hypothesis that MV is reduced following BoNT-A exposure. In the injected gastrocnemius, absolute MV was found to be reduced at all time points relative to baseline, indicating true muscle atrophy. However, a more accurate understanding of the magnitude MV changes over time in paediatric populations comes from assessment of MV change relative to limb growth. Indeed, in TD children we can expect hypertrophy over time, with the absolute MV increasing [21], allowing maintenance of the functional capacity of a muscle as the child grows. Normalising the muscle to bone length allows skeletal growth to be taken into account [13]. When assessing alterations of normalised MV of the injected gastrocnemius, reductions occurred at all time points.
### Table 5.1 6 Minute Walk Test and Timed Up-and-Go changes relative to baseline, with 95% confidence intervals for changes seen. *p<0.05; **p<0.01

<table>
<thead>
<tr>
<th></th>
<th>Ax0 mean (95% CI)</th>
<th>Ax1 mean Δ (95% CI)</th>
<th>Ax2 mean Δ (95% CI)</th>
<th>Ax3 mean Δ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MWT (m)</td>
<td>520.05 (429.52, 610.58)</td>
<td>+26.85 (-12.02, 72.71)</td>
<td>+50.64 (4.56, 96.72)</td>
<td>+52.72 (5.07, 102.37) *</td>
</tr>
<tr>
<td>TUG (s)</td>
<td>5.17 (4.25, 6.09)</td>
<td>-1.07 (-1.99, -0.16) *</td>
<td>-1.08 (-1.99, -0.16) *</td>
<td>-1.15 (-2.02, -0.29) **</td>
</tr>
</tbody>
</table>

### Table 5.2 Absolute changes in muscle volume relative to baseline, with 95% confidence intervals for change seen. Entire sample was used in calculations for muscles of the lower leg. Only those who did not receive upper leg injections were used for calculations of changes in hamstring muscle volume (n=12). *p<0.05; **p<0.01

<table>
<thead>
<tr>
<th></th>
<th>Ax0 ml (95% CI)</th>
<th>Ax1 Δ ml (95% CI)</th>
<th>Ax2 Δ ml (95% CI)</th>
<th>Ax3 Δ ml (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrocnemius</td>
<td>56.32 (44.80, 67.84)</td>
<td>-3.34 (-6.43, -0.25) *</td>
<td>-5.27 (-8.54, -2.01) **</td>
<td>-3.84 (-6.93, -0.74) *</td>
</tr>
<tr>
<td>Soleus</td>
<td>116.28 (94.42, 138.12)</td>
<td>+6.98 (2.09, 11.88) **</td>
<td>+8.90 (3.73, 14.08) **</td>
<td>+12.56 (7.66, 17.47) **</td>
</tr>
<tr>
<td>Plantarflexors</td>
<td>172.56 (140.60, 204.52)</td>
<td>+3.60 (-3.42, 10.67)</td>
<td>+3.63 (-3.83, 11.08)</td>
<td>+8.72 (1.66, 15.79) *</td>
</tr>
<tr>
<td>Tibialis Anterior</td>
<td>21.24 (18.30, 24.18)</td>
<td>+0.05 (-1.46, 1.56)</td>
<td>+1.28 (-0.31, 2.88)</td>
<td>+0.99 (-0.53, 2.50)</td>
</tr>
<tr>
<td>Hamstrings</td>
<td>145.37 (105.57, 185.16)</td>
<td>+3.01 (2.04, 13.83) **</td>
<td>+8.28 (2.37, 14.18) **</td>
<td>+10.18 (4.70, 15.65) **</td>
</tr>
</tbody>
</table>

### Table 5.3 Changes in normalised muscle volume of the lower leg relative to baseline with 95% confidence intervals. Entire sample was used in calculations for muscles of the lower leg. Only those who did not receive upper leg injections were used for calculations of changes in hamstring muscle volume (n=12). *p<0.05, **p<0.01

<table>
<thead>
<tr>
<th></th>
<th>Ax0 ml/mm (95% CI)</th>
<th>Ax1 Δ ml/mm (95% CI)</th>
<th>Ax2 Δ ml/mm (95% CI)</th>
<th>Ax3 Δ ml/mm (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrocnemius</td>
<td>2.24 (1.86, 2.62)</td>
<td>-0.15 (-0.27, -0.03) *</td>
<td>-0.24 (-0.37, -0.12) **</td>
<td>-0.22 (-0.34, -0.10) **</td>
</tr>
<tr>
<td>Soleus</td>
<td>4.61 (3.99, 5.24)</td>
<td>+0.25 (0.07, 0.43) **</td>
<td>+0.28 (0.09, 0.48) **</td>
<td>+0.34 (0.16, 0.53) **</td>
</tr>
<tr>
<td>Plantarflexors</td>
<td>6.85 (5.91, 7.79)</td>
<td>+0.10 (-0.16, 0.37)</td>
<td>+0.04 (-0.24, 0.32)</td>
<td>+0.12 (-0.15, 0.39)</td>
</tr>
<tr>
<td>Tibialis Anterior</td>
<td>0.84 (0.76, 0.92)</td>
<td>0.00 (-0.06, 0.06)</td>
<td>+0.04 (-0.02, 0.10)</td>
<td>+0.02 (-0.04, 0.08)</td>
</tr>
<tr>
<td>Hamstrings</td>
<td>5.27 (4.36, 6.29)</td>
<td>+0.29 (0.07, 0.50) **</td>
<td>+0.20 (-0.02, 0.41)</td>
<td>+0.19 (-0.01, 0.40)</td>
</tr>
</tbody>
</table>
Based on previous research, and understanding that the period of clinical usefulness of BoNT-A is 12-16 weeks, peak atrophy was hypothesised to occur at 13 weeks post injection. The findings support this, with the normalised MV showing the greatest reduction (-10.7%) at 13 weeks. This suggests muscle recovery does occur once the pharmacological effect of the BoNT-A wears off, however full recovery was not achieved by 25 weeks.

While normalised MV provide some insight into the functional volume change, the gastrocnemius is not the only muscle that acts as a plantarflexor. As hypothesised, normalised soleus volume showed significant increase at follow up assessments, resulting in no changes to total plantarflexor volume at any stage. This finding suggests the functional strength capacity of the plantarflexors may be maintained despite gastrocnemius atrophy. However, given the poor relationship between muscle size and measures of muscle strength in children with CP [6] and the impact of BoNT-A exposure on muscle histology [22], further research is required to confirm this.

The hypertrophy of the soleus may be a compensatory mechanism to maintain the role of plantarflexion at the ankle. However, as the gastrocnemius is a biarticular muscle; a reduction in the capacity of the gastrocnemius may also effect its role as a knee flexor [16]. This is the first study to assess alterations in hamstring volume following gastrocnemius injections, and show absolute hypertrophy of the hamstrings at all time points, though normalised MV only demonstrated an increase a 4 weeks. In simulations of healthy adult knee kinetics, a reduction in gastrocnemius force has been found to result in a substantial increase in medial hamstring force to maintain the same knee flexion moment [16]. The results suggest there may be reduced capacity to generate power at the knee as the atrophy in the gastrocnemius (and potential reduction in force generating capacity) is not accompanied by hypertrophy in the hamstrings.

Although the sample size was small, this study met the power required for statistical significance. However, the small sample size of children who received injections to the hamstrings prevented a full statistical analysis of this group, thus limiting the application of these results to single-level BoNT-A cases only. Though the relationships between limbs in children with diplegia was statistically controlled for, the inclusion of both children with diplegia and children with hemiplegia may further limit the generalisability of the results, as research suggests notable differences in response to BoNT-A injections and re-injection schedules with CP subtype [23].

With limited data available in the literature on the natural progression of muscle growth and development in children with CP, this study was also limited by the lack of control group. As such, causal links between BoNT-A exposure and alterations in non-injected muscle groups cannot be established. Clinical outcomes were assessed by clinical reporting of goal based outcomes and functional assessments (TUG and 6MWT), future research would benefit from a more rigorous assessment of functional outcomes including muscle strength.
The findings of this study support previous research showing atrophy in the injected muscle. The magnitude of this absolute reduction was less than that identified in previous research. At four and 13 weeks post injection, the current study identified reductions of 5.9% and 9.4%, compared to 11.2% and 17.6% by Park and colleagues [12], a difference attributed to the measurement method used, with Park and colleagues using muscle thickness as a substitute for MV [12]. At 25 weeks, the 6.8% reduction in MV is notably less than the 13.8% reduction found by van Campenhout and colleagues [11]. This discrepancy may be explained by the different muscle assessed (the psoas muscle, markedly different in its operating characteristics compared to the gastrocnemius) and the ambulatory status of the participants in the study by van Campenhout (which was not stated).

The results of this study suggest a larger magnitude reduction in injected MV compared to previous research by Williams and colleagues [13]. With the muscle of interest and the measurement technique used identical, this may be attributed to participants' BoNT-A history. In the current study, all participants were toxin naïve, while participants in the work of Williams and colleagues had received on average 8.9 (two – 15) injections prior to enrolment. This suggests first exposure to BoNT-A results in greater atrophy compared to subsequent exposure, a finding that is supported by animal models [8]. The larger reduction in injected gastrocnemius in this study was offset by a larger magnitude in normalised soleus volume, resulting in no change in the total plantarflexor volume, replicating the findings of the previous body of work. Williams and colleagues did not report further follow up assessments, so the rate of recovery cannot currently be compared.

No previous research has assessed changes in hamstring muscle volume associated gastrocnemius BoNT-A exposure in children with CP. While absolute hypertrophy (7.0%) was found at 25 weeks, it is notably less than the hypertrophy seen in TD children over the same time period [21]. However, this difference may reflect a reduced rate of muscle growth in children with CP regardless of intervention. While no longitudinal data is currently available on the natural progression of muscle growth in toxin naïve children with CP, cross sectional research has found children with spastic CP demonstrate reduced MV from the age of 15 months [24], and that MV correlates with age [25, 26].

This study has found at 25 weeks MV has not fully recovered, consistent with previous animal [9, 27] and human research [11]. Previously published data by Barber and colleagues has found at 52 weeks, injected gastrocnemius shows a 4.8% increase in normalised volume (calculated based on presented fibula lengths) in toxin naïve children with CP [15]. Combined, these results suggest that gastrocnemius atrophy recovers between 25 and 52 weeks. Indeed, Barber and colleagues reported no significant differences in MV from baseline to 52 weeks post injections in children with CP who received a single BoNT-A injection, compared to those who received 3
injections in the same time period. However, children who received three injections showed slower growth over 52 weeks (1.1% growth compared to 4.8% in single injection group, calculated based on presented fibula lengths) [15].

Understanding when full MV recovery occurs post BoNT-A exposure is important when considering the timing of repeat injections (currently recommended at 26 weeks [17]), and the functional and long term clinical consequences this may have. This has been highlighted by recent research assessing morphological and functional changes in healthy animal muscle following BoNT-A exposure; Minamoto and colleagues (2015) found the effect of subsequent BoNT-A injections is notably stronger when muscles are reinjected prior to complete recovery [28], while Ward and colleagues (2017) identified that muscle mass recovery preceded recovery of torque production by six months [27]. Furthermore, two randomised control trials comparing four-monthly and yearly BoNT-A exposure in children with CP identified no differences in clinical outcomes between the two re-injection schedules [23, 29]. While more research is required into the functional and morphological recovery of muscle in children with CP, the results of this study provide data to further support a conservative re-injection schedule of greater than 26 weeks is preferable.

ACKNOWLEDGMENTS

Thank you to the CP Mobility Services and Diagnostic Imaging teams at PMH (with specific thanks to Martins Spits and Erin Robins), the PMH Foundation, as well as the children and their families who participated in this research.

CA is supported by the Australian Post-graduate Award, the UWA and Havill Shacklock top-up scholarships; SR is supported by a BrightSpark Foundation and Pay-it-Forward Foundation Fellowship; and JV is supported by the RACP AFRM Ipsen Open Research Fellowship.

5.5 REFERENCES


Chapter 6 Statistical methodologies for analysis of time varying waveforms in clinical gait analysis
FOREWORD

Chapters three to five of this thesis have been concerned with the validity of inputs used to develop and scale neuromusculoskeletal models for children with CP. Musculoskeletal models have primarily focused on the analysis of ambulation or gait. Gait analysis is highly complex and results in a large number of variables being available to clinicians, who are required to accurately interpret them in order to determine best clinical management. Thus, attention is now turned to the handling of biomechanical data within clinical gait settings. Due to the data’s inherent complexity, subjective observational analysis is typically employed for the assessment of data in clinical environments. This chapter considers four statistical methodologies currently being applied to biomechanical gait data within the research, and assesses them for their ability to address key challenges inherent in time-varying biomechanical data, and for the applicability to clinical settings, with particular focus on the ease of interpretation and meaningfulness of the results generated. This chapter is intended to provide contextual information to enhance the outcomes and interpretation of the final chapter in this thesis.
6.1 BACKGROUND

The quantitative analysis of gait waveforms is no simple task as they are multifaceted, time varying and dynamic variables. Within clinical settings, whilst objective scores are often estimated in the form of gait indices, such as the Gait Profile Score [1], or Gillette Gait Index [2], clinicians rely heavily on the subjective identification of gait ‘features’, or regions where gait variable for one participant/time point displays differences compared to a normative sample or another time point in the series. As with any method dominated by subjective assessment, identifying differences in this way is prone to bias and error stemming from multiple sources, which include, but are not limited to, the clinicians’ level and type of experience as well as biases (conscious or unconscious) relating to what can, and should, be addressed in the management of gait pathology. In addition, accurately assessing the high variability of gait profiles is incredibly difficult, as such variance can be non-linear and difficult to perceive visually.

Objective analysis, however, is incredibly challenging, thanks to the complexity of gait data. In their review, Chau (2001) [3] identified five key challenges faced when attempting to analyse quantitative gait data.

1) **Correlations between curves.** In his review, Chau describes this challenge in terms of mathematically assessing similarities and differences between two time-varying waveforms, or curves. This is not to be confused with the inherent relationship between variable curves from a single gait dataset.

2) **Temporal Dependence.** Biomechanical data collected during gait, while quasi-periodic, exhibits temporal dependence. Each variable is, by nature, a smooth, time-varying waveform. This smoothness is not trivial, and represents inter-dependence between local data points [4].

3) **High variability.** Variability across the waveform is non-linear, and comes from many sources, including inherent biomechanical variability seen intrasubject and intersubject, as well as variability due to instrumentation of data collection, such as marker placements and soft tissue artefact.

4) **High Dimensionality.** High dimensionality refers to the large volume of variables contained in a gait dataset, including numerous potential inputs (marker trajectories, ground reaction forces, subject anthropometry, electromyography, and metabolic measures such as oxygen consumption) and estimations (joint angles, velocities, moments, powers, internal muscle and joint forces).

5) **Nonlinear relationships.** Dynamics of human movement are intrinsically non-linear. As such, gait variables also relate in a complex manner. However, what is of critical importance within this challenge is the acknowledgment that variables are inter-related and thus cannot be regarded as independent of each other.
The statistical methods frequently employed to analyse gait data [5] are rarely equipped to handle all five challenges identified by Chau [3]. In contrast, summary statistics (such as means, or variance) and waveform parameterisations (such as peak amplitudes, or amplitudes at discrete time points) are not equipped to characterise the waveform as they are not able to correctly model the time varying randomness of waveform data. At best, they provide no more information than that which can be gained by simply observing the bivariate plots [3]. At worst, they result in false identification of differences, or the lack thereof, as variance is modelled as zero-dimensional (0D) [5, 6]. It is widely accepted in the literature that an appropriate statistical tool which models the randomness of time varying data, and addresses the challenges inherent in biomechanical data, is lacking in the field of gait, leaving subjective assessment as the primary mode of analysis within clinical gait settings.

6.1.1 STATEMENT OF THE PROBLEM

In attempting to address the lack of an appropriate statistical tool for use in time varying gait analysis, researchers have explored numerous approaches, drawing on inspiration from a vast array of fields from psychology to engineering. While non-traditional methodologies such as neural networks (machine learning) show future promise, multivariate statistical tools are based on extensive research and are therefore the most widely understood and applied methods [7]. However, to date, the uptake of any of the various methods into clinical practice has been lacking. This may be due to a number of factors including perceived theoretical and computational difficulties in running the statistical analyses. Finally, even if these advanced statistical methods can be used to identify significant effects, many time results lose all clinical relevance as most advanced statistical methods transform data outside of the measurement domain the signal was originally collected, which limits one’s ability to understand them in a clinically or practically meaningful way. Meaningful interpretation of the results may require a significant knowledge shift in order to become common place in clinical practice. The extent of any knowledge shift required, however, must be balanced with the benefits gained from uptake of any given methodology.

6.1.2 CHAPTER AIMS

This chapter aims to review the existing multivariate statistical methods which have been applied to biomechanical data to identify the most appropriate method for application to clinical gait analysis. This method should, along with addressing Chau’s [3] challenges mentioned above, meet the following requirements: a) be freely available for use in any laboratory setting; b) be computationally efficient to run; c) provide inherently meaningful outputs.

6.2 REVIEW OF AVAILABLE METHODS

Four different multivariate statistical methods will be reviewed in detail in this chapter: Bootstrap Confidence Bands, Principal Component Analysis (PCA), Functional PCA and Vector Field
Statistics. Each method addresses at least three of the five challenges defined by Chau [3], as shown in Figure 6.1. Two other multivariate statistical methods were considered for review: Factor Analysis and Multiple Correspondence Analysis. Factor Analysis was excluded due to its primary application to electromyography latency research, and limited scope for application to gait kinematics and kinetics. Multiple Correspondence Analysis was excluded as it is a descriptive multivariate tool, and does not provide statistical data, rather a graphical display that requires high level of interpretation to extract meaningful and clinically relevant data.

Figure 6.1 The statistical methodologies reviewed in this chapter and the challenges they each address

6.2.1 Bootstrap Confidence Bands

6.2.1.1 Conceptual Overview

Bootstrap confidence bands were first introduced and applied to biomechanical data in the 1990’s [8, 9]. Confidence bands are to a gait curve as confidence intervals are to a numerical parameter: they define the region containing the true mean with a predetermined probability. This probability is typically set at 0.95, indicating there is a 95% probability the true mean is within the band limits, while statistically there remains a 5% chance that the observed result occurred by random chance. Establishing of confidence bands for gait data is conducted using either a Gaussian approximation or a bootstrap method. It has been suggested a bootstrap method is the preferred option, as this is a non-parametric procedure, which does not assume a particular model for the data distribution [9]. This reduces the risk of distortion between the presumed and true probability of the waveform data. Indeed, Bootstrap methods for defining confidence bands were found to be more reliable, or less likely to result in a Type I error, than Gaussian approximations [10]. Results
are presented in a plot similar to that in which kinematic curves are presented, allowing for an intuitive interpretation, as shown in Figure 6.2.

![Figure 6.2 Example output of 90% confidence band for knee flexion angle across the gait cycle, adapted from Lenhoff et al (1999).](image)

**Figure 6.2** Example output of 90% confidence band for knee flexion angle across the gait cycle, adapted from Lenhoff et al (1999) [9].

**Application to gait analysis**

As outlined in Figure 6.1, bootstrap confidence bands address three out of the five key challenges for the analysis of gait data: *correlations between curves* (Challenge 1), *temporal dependence* (Challenge 2); and *high variability* (Challenge 3).

*Correlations between curves* (Challenge 1) can be assessed through the generation of confidence bands for the mean difference between the two curves. In this instance, where the entire confidence band exceeds the horizontal line value zero, a significant difference exists. Where the horizontal line value zero is contained within the confidence band, no such difference can be statistically confirmed. Duhamel and colleagues (2004) showcased the use of bootstrap confidence bands by comparing the knee flexion angle across the gait cycle between young and elderly patients. The confidence band for differences between the mean curve of each group showed differences between 64% and 76% of the gait cycle [10] as shown in Figure 6.3.

Unlike a Gaussian approximation, which computes a confidence interval on a point by point basis to generate a band, a bootstrap method constructs bands for the continuous waveform, thus addressing *temporal dependence* (Challenge 2). Furthermore, the use of confidence bands for gait curves has the clear advantage of retaining temporal information without losing clinical meaningfulness.
The high variability (Challenge 3) nature of gait data is directly addressed through the generation of confidence bands using the bootstrap technique. Where complex events, such as peak amplitude, are being studied, the high within- and between-subject variability of the data means it is near impossible to a priori determine the exact time in the gait cycle when this will occur. Bootstrap methods are able to overcome this by computing a confidence band accounting for variability across the entire gait cycle [9].

**Figure 6.3** Example output of bootstrap confidence band for comparing differences between two knee flexion curves. The red bar indicates the portion of statistically significant differences, as the horizontal line value zero is not included within the confidence band. Figure adapted from Duhamel and colleagues (2004) [10]

As demonstrated in Figure 6.2 and Figure 6.3, the outputs of bootstrap confidence bands are inherently meaningful as they are presented directly in relation to the underlying variable dimensions, providing clear direction, magnitude and timing information.

### 6.2.1.3 Limitations

Although retaining clinical meaningfulness and temporal information, bootstrap confidence bands fail to address the high dimensionality (Challenge 4) and non-linear relationship (Challenge 5) challenges associated with the analysis of time varying gait data. Each variable is assessed in isolation, and there is no capacity to do otherwise. In this regard, it offers little more than robust statistical backing (in contrast to discrete statistical analysis on parameterisations) to observed differences in gait curves. While the importance of this is not to be diminished, in that it provides objectivity to clinical gait analysis reporting without interfering or altering the current procedure in any way, the potential application of it as a statistical tool to enhance our understanding of
pathological gait beyond what is currently achievable is rather limited in comparison to other methods.

6.2.2 PRINCIPAL COMPONENT ANALYSIS

6.2.2.1 Conceptual overview
Originating in the 1980’s [11], PCA aims to reduce large numbers of vector and scalar components to a smaller set, representative of the dataset as a whole, thus facilitating interpretation [3]. Assuming linear relationships between smooth data points, PCA transforms data into new orthogonal (i.e. independent and uncorrelated) components. A set of components that account for a pre-determined amount of variance (i.e. researchers may require 95% of variance to be explained) is then selected, and given the term ‘principal components’. The principal components are intended to maximally preserve the variance of the data through setting an appropriately high level of variance to be explained, while reducing the data to a more manageable set for further analysis. The success of PCA depends greatly on the ability to interpret the results. Interpretation requires the meaningful labelling of each principal component, of which there can be many. Within each principal component, multiple dependent variables can reside, with differing levels of correlation. As a result, interpretation is not always straightforward as a researcher’s a priori definition of what is an acceptable level of correlation will influence their interpretation of these data.

6.2.2.2 Application to gait analysis
As outlined in Figure 6.1, PCA addresses two out of the five key challenges of analysing gait data: high variability (Challenge 3); and high dimensionality (Challenge 4). It also partially addresses temporal dependence (Challenge 2).

Ingrained in its theory, PCA directly tackles the high variability (Challenge 3), as it attempts to reduce a large number of variables to a subset that can still adequately describe the variance of the dataset. Within PCA, every data point generated from gait analysis (such as a variable amplitude at a single time point) is considered a component. Researchers, therefore do not have to limit the data analysed a priori, and can select, based on their research questions, all relevant data to include in the PCA.

Temporal dependence (Challenge 2), can be partially addressed through PCA. Independence between data points is not assumed, and retention of meaningful temporal information is possible in some circumstances. For example, Deluzio and colleagues (1997) [12] assessed 13 osteoarthritis patients pre- and post- operatively, compared to 29 healthy controls to identify differences in knee flexion throughout the gait cycle. Two principal components were able to predict 96% of the variability in knee flexion/extension.
Figure 6.4 Example PCA output for two principal components, adapted from Deluzio et al (1997) [12]. a) The amount of variability explained over the gait cycle by PC1 and PC2. The shaded area shows PC1 is associated with greater levels of variability during the stance phase of gait b) the pre-operative score indicates the patient scored low on PC1, with similar PC2 scores to control participants.

A case study was used to demonstrate how temporal characteristics could be extracted using PCA. The variability explained by each principal component (PC1 and PC2) was plotted against the
percentage gait cycle, as shown in Figure 6.4a, indicating PC1 accounted for most variability in stance, while PC2 accounted for most variability in swing. The scores for PC1 and PC2 were then plotted for each control participant, and one osteoarthritis participant pre- and post-operatively (Figure 6.4b). Analysis showed post-operatively, the patient had similar PC1 and PC2 scores compared to the normative cohort. Pre-operatively, the PC2 score was similar, however PC1 was significantly different. Thus, the significant differences in knee flexion/extension pre-operation compared to controls could be attributed to differences in stance phase.

Finally, PCA can be used to address high dimensionality (Challenge 4) through its application to entire 3DGA data sets. Federolf and colleagues (2013) [13] used PCA to assess differences in gait kinematics and kinetics in patients with osteoarthritis compared to healthy controls. A total of 12,432 vector components (made up of 36 markers and GRF, in 3D space, over 112 time points) were analysed. Out of the ten PC’s identified, three had large Cohen’s d effect sizes and were therefore selected to form the resultant discriminant vector. This vector accounted for 26.2% of the variability in gait between osteoarthritis patients and controls, demonstrating the ability to analyse entire 3DGA datasets in a single analysis.

6.2.2.3 Limitations

A PCA assumes linearity in the data’s relationships, and therefore does not address the non-linear relationships (Challenge 5) associated with complex biomechanical data.

Furthermore, PCA does not allow for direct or complete analysis of correlations between curves (Challenge 1). Conditions and populations can be compared using the principal component variable scores using residuals and Mahalanobis distances, as performed by Deluzio and colleagues (1997) in the above example [12]. The insights provided by such analyses are limited, however, primarily due to the reliance on a meaningful principal component label and the collapsing of the differences in the curves into a single scalar score. As such, PCA reveals only global structure within data. While this is adequate for general conclusions, specific details about pathological gait are generally not revealed [3].

The meaningful labelling of the principal components generated from PCA requires further correlations with gait parameters, in combination with expert knowledge on human gait. The limitations of this approach are highlighted in an early study by Olney and colleagues (1998) [14]. Each of the four principal components were identified in assessment of gait deviation among 33 adult stroke patients for correlation with gait parameters (such as stride length or maximum hip power), and then assessed subjectively to identify common themes. Of the variables contained in PC1, the majority related to velocity, leading the authors to conclude PC1 represented ‘speed’. PC2 was identified as limb differences, and PC3 as postural flexion bias. However PC4 significantly correlated with seven variables, though without a meaningful pattern being observed, the authors could not assign a meaningful label to it [14]. Meaningful labelling was an
issue also faced by Federolf and colleagues when interpreting the principal vector components of a PCA analysis through an entire gait cycle. The interpretation of the results was conducted by the subjective examination of stick figures of osteoarthritis patients and controls at various intervals of time, with the authors selecting 13 perceived differences. No statistical analyses were used in selection of these 13 variables, nor were they correlated in any way to the principal vector component identified. The subjective labelling of principal components, particularly when they are numerous, is also a time consuming process which may limit its usability in a clinical setting [3].

Even when temporal information is retained, the interpretation of the results is not directly intuitive or sometimes not possible. As highlighted in the work by Deluzio and colleagues reviewed above, the ease of interpretation is reliant on the smaller number of principal components and the clear separation of the effect on variability throughout the gait cycle. A greater number of principal components, or disjointed areas of their effects on variability, would mean attribution of differences to particular points in the gait cycle would not be feasible [12]. Furthermore, temporal information is lost completely when PCA is expanded to include the full dataset [15].

6.2.3 FUNCTIONAL PRINCIPAL COMPONENT ANALYSIS

6.2.3.1 Conceptual overview

Functional PCA, a component of the broader functional data analysis toolkit, is an extension of PCA, pioneered by Ramsay and Silverman in 1997 [16]. While traditional PCA transforms the smooth data points into orthogonal components for analysis, functional PCA applies PCA to the waveform, treating the waveform as a single entity [17, 18]. Generated functional principal components (fPC) are defined in the same domain as the original functions [18], facilitating interpretation. Functional PCA employs a graphical approach to the illustration of how an individual’s kinematics are altered by scoring high or low on an fPC, by adding or subtracting a multiple of the fPC to/from the mean variable amplitude, as illustrated in Figure 6.5. A high or low score, while showing the direction through the gait cycle, does not show magnitude, and functional interpretation of each component is required. Each participant or group can be compared by taking the integral of the fPC curve to provide an overall scalar, or score.

Functional PCA can also be applied to bivariate functions (two joint angle data, typically presented as angle-angle plots) to extract harmonics, called bivariate fPCs from the data [19]. In addition to the outputs presented in Figure 6.5, a bivariate angle plot of the bivariate fPC is also generated, as shown in Figure 6.6. Open circles are used to represent the speed of movement, while arrows are used to represent the direction and magnitude of the bivariate fPC’s influence on the two variables.
6.2.3.2 Application to gait analysis

As outlined in Figure 6.1, functional PCA addresses three of the five key challenges of analysing gait data: *temporal dependence* (Challenge 2); *high variability* (Challenge 3); and *high dimensionality* (Challenge 4).

As an extension of PCA, functional PCA retains the same ability to address *high variability* (Challenge 3) and *high dimensionality* (Challenge 4) as described in the earlier section on the application to gait analysis for PCA.

Functional PCA offers a more complete solution for addressing *temporal dependence* (Challenge 2) than traditional PCA. Through application of PCA to the entire waveform, and the subsequent presentation of data in the original functional domain (i.e. variable amplitude across the gait cycle), functional PCA both accounts for the time-varying nature of biomechanical data and retains this important feature for meaningful interpretation of differences [17, 18]. For example, Donoghue and colleagues (2008) compared the variability explained by each fPC over the gait cycle for three groups: patients with Achilles tendon injuries running with orthoses (ATO), patients with Achilles tendon injuries running without orthoses (ATNO), and controls. When considering the fPC1 for eversion, controls showed six times greater variation than either AT group at 40% of stance, with the ATO showing increased variation relative to ATNO at the same stage [18]. From this, the authors concluded the increased variation provided by orthoses may assist to relieve injury symptoms.

![Figure 6.5 Example output of functional PCA, adapted from Donoghue et al (2008) [18]](image-url)
Figure 6.6 Example output for a bivariate fPC, adapted from Harrison et al (2007) [19]

Functional PCA can include landmark registration. Landmark registration synchronises the timing of features such as maximum or minimum amplitude, which are variable among and within participants, for easier comparison of variability in these features [17]. The appropriate use of landmark registration can further address high variability (Challenge 3) without compromising temporal dependence (Challenge 2). Ryan and colleagues (2006) used landmark registration to assess variability in knee joint angle at the bottom of the countermovement during a vertical jump movement. Interpretation of the first fPC showed participants who scored high reached the bottom of the countermovement later than those who scored low. From the registered graphs, authors were able to conclude participants who scored high on the first fPC also showed increased knee angle compared to participants who scored low [17].

6.2.3.3 Limitations

As with traditional PCA, functional PCA provides a scalar score which is derived from each fPC. Standard scalar statistical methods, such as ANOVAs are then used to compare differences in fPC scores [17]. As such, functional analysis does not adequately address the correlation between curves (Challenge 1).

The non-linear relationships (Challenge 5) of biomechanical data again are not addressed through functional PCA as this extension of PCA does not alter the linear assumptions made.

Functional PCA provides greater clinical meaningfulness relative to traditional PCA. This is achieved as fPCs are defined in the same domain as the original observations. However, appropriate functional interpretations of each fPC is still required. As with PCA, this interpretation may take significant time and limit its usability in a clinical setting. Furthermore,
while a high or low score can be graphically represented, it does not directly relate to the magnitude of joint differences, again requiring accurate interpretation to deduce meaning [17].

6.2.4 VECTOR FIELD STATISTICS

6.2.4.1 Conceptual Overview

First introduced to biomechanical applications in 2010, vector field statistics utilise statistical parametric mapping (SPM) to analyse large volume, time-varying biomechanical data [4]. Analysis can be conducted on a scalar field (i.e. a single component over time) or a vector field (i.e. a resultant 2D, 3D or nD vector over time) [4]. Scalar field analysis is conceptually the same as uni-variate t-testing, but employs a different probability distribution to account for the time varying nature of the data, considering the entire field simultaneously [20]. Vector field analysis, on the other hand, assess the resultant vector, which is the product of its individual scalar components, thus does not rely on the assumption that components are independent of one another [20].

![Figure 6.7 Example statistical parametric map for a scalar variable over the stance phase, adapted from Pataky (2010)[4]](image)

An example statistical map is displayed in Figure 6.7. The shaded areas represent where the nodal values were greater than the critical threshold, and the likelihood of a random field process producing such a suprathreshold cluster is described in the associated probability values.
A General Linear Model (GLM) is employed to describe the relationship between the experimental observations (n-dimensional sampling of a scalar field) and ‘nodes’, or the discrete measurement points, allowing estimation of the parameters, and a matrix of residuals. Test statistics can then be computed through calculation of nodal variance and then subsequent calculation of the nodal statistic. The nodal statistics of a GLM form a vector, which can be directly viewed in the context of the original data, and is referred to as a statistical ‘map’, and given the annotation SPM(t).

Random field theory (RFT) provides the mathematical basis for conducting topological statistical inferences on an SPM. Briefly, field and nodal smoothness (i.e. resel unit) are estimated using the full-width at half maximum of a Gaussian Kernel. From this, a threshold is set at a suitably high value for the observed SPM(t). Suprathreshold clusters can be corroborated by computing $p$ values for each cluster. The logic behind RFT is that as nodal smoothness increases, spatially broad suprathreshold clusters also increase, but very broad and/or very high clusters are expected to occur with low probability. In this way, a large cluster is the SPM is equivalent of a large univariate $t$ statistic [4].

6.2.4.2 Application to gait analysis

As outlined in Figure 6.1, vector field statistics address four of the five key challenges of analysing gait data: correlations between curves (Challenge 1); temporal dependence (Challenge 2); high variability (Challenge 3); and high dimensionality (Challenge 4).

Vector field statistics directly compare two curves, using a scalar field or vector field equivalent of a student $t$-statistic. In addition, a variance ratio (from ANOVA) is also able to be computed to compare multiple curves simultaneously [20]. The correlations between curves (Challenge 1), is addressed in such a way that it maintains temporal information as the differences over the waveform are not collapsed to a single score.

Vector field statistics addresses temporal dependence (Challenge 2) as it regards data as a field whose values change smoothly in time by considering the co-variance of all scalar components or a vector across the entire domain [4]. In addition, temporal information is retained as the SPM(t) is defined in the same domain as the data field. The subsequent interpretation is implicit and objective, as suprathreshold clusters which indicate significant differences, are defined relative to the time domain. For example, to demonstrate the use of SPM for analysis of kinematic variables, 3D knee rotation data from eight participants performing a side shuffle, and a v-cut manoeuvre were used. Vector field SPM revealed statistically significant differences in the knee kinematic vector at 1%, 10%, 20%, 30-35% and 95-100% of stance between the two conditions [21], highlighting the ability to accurately identify timing of differences.
The computation of the test statistic for SPM involves the calculation of the standard deviation of the data, across the entire waveform. Thus, the high variability (Challenge 3) of data is modelled for during the calculation of the test statistic as well as presented through the graphical display of variability clouds [21].

In addition to accounting for the high variability (Challenge 3) of biomechanical data, vector field analysis also address the high dimensionality (Challenge 4) of gait data. While research has demonstrated its appropriateness in analysing variables of varying dimensions, from 1D (such as time varying knee kinematics), to 2D (such as contact pressure) and 3D (such as bone strain) [4], vector field statistics can be, in theory, applied to vector/tensor fields in nD spaces meaning an individual’s lower limb, upper limb or whole body movement pattern can be modelled and analysed as a single vector.

6.2.4.3 Limitations
As with the other methodologies considered, vector field statistics in its current format does not address the non-linear relationships (Challenge 5) within complex biomechanical data. While vector field statistics does offer a regression model, the canonical correlation analysis, is analogous to linear regression, aiming to determine the strength of the linear relationships between the variables [20]. Vector field statistics, however, is a relatively young methodology within the field of biomechanics. With time and more widespread use, these gaps may be bridged.

A general limitation of vector field statistics is the appropriate registration of data, limiting the application to phasic and time based analyses [22]. Within clinical biomechanics settings, however, registration is standard practice through time normalisation to 100% of the gait cycle.

Finally, while vector field statistics can model nD vector waveforms, large sample sizes are required to yield sufficient statistical power when a large number of dimensions are being considered simultaneously [22]. Attaining large sample sizes may not currently be practical in a clinical setting when working with pathological paediatric populations, thus limiting its ability to handle high dimensionality (Challenge 4) for clinical applications.

6.3 SUMMARY
Though there are many benefits to the statistical tools available in the literature, the use of objective statistical analysis in a clinical setting has not been widely adopted, likely due to computational difficulties and the lack of inherently meaningful outputs from high level time varying analyses. For a statistical method to be appropriate for clinical applications, it must not only be statistically and theoretically robust (in addressing the challenges presented by complex biomechanical data), but must be freely available, should not be computationally cumbersome and provide practical, clinically meaningful outputs that will add value within clinical gait reporting [23].
Following the review of four multivariate statistical methodologies, which have each been applied to biomechanical data in various research settings, none were found to completely address all five challenges associated with biomechanical data analysis. While all four methods failed to account for non-linear relationships (Challenge 5), vector field statistics was the only method to adequately address the remaining four challenges.

Both vector field statistics and bootstrap confidence bands provided inherently meaningful outputs for the comparison of data curves that did not require any further subjective interpretation and retained valuable temporal information. Bootstrap confidence bands, however, are only able to address single variables within each analysis. Vector field statistics has potential to assess data in $n$ dimensions. Although statistical power is limited with higher level dimensions without an increase in sample size, this ability to address the high dimensionality (Challenge 4) of biomechanical data is a significant strength over bootstrap confidence bands.

The statistically robust and clinically meaningful data produced by vector field statistics lend themselves strongly to clinical application [23]. Despite the high level of mathematical complexity, vector field statistics is a streamlined analysis tool that takes mere seconds to complete using freely available pipelines [4]. Vector field statistics therefore, can be recommended as an appropriate statistical technique of clinical gait analysis. Future research is therefore needed to facilitate the integration of vector field statistics into current clinical analysis templates.

6.4 REFERENCES

Chapter 7 Vector field statistics for clinical gait reporting: A case study

ALEXANDER, C.F., DONELLY, C.J., STANAGE, K., REID, S., ROBINSON, M.
FOREWORD

Having determined vector field statistics as an appropriate statistical methodology for clinical gait applications, chapter seven presents a case study using vector field analysis specifically adapted for use in a clinical setting. This report handles kinematic variables that can be derived from traditional 3DGA, as well as neuromusculoskeletal models. As with the findings of the earlier chapters, this has potential for direct and immediate clinical application.
7.1 ABSTRACT

Vector field statistics, through the application of statistical parametric mapping (SPM), offers an objective statistical analysis framework that takes into account the time-varying and inter-related nature of complex biomechanical data, and may thereby enhance the understanding of pathological gait within clinical settings. This study demonstrates the application of vector field statistics through a case study of a 12 year old male with spastic diplegic cerebral palsy. Kinematic data was obtained from 15 trials of the participant walking at a self-selected pace, with comparisons made to a normative sample of 13 typically developing children. Vector field analysis was conducted on the 3D joint vectors and the 2D vector pairs using an SPM Hotelling’s $T^2$ statistic. Scalar field analysis was conducted on the individual 1D scalar components using an SPM student $t$-test. Scalar field results provided objective evidence to support observations made using traditional subjective methods by demonstrating where statistically significant differences existed between the participant’s kinematic profile compared to normative data. The use of vector field analysis on 2D vector pairs provided additional information on joint function, beyond what was provided by analysis of the individual kinematic components. Observational assessment suggested a cross talk artefact was occurring on the right side as a result of poor anatomical coordinate system definition. The 2D vector analysis results supported this, demonstrating similar levels of pathology at the left and right knee, despite different patterns being observed when considering the sides by their individual scalar components. The use of vector field statistics for clinical gait applications would allow more robust, objective analysis of data, reducing the reliance on human experience and accuracy for the collection and interpretation 3DGA data.
7.2 INTRODUCTION

Three-dimensional gait analysis (3DGA) is the quantification of gait variables (i.e. kinematics, and kinetics) from 3D motion capture systems. The uptake of 3DGA as an assessment tool prior to clinical interventions for children with cerebral palsy (CP) has resulted in significant improvements in surgical outcomes [1] compared to 2D video analysis methods and/or visual observations of time varying gait patterns. Positive outcomes, however, are not guaranteed [2, 3].

Traditional gait analysis reporting requires accurate and repeatable interpretation or assessment of 3DGA data. This is typically achieved using feature analysis, in which specific regions of interest within the gait signature are identified subjectively by visual inspection. Though common practice, one limitation is that this analysis method relies heavily on the experience of the individual(s) analysing the data. To overcome potential biases associated with this subjectivity, and to facilitate the interpretation and synthesis of multiple time varying waveforms simultaneously, attempts have been made to summarise gait pathology in an objective, meaningful way using gait indices. Indices such as the Gait Profile Score [4], or the Gait Deviation Index [5], aim to capture differences in gait curves using a single number. While useful in a clinical gait setting, it could be argued summary statistics such as these fail to adequately assess the intricacies of gait pathology.

Reliance on subjective, qualitative methods or summary indices for analysing 3DGA data is likely the result of the complex characteristics of biomechanical data that cannot be adequately captured by traditional discrete (0D) scalar statistical techniques (i.e. t-tests) [6]. Firstly, 3DGA data are time varying waveforms. Parameterisation, or the extraction of discrete parameters (i.e. peak, mean, minimum amplitude) from these signals is unlikely to accurately represent the waveform as a whole, therefore provides little or no additional information than that which can be obtained by observation of bivariate plots alone [7]. As 3DGA in clinical settings is exploratory in nature, the a priori definition of discrete parameters is likely to result in statistical errors [6, 8]. Secondly, components are interdependent. Each joint and joint degree of freedom in the kinematic and kinetic chain are covariant to one another. Failure to account for these two key characteristics of 3DGA can result in both Type I and Type II statistical errors in their exploratory analysis [8, 9], which will impact the clinical interpretation of the data.

Vector field statistics offers clinicians an objective exploratory statistical analysis framework that accounts for both the time-varying and interdependent nature of 3DGA data through the use of Statistical Parametric Mapping (SPM). Conducting SPM analysis is conceptually identical to conducting univariate (0D) t-tests, however SPM analyses consider the entire waveform, covariance with other waveforms, field smoothness and size and random field behaviour when computing the test statistics and critical threshold for a given alpha [6]. All of these factors protect SPM from biases, which can lead to the aforementioned statistical errors. While a clinician’s subjective opinion and clinical interpretation of clinical gait reports will always play a critical role...
within the clinical decision making process, vector field statistics may offer an objective statistical framework for the assessment of pathological gait for a clinician to build from when formulating, and/or assessing the efficacy of a clinical care plan. The objective of this study is to present a case study demonstrating the application of vector field statistics to 3DGA kinematic outputs that align with current 3DGA clinical reporting practices.

7.3 METHOD

The participant was a 12 year old male with spastic diplegic CP, classified as Gross Motor Function Classification System level III, with no history of pharmacological or orthopaedic interventions. The participant has a Functional Mobility Scale score of 5-4-1. The 3DGA was conducted three days prior to onabotulinum toxin injections to the bilateral gastrocnemius, medial hamstrings and rectus femoris muscles. Normative data from 13 typically developing children (five females, eight males; age 4.1 – 10.2 years) were used for comparison. Data was collected at the University of Western Australia using the same protocol as for the participant with CP (as outlined below), with the mean left and right sides for each participant being included for analysis, proving a normative sample of 26 trials.

The 3DGA was conducted using an eight infra-red camera Vicon Motion Capture System (Oxford Metrics, Oxford, UK) sampling at 100Hz and processed using Nexus software (v1.8, Oxford Metrics, Oxford, UK). The participant walked barefoot, using their usual assistive devices, at a self-selected walking pace on a 10m track. Fifteen trials were used for analysis. Retro reflective kinematic markers were placed on the pelvis and lower limbs in accordance with the Calibrated Anatomical System Technique (CAST) [10]. Harrington regressions were used to determine the hip joint centres, as the participant’s range of motion and ability to perform functional calibration unassisted was impaired [11]. The knee joint centre was taken as the midpoint between the femoral condyles. The anatomical coordinate system was defined using the vector between the hip and knee joint centres and the vector between the femoral condyles [12].

Three anatomical degrees of freedom were analysed for each joint (pelvis, hip, knee and ankle) [12]. Aligning with clinical 3DGA reporting practices, foot progression (foot ab/adduction relative to global) was also included. Stance phases were defined visually using foot marker trajectories, and data was normalised to 100% gait cycle.

Vector field analysis was conducted on the 3D joint vectors and the 2D vector pairs using a Hotelling’s $T^2$ statistic (SPM($T^2$)). Scalar field analysis was conducted on the individual 1D scalar components using a student t-test (SPM(t)). To be correct from a statistical perspective, it is recommended to run vector field analysis prior to scalar field analysis. However, to align with current clinical templates and reporting practices, 1D scalar analyses are presented first, followed by 2D and 3D vector analyses.
Briefly, for the calculation of \( SPM\{T^2\} \) and \( SPM\{t\} \), an SPM analysis utilises a mass-univariate General Linear Model (GLM) to explain a large proportion of the variability in the data by assessing the relationship between experimental design and observations. The test statistics computed from a GLM are reshaped to form a vector that is viewed in the context of the underlying data, providing a statistical ‘map’ of the waveform data being analysed. Random field theory is then used to determine the critical threshold above which only 5% of data would cross by random chance (alpha of 0.05) [13]. Full mathematical procedures for SPM analysis can be found in works by Pataky (2010) and Pataky et al (2013) [6, 14].

Statistical plots are scaled to the maximum \( t\)- and \( T^2\)-statistics across a given analysis level (3D, 2D or 1D). For each analysis conducted, an effect magnitude was calculated as the integral of the absolute \( SPM\{T^2\} \) or \( SPM\{t\} \) trace divided by 1,000. This analysis is equivalent to 0D effect size calculation [15]. Relative effects were calculated as each component’s effect magnitude divided by the maximum effect magnitude seen in the analysis level (1D, 2D or 3D). Effects were also ranked within each analysis level. Full template codes for complete analyses can be found in Appendix H – Vector field statistics codes.

7.4 RESULTS

Kinematic outputs are presented in Figure 7.1. The patient demonstrates a classic ‘double bump’ pattern in pelvic tilt, with hip flexion increased bilaterally during stance and late swing. Increased knee flexion is evident bilaterally during stance (more pronounced on the left), along with reduced range, and a delayed and reduced peak knee flexion during swing. Marked increase in ankle dorsiflexion is observed bilaterally throughout the gait cycle, again with reduced range evident. Increased pelvic obliquity is demonstrated on the left, with an unusual pattern of increasing obliquity during swing. A slight decrease in hip adduction is observed on the left, and corresponding increase on the right. Knee adduction is increased throughout the gait cycle, with an exaggerated range. This is notably more pronounced on the right side. Ankle inversion is increased bilaterally throughout the gait cycle. High levels of variability are observed in pelvis rotation, as indicated by the standard deviation bands. Hip internal rotation is increased bilaterally, though this is more pronounced on the right side. Knee rotation demonstrates an unusual pattern, moving into internal rotation during swing, however the range is within normal limits. Increased ankle abduction is seen bilaterally throughout the gait cycle, while the left foot is in a greater degree of external foot progression throughout the gait cycle on the left side.

The \( SPM\{t\} \) outputs for the scalar field analysis of the kinematic components are displayed in Figure 7.2. For each plot, the critical threshold is shown as a dashed line. Any suprathreshold clusters are shaded in the appropriate colour (red = left, blue = right), indicating statistically significant differences between the patient data and normative data for that component. A positive \( SPM\{t\} \) indicates the kinematic variable is increased in the patient data compared to the normative
data. Effect magnitudes, the relative effects and the rank order for the effects across the scalar analysis are presented in Table 7.1. The largest differences are found in right ankle plantar-/dorsi-\text{-}flexion, both in terms of maximum SPM{t} and effect magnitude, followed by left ankle plantar-\text{/}dorsi- \text{-} flexion, with statistically significant increases found in the participant compared to normative data across the gait cycle. Bilaterally, hip internal rotation is increased throughout the gait cycle, with a greater relative effect on the right (RE = 0.67) compared to left (RE = 0.40). Knee flexion shows increases bilaterally at 0-55% and 80-100%, with a greater peak SPM{t} evident on the left. Reduced knee flexion compared to normative data is observed at 70% gait cycle bilaterally, though of greater magnitude and duration on the right side. Overall there is a greater relative effect on knee flexion/extension on the left (RE = 0.53) compared to right (RE = 0.43). Knee abduction is significantly increased on the right relative to normative data throughout the gait cycle, with a relative effect of 0.55, ranking it the sixth most affected scalar variable assessed. Foot progression is significantly more external at 0-20% and 80-100% of the gait cycle on the left, although the relative effect is small at 0.12. No significant differences identified on the right, with a relative effect of 0.06 being observed.

The SPM\{T^2\} outputs for the vector field analysis on the 2D vector pairs are presented in Figure 7.3. The 2D vector pairs are the vector produced by two components. SPM\{T^2\} plots are presented in the same manner as SPM{t} plots. Effect magnitudes, relative effects and ranks are displayed in Table 7.1. All vector pairs demonstrate statistically significant differences between the participant and normative bands. Ankle plantarflexion/ abduction (Ank xz) on the right demonstrates the largest peak SPM\{T^2\} and the largest effect magnitude, with differences evident across the gait cycle. Differences are apparent bilaterally throughout the full gait cycle for knee flexion/abduction (Knee xy), with similar peak SPM\{T^2\} on the left and right sides. Relative effects were also similar with 0.27 on the left, and 0.33 on the right, ranking this vector pair tenth and eighth most affected respectively.

The SPM\{T^2\} outputs for the vector field analysis on the 3D vector are presented in Figure 7.4, with effect magnitudes, relative effects and ranks displayed in Table 7.1. The 3D vector is the resultant vector representing the overall joint motion. The 3D ankle vector show similar patterns of differences between the participant and normative data on both sides. The peak SPM\{T^2\} and effect magnitude is larger on the right side, with the left side displaying a relative effect for the ankle vector of 0.82, with a rank of second. The right knee and hip 3D vector effects are ranked third and fourth, with relative effects of 0.31 and 0.28 respectively.
Table 7.1 Effect magnitude, relative effect and rank order for the scalar, 2D vector and 3D vector analyses

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Figure 7.1 Kinematic outputs for left (red) and right (blue) sides along with the normative band (grey). Solid lines indicate mean curve, while the shaded bands indicate the associated variability. Vertical lines indicate mean foot off for left and right sides.
Figure 7.2 A) SPM[t] outputs for scalar components comparing the left side with normative data. Dashed horizontal lines indicate critical threshold for statistical significance at alpha level 0.05. Suprathreshold clusters, indicating statistically significant differences, are shaded in red. EM = effect magnitude.
Figure 7.2 B) SPM(t) outputs for scalar components comparing the right side with normative data. Dashed horizontal lines indicate critical threshold for statistical significance at alpha level 0.05. Suprathreshold clusters, indicating statistically significant differences, are shaded in blue. EM = effect magnitude.
Figure 7.3 A) SPM(T^2) outputs for vector pairs on the left side relative to normative bands. Dashed lines represent the critical threshold, while shaded clusters indicate areas of statistically significant differences. EM = effect magnitude.
Figure 7.3 B) SPM($T^2$) outputs for vector pairs on the right side relative to normative bands. Dashed lines represent the critical threshold, while shaded clusters indicate areas of statistically significant differences. EM = effect magnitude.
Figure 7.4 SPM(T²) outputs for the 3D vectors of the left (red) and right (blue) sides relative to normative bands. Dashed lines represent the critical threshold, while shaded clusters indicate areas of statistically significant differences. EM = effect magnitude.
The results of this study support the use of SPM in providing objective statistical corroboration to subjective, qualitative assessment of gait differences in a clinical setting, which aligns with previous research [16]. The results of the scalar field analysis provide statistical verification to many of the differences that would be observed from the kinematic traces using traditional feature observation methods. Significant differences of a large magnitude were identified in ankle plantarflexion by SPM analysis, whereby peak SPM(t) and effect magnitudes indicating the right side was more affected than the left. This finding supports the observational assessment of the kinematic waveforms. Just as important, SPM was also able to verify where statistical differences did not occur. In this case study, the observation of kinematic traces suggested foot progression on the left side demonstrated greater external progression angle throughout the gait cycle. Statistically significant differences, however, were only identified at 0-20% and 80-100% of the gait cycle, with a small relative effect. This finding highlights the potential for SPM to prevent Type I error, or the incorrect identification of a difference where one does not exist. Within a clinical decision making setting, this may have large and significant impacts on treatment plans developed with 3DGA reports, and future research is warranted to verify this impact.

Scalar field analyses are the most intuitive way to interpret gait data, as it aligns with the current and established practices of feature analysis, thus has been presented first in this case study report. Analysis using SPM allows the clear identification of statistical differences between waveforms, with information regarding the timing, direction and magnitude of difference inherent in the graphical presentation, providing an objective starting point for interpretation of 3DGA data. By providing reliable feature identification, SPM may aid in resolving potential differences in opinions between individuals on a clinical reporting team. Vector field analysis for clinical reporting has been supported by previous research which compared subjective feature analysis to an SPM based analysis approach. That research demonstrated while two experienced clinicians agreed with each other only 61% of the time, SPM identified the same differences as at least one clinician more than 80% of the time [16], indicating a clinical utility to the statistical information provided.

Vector field statistics offer additional information beyond scalar components which can add further detail on a patient’s movement pathology. This application has been highlighted within this case study when considering the results of the scalar components together with the 2D vector pair results at the knee. Scalar field analysis identified a similar pattern of differences in knee flexion/extension on the left and right sides, however the relative effect was greater on the left (RE = 0.53) compared to the right (RE = 0.43). When considering knee abduction/adduction compared to the normative data, a significant difference was observed on the right with a relative effect of 0.55. Differences were also observed on the left, however of smaller magnitude, with a relative effect of 0.26. There are two possible reasons for this: 1) there is a true biomechanical
increase in abduction/adduction on the right side in this patient and/or 2) human error in marker placement resulting in the anatomical coordinate system of the knee not being defined correctly, resulting in flexion/extension being measured as abduction/adduction and vice versa. While this error is an accepted limitation of defining anatomical coordinate systems using external marks, it commonly leads to reservations about reliable interpretation of the knee abduction/adduction data in clinical settings. Typically, when questions around marker placement arise, clinical analysts will assess the hip rotation graphs for abnormalities. In this case, the right hip displayed a greater level of internal rotation relative to the left (relative effect 0.67 on right compared to 0.40 on left). In combination with clinical assessments which indicate similar levels of internal hip rotation on each side, this suggests increased adduction was the result of poor marker placement.

When concerns over the scalar variables such as this arise, the vector pairs may offer valuable objective information into the true biomechanical differences to support the observations of the clinical analysts, as vector pairs and 3D vectors are independent of the coordinate system definition [6]. In this instance, the right knee vector pair of flexion/extension and abduction/adduction demonstrate a moderate difference compared to normative data with a relative effect of 0.33. The magnitude of this difference is comparable to the difference observed in the same vector pair on the left, which showed a relative effect of 0.27. These results indicate the combination of knee flexion/extension and abduction/adduction shows a similar level pathology on the left and the right sides, despite different patterns being observed when considering the sides by their individual scalar components (i.e. flexion/extension and abduction/adduction). Vector field analysis can, in theory, be conducted on any relevant pair of components. For this case study, the pairs selected were the three pairs contained within each joint. Future applications however, could assess the vector pair of knee abduction/adduction and hip rotation to provide further insight into the collinearities between waveforms and potential cross talk from poor anatomical coordinate system definition.

Vector field statistical analysis on the 3D vectors allows for the assessment of differences between the joints motion that is completely independent of the coordinate system definition [6]. All formal biomechanical training breaks a joint’s motion down into independent degrees of freedom. The origins of this reach as far back as the work of Muybridge (1887) [17], who sought to quantify movement when only 2D measurement techniques were available, and are maintained today through the International Society of Biomechanics recommendations for anatomical coordinate system and degree of freedom definitions [12]. However, these planes are not biologically discrete, and assuming so may lead to errors in the time varying interpretation of these waveform data as joints’ degrees of freedom are known to be collinear and interdependent [6, 16]. In the context of clinical 3DGA, 3D vectors can be used to objectively identify the joints which are most affected compared to normative data bands or following intervention. In this case study, the 3D vectors, and associated effect magnitudes, reveal the right ankle joint to show the largest
differences compared to normative bands, followed by the left ankle. The next two joints demonstrating the largest differences are the right knee and right hip, indicating this patient may be asymmetrically affected by CP. Objective information on the relative effects on each joint may contribute to enhanced understanding of an individual’s gait pathology, which could positively influence clinical decision making and ultimately patient outcomes.

The introduction of effect magnitudes as an additional statistic provided by SPM analysis, further facilitates the understanding of the patient’s gait pathology by quantifying the overall differences for each scalar component, 2D or 3D vector, irrespective of statistical significance. There is potential for the development of robust thresholds to classify effects as ‘small’, ‘moderate’ or ‘large’ and further enhance its usability, although this will require further research with large sample sizes. Such research would be expedited by multi-disciplinary, multi-centre projects. Furthermore, as a descriptive statistic, the effect magnitudes could be considered to be similar to gait indices currently used within clinical settings. The advantage of effect magnitudes over traditional gait indices, however, is the level of detail provided in the SPM{t} and SPM{T{2}} that generates each effect magnitude statistic. Future research comparing effect magnitudes to gait indices, such as the Gait Deviation Index [5] or Gait Profile Score [4], to establish construct validity would be a worthwhile future research endeavour.

The application of SPM to clinical 3DGA may be limited by the sample sizes required to achieve stable variance and an appropriate level of statistical power [18]. Ideally, 5 – 10 trials are required per vector component. Thus, for simple scalar field analysis, 5 – 10 trials is sufficient, but for full 3D vector field analysis, 15 – 30 trials are required. For analysis of nD vectors for assessment of joint synergies, the number of trials required increases proportionally. Attaining a sufficiently large number of trials for 3D – nD vector analyses may not be feasible in clinical settings, particularly among paediatric and pathological populations whom fatigue quickly. This is particularly true when considering kinetic analysis, where each trial requires successful foot strike on a force plate meaning not every trial performed in the laboratory will meet the requirements for modelling and subsequent analyses.

The use of vector field statistics for clinical 3DGA may provide potential benefits to the interpretation and assessment of a patient’s gait pathology, and ultimately patient outcomes, directly enhancing what is considered best clinical practice. The objective analysis framework developed for specific application to clinical 3DGA automates the identification of gait features including timing, magnitude and direction of statistical differences between waveforms independent of the experience of clinicians’, while reducing the time required for report generating. Valuable information is provided by vector field analysis on 2D and 3D vectors for enhanced understanding of gait pathologies. By modelling collinearities, vector analysis has potential to remove the need for a priori definition of anatomical or joint coordinate systems.
Vector analysis, therefore, provides an objective, statistical methodology to overcome potential errors in the modelling of joint degrees of freedom, and in turn their time varying signals. This research has developed a vector field statistics framework that overlays statistical inference directly on to existing 3DGA clinical templates, meaning the results are inherently meaningful, and have immediate utility in a clinical setting for clinicians and biomechanists of all levels of experience and training. Through further research, robust thresholds and construct validity can be established for effect magnitudes, further increasing the utility of vector field statistics in clinical settings. While a clinician’s experience and expertise will also play a critical role in the development of treatment plans from 3DGA reports, vector field statistics offers a statistically robust and clinically meaningful framework on which clinical management decisions can be built.

7.6 REFERENCES

Chapter 8 Synthesis of results and conclusions

8.1 SUMMARY

Cerebral palsy (CP) is the most common disability treated within paediatric rehabilitation programmes. With no known cure, treatment aims to maintain or improve function [1]. As such, gait function is frequently a goal of interventions targeting the primary and secondary musculoskeletal features of the disorder. The variability and complexity of CP means treatment outcomes are often hard to predict, and successful intervention results are not guaranteed [2, 3]. The uptake of conventional 3D gait analysis (3DGA) into clinical decision making, however, has led to significant improvements in treatment outcomes [4].

Neuromusculoskeletal modelling is a sophisticated methodology that combines 3DGA kinematic and kinetic data with subject specific musculoskeletal parameters [5]. It can be used to estimate internal muscle and contact forces, and provides a greater level of detail on the potential underlying causes of pathological gait [6]. In 2017, Sartori, Fernandez and colleagues eloquently stated:

“the translation of neuromusculoskeletal modelling pipelines to the clinical level is a promising avenue for deriving a new class of biomarkers that directly correlate to a patient’s impairment and subsequent recovery” (pg. 16) [7].

The uptake of neuromusculoskeletal modelling into clinical practice has been hampered by the lack of models with appropriate specificity for children with CP. CP can affect patients’ musculoskeletal functioning in a multitude of ways, including abnormal muscle activation patterns, altered muscle histology, and bone and joint deformities [1]. Furthermore, the presentation of CP is highly variable, and is affected by many factors including brain lesion location, extent and timing; age; and previous interventions. Implementation of neuromusculoskeletal modelling for children with CP within clinical settings requires the development of CP specific models that have the capacity to be highly subject specific to reflect this complexity and variability of musculoskeletal parameters that exist across this pathological population. This research aimed to build on the repertoire of knowledge that will collectively develop such models by the assessment of musculoskeletal geometry and muscle tendon structure, in a way that also facilitates transfer of knowledge directly into clinical practice.

Biomechanical gait data, whether derived from conventional 3DGA or neuromusculoskeletal models, is universally accepted as being smooth, time-varying waveforms. However it is rarely treated as such when being analysed statistically for research purposes. Furthermore, when developing treatment plans in clinical settings, statistical analysis rarely occurs. Instead,
subjective identification of gait features is common practice, relying on the experience of the clinician(s) assessing the time varying data [8]. A sophisticated, patient specific neuromusculoskeletal model could offer a wealth of information on the mechanisms behind pathological gait, however its full potential would remain restricted without the ability to appropriately handle the data outputs. Therefore, the second aim of this research was to develop the application of an appropriate statistical method for the handling of the complex time varying biomechanical outputs from neuromusculoskeletal modelling that could also be utilised directly in clinical 3DGA practices.

The research questions outlined above were addressed using five interrelated studies. This chapter will summarise the findings of each of these studies with respects to the hypotheses outlined in Chapter One, draw conclusions based on the results of each of these studies, and make recommendations for clinical implications and future research.

8.1.1 CHAPTER THREE
A SIMPLE BUT RELIABLE METHOD FOR MEASURING 3D MOMENT ARM GEOMETRY FROM A SINGLE, STATIC MAGNETIC RESONANCE SCAN.

While the assessment of Achilles tendon moment arm (ATMA) geometry can be achieved using simple, cost-effective 2D methods, research suggests 2D estimates are not valid representations of the 3D in-vivo geometry [9]. However, 3D methods typically involve multiple static magnetic resonance imaging (MRI) scans, or the use of dynamic MRI scans [9-11]. Both options are time- and cost- expensive and may not be achievable for individuals with reduced range of motion at the ankle. Chapter three aimed to develop a simple and reliable participant specific in-vivo 3D ATMA method from a single static clinical MRI scan and validate this method against a previously validated 3D dynamic method.

The first hypothesis, that:

_The novel method will be repeatable at estimating in-vivo 3D ATMA from a single clinical MRI scan_

was supported. The novel 3D ATMA method showed excellent intra-rater repeatability in healthy adults with an intra-class correlation coefficient (ICC) of 0.996 (p<0.001). The mean measurement error for intra-rater repeatability was -0.1mm, with a limits of agreement (95% confidence interval) of -1.1mm to +0.9mm. This method also possessed excellent inter- and intra-tester repeatability with an ICC ≥ 0.985 (p<0.001; 1 – β = 99.9%) in typically developing children.

The second hypothesis, that:

_The method will be valid compared to a previously validated method_
was supported. No difference (p=0.21) in ATMA length was identified when assessed using the current method compared to the previously validated dynamic method in healthy adults, with the two techniques demonstrating very strong agreement, with an ICC of 0.912 (p<0.001). The measurement error of the current method compared to the previously validated method (1.0mm ± 2.2mm) is less than that reported between the previously validated method and physical measurements (2.9mm ± 2.1mm) [10], while the variability is comparable. The maximum inter-model measurement error found in the current study, of 8.9%, is smaller than the maximum measurement error found in the earlier reported study validating the dynamic method against physical measurements (13-22%) [10].

To the authors’ knowledge, this is the first study of its kind to validate 3D ATMA from a single, static MRI scan sequence. It presents a feasible option for measuring subject specific, 3D ATMA in clinical and research settings for subjects with limited or no ankle range of motion, such as patients with joint contractures. It should be noted the validation of this technique was carried out using a sample of healthy adults, and further validation in children with CP would be beneficial. This technique, however, may offer a pragmatic solution to implement subject specific measurements of ATMA into musculoskeletal modelling in children with reduced range of motion at the ankle. This has direct application for clinical use of modelling, where medical imaging is commonly used to inform surgical decision making.

8.1.2 CHAPTER FOUR

CHILDREN WITH CEREBRAL PALSY HAVE LARGER IN-VIVO AND LINEARLY SCALED ACHILLES Tendon Moment Arms Than Typically Developing Children.

While children with CP are frequently found to have reduced plantarflexor function [12, 13], and altered muscle morphology and histology [1], there has been very little attention paid to the potential role of the ATMA in plantarflexor function. To date, only one study, to the authors’ knowledge, has compared ATMA geometry in children with CP and typically developing (TD) controls [14]. That study, however, utilised a 2D method for estimating ATMA. Furthermore, no research has compared subject specific, in-vivo ATMA measurements to scaled ATMA from neuromusculoskeletal models. Chapter four aimed to compare in-vivo 3D ATMA estimates between adults, TD children and children with CP. The secondary aim of this chapter was to compare 3D ATMA derived from an established musculoskeletal model linearly scaled to tibia length with participant specific in-vivo 3D ATMA for adults, TD children, and children with CP.

The first hypothesis, that:

*Adults will possess the largest 3D ATMA estimates, while children with CP will have the smallest*
was not supported. The mean predicted ATMA at 20° plantarflexion was 52.8mm ± 5.6mm for adults, which was significantly larger than for TD children, who had a mean ATMA of 41.8mm ± 5.9mm for TD. However, children with CP demonstrated a mean predicted ATMA of 47.1mm ± 3.5mm which was not significantly different to either group. When normalised to tibia length, however, adults (12.5% ± 0.8%) were found to have significantly smaller ATMA compared to both paediatric groups. Children with CP (17.2% ± 2.0%) were found to have ATMA significantly larger than their TD counterparts (15.2% ± 1.2%).

The second hypothesis, that:

*No differences in 3D ATMA estimates will be observed between methods for the adult population; however significant differences will be found between methods for both paediatric groups*

was partially supported. The effect of ATMA method (in-vivo or scaled) was significantly different among adults compared to the paediatric groups, while the effect of method was not different between children with CP and TD children. Specifically, *in-vivo* 3D ATMAs were significantly larger than the linearly scaled 3D ATMA at the same MRI derived joint angles for all groups. The mean difference however was smaller for adults, at -5.8mm (10.7%) for adults than for TD children at -11.0mm (26.2%) and children with CP -15.7mm (34.7%). Although significant differences were found between the methods for adults, it was within the error range accepted in the literature as being appropriate for neuromusculoskeletal models.

To the knowledge of the authors, this is only the second study directly comparing ATMA in children with CP and TD children. The results of this study demonstrate that children with CP have larger 3D ATMA, which is in contrast to the previously reported findings [14]. This difference may be attributed to the use of 3D estimates compared to 2D estimates, however further research is required to confirm this. The finding is significant as it suggests ATMA geometry may offer a mechanical advantage to potentially compensate for muscular weakness/poor activation, which has direct implication for surgical interventions that target structures that influence ATMA geometry at the ankle. While many musculoskeletal models do not have AMTA as an input parameter, these findings can be used to assess the validity of current models. Comparison of subject specific *in-vivo* estimates of 3D ATMA geometry to scaled estimates from neuromusculoskeletal modelling highlights the deficiencies of current models to accurately reflect children with CP.

8.1.3 Chapter Five

Muscle Volume Alterations in Children with Cerebral Palsy Following the First Botulinum Toxin Treatment: A 6 Month Prospective Cohort Study.
Muscle volume, already reduced in children with CP [15], is found to demonstrate further atrophy following exposure to BoNT-A in children who have received multiple injections [16-18]. Research shows, however, compensatory hypertrophy occurs in muscles synergistic to the injected muscle [16]. Animal models suggest the first exposure of BoNT-A has the greatest impact upon muscle atrophy [19, 20] although to date, this has not been investigated in children with CP. Chapter five aimed to assess muscle volume change, using MRI, of the injected and synergistic muscles, following the first exposure to BoNT-A in children with CP.

The first hypothesis, that:

*The injected gastrocnemius muscle will show atrophy at each time point, with the greatest atrophy expected at 13 weeks post injection*

was supported. The injected gastrocnemius displayed significant reductions in normalised muscle volume of 6.7%, 10.7% and 9.8% at 4, 13 and 25 weeks respectively. This finding suggests the muscle volume does show recovery following the ‘period of clinical usefulness’ of the intervention. However, as atrophy was still evident at 25 weeks post injection, the results demonstrate that muscle volume has not fully recovered at the recommended timing of reinjection. No comparisons were made to children who had received multiple injections, although findings from previous literature suggests a greater magnitude of atrophy was observed in toxin naïve children within this study.

The second hypothesis, that:

*Hypertrophy will be found in the synergistic muscles of the soleus and medial hamstring group at each time point*

was partially supported. As hypothesised, soleus displayed significant increases in normalised MV of 5.4%, 6.1% and 7.4% at 4, 13 and 25 weeks respectively. This finding replicates that found in previous research, which also demonstrated compensatory hypertrophy in children with CP who had previously received multiple injections [16]. Contrary to the hypothesis, the hamstring group showed significant increase in normalised muscle volume at the 4 week follow up. Together these findings suggest that while total plantarflexor volume is maintained, total knee flexor volume is not. Given the particular nature of the gastrocnemius, this may have functional implications which warrant further investigation.

This is the first research undertaken to track muscle volume alterations over a six month time frame in toxin naïve children with CP. The findings replicate previous work in children who have received multiple injections, demonstrating significant atrophy in the injected muscle five weeks following BoNT-A exposure. They contribute new knowledge, demonstrating that peak atrophy occurs at 13 weeks post injection, and significant atrophy is still apparent at 25 weeks post
injection. This has implications for clinical reinjection schedules, which current recommendations suggest can occur from six months [21]. This study demonstrates the variability in muscle-tendon parameters that can occur within a child with CP over time, in response to the interventions received, highlighting the importance of subject specificity in neuromusculoskeletal models for this population. Finally, this study has highlighted the potential for synergistic muscles not targeted for intervention, to also be altered as a response to treatment. In this case, a potentially compensatory affect was observed in synergistic muscles. This change in relative muscle size of muscles within the same functional group has implications in solving force optimisations, used to estimate muscle forces in neuromusculoskeletal models [7]. This reinforces the need for high levels of subject specificity to optimise model estimations.

8.1.4 CHAPTER SIX
STATISTICAL METHODOLOGIES FOR THE ANALYSIS OF TIME VARYING WAVEFORMS IN CLINICAL GAIT ANALYSIS

Current techniques for analysing 3DGA for clinical purposes rely heavily on expert opinion to subjectively identify differences and areas of interest. Within a clinical research setting, simple discrete statistical methods are employed, resulting in the increased risk of Type I and Type II errors [22, 23]. This chapter provided evidence for the use of four statistical methodologies to address the key challenges presented by complex biomechanical data [24] and their potential viability in clinical settings. While all four methodologies do not adequately address the fifth challenge of non-linear relationships that exist in biomechanical data, vector field statistics were found to overcome the remaining four challenges: correlations between curves, temporal dependency, high variability and high dimensionality. The inherently meaningful outputs generated by vector field statistics lend themselves strongly to clinical applications, leading to the recommendation for vector field statistics to be appropriate clinical settings.

8.1.5 CHAPTER SEVEN
VECTOR FIELD STATISTICS FOR CLINICAL GAIT REPORTING: A CASE STUDY

Vector field statistics for analysis of time varying biomechanical data offers a pragmatic solution to address many of the limitations of current analysis techniques in both clinical and research settings. Briefly, vector field statistics utilises Statistical Parametric Mapping (SPM) to generate a statistical map showing statistically significant differences between curves throughout the time varying waveform. Analysis can be run on nD vectors or scalar components. Thus, along with providing valuable temporal and magnitude information on the observed differences, vector field statistics accounts for interdependence between the scalar components, and is independent of the coordinate system definition. By addressing many sources of statistical and procedural bias, vector field statistics offers a comprehensive, objective framework for identifying differences
between curves in a clinical 3DGA setting. Chapter seven presents a case study demonstrating the use of vector field statistics for a full lower limb kinematic 3DGA dataset in a clinical setting. Along with demonstrating the use of scalar field analysis for providing objective evidence to support observed differences in kinematic traces, the case study presented highlights the potential use of vector field statistics to overcome assumptions around the definition of anatomical coordinate systems. The case study was a 12 year old boy with spastic diplegia who had a 3DGA prior to BoNT-A treatment.

The first hypothesis, that

*Vector field statistics will provide objective support to subjective interpretation of kinematic variables*

was supported. Subjective observation of the kinematic waveforms found the right ankle plantar-/dorsi- flexion showed greater deviation from normative data than the left side. This was supported by significant differences of a large magnitude were identified in ankle plantarflexion by SPM analysis, with peak SPM(t) and effect magnitudes indicating that the right side was more affected than the left. Analysis with SPM was also able to verify where differences did not exist statistically, despite being identified during subjective assessments. Foot progression on the left side was observed to be reduced throughout the gait cycle, however statistically significant differences were only identified at 0-20% and 80-100% of the gait cycle, with a small relative effect.

The second hypothesis, that:

*Vector field statistics will provide additional information, of clinical relevance, beyond what was obtained by scalar field analysis*

was supported. Visual examination of the kinematic flexion/extension and abduction/adduction curves suggest some concern over the joint axis definition for the right knee. Scalar analysis revealed larger magnitude differences on the left side for knee flexion/extension. However, vector analysis of the flexion/extension, abduction/adduction vector pair, suggest the left and right side show similar magnitude deviations from the normative band. This finding suggests the differences in the scalar components may be due to cross talk.

This chapter also introduces effect magnitudes as a means for quantifying differences over the waveform in a single statistic. This has significant potential to increase the usability of vector field statistics without compromising on detail as it is presented with full waveform statistical analysis. With further research and validation, effect magnitudes may be used in a similar manner to traditional gait indices, allowing the vector field statistics template to present a full analysis report for expert interpretation. Uptake of vector field statistics into clinical settings would allow
more robust, objective analysis of 3DGA data, reducing the reliance on human experience and accuracy in both collecting and interpreting 3DGA data.

8.2 CONCLUSIONS

This thesis has focused upon building the repertoire of neuromusculoskeletal models in a way that facilitates direct clinical application from the individual studies. For the first time, 3D ATMA was directly measured among children with CP using a novel technique that is feasible for populations with reduced range of motion at the ankle, as well as being practical for use in clinical settings. This research confirmed the hypothesis that linear scaling of currently available generic neuromusculoskeletal models is not appropriate for children with CP. However, contrary to the hypothesis, children with CP displayed larger 3D ATMA compared to their TD counterparts. This contrasts with the only other known study to assess ATMA in children with CP [14]. This discrepancy may be explained by the different measurement techniques used, with previous research employing a 2D method to estimate ATMA. While the effects of this have not been investigated in children with CP, in healthy adults 2D estimates are found to be significantly different from 3D ATMA measurements [9]. The results have significant implications for our understanding of muscle function in children with CP. These data indicate that at any given joint moment, the underlying muscle force required to generate that moment would be proportionally reduced relative to that in a TD child. Altered ATMA may therefore serve a mechanical compensation to a weaker muscle with poor innervation, allowing maintenance of moment production around the joint to facilitate ambulation.

Along with musculoskeletal geometry, this thesis assessed alterations in muscle-tendon unit parameters in response to a targeted intervention. As was hypothesised, alterations in both the BoNT-A targeted muscle, and synergistic muscles were apparent. The improvement of functional and clinical outcomes over the course of the study are positive indications that the targeted muscle atrophy was not detrimental in the time frame assessed, although the change in muscle volume proportions of the plantarflexor group may have interesting longer term implications. Factoring this proportional change into a neuromusculoskeletal model would allow more accurate muscle forces to be ascertained, and facilitate a better understanding of how functional gains are generated to better predict the long term effects of select atrophy and compensatory hypertrophy within a muscle group. It is important to note however, that a simple change in muscle volume within a generic neuromusculoskeletal model will not reflect the true adaptations occurring. Injection of BoNT-A into a muscle not only results in atrophy, but also in reduction in spasticity, and these altered innervation patterns impact the underlying muscle histology, all of which will play an important role in the force production, and must be modelled accordingly.

Following the exploration of two key neuromusculoskeletal model inputs, attention then turned to model outputs, specifically the analysis of outputs. It has been widely accepted in the literature
that the current methods for analysing biomechanical data arising from neuromusculoskeletal models and traditional 3DGA are not adequate to reflect the complexity of the data [25]. However, methods proposed to address this have been equally complex in their presentation and therefore interpretation, making them impractical for clinical settings. This research has applied vector field statistics, a statistical approach that has fast gained momentum in the general biomechanics field, to this clinical application. Allowing for exploratory analysis and providing intuitive results, vector field statistics are perfectly suited for clinical applications. The clinically focused template, and integration of effect magnitudes, facilitates its accessibility and therefore potential for immediate use in research and clinical practice.

8.2.1 LIMITATIONS
The small sample size of the studies included in this thesis is a limiting factor in this research. While statistically significant differences were identified in all studies, the small sample size means only a small portion of the heterogeneous population was represented, limiting the generalisability of the results to the wider CP population. In the assessment of 3D ATMA, results can be applied only to ambulant patients, with no history of orthopaedic surgery and no indications for orthopaedic surgery. In the assessment of the impact on BoNT-A on toxin naïve children, results can be applied only to ambulant children over the age of 4 years.

As BoNT-A is evidence based, best practice care, more rigorous research methodologies, such as randomised control trials, were not ethically possible. Therefore, the lack of control group limits the generalisability of the research outcomes.

Though conventional 3DGA as operationally defined in this thesis includes both joint kinematic and kinetic analysis, vector field statistics were applied to kinematic outputs only in chapter seven. The required number of trials to achieve stable variance and appropriate statistical power for 3D vector analysis was not achievable for kinetic outputs in the laboratory setting used for data collection, which included two in-ground force plates. Given the low rate of successful foot strikes, and therefore low rate of attaining successful trials for kinetic analysis, within the population sampled, the 15 – 30 trials required were not achievable. While this research has not explicitly developed templates for the use of vector field statistics on joint kinetics, the templates developed could be easily extended to include joint kinetics for future applications.

While chapters three to five of this thesis are concerned with musculoskeletal parameters that are likely to impact on strength, this study is limited by the exclusion of strength measurements. Strength was excluded due to the lack of a reliable and valid measurement technique for assessing plantarflexor function. This exclusion poses a significant limitation in understanding how the measures conducted (3D ATMA and muscle volume) relate to function, and how these measures may be included in a CP specific neuromusculoskeletal model.
8.2.2 Future Research

In regards to the increased 3D ATMA identified through this research, future research is recommended to establish why these findings are different to the previous work showing ATMA when measured in 2D is smaller in children with CP. This may involve full validation of the 3D ATMA technique in children with CP, and throughout the range of motion. Upon confirmation of the direction and magnitude of differences between children with CP and TD children, it is highly recommended that further research investigating the mechanisms behind altered 3D ATMA be carried out. This would greatly enhance our understanding of any potential implications surgical interventions may have on ATMA and therefore moment production about that joint, with direct implications for neuromusculoskeletal model inputs.

With respect to the effect of BoNT-A on muscle morphology, future research assessing the impact of the timing of subsequent injections is warranted. Ideally, a randomised control trial would be used to assess muscle volume alterations in children who receive repeat injections at the clinically recommended minimum six month time frame, compared to children who receive a delayed repeat injection, thus potentially allowing for greater muscle volume recovery. Furthermore, future research should aim to assess functional outcomes alongside morphology. Longitudinal research assessing the long-term morphological and functional changes, and cumulative effect of multiple injections is also recommended. When considering the potential use of muscle volume as a factor in muscle optimisation for force estimates in neuromusculoskeletal models, there is extensive further research to be done. Models that more accurately reflect the complexity of children with CP, including abnormal muscle activation patterns and histology (both of which are impacted by BoNT-A exposure) are required before application of changes in muscle volumes is implemented to full effect within neuromusculoskeletal models.

Finally, the potential of the effect magnitude statistic will not be realised without a large body of evidence to develop appropriate guidelines for its interpretation. The strength of traditional effect sizes, and of gait indices, is in the numeric simplicity in combination with clear guidelines for their interpretation. Effect magnitudes have the numeric simplicity, and come from a strong mathematical and clinical foundation, however they currently do not have the evidence to determine what is a ‘large’ or what is a ‘small’ effect. This will come with either widespread use and subsequent collation of data, or from a large scale study, likely a retrospective study assessing previously collected 3DGA data from a wide variety of participants.

8.2.3 Significance of this Research

Clinical research can be used to inform neuromusculoskeletal modelling, just as neuromusculoskeletal modelling research can have direct clinical meaning. It is through multifaceted aims that research will have the greatest impact on the lives of children with CP,
which is the overriding goal of all clinical and research endeavours, including the work contained in this thesis.

While further research is required to substantiate the mechanisms behind altered ATMA in children with CP, the potential for ATMA to be a mechanical compensation for a weaker muscle should be acknowledged when considering any surgical interventions that may impact upon the ankle geometry. The findings arising from the study assessing alterations in muscle volume following first exposure to BoNT-A have potential to greatly impact on the longer term spasticity management plans for children with CP in regards to timing of subsequent injections. Data on volume alterations demonstrates that muscle recovery starts to take place between three and six months, however is not complete by six months. This suggests the current clinical guidelines recommending repeat injections at six months should be considered a minimum time frame until further research establishes the optimum time frame for balancing muscle volume alterations and functional outcomes, along with the child’s and family’s goals. The finding that functional and clinical outcomes were improved at every time point following injection was as expected for the standard best practice care. However, continued monitoring of musculoskeletal alterations as a result of BoNT-A exposure is warranted.

The vector field statistics template for clinical gait reporting was developed specifically to have direct clinical application. The use of vector field statistics in biomechanical research has seen rapid acceptance since its introduction in 2010 by Pataky and colleagues [25], however has not yet been integrated into clinical settings. The template presented in this research facilitates that integration by providing clinicians and clinical biomechanists a free, simple, robust tool for the objective analysis of 3DGA data.

The development of a neuromusculoskeletal model for children with CP is an incredibly complex task that will require contribution and collaboration from the international research community. The studies contained within this thesis add to that repertoire of neuromusculoskeletal modelling through exploration of parameters within the elements of musculoskeletal geometry and muscle-tendon unit.

8.3 REFERENCES


Appendices

APPENDIX A – PUBLICATIONS

A SIMPLE BUT RELIABLE METHOD FOR MEASURING 3D ACHILLES TENDON MOMENT ARM GEOMETRY FROM A SINGLE, STATIC MAGNETIC RESONANCE SCAN

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ABSTRACT

Current methods for measuring in vivo 3D muscle-tendon moment arms generally rely on the acquisition of magnetic resonance imaging (MRI) scans at multiple joint angles. However, for patients with musculoskeletal pathologies such as fixed contractions, moving a joint through its full range of motion is not always feasible. The purpose of this research was to develop a simple, but reliable in vivo 3D Achilles tendon moment arm (3D ATMA) technique from a single, static MRI scan. To accomplish this, for nine healthy adults (5 males, 4 females), the geometry of a cylinder was fit to the 3D form of the tali dome, which was used to estimate the talonavicular flexion/extension axis, and a fifth-order polynomial fit to the line of action of the Achilles tendon. The single static scan in vivo 3D ATMA estimates were compared to estimates obtained from the same subjects at the same ankle joint angles using a previously validated 3D dynamic MRI based in vivo ATMA measurement technique. The ATMA estimates from the single scan in vivo 3D method (52.5 mm ± 5.6 mm) were in excellent agreement (ICC = 0.912) to the validated in vivo 3D method (51.5 mm ± 5.1 mm). These data show reliable in vivo 3D ATMA can be obtained from a single MRI scan for healthy adult populations. The single scan, in vivo 3D ATMA technique provides researchers with a simple, but reliable method for obtaining subject-specific ATMAs for musculoskeletal modelling purposes.

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1. Introduction

Subject-specific musculoskeletal models offer the ability to estimate an individual’s muscle mechanics and dynamics, allowing enhanced understanding of healthy and pathological movement. This information may be beneficial for the development and implementation of patient-specific treatment plans. Subject-specific muscle mechanics are estimated using many model parameters, including three dimensional (3D) moment arms. When unknown, these parameters are estimated by linearly scaling the model to subject-specific anthropometric measurements (e.g. segment lengths). Reliance on musculoskeletal model scaling comes into question when considering pathological and/or paediatric populations, for whom standard assumptions are more likely to be violated (Nicks et al., 2009; Regunji et al., 2013; Waugh et al., 2011).

As such, much research has focused on the development of reliable and valid methods for obtaining subject-specific muscle parameters like 3D musculotendon moment arms. There are currently many in vivo methods available for the measurement of musculotendon moment arms, such as the Achilles tendon moment arm (ATMA). Surface measurements, while appealing in their simplicity, do not have the accuracy and repeatability associated with medical imaging techniques such as ultrasound (Fath et al., 2010; Maganaris et al., 2000; Manal et al., 2013; Waugh et al., 2011) and magnetic resonance imaging (MRI) techniques (Fath et al., 2010; Hashizume et al., 2012; Maganaris et al., 2000; Aug et al., 1999; Sheehan, 2012). For most measures of the ATMA the axis of rotation must be defined, which is complex, as there are no surface landmarks to define its anatomical axis of rotation.
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2. Method

From a previously published data set of 10 healthy adults (Clarke et al., 2015), one participant (24 years, 2.46 m, 75.8 kg) was paired for the current investigation. The participant data not used enrolled as data set was composed while in a data image. After informed consent was provided by all participants, and methods were approved by the Human Research Ethics Committee of the University of New South Wales.

Participants lay prone in a 3T MRI scanner (Philips Achieva, Netherlands) with the thighs and legs supported in neutral positioning. The knees were held in a flexed position between '9' and '10'. A flexible surface coil was wrapped around the ankle.

High-resolution anatomical scans in the sagittal plane of the right ankle were used to create the new 3D AAM for each participant. The high-resolution scans used the following parameters: 30 °1 weighing T2, 50° slice angle; 30° x 320 maximum, 100 x 100 FID, 35.5/16.66 ms TR/TE, and 1.0 mm slice thickness.

The atlas-based validation process was a series of high-resolution dynamic sagittal scans of the right ankle. Parameters used in the low-resolution scans were

\[ \Delta(\theta) = \frac{\theta + 2}{(S, S)} \]

\[ \Delta(\theta) = \frac{\theta - 2}{(S, S)} \]

Lastly, the in vivo 3D AAM estimates were only validated to the 3D AAM estimates published by Clarke et al. (2013) for two reasons: (1) these data are available to research groups, which allows for direct comparisons; and (2) the 3D AAM approach, which would be directly mapped to the specifically defined talo-tibial joint angles estimates.

3. Results

The current AAM method showed excellent intra-rater repeatability with an ICC of 0.996 (p < 0.001; 1 − β = 99.9%). The mean measurement error was 0.001° (0.001° to 0.05°) and LoA of −0.09° to +0.09°.

Additionally, when comparing the validated 3D AAM method, there was a significant difference in tibial-soleus angle (p < 0.02) at which the AAM were compared. Descriptive statistics demonstrated a mean difference of 1.3° ± 4°.

There was no difference in (p > 0.02) in AAM length when assessed using the current method compared to the validated 3D AAM method. The mean AAMs calculated by the current method and validated 3D AAM were 52.5 mm (±6.6 mm) and 51.5 mm (±1.3 mm), respectively. Descriptive statistics showed a mean AAM difference of 1.0 mm (±2.2 mm) across a range of −3.2 mm to +5.2 mm (Fig. 2). The mean and maximum measurement error of 1.5° and 4.0º, respectively.
Fig. 1. (A) Sample slice showing manual digitisation of talus and location of point markers. (B) 3D reconstructed talus (green) with manually fit cylinder. Calcaneus (yellow) and tibia and fibula (pink) are presented for display purposes only. (C) Graphical representation of moment arm calculation, where the joint axis is shown in green, the ATMA modelled as a polynomial curve is shown in red, and the 3D ATMA in dark blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. (A) Bland-Altman plot comparing the current method for ATMA and the validated 3D method ATMA demonstrating a small mean measurement error and tight limits of agreement. (B) Comparison of the measured ATMA using the current method and the validated 3D ATMA method for each of the 5 participants.

4. Discussion

The current method is a reliable way to calculate in vivo 3D ATMA from a single, static MRI scan, exhibiting excellent repeatability and accuracy. The intra-tester measurement error of the current ATMA method (error 1.0 mm ± 2.2 mm) is less than that reported by previous literature, which compared the validated 3D ATMA estimates to a physical measurement (error 2.0 mm ± 2.3 mm). The maximum inter-model measurement error observed in the current study was 8.9%, which is smaller than the maximum measurement error observed from a validated in vivo 3D ATMA method when compared against physical measurements (13–25). What these results and previous literature show is that MRI based in vivo 3D ATMA models appear to be repeatable, with typical errors of 1.5–1.6 mm (Hopkins, 2000).

As shown in Fig. 3, the in vivo 3D ATMA measured using the current method displays measuring similarity with 3D ATMA measurements among healthy adults published previously in the literature (Clarke et al., 2015; Fath et al., 2016; Hashizume et al., 2012; Maganaris et al., 2000; Rugg et al., 1990; Sheehan, 2012). The exception for this is the data published by Hashizume et al. (2012) which shows smaller ATMA compared to both 2D centre of rotation estimates and other 3D estimates. It is also worth noting the mean error and variance of error in the in vivo 3D ATMA method presented in the manuscript is within the accepted errors margins presented previously in the literature (Clarke et al., 2015; Fath et al., 2010; Hashizume et al., 2012; Maganaris et al., 2000; Rugg et al., 1990; Sheehan, 2012).

One limitation of the study was the measurement of talo-tibial joint angles to make direct comparisons between the high and low resolution scans for validation purposes. Foot-tibia joint angles were not measured externally during scanning and the base of the foot was not visible in the static scans, which is why joint kinematics were estimated from the bony geometry of the talus body.

The talo-tibial angle was deemed appropriate for comparison purposes as it could be measured objectively and repeatedly (ICC = 0.939; p = 0.003) from both static and dynamic scan sets. Though a practical method to obtain ankle joint kinematics for comparison purposes, this method was limited as the motion of the talus during the dynamic was prone to perspective error, meaning the bony body, at times was observed to move in and out of the scan plane. Future applications of this technique would benefit from the use of MRI compatible motion capture markers to directly calculate foot-tibia angles. External kinematic markers would also allow researchers to place a given musculoskeletal model into the correct posture during model scaling to ensure its ATMA matches the participant's in vivo estimates with the highest fidelity possible.

An inherent limitation of this current method is that it is restricted to the measurement of ATMA at one posture. ATMA length is known to change with change in joint kinematics, and while this pattern of variability is frequently modelled as predictable in musculoskeletal calculations and simulations (Sherman et al., 2013) it may be highly subject-specific even among healthy adult populations (Clarke et al., 2015). While a single static posture, it is also difficult to standardise muscle tendon force estimates, which may add additional errors to ATMA estimates. While these limitations are acknowledged, it is important to keep in mind the population for whom this method had been developed: individuals with pathologies which prevent or impede their ability to move joints through full or partial ranges of motion. In such populations, these limitations are mitigated as the method currently provides the only feasible method for reliable 3D assessment of ATMA, and/or given the reduced range of motion available, less extrapolation is required to estimate ATMA at the end range of motion for the joint, thereby reducing potential errors.

While the development and validation of the current method was performed using high-resolution scans, the nature of the technique (where a simple geometric shape is being fitted to a full bone, without reliance on precise identification of specific landmarks) suggests lower resolution scans might produce 3D ATMA measurements of nearly comparable high quality. Furthermore, the scan plane is not required to be aligned with the sagittal plane of the ankle, as the model is a true 3D model, using 3D forms and coordinates.

The current method has many advantages as a model to measure in vivo 3D ATMA. To the authors’ knowledge, this is the first method that provides validated measurements of 3D ATMA from a single, static MRI scan. It may be a feasible option for measuring subject-specific 3D ATMA in clinical and research settings for subjects with limited ankle range of motion, offering a pragmatic solution for implementing subject-specific measurements of ATMA for musculoskeletal modelling purposes.

Ethical approval

Ethical approval was attained for this project from the University of NSW (09179) and the University of Sydney (12192).

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgments

The authors would like to thank David Lloyd, Chris Carter, Jane Valentine and Catherine Elliott for useful discussions and support. CA is supported by the Ernest and Evelyn Havill Shacklock fellowship for her PhD candidature. IC and RH are supported by NHMRC research fellowships. The scans for this study were collected as a pilot project, funded by a grant from the Sydney Medical School, University of Sydney.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bonechem.2017.01.018.
References


Vector-field statistics for the analysis of time varying clinical gait data

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ARTICLE INFO

Received 20 March 2016
Accepted 28 November 2016

Keywords:
Kinematics
Lower limb
Biomechanics
Statistical-parametric mapping

ABSTRACT

Background: In clinical settings, the time varying analysis of gait data relies heavily on the experience of the individual(s) assessing these biological signals. Though three-dimensional kinematics are recognised as time varying waveforms (1D), exploratory statistical analysis of these data are commonly carried out with multiple discrete or OD dependent variables. In the absence of an a priori hypothesis, clinicians are at risk of making type I and II errors in their analysis of time varying gait signatures in the event statistics are used in concert with preferred subjective clinical assessment methods. The aim of this communication was to determine if vector field waveform statistics were capable of providing quantitative corroborations to practically significant differences in time varying gait signatures as determined by two clinically trained gait experts.

Methods: The case study was a left hemiplegic cerebral palsy (CWP) patient following a botulinum toxin (BTX-A) injection to their left gastrocnemius muscle.

Findings: When comparing subjective clinical gait assessments between two testers, they were in agreement with each other for 61% of the joint degrees of freedom and phases of motion analysed. For tester 1 and tester 2, they were in agreement with the vector field analysis for 78% and 53% of the kinematic variables analysed. When the subjective analysis of tester 1 and tester 2 were pooled together and then compared to the vector field analysis, they were in agreement for 83% of the time varying kinematic variables analysed.

Interpretation: These outcomes demonstrate that in principle, vector field statistics corroborates with what a team of clinical gait experts would classify as practically meaningful pre-versus post time varying kinematic differences. The potential for vector field statistics to be used as a useful clinical tool for the objective analysis of time varying clinical gait data is established. Future research is recommended to assess the usefulness of vector field analyses during the clinical decision making process.

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1. Introduction

There is little argument that three dimensional joint kinematics and force data are time varying (1D) vector waveforms. In clinical settings, the commonplace analysis of time varying clinical gait data is subjective, relying heavily on the experience of the individual(s) assessing these biological signals. Though recognised as waveform data, the exploratory statistical analysis of clinical gait data is generally carried out using a variety of discrete, zero-dimensional (0D) dependent variables (i.e., min, max, mean, etc.) in an attempt to best model the time varying (1D) characteristic of these signals.

When gait waveforms are objectively assessed to determine the efficacy of a treatment in a research setting, the statistical analysis of these three dimensional or multi-component vectors are generally modelled with 00 variance about fixed means within predefined joint degrees of freedom and phases of the gait cycle (Bert et al., 2013). From a scientific viewpoint, if no a priori hypothesis is presented, and the 1D gait waveform is modelled with 00 randomness, researchers are predisposed to making regional focus fitness in their statistical analysis (Pataky et al., 2013) and virtually guaranteed to make type I errors in their assessment of discrete (0D) time points within the waveform (Pataky et al., 2016b). They are also at risk of making type II errors at very other time point within the time series (Pataky et al., 2013). This places practical limitations on the type(s) of quantitative analyses a clinician can use to formulate reliable clinical assessments associated with the effectiveness or efficacy of a given treatment or intervention.

Following the development of vector-field analysis for the mapping of human brain activity and anatomy (Pristo et al., 1995; Pristo et al., 2007), these statistics have been validated for the assessment of three dimensional, time varying (1D) kinematic and force vectors (Pataky, 2016b) in research settings. From a research standpoint, the development of vector-field statistics for the analysis of gait can mitigate the probability
of making type I and II errors in the statistical assessment of human movement data (Patel et al., 2013). The utility of vector-field statistics for the analysis of time-varying gait data within clinical gait settings is also apparent. Specifically, vector-field statistics have the potential to assist in the objective analysis of these complex time-varying signals (i.e., pre-versus post-versus normative), helping to improve the inter- and intra-clinician analysis reliability of these data.

The primary aim of this communication was to compare the subjective analysis of pre-versus post-clinical gait data between two trained clinical gait experts and a vector-field statistical method. We predict vector-field statistics will corroborate with the subjective clinical analysis of both clinical gait experts as the statistical methodology considers the within-dataset time varying variability in its entirety. A secondary aim of this communication was to conduct exploratory analyses of the same data using a pre-versus post-Biostat scatter plot. The motivation for these secondary analyses are for completeness, and to highlight some potential limitations associated with the 100 analysis of clinical gait data in an exploratory type setting.

2. Methods

A single paediatric participant (4.4 years, 1.21 m, 26.4 kg) classified as spastic type left hemiplegic Cerebral Palsy (GMDR-I) was the case study chosen for these analyses. A sensor camera motion capture system operating at 100 Hz (Vicon MX) recorded three dimensional (3D) kinematic marker trajectories during walking gait four days prior to and four weeks following a single botulinum toxin (BoNT-A) injection to the left gastrocnemius muscle. During each testing session, 20 individual trials were recorded at the participant’s self-selected walking speed.

The kinematic marker set and three dimensional lower-limb kinematic modeling procedures, which used a Calibrated Anatomical System Technique (CAST) and functional hip and knee joint axes and/or centres was used to estimate the participant-specific lower limb kinematics. Full modeling procedures have been described previously (Bester et al., 2005). Aligning with Biostat recommendations, the anatomical degrees of freedom for each joint were flexion/extension, abduction/adduction and internal/external rotation (Bester et al., 2009; Ellen et al., 2011). For simplicity, a condensed clinical gait report, which contained the three dimensional kinematics of the left and right hip, knee and ankle separated into their anatomical degrees of freedom (n = 10) was used for analyses (Fig. 1, pane 1). All data were time-normalized to 100% stride. See Appendix A for full three dimensional kinematic gait report.

Three analyses were performed. First, two testers with 11 and 7 years’ experience analysing paediatric cerebral palsy gait independently assessed the mean time varying joint kinematics of the participant pre-versus post-BoNT-A injection (Fig. 1, pane 1). The testers were instructed to report all clinically meaningful kinematic differences within the stance and swing phase of the gait cycle. They were also asked to report when within the normalized gait cycles these differences were observed, as well as the direction of these changes. See Appendix B for written instructions provided to testers.

Second, statistical parametric mapping (SPM), specifically a Hotelling’s T2 test (α = 0.05) were used to assess the three dimensional (i.e., 3-Component) time varying (1D) vectors of the hip, knee and ankle joint. By modelling the hip, knee and ankle as a 3-Component vectors, the flexion/extension, abduction/adduction, internal/external kinematic waveforms, as well as co-linearity between them are all modelled statistically. If significant differences were observed,

![Fig. 1. Pane 1 represents the time varying kinematics of the ankle, knee and hip joint, separated into their anatomical degrees of freedom (flexion/extension, abduction/adduction, and internal/external rotation), pre-versus post-BoNT-A injection. Positive values for the hip, knee and ankle represent flexion/extension, abduction/adduction, and internal/external rotation or abduction/adduction (ankle) Pre-Post represents the three components: 1D vector analysis of the hip, knee and ankle pre-versus post-BoNT-A injection. In pane 2, where the P-statistic (blue or red lines) is greater than the critical P-threshold (red dotted line) (α = 0.05), a significant pre-versus post-difference exists in the BoNT-A injection is observed. It should be noted that the 1D statistic can be interpreted as an effects size. The factors that the P-statistic deviates from the critical P-threshold defined by the experimental alpha level, the larger the relative effect. Pane 3 represents the scaled 1D analysis of the ankle, knee and hip joint, separated into their anatomical degrees of freedom pre-versus post-BoNT-A injection. For simplicity, regions of statistical difference (α = 0.05) are highlighted in red or blue. The direction of the difference is reported for clinically meaningful differences within the shaded regions. For interested readers, the t-statistic and critical t-threshold for the 1D analysis of the ankle, knee, and hip joint are presented in Fig. 5.6 of the Supplementary materials.](image-url)
the three dimensional time varying (1D) vector was separated into its vector components, and analysed as time varying (1D) scalar waveforms. Conceptually, these analyses would be comparable to using a post hoc analysis when a main effect is identified with a three factor ANOVA. See Appendix C for a two component time varying (1D) vector analysis.
Agreement between both testers and vector-field analysis were assessed throughout the stance and swing phase of a stride. Agreement was operationally defined as when the same pre-versus post-kinematomic difference was observed, when the observed difference was in the same direction and when the timing of this difference was in agreement (90% of the observed difference).

Third, the discrete OD statistical analysis of 18 independent kinematic waveforms pre-versus post-BoNT-A injection were performed. To accomplish this, the local maximum and minimum of each kinematic waveform within the stance and swing phases of the gait cycle were analysed. All OD scalar variables were analysed using independent sample t-tests (t = 0.05). As these analyses were exploratory in nature without any a priori hypotheses, protected post hoc adjustments for multiple comparisons were not made. It should be noted that pre-versus normal and post-versus normal statistical analyses can be performed. Additionally, any alpha level can be chosen for these statistical analyses of these waveform data (i.e., α = 0.05, 0.01, 0.001). By using random field theory within the vector field statistical approach, alpha is protected for the analysis of time varying and three dimensional or 3-Component vector waveforms.

3. Results

The time varying (1D) vector analysis of the three dimensional (i.e., 3-Component) vectors for the left and right hip, knee and ankle were statistically difference pre-versus post-BoNT-A injection (Fig. 1, panel 2). The time varying (1D) scalar analysis of each joint degree of freedom, pre-versus post-BoNT-A injection (Fig. 1, panel 3) revealed statistical differences for all but two lower limb joint degrees of freedom. These include left hip flexion/extension and right ankle plantar/dorsiflexion.

When comparing the subjective assessments of the two testers, they were in agreement with each other for 61% of the joint degrees of freedom and phases of motion assessed (Table 1). For tester 1, they were in agreement with the vector-field analysis for 38% of the variables analysed. For tester 2, they were in agreement with the vector-field analysis for 33% of the variables analysed. When the subjective analyses of tester 1 and tester 2 were pooled together, they were in agreement with the vector-field analysis for 38% of the time varying kinematic variables analysed. This is practically significant as clinical gait reports are generally analysed in teams or with two more gait experts.

For the OD analysis, only three of the 72 discrete variables assessed did not report pre-versus post statistical differences (Table 1). These included left ankle inversion/eversion and right ankle planar dorsiflexion during stance, and right knee internal/external rotation during swing. For 22 of the 36 gait phase and joint degree of freedom combinations analysed, both the local minimum and local maximum were significantly different.

4. Discussion

Results showed that over 85% of the lower limb kinematic variables analysed, one of the two clinical gait expert subjective analyses of these data were in agreement with the SPH vector analyses. We find this is a practically meaningful result as clinical gait case studies are generally analysed in teams of two or more gait experts. We acknowledge that the kinematic variables the testers did not agree upon during their analysis may not have translated to differences in their clinical interpretation(s)/recommendation(s) of the data. These results clearly show that vector-field metrics can provide statistical, clinically meaningful information for the analysis of time varying kinematic data. We feel this is a meaningful step forward for the objective, exploratory analysis of clinical gait data, as clinicians are provided an objective statistical tool from which best practice clinical decision making can be built from.

Vector-field statistics offers clinicians an objective analysis framework to work from when formulating conclusions and/or making clinical decisions from pre-versus post-versus normal normative clinical gait data. What is interesting to note is that both researchers, and NPM reported increases in left ankle dorsiflexion following the BoNT-A injection, which aligns with previous research studies utilizing a vector field statistical approach to assess the influence of BoNT-A as a clinical treatment for a similar age population (Nimeshwnayya et al., 2016). However, SPH did not identify the same differences in lower extremity kinematics, which have been documented previously (Nimeshwnayya et al., 2016). In addition, for this clinical case study, SPH identified statistical difference at the hip, knee and ankle, which were not observed previously (Nimeshwnayya et al., 2016). These results highlight the importance of using an exploratory time varying analysis method like vector field statistics in clinical settings, as each case study is unique and statistical analyses can be performed.

We appreciate that vector field statistics may be initially perceived by some researchers or clinicians as a comparatively cumbersome or a time expensive analysis tool. In reality, vector field statistics simplifies the analysis of time varying data. Therein, 1D analyses of a time varying waveform can be performed in single step versus researchers attempting to pull out multiple 0D variables that best characterizes the time varying behaviour of the signal. An additional, and underappreciated benefit for using vector field statistics method over a 0D statistical approach is that time does not need to be spent consciously deliberating on the rational/method(s) to protect, or not protect alpha. For example, it could be argued that for the OD scalar analysis presented in this manuscript, alpha should have been protected for four comparisons (i.e., two maximum and two minimum). Our rational for not protecting alpha is that clinical gait analysts are exploratory in nature. This type of argument is avoided when using SPH, as random field theory and the temporal smoothness of the time varying signals are used to define critical-t thresholds while protecting alpha.

As the focus of this communication was to explore vector-field statistics as a clinical gait analysis tool, future research is recommended to assess whether this statistical approach may alter or influence the clinical decision making for, and or assessment of, interventions like orthopaedic surgery, BoNT-A treatment, casting, etc. In addition, we encourage researchers to investigate the utility of vector field statistics for the clinical assessment of joint moments, joint power and joint work pre-versus post-intervention(s).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clinbiomech.2016.11.008.

References

Papale, T.C., Hua, R-Y. BIDT: search one-dimensional random forest superposing probabilities in gait, J. Vis. Syst. (in press).
Neuromuscular electrical stimulation-assisted gait increases muscle strength and volume in children with unilateral spastic cerebral palsy

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AIM: To determine if neuromuscular electrical stimulation (NMES) applied to the ankle dorsiflexors during gait improves muscle volume and strength in children with unilateral spastic cerebral palsy (CP).

METHODS: Thirty-two children (16 females, 17 males, mean age 10.5y; 80% with unilateral spastic CP and a Gross Motor Function Classification System of level I or II) were randomly assigned to either the 8-week daily NMES intervention group or control group (usual or conventional treatments). Outcomes at week 8 (post-NMES) and week 14 (carryover) included magnetic resonance imaging for muscle volumes (tibialis anterior, anterior compartment, and gastrocnemius), strength (hand-held dynamometry for isometric dorsiflexion strength and heel raises for functional strength), and clinical measures for lower limb selective motor control.

RESULTS: At week 8, the treatment group demonstrated significantly (p = 0.05) increased muscle volumes for tibialis anterior, anterior compartment, medial and lateral gastrocnemius, and dorsiflexion strength not only when compared to their baseline values but also when compared to the control group at week 8. At week 14, both tibialis anterior and lateral gastrocnemius volumes in the treatment group remained significantly increased when compared to their baseline values. However, only lateral gastrocnemius volumes had significantly greater values when compared to the control group at week 14. There were no between-group differences in the clinical measures for lower limb selective motor control at week 8 and 14.

INTERPRETATION: Eight weeks of daily NMES-assisted gait increases muscle volume and strength of the stimulated ankle dorsiflexors in children with unilateral spastic CP. These changes are dose-dependent and do not carry over after the 8-week treatment period. Gastrocnemius volume also increased post-treatment with carryover at week 14.

Problems with ankle selective motor control (SMC), atrophy, and weakness to the muscles around the ankle joint are well-documented impairments in children with unilateral spastic cerebral palsy (CP).

While the majority of children with unilateral spastic CP are ambulant, these impairments contribute to optimus when walking and result in the recruitment of compensatory strategies or incidences of trips and falls.

Interventions such as strength training are often employed to address these impairments but these efforts can be hampered by poor SMC and insufficient training volume to achieve clinically meaningful changes.

Investigating effective methods that aim to ameliorate these impairments are necessary to provide immediate functional benefit as well as for the maintenance of long-term mobility.

Neuromuscular electrical stimulation (NMES) is the application of an external electrical impulse to initiate a limited voluntary skeletal muscle contraction. A muscle contraction is elicited when an electrical current is delivered through electrodes placed over the skin of the target muscle or nerve to activate intact motor units by inducing an action potential. A unique feature of NMES is that it can be employed even if there are problems with SMC. Reduced SMC refers to the ‘impaired ability to isolate the activation of muscles in a selected pattern in response to demands of a voluntary posture or movement.’ For this reason, NMES-assisted gait (also commonly referred to as functional electrical stimulation) has been used as a rehabilitation modality in adult stroke rehabilitation to address...
the functional consequences of equinus.11,17 More recently, NMES-assisted gait has been applied during walking in children with spastic CP to similarly overcome problems with equinus.13,14 The predictable and repetitive nature of a gait pattern enables NMES of the ankle dorsiflexors during swing to be triggered by predictable phases of gait, such as the swing or stance phase at toe-off. Such technology has enabled NMES-assisted ankle gait to be applied in the child’s own environment, which advantageously enables high dose intervention to be embedded within activity.

So far, the literature describes NMES applied to the ankle dorsiflexors during gait to be well tolerated,13,15 with compelling improvements in ankle kinematics,14,16 thus producing an orthotic effect, i.e., stimulation of tibialis anterior to clear the foot during swing phase of gait.17 Further, it has also been recognized that the orthotic effect of NMES-assisted gait can have the additional benefit of improving the muscle volume, strength, and ankle SMC of the stimulated muscle tibialis anterior in children with unilateral spastic CP.11,19

While the recent emergence of these studies provides compelling results to support the efficacy of NMES-assisted gait in children with CP, one limitation to these pioneering studies is that control groups were not included. The inclusion of control groups is particularly relevant in paediatric populations to enable distinctions between training-induced hypertrophy and natural development or growth.7 Therefore, there is a need to evaluate the effect of NMES-assisted gait in a randomized controlled trial to not only investigate its effect over time but also when compared to a control group undergoing conventional therapy. The investigation of potential carryover effects (also referred to as therapeutic effects) following the discontinuation of NMES is also essential to improve our understanding of this intervention in children with CP.

The aim of this study is to conduct a randomized controlled trial to evaluate the effectiveness of an 8-week community-applied NMES-assisted gait programme on muscle strength and volume in children with unilateral spastic CP. We hypothesized that children undergoing an 8-week NMES-assisted gait treatment period would demonstrate a greater increase in ankle dorsiflexion strength and muscle volume compared to children without NMES. We also hypothesized that children who received 8 weeks of NMES-assisted gait would maintain the muscle hypertrophy and strength improvements at the 6-week follow-up compared to children without NMES.

**METHOD**

**Design**

The study design was a randomized controlled trial to investigate the effect of an 8-week daily community-applied NMES-assisted gait programme to the ankle dorsiflexors compared with usual or conventional care (control group).

**Participants**

Thirty-two children (17 females, 15 males; mean age 10y 8mo, SD 3y 3mo) with unilateral spastic CP, in Gross Motor Function Classification System (GMFCS) level I or level II, were recruited for the study. Table 1 demonstrates that there were no significant group differences in participant characteristics at baseline. Participants were referred to the study from physiotherapists and paediatric rehabilitation consultants. Participant inclusion criteria included children with unilateral spastic CP, GMFCS levels of I or II between the ages of 5 and 18. Participants needed to have at least 5 degrees of passive ankle dorsiflexion (with the knee extended) and full knee extension. Participants had to be able to co-operate with assessment procedures and be willing to use the NMES-assisted gait device daily for 8 weeks.

The schedule for study commencement was dictated by current clinical care involving botulinum toxin-A (BoNT-A). BoNT-A is injected at 6-monthly intervals if clinically indicated. With the exception of four children who do not have routine BoNT-A injections (two children in the treatment group and two children in the control group), all remaining children have 6-monthly BoNT-A injections. For these children, baseline measures commenced 3 months after injections, which is widely accepted to be after the peak technical response caused by motor end plate regeneration.21

**Table 1: Characteristics of participants**

<table>
<thead>
<tr>
<th></th>
<th>Treatment (n=16)</th>
<th>Control (n=16)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>38.5 (15.2)</td>
<td>37.4 (15.9)</td>
<td>0.890</td>
</tr>
<tr>
<td>Sex</td>
<td>Male: 9</td>
<td>Male: 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female: 7</td>
<td>Female: 8</td>
<td></td>
</tr>
<tr>
<td>Side of hemiplegia</td>
<td>Right: 11</td>
<td>Right: 12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Left: 5</td>
<td>Left: 4</td>
<td></td>
</tr>
<tr>
<td>GMFCS (r)</td>
<td>1-10</td>
<td>1-10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Age, y (SD)</td>
<td>10(3.13) (10)</td>
<td>10(2.68) (10)</td>
<td>0.950</td>
</tr>
<tr>
<td>Orthoses (n)</td>
<td>Anterior AFO</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fixed AFO</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>History of BoNT-A (n)</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;10 injections</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>11-20 injections</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>&gt;21 injections</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Muscle volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>0.68 (0.21)</td>
<td>0.57 (0.18)</td>
<td>0.890</td>
</tr>
<tr>
<td>Symmetry ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strength</td>
<td>0.11 (0.09)</td>
<td>0.12 (0.07)</td>
<td>0.620</td>
</tr>
<tr>
<td>Dorsiflexion (normalised)</td>
<td>3 (2.3)</td>
<td>3 (3.2)</td>
<td>0.888</td>
</tr>
<tr>
<td>Axle motor control</td>
<td>Ankle SMC (Ordinal scale 0-4)</td>
<td>3 (2.3)</td>
<td>3 (3.2)</td>
</tr>
</tbody>
</table>

*Note: *Mann Whitney U test, GMFCS, Gross Motor Function Classification System; AFO, ankle foot orthosis; BoNT-A, botulinum toxin A; SMC, selective motor control.
Participants were excluded if they had orthopaedic surgery on the affected side in the past 12 months, had orthopaedic metalwork at the site of stimulation, or had uncontrolled seizure disorder.

Study recruitment took place between June and July 2013 in Perth, Australia from clinics of the Cerebral Palsy Mobility Service at Princess Margaret Hospital for Children and The Centre for Cerebral Palsy, with the final assessments completed by April 2014. Ethics committees at Princess Margaret Hospital for Children and The University of Western Australia approved the registered trial (ACTRN12614000946846) and the committee’s recommendations were adhered to, with written and informed consent for participation and publication obtained.

Procedure

An initial appointment with the principal investigator was scheduled to determine NMES tolerance and introduce the study protocol. Randomization to either the treatment or control group was achieved through a coin toss, performed by an individual uninvolved with the study, once two matched participants were enrolled. Criteria for matched participants were: (1) within 2 years of age for children aged between 5 years and 10 years and within 6 years of age for children aged between 11 years and 18 years, and (2) in the same GMFCS level. This method was applied to improve the homogeneity of each group in terms of age and gross motor function.

Outcome measures were assessed at baseline, week 8, and week 14. Participants in both groups were asked not to participate in any new sporting activities during the study and to maintain pre-existing conventional therapy (including stretching, neurodevelopmental therapy, and strengthening programmes) throughout the 14-week study period to better isolate the effects of NMES treatment.

Outcome measures

This randomized controlled trial of community-based NMES assessed outcomes across all domains of the International Classification of Functioning, i.e. body structure and function, activity, and participation. The results on activity and participation are reported elsewhere. This paper reports on the results pertaining to body structure and function, i.e. muscle volume, strength, and motor control.

Calculation of overall muscle volume was achieved using magnetic resonance imaging (MRI). Bilateral MRI of the lower limbs were taken at the Department of Diagnostic Imaging at Princess Margaret Hospital for Children, Perth, Australia. T1-weighted spin echo sequence was used following standardized protocols, with a slice thickness of 5mm and mean inter-slice gap between 5mm and 7mm. Images were analyzed using Mimics visualization software (Version 16.6; Materialise, Leuven, Belgium) following a standardized procedure described previously. All volumes were normalized to tibia length to account for differences in participant stature and/or growth between scans.

Muscle volumes are also expressed as a symmetry ratio (with a value of 1 indicating perfect symmetry) in relation to the unaffected side. This enables quick and meaningful interpretation of the data while considering the effect of growth or activity in the unaffected limb. In addition to tibialis anterior, the volume of the anterior compartment (tibialis anterior, extensor hallucis longus, extensor digitorum longus, and peroneus tertius) was also included in the analysis because of its contribution to ankle dorsiflexion. The volumes for soleus and gastrocnemius were also obtained. The inter- and intrarater (to ensure consistency of technique with investigators in previous studies) reliability of the muscle volume measurements was high, with intraclass correlation coefficient values of 0.99 calculated by testing a random selection of five scans of all muscles (55 muscles in total).

Strength was assessed using hand-held dynamometry for ankle dorsiflexion. Maximum isometric ankle dorsiflexion strength was measured three times using hand-held dynamometry (Lafayette Nicolas Manual Muscle Tester Model 01160) using the stabilization test position following Crompton et al.’s protocol, i.e. supine with the knee stabilized in extension, foot held in plantarflexed position with resistance applied to the dorsal surface of the metatarsal heads. To enable equitable comparisons for children of different foot length and body size, the median value was normalized to weight and foot length. The maximum number of single limb heel raises was used as a functional ankle plantarflexion strength. The method and procedure for a successful heel raise followed Yocum et al.’s description and that of our pilot work.

SMC was assessed using two common clinical tools. Boyd and Graham’s ordinal scale for ankle SMC assessment was used because of its relevance to clinical practice and applicability in young children. This 5-point ordinal scale ranges from 0 describing no active movement to 4, which describes valgus ankle dorsiflexion through full available range of motion with the knee extended. A score change of 1 was considered to be clinically meaningful.

The Selective Control Assessment of the Lower Limb (SCALE) was also employed because of its established validity and reliability. Although the SCALE measures SMC of all lower limb joints (providing a score out of 10), it has a greater weighting for the ankle and foot. Only the affected limb score was used for analysis and we considered a score change of 2 to be clinically meaningful.

The assessments were all performed on the same day. An experienced physiotherapist and research assistant followed the outlined protocols for the strength and SMC assessments. MRI was randomized, then processed and analyzed by one assessor. The assessor was blinded to the assessment time point and group allocation.

NMES-assisted gait intervention

Participants in the treatment group received the NMES-assisted gait device after the baseline assessment. The Walk Aide (Innovative Neurotronics, Austin, TX, USA) is a...
small (8.2cm x 6.1cm x 2.1cm, 8.7g) device that delivers asymmetrical biphasic surface electrical stimulation triggered by tibial motion to enable toe clearance by stimulating active dorsiflexion during the swing phase of gait. It is attached to the participant’s leg by a cuff that sits just under the knee on the affected side. One electrode is placed on the muscle belly of the tibialis anterior and the other on the common peroneal nerve, which innervates tibialis anterior and other ankle dorsiflexors (extensor digitorum longus, peroneus tertius, and extensor hallucis longus). Before the application of the Walk Aide in the gait cycle, electrode position, amplitude, and pulse widths were first determined with the participant in long sitting. This process enabled individual settings to be established by balancing tolerance to the stimulation and in the attainment of dorsiflexion without excessive ankle movements into inversion or eversion (this limitation meant that only 5 degrees could be achieved in some children).

During a gait cycle, the Walk Aide is triggered by an individualized programme detecting changes in tibial angle to stimulate ankle dorsiflexion. The set-up procedure followed that described in our pilot study. Participants and parents were supported so that they were confident and independent with the NMES-assisted gait device, ensuring balanced dorsiflexion with every use. Weekly to fortnightly visits at home or school were necessary for education to support daily use, adjust parameters to ensure adequate dorsiflexion, monitor electrode integrity, and inspect for any adverse events.

Participants were asked to use the Walk Aide for at least 4 hours per day, 6 days per week during the 8-week treatment period. This was monitored through the usage log on the device itself. To enable participants an opportunity to accommodate to the device, they were asked to gradually build up to the required dosage over the first week. Participants in the NMES treatment group did not wear their ankle-foot orthoses (AFOs) throughout the study. They were provided with customized in-shoe orthoses at the commencement of the study to support foot posture and account for leg length discrepancies particularly in the absence of AFOs. Participants in the control group were asked to continue with their usual orthotic treatments.

Statistical analysis

Based on effect sizes observed in our pilot study of NMES-assisted gait use, a one-tailed alpha of 0.05 and power of 80% power analysis suggested that each group required at least 15 participants per group to detect a clinically meaningful change of six heel raises.27

Normality was established through examining distributional plots, Q-Q plots, and the Shapiro-Wilk test. For normally distributed interval data (muscle volume symmetry ratio, normalized dorsiflexion strength, and SCALE), repeated measures analysis of variance was used to establish within and between group differences. To examine between group differences at week 8 and week 14, baseline measures were entered as a covariate, resulting in a repeated analysis of the covariance model. If a significant main effect for group and time or an interaction of these was found, post hoc Tukey’s analysis was applied. This enabled adjustments for multiple comparisons, calculation of mean differences, and 95% confidence intervals. Normalized muscle volume data was transformed using the natural log because of skewed distributions. Following the analysis, data was back-transformed by taking the exponential, with the interpretation in terms of percentage change. Back-transformation outputs are expressed as ratios of the geometric mean, with any back-transformed coefficients with a 95% confidence interval (CI) crossing the value 1, indicating a non-significant result. Effect sizes were determined for statistically significant comparisons by using Cohen’s $d$ calculation, with a value of 0.8 considered a large effect, 0.5 to be a medium, and 0.2 to be a small effect.11

Between group differences for ordinal data (ankle SMC) and when assumptions for normality even after transformation were not met (heel raises), the Mann-Whitney $U$ test was applied to change scores. Correspondingly, the Wilcoxon signed-rank test was used for examining within group changes over time. Relationships between the variables were examined using scatterplots and correlation coefficients. Intention to treat principle was applied. For all statistical tests, significance was $p<0.05$ with analyses performed using Stata version 12.1 (StataCorp, College Station, TX, USA). Given the heterogeneity of CP, individual intervention effectiveness was also explored by reviewing individual graphs. We reasoned that this might provide clinicians with an understanding of the variations in individual response.

RESULTS

All participants who provided informed consent entered and completed the study in their original group assignment. The baseline MBI for one participant in the control group was eliminated because of movement artifact. The clinical measures of strength and SMC were complete for all 32 participants.

All participants had a frequency set at 133 Hz with pulse width ranging from 25 to 100 μs. Participants used NMES-assisted gait daily for a mean of 6.2 (SD 3.2) hours over the 8-week intervention period. There were no reported unintended effects or adverse events using the NMES device.

Muscle volume

The treatment group demonstrated significantly increased volumes (for both normalized volumes and volume symmetry ratio) in all muscles except for soleus at week 8 when compared to baseline measures (Table II and Fig. 1). Notably, as indicated in Table III, the treatment group’s normalized tibialis anterior volume had significantly increased by 23% (95% CI 14-31; $p<0.001$; $d$=0.62) at week 8 when compared to baseline. At week 14, there were significant increases in lateral gastrocnemius muscle vol-

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Table II: Mean (SD) of groups for muscle volume, ankle dorsiflexion strength, and Selective Control Assessment of the Lower Extremity (SCALE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Week 0</th>
<th>Week 8</th>
<th>Week 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rx</td>
<td>Con</td>
<td>Rx</td>
</tr>
<tr>
<td>Normalized muscle volume (affected side muscle volume/tibia length)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tib anterior</td>
<td>1.60 (0.29)</td>
<td>0.72 (0.29)</td>
<td>1.00 (0.34)</td>
</tr>
<tr>
<td>Anterior comp.</td>
<td>1.60 (0.49)</td>
<td>1.57 (0.83)</td>
<td>2.00 (0.28)</td>
</tr>
<tr>
<td>Medial gastrocnemius</td>
<td>1.54 (0.29)</td>
<td>1.54 (0.88)</td>
<td>1.71 (0.96)</td>
</tr>
<tr>
<td>Lateral gastrocnemius</td>
<td>0.99 (0.36)</td>
<td>1.92 (0.44)</td>
<td>1.08 (0.49)</td>
</tr>
<tr>
<td>Soleus</td>
<td>3.93 (1.25)</td>
<td>3.89 (1.22)</td>
<td>3.86 (1.31)</td>
</tr>
<tr>
<td>Muscle volume symmetry ratio (affected/unaffected muscle volume)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tib anterior</td>
<td>0.99 (0.25)</td>
<td>0.97 (0.13)</td>
<td>0.72 (0.22)</td>
</tr>
<tr>
<td>Anterior comp.</td>
<td>0.65 (0.16)</td>
<td>0.62 (0.12)</td>
<td>0.74 (0.14)</td>
</tr>
<tr>
<td>Medial gastrocnemius</td>
<td>0.65 (0.22)</td>
<td>0.69 (0.21)</td>
<td>0.75 (0.25)</td>
</tr>
<tr>
<td>Lateral gastrocnemius</td>
<td>0.64 (0.19)</td>
<td>0.70 (0.16)</td>
<td>0.71 (0.16)</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.76 (0.18)</td>
<td>0.76 (0.15)</td>
<td>0.74 (0.15)</td>
</tr>
<tr>
<td>Strength and selective motor control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsiflexion (normalised)</td>
<td>0.11 (0.09)</td>
<td>0.13 (0.07)</td>
<td>0.21 (0.13)</td>
</tr>
<tr>
<td>Heat raised*</td>
<td>0.0 (0.34)</td>
<td>0.0 (0.2)</td>
<td>0.0 (0.34)</td>
</tr>
<tr>
<td>SCALE score out of 10</td>
<td>4.94 (1.12)</td>
<td>5.4 (1.64)</td>
<td>6.06 (1.53)</td>
</tr>
</tbody>
</table>

*Reported as median and interquartile range. Rx: treatment; Con: control; tib: tibialis; comp: compartment; gastro: gastrocnemius.

For the treatment group when compared to baseline, with a mean increase of 14% (95% CI 7–21; p=0.017; d=0.31). There was a significant increase in the normalized volume for tibialis anterior in the treatment group at week 14 when compared to baseline (p=0.039) and this approached significance when expressed as a muscle volume symmetry (p=0.005). There were no significant changes for the control group over time when compared to baseline.

Between groups at week 8 (Table III), the treatment group demonstrated significantly increased volumes (for both normalized volumes and volume symmetry) for all muscles except for soleus when compared to controls. There was a large effect for tibialis anterior, with a mean difference of 18% (95% CI 6–34; p=0.001; d=0.87). There was a medium effect for the anterior compartment, with a mean difference of 18% (95% CI 7–30; p<0.001; d=0.67). Medial gastrocnemius had a small effect with a mean difference of 10% (95% CI 2–20; p=0.014; d=0.29). Lateral gastrocnemius also had a small effect with a mean difference of 14% (95% CI 2–27; p=0.017; d=0.15). At week 14, the treatment group had significant increases in lateral gastrocnemius muscle volume only when compared to controls with a mean difference of 15% (95% CI 3–29; p=0.009; d=0.19).

**Strength**

The treatment group demonstrated significantly greater ankle dorsiflexion strength at week 8 (mean difference 0.1, 95% CI 0.06–0.14; p=0.001; d=0.89) and at week 14 (mean difference 0.09, 95% CI 0.06–0.13; p=0.001; d=0.85) when compared to baseline measures. The control group demonstrated significant gains in strength at week 14 only when compared to baseline (mean difference 0.04, 95% CI 0.003–0.08; p=0.032; d=0.57). Between groups, the treatment group had significant increases in ankle dorsiflexion strength when compared to controls at week 8 (mean difference 0.09, 95% CI 0.03–0.15; p=0.002; d=0.7) but not at week 14 (mean difference 0.08, 95% CI 0.01–0.12; p=0.16) (Fig. 1).

There were significant within-group improvements over time in the number of heel raises for both the treatment (week 8 median difference =3.5, interquartile range [IQR] 1–6; p=0.002) and control group (week 8 median difference =3, IQR 0–7.5; p=0.008) and control group (week 8 median difference =0, IQR 0–3; p=0.03) week 14 median difference =1, IQR 0–7; p=0.05). Although these median changes did not represent clinically meaningful changes (defined as a minimum change of six heel raises33), individual analysis identified that there was a trend for more participants in the treatment group (5/16) than the control group (1/16) to achieve clinically meaningful improvements at week 8. Of note, seven out of 16 participants in the treatment group were just short of achieving a clinically meaningful improvement. At week 14, there was a trend for more participants in the treatment group (6/16) than in the control group (3/16) to achieve clinically meaningful improvements in the number of heel raises performed. There were no significant between group differences at week 8 (p=0.08) and at week 14 (p=0.3) for heel raises.

**Selective motor control**

The treatment group demonstrated significant improvements in ankle SMC and SCALE scores at week 8 (ankle SMC median difference =1, IQR 0–3; p=0.002, SCALE mean difference =1.1, 95% CI 0.62–1.63; p=0.001; d=0.85) and week 14 (ankle SMC median difference =0.5, IQR 0–1; p=0.048, SCALE median difference =0.01, 95% CI 0.3–1.32; p=0.001; d=0.07) when compared to baseline measures. The control group demonstrated no significant improvements in ankle SMC at week 8 (median difference =0, IQR 0–1; p=0.1) and at week 14 (median difference =0, IQR 0–1).
Figure 1: Mean symmetry ratios of muscle volumes and normalized isometric dorsiflexion strength for the treatment (Rx) and control group across all time points. *Between group difference p<0.05; †Within group difference compared to baseline p<0.05.

$p=0.65$) when compared to baseline scores. The control group demonstrated significant SCALE improvements at week 8 (mean difference = 0.67, 95% CI 0.14–1.19; $p=0.007$; $d=0.41$) but not at week 14 (mean difference = 0.47; 95% CI = –0.06 to 0.99; $p=0.098$) when compared to baseline measures. Further, individual analysis revealed that at week 8, there was a trend for more participants in the treatment group (9/16 in ankle SMC, 6/16 on the SCALE) than in the control group (5/16 in ankle SMC, 2/16 on the SCALE) to make clinically meaningful score changes. To note, six out of 16 participants in the treatment group made no changes in ankle SMC at week 8. At week 14, there was a trend for ankle SMC improvements only, with more participants in the treatment group (8/16) than in the control group (3/16) to maintain clinically meaningful score changes. There were no statistically significant differences between the groups at week 8 (ankle SMC $p=0.10$ and SCALE $p=0.67$) and week 14 (ankle SMC $p=0.16$ and SCALE $p=0.86$).

Scatterplots demonstrated that at week 8 in the treatment group, there was no relationship between change in tibialis anterior muscle volume and ankle dorsiflexion strength ($p=0.09$, $p=0.75$) or change in anterior compartment MRI muscle volume and ankle dorsiflexion strength ($p=0.37$, $p=0.13$). There was a strong positive relationship between ankle dorsiflexion strength and SMC with a Spearman’s correlation coefficient at baseline $p=0.79$ ($p<0.001$), week 8 $p=0.74$ ($p<0.001$), and week 14 $p=0.63$ ($p<0.001$).

**DISCUSSION**

Supporting our first hypothesis, NMES-assisted gait significantly increased muscle volume and ankle dorsiflexion strength following an 8-week intervention. The inclusion
Table III: Mean difference (95% confidence interval) within and between groups for muscle volume, ankle dorsiflexion strength, and Selective Control Assessment of the Lower Extremity (SCALE)

<table>
<thead>
<tr>
<th></th>
<th>Difference within groups</th>
<th>Difference between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 8-0</td>
<td>Wk 14-0</td>
</tr>
<tr>
<td></td>
<td>Rx</td>
<td>Con</td>
</tr>
<tr>
<td>Normalized muscle volume (back-transformed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>1.23 (1.14-1.33)</td>
<td>1.06 (0.98-1.13)</td>
</tr>
<tr>
<td>Anterior comp</td>
<td>d=0.02</td>
<td></td>
</tr>
<tr>
<td>Medial gastrocnemius</td>
<td>1.44 (1.12-1.77)</td>
<td>1.01 (0.96-1.06)</td>
</tr>
<tr>
<td>Lateral gastrocnemius</td>
<td>2.22 (1.06-1.33)</td>
<td>1.01 (0.94-1.09)</td>
</tr>
<tr>
<td></td>
<td>d=0.34</td>
<td></td>
</tr>
<tr>
<td>Muscle volume symmetry ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>0.12* (0.09-0.16)</td>
<td>0.02 (0.01 to 0.03)</td>
</tr>
<tr>
<td></td>
<td>d=0.05</td>
<td></td>
</tr>
<tr>
<td>Anterior comp</td>
<td>0.11* (0.07-0.15)</td>
<td>0.01 (-0.03 to 0.05)</td>
</tr>
<tr>
<td>Medial gastrocnemius</td>
<td>0.09* (0.05-0.12)</td>
<td>0.005 (-0.03 to 0.04)</td>
</tr>
<tr>
<td></td>
<td>d=0.34</td>
<td></td>
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<tr>
<td>Lateral gastrocnemius</td>
<td>0.14* (0.06-0.14)</td>
<td>0.01 (-0.04 to 0.06)</td>
</tr>
<tr>
<td></td>
<td>d=0.42</td>
<td></td>
</tr>
<tr>
<td>Strength and selective motor control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsiflexion (normalized)</td>
<td>0.10* (0.06-0.16)</td>
<td>0.01 (-0.03 to 0.05)</td>
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<tr>
<td></td>
<td>d=0.09</td>
<td></td>
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<tr>
<td>SCALE (score out of 10)</td>
<td>7.13* (6.8-7.63)</td>
<td>0.41* (0.3-1.32)</td>
</tr>
</tbody>
</table>

*p=0.05, d=Cohen’s d for statistically significant differences; Rx, treatment group; Con, control group; comp, compartment; gastrocnemius, gastrocnemius.
of a control group in this randomized controlled trial provides clinicians with the necessary clarity in the use of NMES-assisted gait within a community setting. Not only do the results provide evidence of further substantiating previous uncontrolled trials\textsuperscript{12,13,19} with benefits in muscle strength and volume, but that the effects are also more superior than conventional therapy. Traditionally, functional applications of NMES such as NMES-assisted gait is not used for muscle strengthening because impulses are generally not delivered at a high enough intensity to provide muscular improvements.\textsuperscript{22} However, the advantage of NMES-assisted gait is the high dosage that is offered through repetitive, non-selective recruitment of muscle fibres within functionally relevant and activity-based contexts.\textsuperscript{13,14} The non-selective recruitment ensures that all fibres regardless of type can be activated during everyday walking despite problems with SMC.\textsuperscript{34} The advantage of functionally applied NMES has similarly been reported in the spinal cord injury rehabilitation literature where muscle volume improvements were accompanied by greater strength gains when compared to muscles stimulated using NMES in non-functional contexts, i.e. in standardized positions.\textsuperscript{21} Hence NMES-assisted gait can be considered to be a viable option in the management of children with unilateral spastic CP to facilitate muscular changes while having the advantage of being integrated within individualized contexts.

The treatment group's significant increase in ankle dorsiflexion strength and muscle volume was not maintained at week 14 when compared to the control group, thus rejecting our second hypothesis. To note, the treatment group did demonstrate significant improvement in ankle dorsiflexion strength and volume at week 14 compared to baseline; however, the changes were not significantly greater than the control group who maintained conventional or usual therapies only. Hence the inclusion of a control group was beneficial to account for muscular changes from growth or conventional therapy input. The results from this time point support current literature that muscular changes following NMES are use-dependent\textsuperscript{26} and do not facilitate long-term or prolonged lasting neuro-muscular adaptations. While it may be preferable to continue to use NMES to maintain the muscle hypertrophy and strength changes, ongoing use could increase dependence on an external stimulus.\textsuperscript{19} To limit this, devices should only be used for as long as needed in order to promote movement control.\textsuperscript{36}

One strategy could be to alternate between a period of 8 weeks of use and a period of non-use (as used in the current study). Examination of individual responses in ankle SMC post-treatment provides some indication for the appropriateness of alternating between a period of NMES use and non-use. Although there were no statistically significant changes in SMC between the groups, the participant-specific responses provide useful clinical information. In the current study, nearly 40% of the participants in the treatment group made no SMC changes at week 8. For this group of children, NMES is needed to address impairments in SMC, hence a period of non-use may not be warranted. In contrast, 50% made clinically meaningful improvements at week 8 and week 14. For this group, a period of non-use would be appropriate for the therapeutic effect in SMC and to limit NMES dependence. Therefore, goals of treatment would be to either: (a) achieve muscle volume and strength gains and maintain the orthotic effect of NMES-assisted gait, or (b) achieve muscle volume and strength gains and obtain a therapeutic effect in SMC. This individual responsiveness may account for the limited and inconsistent evidence of therapeutic effects reported in the literature.\textsuperscript{52,57}

There were significant differences in medial and lateral gastrocnemius muscle volume at week 8 and for lateral gastrocnemius volume at week 14. The trend towards improvement in strength supports the results from our previous work\textsuperscript{27} and addresses previous concerns that stimulating only one muscle group at a joint would exacerbate muscle imbalances.\textsuperscript{22} To our knowledge, the volumetric improvement in medial and lateral heads of gastrocnemius following NMES to the ankle dorsiflexors has not been reported. These changes suggest that NMES could be a viable supplement to targeted strength training, especially if there are challenges with exercise compliance. Further, increases in gastrocnemius volume may be advantageous for children who undergo serial BotNT-A because of the documented volumetric loss that occurs soon after injections.\textsuperscript{25}

Given that the control group had no within-group changes over time, it is likely that the improvements in both volume and strength of gastrocnemius is attributable to the removal of AFOs and the advantage of the NMES' orthotic effect to enable greater gastrocnemius contribution in mid- to late stance for push-off.\textsuperscript{12,13,19} Also, these muscle changes occurred after just 8 weeks of intervention, thus NMES may offer controlled breaks from AFO use. These breaks may provide an opportunity to facilitate strengthening and prevent atrophy from serial BotNT-A. For ambulant children with CP, addressing problems with muscle strength and volume may have long-term implications, particularly with the management of pain and reports of walking deterioration with increasing age.\textsuperscript{8,6} Given the significant muscular improvements noted in the present study, integrating NMES-assisted gait into current therapeutic management is superior to conventional therapy alone and may potentially have a role in forward planning for people with unilateral spastic CP. Further longitudinal investigation is therefore warranted.

While this study has provided further support for the use of NMES in children with CP in a randomized controlled trial, there are some limitations to note. One limitation is that participants actively sought to be in the study and this may account for the high compliance with use of the intervention. Another limitation is that funding restrictions meant that the assessor also provided the intervention.
Therefore it was not possible for the assessor to be blinded to group or assessment time period for the clinical measures. This apparent adverse effect prompted us to randomize scans in randomized time points, blinded for group and time with rigorous methods implemented to ensure that the assessor could not reference previous clinical results.

A further limitation is that all but four participants had a history of BotNT-A. This group was too small to enable sub-group analysis to yield adequate power. However, the application of analysis of covariance with the baseline as the covariate enabled adjustments for any baseline variation. Finally, there were limitations in the range of frequency parameters available on the WalkAide. Higher frequencies reportedly have a greater effect on the central nervous system and this may be associated with more prolonged neuroplastic adaptations. Future studies should consider the use of devices that enable a greater selection of parameters to elicit neuroplastic changes for a longer lasting effect. The strength of this study was the inclusion of a control group that was adequately powered with all participants completing the study.

Our results support the efficacy of NMES-assisted gait as a viable treatment option by providing the opportunity for targeted intervention to address the known problems in ankle dorsiflexion: SMG to improve muscle volume and strength in children with unilateral spastic CP. Although improvements in muscle volume and strength are use-dependent, clinicians should evaluate individual responses in ankle SMG after 8 weeks of NMES-assisted gait to determine an appropriate wear regime, i.e. ongoing NMES use or alternating between periods of use and non-use to prevent SMG.

ACKNOWLEDGEMENTS
This study was supported financially by the Princess Margaret Hospital Foundation, Perth, Australia. Orthopedic Appliances Pty Ltd donated 10 WalkAides to The Centre For Cerebral Poly which were subsequently used for this study. We thank Martin Spitt from the Department of Diagnostic Imaging at Princess Margaret Hospital for Children for his time and expertise in performing the MRI. The authors have stated that they had no interests that might be perceived as posing a conflict or bias.

REFERENCES
10 Developmental Medicine & Child Neurology 2015

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APPENDIX B – ACCEPTED ABSTRACTS

MUSCLE VOLUME ALTERATIONS IN CHILDREN WITH CEREBRAL PALSY FOLLOWING THE FIRST BOTULINUM TOXIN TREATMENT: A 6 MONTH PROSPECTIVE COHORT STUDY.

Alexander, C.F.1, Elliott, C.2, Bear, N.3, Valentine, J.2, Stannage, K.4, Donnelly, C.J.1, Shipman, P.3, and Reid, S.1

1 School of Sport Science, Exercise and Health, UWA
2 Department of Paediatric Rehabilitation, PMH
3 Clinical Research and Education, PMH
4 Department of Orthopaedics, PMH
5 Department of Diagnostic Imaging, PMH

Presented at the Australasian Academy of Cerebral Palsy and Developmental Medicine Conference, 2018, Auckland, New Zealand

Objective: Focal spasticity, a common impairment for children with cerebral palsy (CP), is frequently managed with botulinum toxin (BoNT-A). BoNT-A has well documented clinical and functional benefits as well as an established safety profile. The impact of BoNT-A on muscle volume (MV), which is well documented as smaller in children with CP and contributes to reduced strength1 has been shown to be reduced following repeat BoNT-A injections2. This impact is an important consideration in the long term spasticity management plans and timing of repeat injections. This study aimed to identify alterations in muscle of children with CP following their first exposure to BoNT-A over a six month period

Design: Longitudinal, observational cohort design.

Method: Eleven children with CP (male n=8), aged 8.8 (SD3.1) years, of GMFCS levels I (n=7) and II (n=4) were recruited from the CP Mobility Service at Princess Margaret Hospital. All participants received BoNT-A injections to the affected gastrocnemius. MV of the gastrocnemius, soleus, tibialis anterior and hamstrings were measured using T1-weighted spin echo sequence MRI scans. MV was normalised to bone length, and changes analysed relative to baseline volumes. Assessments were conducted 1 week prior to BoNT-A treatment, and 5 weeks, 13 weeks and 25 weeks post treatment.

Results: All children demonstrated positive clinical and functional gains. MV of the injected gastrocnemius was found to be significantly reduced at 5 weeks (-0.15ml/mm; 95% CI -0.27, -0.05), 13 weeks (-0.24ml/mm; 95% CI -0.37, -0.12) and 25 weeks (-0.22ml/mm; 95% CI -0.34, -0.10). These changes corresponded to average reductions of 6.7%, 10.7% and 9.8% respectively.
Significant increases in normalised soleus MV were identified at each follow up, while hamstrings showed significant increase at 5 weeks only. Total plantarflexor volume showed no changes.

Conclusion: Normalised MV of the injected gastrocnemius reduces following first BoNT-A exposure, and does not return to baseline volume by 25 weeks. Compensatory hypertrophy is seen in the soleus (synergist to the gastrocnemius for plantarflexion) up to 25 weeks, but not in the hamstrings (synergist to the gastrocnemius for knee flexion). While further research tracking changes beyond 25 weeks would be useful to determine an optimal re-injection schedule (to allow muscle volume to return to baseline while maintaining the clinical and functional gains), the results of this study contribute further to the knowledge on BoNT-A and muscle morphology.

References

1Reid et al. (2015). Disability and Rehabilitation, 37(7).
Children with cerebral palsy have larger in-vivo MRI derived Achilles tendon moment arms than typically developing children.

Alexander, C.F.¹, Donnelly, C.J.¹ Reid, S.¹, Dwyer, B.², and Stannage, K.³,

¹ School of Sport Science, Exercise and Health, UWA
² Department of Diagnostic Imaging, PMH
³ Department of Orthopaedics, PMH

Presented at the Australasian Academy of Cerebral Palsy and Developmental Medicine Conference, 2018, Auckland, New Zealand

Objective: Children with cerebral palsy (CP) have impaired plantarflexion function, which is frequently attributed to impaired muscle structure and activation patterns. There is limited research investigating the role the Achilles tendon moment arm (ATMA) may play in modulating the muscles ability to generate a moment. A smaller ATMA in children with CP would contribute to impaired plantarflexion function. This study aimed to compare 3D in-vivo estimates of ATMA in children with CP and typically developing (TD) children. We hypothesised that ATMA among children with CP will be smaller than TD children.

Design: Cross sectional, observational cohort design.

Method: Eight children with CP (male n=7), aged 9.7 (SD2.6) years, of GMFCS levels I (n=4) and II (n=4) were recruited from the CP Mobility Service at Princess Margaret Hospital. Nine TD children (male n=5), aged 8.7 (SD2.3) years were recruited for comparison. Static T1-weighted spin echo sequence MRI scans of the dominant (TD) or most affected ankle (CP) were taken with the ankle held passively at a comfortable resting angle. Validated in-vivo ATMA’s were calculated as the perpendicular distance between the 3D coordinates of the AT path, and the bipolar axis of a cylinder fit to the 3D reconstructed talus dome (Alexander et al., 2017). OpenSim (simtk.org) musculoskeletal modelling framework was used, where subject specific ATMAs at a plantarflexion angle of 20° were estimated using an established lower limb model (Arnold et al., 2010). ATMA lengths were normalised to MRI derived tibia lengths for comparison.

Results: No differences were identified in age (p=0.911) or tibia length (p=0.914) between the groups. Children with CP had significantly larger MRI derived joint angles (p=0.003). At 20° plantarflexion, ATMA among children with CP (17.2% SD1.2) were significantly (p=0.013) larger than TD children (15.2% SD2.0).

Conclusion: Contrary to our hypothesis, children with CP have larger 3D ATMA when compared to TD children. This suggests the ATMA geometry may not be a contributing factor to reduced
plantarflexor moment production. Larger ATMA may in fact be a mechanical compensation for impaired muscle function. The mechanisms for altered ATMA geometry among children with CP were not explored in this study, but may be attributed to bony deformities frequently observed among children with CP. Further research is required into this, and the potential significant implications for interventions at the ankle.

References:

Muscle volume alterations in children with cerebral palsy following the first botulinum toxin treatment: A 6 month prospective cohort study.

Alexander, C.F.¹, Elliott, C.², Valentine, J.², Stannage, K.³, Donnelly, C.J.¹, Shipman, P.⁴, and Reid, S.¹

¹ School of Sport Science, Exercise and Health, UWA
² Department of Paediatric Rehabilitation, PMH
³ Department of Orthopaedics, PMH
⁴ Department of Diagnostic Imaging, PMH

Presented at the Australasian Academy of Cerebral Palsy and Developmental Medicine Conference, 2016, Adelaide, SA, Australia

Objective: Onabotulinum Toxin Type A (BoNT-A) is best practice care for children with spastic cerebral palsy (CP) with well documented clinical and functional outcomes. However, concerns have been raised about the impact of the toxin on muscle morphology, with recent studies demonstrating repeat BoNT-A injections having immediate impact on muscle volume in children with spastic CP. We aimed to track changes in lower limb muscle volume over 6 months following the first BoNT-A injection in ambulatory children with spastic CP.

Design: Prospective Cohort study.

Methods: A total of 10 children, 8 boys and 2 girls, with spastic CP (GMFCS I and II) were recruited. Six children were classified as diplegic and four hemiplegic. All children were BoNT-A naïve on enrolment and the average age at first BoNT-A injection was 8.93 years (SD=2.57). All 10 children received injections in affected gastrocnemius, and 2 children also received hamstring injections. Participants were recruited from Princess Margaret Hospital for Children, Western Australia.

Muscle volume was assessed using MRI within 1 week prior to first BoNT-A injection, with follow up assessments at 4, 12 and 26 weeks post injection. MRI scans were obtained using a 1.5T whole body unit with a T1 weighted spin echo sequence. Mimics® software was used for segmentation and volume calculations. All volumes were normalised to tibia length to account for growth and expressed as a percentage of baseline. Muscles assessed included; medial and lateral gastrocnemius, soleus, tibialis anterior, and three muscles of each the quadriceps and hamstrings.

Results: All participants demonstrated a positive clinical response to BoNT-A. The injected medial gastrocnemius showed a significant reduction in muscle volume at 12 weeks [-10.91%
(5.65), p<0.001] and 26 weeks [-10.84% (15.76), p=0.014]. Lateral gastrocnemius volume was also significantly reduced at 12 weeks post injection [-16.75% (11.47), p<0.001], with a reduction approaching significance at 4 weeks [-9.40% (21.26), p= 0.068]. Soleus muscle volume showed significant hypertrophy at 4 weeks [+6.32% (6.26), p= 0.002], 12 weeks [+5.12% (5.07), p= 0.004] and 26 weeks [+6.63% (10.48), p= 0.021]. No significant differences in total plantarflexor volume, or tibialis anterior volume was found.

**Conclusion:** BoNT-A injections in toxin naïve children with spastic CP are associated with significant atrophy of target muscles that is sustained up to 26 weeks after injection. However, it was also associated with functional gains and significant hypertrophy in the synergistic soleus muscle, suggestive of a compensatory mechanism to maintain total plantarflexor volume.
ACHILLES TENDON MOMENT ARM GEOMETRY IN TYPICALLY DEVELOPING CHILDREN

Lum, I.1, Donnelly, C.2, Reid, S.2, Alexander, C.F2, Elliott, C.3, Valentine, J.3, El-Shallam, A.3

1School of Sports Science, Exercise and Health, University of Western Australia, Singapore, Singapore,
2School of Sports Science, Exercise and Health, University of Western Australia,
3Department of Paediatric Research, Princess Margaret Hospital for Children, Perth, Australia

Accepted for presentation at XXV Congress of the International Society of Biomechanics, 2015, Glasgow, Scotland

Introduction and Objectives: The ankle is one of the most important joints to absorb and generate forces and moments of force during gait. Surgeries are frequently used to correct observed differences in a patient’s ankle mechanics during gait. To inform researchers and clinicians on specific mechanical characteristics of pathological gait pattern prior to surgeries, musculoskeletal models of the ankle are sometimes used. To accurately model muscle forces and joint moments about the ankle, reliable subject-specific in-vivo moment arm estimations are required. Current 3D in-vivo models use multiple magnetic resonance imaging (MRI) scans across the ankle’s full range of motion, which is extremely time consuming with pragmatic limitations. Our aim was to develop a reliable MRI based, 3D, in-vivo method to estimate Achilles tendon (AT) moment arms in typically developing children from a single scan sequence at a single joint posture. The secondary aim was to develop a regression equation to predict AT moment arm lengths using specific anthropometric variables.

Methods: A single time point cross-sectional design was used. Fifteen typically developing children, aged 4-12 years (mean 8.1 years, standard deviation (SD) 2.3 years), with no history of musculoskeletal conditions or injuries participated. A T1 spin echo MRI scan sequence of the lower limb was performed at Princess Margaret Hospital to obtain images of the AT and talocrural joint in the transverse and sagittal planes. Images were collected using a repetition time of 572ms, echo time of 13ms, slice thickness of 3mm, and mean inter-slice gap of 0.3mm. MR images were processed using Mimics and six model combinations were used to estimate AT moment arms of participants. These include three definitions of the ankle’s flexion-extension (F/E) axis of rotation (the Two-marker method (TMM), 2-point Talus method, and 3D Geometric model) (Fig. 1) each matched with two definitions of the AT line of action (the linear and polynomial methods). 3D coordinates of the talocrural joint’s F/E axes and the AT were exported from Mimics and processed using MATLAB to calculate the AT moment arms from each model combination.

To test the intra- and inter-rater reliability of all six AT moment arm model combinations, the first researcher processed the scans and calculated the AT moment arms twice (one week apart),
and a second researcher did this once. Both researchers were blinded to the identities of participants. Intraclass correlations (ICC) and Bland-Altman plots were used to assess intra- and inter-rater reliability of all AT moment arm models. Bland-Altman plots were used to compare relative differences between methods and the limits of agreement (LoA) of their respective measurement errors (±2 SD). A stepwise regression analysis was used to formulate a regression equation to predict AT moment arm length using the anthropometric variables foot length, tibia length and ankle plantar-flexion angle calculated during the scan sequence.

Results: Irrespective of the modelling approach, all methods displayed high intra- and inter-rater reliability (ICC = 0.957-0.990), low mean errors (-1.97-0.98) and tight LoA (1.57-2.72). A difference of 9.8mm in AT moment arm measurement was observed between the 3D Geometric model and TMM. There were minimal differences between the AT moment arm measured by the 3D Geometric model and 2-Point Talus method when calculated with the AT modeled as both a straight line and fifth order polynomial. Using the 3D Geometric model and polynomial method, foot length (p = 0.002) and tibia length (p = 0.002) were significant predictors of AT moment arms, accounting for 92% (R2 = 0.92) of the variance in AT moment arm length.

The Two-marker method, 2-Point Talus method, and 3D Geometric model

Conclusion: All models for estimating 3D AT moment arms in typically developing children had high intra- and inter-rater reliability. The 3D Geometric/Polynomial model is recommended as the method of choice for three reasons; 1) it accounts for the geometry of the talus when defining the F/E axis of the talocrural joint; 2) a polynomial better represents the curved geometry of the AT, particularly during non-weight bearing tasks; and 3) a geometric cylinder to define the F/E axis of the talocrural joint may be more appropriate for populations with bony deformity where specific anatomical landmarks may be difficult to define. The current regression model predicted AT moment arm length across a wide range of foot and tibia lengths regardless of ankle position, potentially removing the need for MR images to estimate the AT moment arm in typically developing children. We recommend that the 3D Geometric/Polynomial model be tested for the
measurement of AT moment arms among pathological paediatric populations with foot and ankle deformities. The current findings will have clinical relevance if used for the development of subject-specific musculoskeletal ankle models in a clinical paediatric population.

Disclosure of Interest: None Declared
APPENDIX C – HUMAN RESEARCH ETHICS APPROVALS

Government of Western Australia
Department of Health
Child and Adolescent Health Service

Dr Jane Valentine
Department of Paediatric Rehabilitation
Princess Margaret Hospital for Children
Roberts Road
SUBLACO WA 6008

Dear Dr Valentine

REGISTRATION NUMBER: 2013085EP
TITLE: Functional and morphological changes in muscle after first injection with botulinum toxin in children with cerebral palsy
MEETING DATE: 17 October 2013

RGO and Ethics requirements satisfied 5 November 2013

The Princess Margaret Hospital for Children Ethics Committee and the Research Governance Office consider that the study protocol conforms to the requirements of the NHMRC Statement on Ethical Conduct in Human Research (National Statement) and resolved at the meeting to recommend the protocol for approval to the Chief Executive. This recommendation has been ratified by the Child and Adolescent Health Service.

The Ethics Committee does however wish to be informed immediately of:

I. any untoward effects experienced by any participant in the trial where those effects in degree or nature were not anticipated by the researchers, and steps taken to deal with these,

II. substantive changes in the research protocol together with an indication of ethical implications, and

III. other unforeseen events.

The Ethics Committee has been charged with the responsibility of keeping the progress of all approved research under surveillance. A copy of the final result must be forwarded to the Committee upon completion of the research or if the research is not completed within twelve months you are asked to submit a progress report and annually thereafter. This information should include:

Princess Margaret Hospital for Children
Roberts Road Subiaco WA 6008
GP0 Box 1714 Perth WA 6840
Tel: (08) 9340 8222 Fax: (08) 9340 8111
www.cahs.health.wa.gov.au

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a) The status of the project (completed/under progress/abandoned/not commenced). In the event that a project does not commence within 12 months of being approved by the Ethics Committee the study must be resubmitted to the Committee for approval.

b) Compliance with conditions of ethical approval, including security of records and procedures for consent.

c) Compliance with any special conditions stated by the Ethics Committee as a condition of approval.

d) Results from the study to date, including outcome.

Please note that approval for studies is for three years and if the research is not completed within that period of time, a request for an extension of time should be submitted for consideration. In the event that a project does not commence within 12 months of being approved by the Ethics Committee, the study must be resubmitted to the Committee for approval.

In accordance with the NHMRC National Statement on Ethical Conduct in Human Research Chapter 5.5.3, researchers have a significant responsibility in monitoring and must submit the following to the Ethics Committee:

- Annual Reports on the anniversary of the approval date of the study
- Adverse event reports as received
- Amendments and extensions to the study to be requested in adequate time

Please quote the above registration number on all correspondence.

Yours sincerely,

[Name Redacted]

Executive Director
Medical Services

21 November 2013
Government of Western Australia
Department of Health

Child and Adolescent Health Service

Our Ref: 173/01.03/SEP

Dr Jane Valentine
Department of Paediatric Rehabilitation
Roberts Road
Subiaco 6008

Dear Dr Valentine

RE: AMENDMENT OF TRIAL APPROVAL
HUMAN RESEARCH ETHICS COMMITTEE (HREC)

HRREC Ref 2013035SEP
Study Title Functional and morphological changes in muscle after first injection with botulinum toxin in children with cerebral palsy

Thank you for your letter received by this office on 17/04/2014 enclosing the following amendment:

Protocol

The PMH HREC reviewed your request for the abovementioned amendment at its meeting on 17/04/2014 and recommended the amendment for approval.

It should be noted that all other aspects of the approval remain unchanged. Particularly in relation to the progress reports required, as in National Statement Chapters 5.5 & 5.7, and any further amendments to the protocols.

Please quote the above trial number 2013035SEP on all correspondence associated with this trial.

Yours sincerely

Dr Mark Salter
Executive Director
Medical Services

23/04/2014

* The Ethics Committee is constituted, and operates in accordance with the National Health and Medical Research Council's National Statement on Ethical Conduct in Research Involving Humans
Dear Professor Reid

HUMAN RESEARCH ETHICS OFFICE – RECOGNITION OF ETHICS APPROVAL FROM ANOTHER HUMAN RESEARCH ETHICS COMMITTEE

Project: Functional & Morphological Changes in Muscle After First Injection with Botulinum Toxin in Children with Cerebral Palsy - Recognition Princess Margaret Hospital for Children Ethics Committee Approval 2013005

Thank you for your correspondence enclosing the necessary documents to facilitate recognition of the ethics approval for the above project granted by an external Human Research Ethics Committee (HREC) registered with the National Health and Medical Research Council (NHMRC).

It is noted that you have ethics approval from Princess Margaret Hospital, approval number 2013005.

The UWA students and researchers identified as working on this project are:

UWA Researchers:

<table>
<thead>
<tr>
<th>Name</th>
<th>Faculty / School</th>
<th>Role</th>
</tr>
</thead>
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<tr>
<td>Assistant Professor Siobhan Reid</td>
<td>School of Sport Science, Exercise &amp; Health</td>
<td>Chief Investigator</td>
</tr>
<tr>
<td>Associate Professor Catherine Elliott</td>
<td>School of Pediatrics &amp; Child Health</td>
<td>Co-Investigator</td>
</tr>
<tr>
<td>Assistant Professor Cyril Donnelly</td>
<td>School of Sport Science, Exercise &amp; Health</td>
<td>Co-Investigator</td>
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Student(s): Caroline Davis

Although The University of Western Australia reserves the right to subject any research involving its staff and students to its own ethics review process, in this case, the Human Research Ethics Office has recognised the existing approval of the external HREC. The project is exempt from ethics review at UWA and the involvement of the above-listed researchers has been authorised. Any conditions for the recognition of the external HREC’s existing approval are listed below:

Special Conditions

1. Approving HREC to receive annual reports, amendments and notification of adverse events

You are reminded that it will be the responsibility of the approving HREC to ensure compliance with all ethics requirements and to monitor and report on the project. However, should any relevant ethics issues arise during the course of the project, you should inform the Human Research Ethics Office of The University of Western Australia.

If you have any queries, please contact the HREO at hreo-research@uwa.edu.au.

Please ensure that you quote the file reference – RA/4/1/6780 – and the associated project title in all future correspondence.

Yours sincerely,
APPENDIX D – INFORMATION SHEETS AND CONSENT FORMS

PARENT INFORMATION SHEET

Functional and morphological changes in muscle after first injection with botulinum toxin in children with cerebral palsy

**Why are we doing the study?**
Children with cerebral palsy have tight muscles that interfere with how the muscle functions. This tightness in the muscle is called spasticity, and is treated with botulinum toxin.

Muscles are very important in how we move everyday, how strong our muscles are and how they work is determined by their structure. We can look at muscle structure with special forms of imaging called MRI.

We want to understand how spasticity and botulinum toxin alter the muscle structure. To do this we need to take a picture of the muscle structure using MRI before and after they have botulinum toxin. To look at how much spasticity there is in the muscle and how it effects function we will look at how your child walks and how their muscles work while walking.

**Who is carrying out the study?**
This study is being carried out by the Paediatric Rehabilitation Department of Princess Margaret Hospital together with the School of Sport Science, Exercise and Health at the University of Western Australia.

**What will the study tell us?**
Currently we know about the effect repeated botulinum toxin has on muscle size, strength and gait in children who have already received botulinum toxin before. This study may tell us more about the effect the first botulinum toxin has on muscle structure and function, and may reveal more about the mechanisms by which botulinum toxin influences muscles.

**Does my child have to take part?**
No, participation in this research is voluntary and you are free to withdraw your child from the study at any time, for any reason, without prejudice in any way. You need not give a reason or justification for such a decision. Should you withdraw from the research, records of your child will be destroyed.

If you withdraw from the study this will not prejudice your status and rights as a patient of Princess Margaret Hospital. Your child’s participation in this study does not
prejudice any right to compensation, which you may have under the statute of common law.

**What will you be asked to do if you decide to take part in this study?**
Your child’s participation will require you and your child to attend testing sessions at Princess Margaret Hospital (Department of Radiology) and at the School of Sport Science, Exercise and Health, at The University of Western Australia (UWA). There will be 5 different testing sessions, spaced out over one year around when your child receives their first injection of botulinum toxin.

The testing sessions at Princess Margaret Hospital will take 30mins, while the testing session at The University of Western Australia will take about 2 hours.

**Assessment Procedures**
1. **MRI** is short for Magnetic Resonance Imaging. MRI is an advanced technology that provides images of muscles and joints. The purpose of the scan is for research only and will not be used diagnostically. Your MRI appointment in the Department of Radiology at PMH will take approximately 30mins. Your child can take along a favourite CD or DVD to watch whilst the scan is happening, and you can stay in the room the whole time. You child will have four scans each taking approximately 3 minutes. During this time your child will have to lie very still. One scan will take pictures of the top of the legs and the second scan will take pictures of the bottom of the legs.

2. At PMH a full physiotherapy clinical assessment will be completed to measure lower limbs (lengths, breadths and circumferences), range of movement and spasticity. You and your child will also be asked to complete some questionnaires relating to the physical abilities of your child and how that relates to quality of life, participation and activity.

3. The testing sessions at UWA will include a walking assessment called 3 dimensional gait analysis that allows us to calculate how the joints and muscles are moving by recording special reflective markers that we place on the skin with a specialised near-infrared camera system. Your child will be asked to walk 10 metres (a number of trials will be conducted) in a testing area with 7 cameras recording their lower limb movement. Muscle electrical activity (EMG) will also be measured during gait assessments. Small sticky markers called electrodes will be placed on the skin over the muscles on the front and back of the leg to record the amount of muscle activity that occurs during the gait assessment.

4. At UWA, some functional assessments of strength and endurance will also be completed. These involve seeing how many times your child can stand up to their tippy-toes, how far they can walk in 6 minutes, and how quickly they can stand up from a chair and walk 3m then return.

**What does my child need to do to be in the study?**
All participants who meet the following criteria are eligible for this study:
- Children with spastic cerebral palsy, aged between 4 and 18 years
- Children of GMFCS level I, II or III
- Children who have first pre BoNT-A appointment scheduled
- Children who have never had orthopaedic surgery on their legs, or botulinum toxin in their legs before.
Is there likely to be a benefit to my child?
Your child’s MRI scans will be in record at PMH for future reference for your child’s clinician. The data from the gait analysis will also be made available to clinicians at PMH.

Is there likely to be a benefit to other people in the future?
Every day children with Cerebral Palsy cope with their physical limitations. Recent research has shown how muscle structure can influence function but little is known about structure of muscle and spasticity and the first botulinum toxin treatment. This research may help with the development of ‘best practice’ standards of care for the use of botulinum toxin in children with Cerebral Palsy. The research has the potential to improve current treatment options available to children with Cerebral Palsy.

What are the possible risks and/or side effects?
There is a slight risk of skin irritation from the EMG electrodes and the adhesive tape that attaches markers to the skin. The electrodes and the tape are low-allergenic and any irritation experienced will be short term. A very small percentage of children experience these skin reactions.

MRI is very safe and has no known harmful effects. It is important to know that MRI will not expose you or your child to radiation.

Because MRI machines use a strong magnetic field, certain metal objects can be attracted to the magnetic fields. It is important that your child does not have any jewelry on them when they do the scan or any metal on their clothing (zips, studs or glittery writing). If they do have metal on their clothing they will be supplied with a gown to change into before the scan.

Children who have any of the following should discuss their suitable to participate with the research coordinator study.

- Pacemaker
- Aneurysm clips
- Cochlear implant
- A neuron-stimulator
- Metal implants
- Steel surgical staples or clips
- An implanted drug infusion device
- Any implant made partially or wholly or iron or steel

If you would like to be in the room with your child while they have a scan you must also let the radiologist know if you have any of the above and make sure you have no metal objects on. There are no restrictions for your child after the scan and they can go right back to their everyday activities.

What are the possible discomforts and/or inconveniences?
The MRI scan does not produce any discomfort and your child will not feel the scan. It is quite noisy, but your child will be wearing headphones and be listening to a DVD or radio show of their choice. If you choose to be in the MRI scan room you also will be provided with earmuffs.
Your child may complain that their muscles are a bit sore after the walking and heel raise exercises; this is completely normal after a good muscle workout and won’t last very long. The sticky markers may result in some discomfort when we remove them following testing, but every effort is made to minimise this.

**Where is your information kept?**
All information is kept on a password secured computer in the private office of the investigator at UWA. This office is locked at all times when not in use and is only accessible by the researcher.

**What about my privacy?**
Participant confidentiality will be respected at all times. Whilst video recordings will be made of your child performing some of the functional assessments, the project investigators will have sole access to this data, and it will be stored in a locked room at UWA. The results of this research may be published, however no identifying information shall be revealed. All data will be coded so as to preserve the identity and confidentiality of your child.

**Who has approved the study?**
The Princess Margaret Hospital Ethics committee has approved this research. Ethics number 2013085.

**Who to contact if you have any concerns about the organisation or running of the study?**
If you have any concerns or complaints regarding this study, you can contact the Director of Medical Services at PMH (Telephone No: (08) 9340 8222). Your concerns will be drawn to the attention of the Ethics Committee who is monitoring the study.

**What to do next if you would like your child to take part in this research:**
If you would like to take part in this research study, please read and sign the consent form provided.

**THANK YOU FOR YOUR TIME**

**Who to contact for more information about this study:**
If you would like any more information about this study, please do not hesitate to contact one of the research team. They are very happy to answer your questions.

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CHILD INFORMATION SHEET

Functional and morphological changes in muscle after first injection with botulinum toxin in children with cerebral palsy

Why?
Botulinum toxin helps to improve muscle tightness in children with cerebral palsy, to make it easier to walk, and join in with activities with friends and family. This study will show us how Botulinum toxin helps the muscles in children with cerebral palsy.

Who is carrying out the study?
There is a team at Princess Margaret Hospital, and also at the university called UWA who are working together on this study.

Who can take part?
Children with cerebral palsy who are 4 - 18 years old who are going to have their first botulinum toxin treatment in their legs.

Do you have to take part?
No. You do not have to participate in this research. If you start the study, you are free to pull out from the study at any time for any reason. You do not need to give a reason for pulling out. All your information will only been seen by us, we will not share it with other people. If we publish the results of this research we won’t use your name.

What will happen?
Twice before, and three times after you have your first botulinum toxin treatment we will take special pictures of your muscles and special videos of your walking. To do this we will use a very special machine called a Magnetic Resonance Imaging machine (MRI) and then do a gait analysis (gait means walking!)

How does MRI work?
MRI’s use very big magnets to take special pictures of muscles and bones. We will teach you all about MRI’s before you have one. You will come to PMH and have some scans done on your legs, to do this you will lie in the MRI tunnel like this photo. The MRI is quite noisy, so you will wear head phones, but it doesn’t hurt at all – in fact you won’t feel a thing. You will just need to lie very still while you are having your pictures taken, and you even get to watch a DVD! The MRI scans will take about 30mins. After your scan, a physiotherapist will look at how tight your leg muscles are and how much they move.
**How does the gait analysis work?**

You and your parent / guardian will come to the School of Sport Science, Exercise & Health at UWA to do some fun and interesting things. You will have shiny markers put on your legs, so we can understand how you walk. We also put some sticky dots that will tell us how much your muscles are working. These are called EMG. You will do some short walks. With the help of your parent / guardian you will fill out two forms about the different activities you do. You will spend about two hours at UWA.

**Will anything hurt?**

The pictures we take of your legs won’t hurt. You might find the MRI machine a bit noisy, but you will be wearing headphones and listening to your DVD. Some children, not many, find the shiny markers itchy. The tape used to stick the markers is special skin tape and if it does itch it will not last for long.

**Is there likely to be any benefit?**

If you participate in this study you will be provided with a report of your muscle tightness and how you walk before and after your botulinum toxin. Your results will also help improve our understanding of how Botulinum toxin helps children with cerebral palsy.

If you would like any more information about this study please feel free to contact one of the research team. We are happy to answer your questions.

Yours sincerely,

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TEEN INFORMATION SHEET

Functional and morphological changes in muscle after first injection with botulinum toxin in children with cerebral palsy

Why?
Botulinum toxin helps to improve muscle tightness in children with cerebral palsy. Reduced muscle tightness makes it easier to walk, play sports, and join in with activities with friends and family. This study will show us how Botulinum toxin helps the muscles in children with cerebral palsy, and may lead to improvements in how we use it.

Who is carrying out the study?
There is a team at Princess Margaret Hospital, and the University of Western Australia who are working together on this study.

Who can take part?
Children with cerebral palsy who are 4-18 years old who are going to have their first botulinum toxin treatment in their legs.

Do you have to take part?
No. You do not have to participate in this research. If you start the study, you are free to pull out from the study at any time for any reason. You do not need to give a reason for pulling out. All your information will only been seen by us, we will not share it with other people. If we publish the results of this research your name or identity will not be revealed.

What will happen?
This study takes place over a 12 month time frame around your first Botulinum toxin treatment. Twice before, and three times after you have your first injection we will take a scan of the muscles in your legs using an MRI machine and 3D videos of your walking.

How does MRI work?
MRI’s use very big magnets to image your muscles and bones. We will give you a lot more information about the MRI before you have it, and you can ask any questions you may have. MRI is very safe and doesn’t cause any harm. You cannot feel the scan at all when you have it, but it can be quiet noisy. You will just need to lie very still while you are having your pictures taken, and you get to watch a DVD (you can chose from our selection or bring your own from home). The
MRI scans will take about 30-45mins. After your scan, a physiotherapist will assess your range of motion in your legs, and test your muscles for spasticity.

**How does the gait analysis work?**
You and your parent / guardian will come to the School of Sport Science, Exercise & Health at the University of Western Australia to assess how you walk and how the muscles in your legs work while you walk. A physiotherapist will assess your range of motion in your legs and spasticity. Then you will have shiny markers put on your legs, which are seen by our 3D cameras. We also put some special jelly dots on that will read how much activity is happening in your muscles. These are called EMG. You will do some short walks. With the help of your parent / guardian you will fill out two forms about the different activities you do. You will spend about two hours at the University of Western Australia.

**Will anything hurt?**
The MRI scan won’t hurt. You might find the MRI machine a bit noisy, but you will be wearing headphones and listening to your DVD. Some children, not many, find the shiny markers itchy. The tape used to stick the markers is low allergenic and any itch won’t last for long.

**Is there likely to be any benefit?**
If you participate in this study you will be provided with a report of your muscle tightness and how you walk before and after your botulinum toxin. Your results will also help improve our understanding of how Botulinum toxin helps children with cerebral palsy.

If you would like any more information about this study please feel free to contact one of the research team. We are happy to answer your questions.

Yours sincerely,

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PARENT INFORMATION SHEET

Typically developing control group for the study titled:

**Functional and morphological changes in muscle after first injection with botulinum toxin in children with cerebral palsy**

**Why are we doing the study?**
Children with cerebral palsy have tight muscles that interfere with how the muscle functions. This tightness in the muscle is called spasticity, and is treated with botulinum toxin.

In this research we are aiming to understand how spasticity and botulinum toxin alter the structure and function of muscles and joints in children with cerebral palsy. To do this, it is important to know about the structure and function of muscles and joints in typically developing children.

**Who is carrying out the study?**
This study is being carried out by the Paediatric Rehabilitation Department of Princess Margaret Hospital together with the School of Sport Science, Exercise and Health at the University of Western Australia.

**What will the study tell us?**
Knowledge about typically developing children is important for us to understand normal muscle growth so we can have a better understanding of the changes we may see in children who have cerebral palsy. Understanding these changes for children with cerebral palsy is important to maximise the benefits they get from muscle treatments.

**Does my child have to take part?**
No, participation in this research is voluntary and you are free to withdraw your child from the study at any time, for any reason, without prejudice in any way. You need not give a reason or justification for such a decision. Should you withdraw from the research, records of your child will be destroyed.

**What will you be asked to do if you decide to take part in this study?**
Your child’s participation will require you and your child to attend a testing session at Princess Margaret Hospital (Department of Diagnostic Imaging) and at the School of Sport Science, Exercise and Health, at The University of Western Australia (UWA).

The testing session at Princess Margaret Hospital will take 30-45 mins, while the testing session at The University of Western Australia will take about 2 hours.

**Assessment Procedures**
5. MRI is short for Magnetic Resonance Imaging. MRI is an advanced technology that provides images of muscles and joints in the leg. The purpose of the scan is for
research only and will not be used diagnostically. Your MRI appointment in the Department of Diagnostic Imaging at PMH will take approximately 30-45mins. Your child can take along a favourite CD or DVD to watch whilst the scan is happening, and you can stay in the room the whole time. Your child will have six scans, each taking between 2 and 5 minutes. During this time your child will have to lie very still.

6. The testing sessions at UWA will include a walking assessment called 3 dimensional gait analysis that allows us to calculate how the joints and muscles are moving by recording special reflective markers that we place on the skin with a specialised near-infrared camera system. Your child will be asked to walk 10 metres (a number of trials will be conducted) in a testing area with 14 cameras recording their lower limb movement. Muscle electrical activity (EMG) will also be measured during gait assessments. Small sticky markers called electrodes will be placed on the skin over the muscles on the front and back of the leg to record the amount of muscle activity that occurs during the gait assessment.

7. At UWA, some functional assessments of strength and endurance will also be completed. These involve seeing how many times your child can stand up to their tippy-toes, how far they can walk in 6 minutes, and how quickly they can stand up from a chair and walk 3m then return.

What does my child need to do to be in the study?
All participants who meet the following criteria are eligible for this study:
 Children who have no known neuromuscular conditions
 Children aged between 4 and 18 years old

Is there likely to be a benefit to my child?
Your child’s MRI scan and gait analysis information will be provided to you for your interest.

Is there likely to be a benefit to other people in the future?
Every day children with cerebral palsy cope with their physical limitations. Recent research has shown how muscle structure can influence function, but little is known about structure of muscle with spasticity and the first botulinum toxin treatment. This research may help with the development of ‘best practice’ standards of care for the use of botulinum toxin in children with cerebral palsy. The research has the potential to improve current treatment options available to children with cerebral palsy.

What are the possible risks and/or side effects?
There is a slight risk of skin irritation from the EMG electrodes and the adhesive tape that attaches markers to the skin. The electrodes and the tape are low-allergenic and any irritation experienced will be short term. A very small percentage of children experience these skin reactions.

MRI is very safe and has no known harmful effects. It is important to know that MRI will not expose you or your child to radiation.

Because MRI machines use a strong magnetic field, certain metal objects can be attracted to the magnetic fields. It is important that your child does not have any jewellery on them when they do the scan or any metal on their clothing (zips, studs or
If they do have metal on their clothing they will be supplied with a gown to change into before the scan.

Children who have any of the following should discuss their suitability to participate with the research coordinator study.

- Pacemaker
- Aneurysm clips
- Cochlear implant
- A neuron-stimulator
- Metal implants
- Steel surgical staples or clips
- An implanted drug infusion device
- Any implant made partially or wholly or iron or steel

If you would like to be in the room with your child while they have a scan you must also let the radiologist know if you have any of the above and make sure you have no metal objects on. There are no restrictions for your child after the scan and they can go right back to their everyday activities.

**What are the possible discomforts and/or inconveniences?**
The MRI scan does not produce any discomfort and your child will not feel the scan. It is quite noisy, but your child will be wearing headphones and be listening to a DVD or radio show of their choice. If you choose to be in the MRI scan room you also will be provided with earmuffs.

The sticky markers that children wear during the gait analysis may result in some discomfort when we remove them following testing, but every effort is made to minimise this.

**Where is your information kept?**
All information is kept on a password secured computer in the private office of the investigator at UWA. This office is locked at all times when not in use and is only accessible by the researcher.

**What about my privacy?**
Participant confidentiality will be respected at all times. Whilst video recordings will be made of your child performing some of the functional assessments, the project investigators will have sole access to this data, and it will be stored in a locked room at UWA. The results of this research may be published, however no identifying information shall be revealed. All data will be coded so as to preserve the identity and confidentiality of your child.

**Who has approved the study?**
The Princess Margaret Hospital Ethics committee has approved this research.

**Who to contact if you have any concerns about the organisation or running of the study?**
If you have any concerns or complaints regarding this study, you can contact the Director of Medical Services at PMH (Telephone No: (08) 9340 8222). Your concerns will be drawn to the attention of the Ethics Committee who is monitoring the study.
What to do next if you would like your child to take part in this research:
If you would like to take part in this research study, please read and sign the consent form provided.

THANK YOU FOR YOUR TIME

Who to contact for more information about this study:
If you would like any more information about this study, please do not hesitate to contact one of the research team. They are very happy to answer your questions.

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CHILD INFORMATION SHEET

Typically developing control group for the study titled:

**Functional and morphological changes in muscle after first injection with botulinum toxin in children with cerebral palsy**

Why?
Some children have a condition called cerebral palsy, which makes it very difficult to walk, play and do fun activities. They get special medicine in their legs to make these things easier. We are doing a study on children with cerebral palsy and the medicine they get, but to understand that we first need to understand about the muscles and bones in children without cerebral palsy.

Who is carrying out the study?
There is a team at Princess Margaret Hospital, and also at the university called UWA who are working together on this study.

Who can take part?
Children who are 4 - 18 years old who do not have any muscle or bone conditions

Do you have to take part?
No. You do not have to participate in this research. If you start the study, you are free to pull out from the study at any time for any reason. You do not need to give a reason for pulling out. All your information will only been seen by us, we will not share it with other people. If we publish the results of this research we won’t use your name.

What will happen?
We will take some special pictures of your muscles and special videos of your walking. To do this we will use a very special machine called a Magnetic Resonance Imaging machine (MRI) and then do a gait analysis (gait means walking).

How does MRI work?
MRIs use very big magnets to take special pictures of muscles and bones. We will teach you all about MRIs before you have one. You will come to PMH and have some scans done on your legs. To do this you will lie in the MRI tunnel like this photo. The MRI is quite noisy, so
you will wear head phones. You will just need to lie very still while you are having your pictures taken, and you even get to watch a DVD. The MRI scans will take about 30mins.

**How does the gait analysis work?**
You and your parent / guardian will come to the School of Sport Science, Exercise & Health at UWA to do some fun and interesting things. You will have shiny markers put on your legs, so we can understand how you walk. We also put some sticky dots that will tell us how much your muscles are working. These are called EMG. You will do some short walks.

**Will anything hurt?**
The pictures we take of your legs won’t hurt. You might find the MRI machine a bit noisy, but you will be wearing headphones and listening to your DVD. Some children, not many, find the shiny markers itchy. The tape used to stick the markers is special skin tape and if it does itch it will not last for long.

**Is there likely to be any benefit?**
Your results will also help improve our understanding of how the muscle medicine helps children with cerebral palsy, so we can help them walk and play easier, just like you like to do.

If you would like any more information about this study please talk to one of the research team. We are happy to answer your questions.

Yours sincerely,

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TEEN INFORMATION SHEET

Typically developing control group for the study titled:

**Functional and morphological changes in muscle after first injection with botulinum toxin in children with cerebral palsy**

**Why?**
Some children have a condition called cerebral palsy. Children with cerebral palsy have tight muscles, which make it difficult to walk, play sports and join in with activities at school and home. Botulinum toxin helps to improve muscle tightness in children with cerebral palsy. We are doing a study to show us how botulinum toxin helps the muscles in children with cerebral palsy, and may lead to improvements in how we use it.

For us to really understand how botulinum toxin works for children with cerebral palsy, we first need to have a good understanding of the muscles and joints in typically developing children (children who do not have a muscle or bone condition)

**Who is carrying out the study?**
There is a team at Princess Margaret Hospital, and the University of Western Australia who are working together on this study.

**Who can take part?**
Children who are 4-18 years old and do not have a muscle or bone condition.

**Do you have to take part?**
No. You do not have to participate in this research. If you start the study, you are free to pull out from the study at any time for any reason. You do not need to give a reason for pulling out. All your information will only been seen by us, we will not share it with other people. If we publish the results of this research your name or identity will not be revealed.

**What will happen?**
We will take a scan of the muscles in your legs using an MRI machine and 3D videos of your walking.

**How does MRI work?**
MRIs use very big magnets to image your muscles and bones. We will give you a lot more information about the MRI before you have it, and you can ask any questions you may have. MRI is very safe and doesn’t cause any harm. You cannot feel the scan at all when you have it, but it can be quiet noisy. You will just need to lie very still while you...
are having your pictures taken, and you get to watch a DVD (you can choose from our selection or bring your own from home). The MRI scans will take about 30-45mins.

**How does the gait analysis work?**
You and your parent / guardian will come to the School of Sport Science, Exercise & Health at the University of Western Australia to assess how you walk and how the muscles in your legs work while you walk. You will have shiny markers put on your legs, which are seen by our 3D cameras. We also put some special gel dots on that will read how much activity is happening in your muscles. These are called EMG. You will do some short walks. With the help of your parent / guardian you will fill out two forms about the different activities you do. You will spend about two hours at the University of Western Australia.

**Will anything hurt?**
The MRI scan won’t hurt. You might find the MRI machine a bit noisy, but you will be wearing headphones and listening to your DVD. Some children, not many, find the shiny markers itchy. The tape used to stick the markers is low allergenic and any itch won’t last for long.

**Is there likely to be any benefit?**
We will give you images from your MRI scan, so you can see what the muscles and bones look like inside your legs. We will also give you a report that tells you about your walking. Importantly, your results will also help improve our understanding of how Botulinum toxin helps children with cerebral palsy so we can help make moving a lot easier for them!

If you would like any more information about this study please feel free to contact one of the research team. We are happy to answer your questions.

Yours sincerely

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FORM OF CONSENT  
(For Parent/Guardian)

PLEASE NOTE THAT PARTICIPATION IN RESEARCH STUDIES IS VOLUNTARY AND SUBJECTS CAN WITHDRAW AT ANY TIME WITH NO IMPACT ON CURRENT OR FUTURE CARE.

I ............................................................................................................................ have read
Given Names                                                             Surname

the information explaining the study entitled

**Functional and morphological changes in muscle after first injection with botulinum toxin in children with cerebral palsy**

I have read and understood the information given to me. Any questions I have asked have been answered to my satisfaction.

I agree to allow

.............................................................................................................................
(full name of participant and relationship of participant to signatory)

to participate in the study.

I understand my child may withdraw from the study at any stage and withdrawal will not interfere with routine care.

I agree that research data gathered from the results of this study may be published, provided that names are not used.

Dated .................. day of ............................................................ 20 ..........

Child’s Signature .................................................................
(Where appropriate)

Parent or Guardian’s Signature ..................................................

I, ................................................................. have explained the above to the
(Investigator’s full name)

signatories who stated that he/she understood the same.

Signature .............................................................................................................
FORM OF CONSENT
(For Parent/Guardian)

PLEASE NOTE THAT PARTICIPATION IN RESEARCH STUDIES IS VOLUNTARY AND SUBJECTS CAN WITHDRAW AT ANY TIME WITH NO IMPACT ON CURRENT OR FUTURE CARE.

I ........................................................................................................................... have read

Given Names ................................................................. Surname

the information explaining the study entitled

*Functional and morphological changes in muscle after first injection with botulinum toxin in children with cerebral palsy*

I have read and understood the information given to me. Any questions I have asked have been answered to my satisfaction.

I agree to allow

................................................................................................................

(full name of participant and relationship of participant to signatory)

to participate in the typically developing control group for this research.

I understand my child may withdraw from the study at any stage.

I agree that research data gathered from the results of this study may be published, provided that names are not used.

Dated .................................. day of .................................................. 20 ........

Child’s Signature ........................................................................

(Where appropriate)

Parent or Guardian’s Signature ......................................................

I, ................................................................................ have explained the above to the

(Investigator’s full name)

signatories who stated that he/she understood the same.

Signature .........................................................................................
APPENDIX E – FEEDBACK TO FAMILIES

The University of Western Australia and Princess Margaret Hospital present:

The Moment Arms
of children with cerebral palsy

Children with cerebral palsy can find it hard to move their feet and ankles.

The way the bones and muscles of the ankle are shaped and arranged might affect how they move.

Some children had a special scan taken of their ankles so we could measure their moment arms.

There is a special measurement we can take called a moment arm – it tells us how effective the muscle will be.

Children with CP have big moment arms!

This means the muscles and bones in their ankles are arranged in a way that helps the muscles do their job better.

We need to do more research to understand why children with CP have bigger moment arms.

This is important because we know that the muscles of children with CP are not as strong, so having big moment arms is really helpful.

This will help us understand why children with CP might still find it difficult to move so we make decisions about treatments.

This research was conducted by the University of Western Australia and Princess Margaret Hospital for Children, in Perth, Western Australia.

It was approved by the UWA and PMH Human research ethics departments.

If you have any questions on this research please contact: catherine.elliott@health.wa.gov.au

Government of Western Australia
Department of Health
Child and Adolescent Health Service

The University of Western Australia
delivering international excellence
BOTULINUM TOXIN AND MUSCLE SIZE

CHILDREN WITH CEREBRAL PALSY HAVE SMALLER MUSCLES compared to their typically developing peers.

WHEN THEY RECEIVE BOTULINUM TOXIN FOR THE FIRST TIME their function improves and they achieve their goals, but we also see changes in the muscle size until about 3 months after the injection, then it starts getting bigger again.

THE MUSCLE INJECTED GETS SMALLER until about 3 months after the injection, then it starts getting bigger again.

THE MUSCLES THAT WERE NOT INJECTED GET BIGGER The other muscles that help the injected muscle do its job get bigger. This is possibly why there is a functional improvement.

MORE RESEARCH IS NEEDED To understand the best timing of botulinum toxin injections so we can continue to see the benefits but also let the muscles grow as much as possible.

This research was conducted by the University of Western Australia and Princess Margaret Hospital for Children, in Perth, Western Australia.

It was approved by the UWA and PMH Human research ethics departments.

If you have any questions or would like to know more about this research, please contact: catherine.elliott@health.wa.gov.au
 APPENDIX F – 3D ATMA CODE

MATLAB CODE FOR CALCULATING 3D ATMA FROM ACHILLES TENDON 3D COORDINATES AND CYLINDER BIPOLAR AXIS 3D COORDINATES

clear all
c1c
close all

%%%%
% curve AT
XYZ_c = [
51.378 64.202 -36.0117
52.9718 61.8949 -22.3932
54.1092 60.7362 -11.0444
55.1852 59.5622 -0.5686
56.1039 59.0702 10.0818
56.5995 58.653 15.3197
57.0528 58.4055 20.5576
];

xc = XYZ_c(:,1);
yc = XYZ_c(:,2);
zc = XYZ_c(:,3);

tc = 0:length(xc)-1;

axc = polyfit(tc(:),xc(:),3);
ayc = polyfit(tc(:),yc(:),3);
azc = polyfit(tc(:),zc(:),3);

%%%%
% interpolated curve
resolution = 0.05;

tcn = -max(tc)/4:resolution:1.25*max(tc);

xcn=polyval(axc,tcn);
ycn=polyval(ayc,tcn);
zcn=polyval(azc,tcn);

%%%%
% line JA
XYZ_l = [
47.8088 14.5427 -25.0918
75.7879 24.5697 -26.2459
];

xl = XYZ_l(:,1);
yl = XYZ_l(:,2);
zl = XYZ_l(:,3);

tl = 0:length(xl)-1;

axl = polyfit(tl(:),xl(:),1);
ayl = polyfit(tl(:),yl(:),1);
azl = polyfit(tl(:),zl(:),1);
% interpolated line
tln = -max(tl)/2:resolution: 1.5*max(tl);

xln=polyval(axl,tln);
yln=polyval(ayl,tln);
zln=polyval(azl,tln);

% minimum distance between Talus line and the AT curve

[-,d1]= dsearchn([xln(:) yln(:) zln(:)],[xcn(:) ycn(:) zcn(:)]);
[min_d1, idx1] = min(d1);
pc = [xcn(idx1) ycn(idx1) zcn(idx1)];

[-,d2]= dsearchn([xcn(:) ycn(:) zcn(:)],[xln(:) yln(:) zln(:)]);
[min_d2, idx2] = min(d2);
pl = [xln(idx2) yln(idx2) zln(idx2)];

d = min([min_d1 min_d2]);

[min_distance_Talus_AT_Curve, idx] = min(d);
disp('Minimum distance (blue) between the (green - Talus) line and the (red - AT) curve = ');
min_distance_Talus_AT_Curve

%% fitting line between AT points 1, 5

% xlc = XYZ_c([1 5],1);
% ylc = XYZ_c([1 5],2);
% zlc = XYZ_c([1 5],3);
% tlc = 0:length(xlc)-1;
% axlc = polyfit(tlc(:),xlc(:),1);
% aylc = polyfit(tlc(:),ylc(:),1);
% azlc = polyfit(tlc(:),zlc(:),1);
% % interpolated line
% tlcn = -max(tlc)/4:resolution: 1.5*max(tlc);
% xlc=polyval(axlc,tlcn);
% ylc=polyval(aylc,tlcn);
% zlc=polyval(azlc,tlcn);
% % minimum distance between Talus line and the AT line from point 1 to 5
% [-,d3]= dsearchn([xln(:) yln(:) zln(:)],[xlc(:) ylc(:) zlc(:)]);
% [min_d3, idx3] = min(d3);
% pc3 = [xlc(idx3) ylc(idx3) zlc(idx3)];
% [-,d4]= dsearchn([xlc(:) ylc(:) zlc(:)],[xln(:) yln(:) zln(:)]);
% [min_d4, idx4] = min(d4);
% pl4 = [xln(idx4) yln(idx4) zln(idx4)];
% d = min([min_d3 min_d4]);
% [min_distance_Talus_AT_line, idx]  = min(d);
% disp('Minum distance (yellow) between the (green - Talus) line and the (cyan - AT line points#1,5)=')
% min_distance_Talus_AT_line
% disp('difference between two distances ( using line to curve minus line to line) = ')
% min_distance_Talus_AT_Curve - min_distance_Talus_AT_line
%
fig = figure;
set(fig,'defaultlinelinewidth',2)
plot3(xc,yc,zc,'ro')
hold on
plot3(xl,yl,zl,'go')
plot3(xcn,ycn,zcn,'r')
plot3(xln,yln,zln,'g')
plot3(xlcn,ylcn,zlcn,'c')
plot3(pl(1),pl(2),pl(3),'k*')
plot3(pc(1),pc(2),pc(3),'k*')

line([pl(1) pc(1)],[pl(2) pc(2)],[pl(3) pc(3)])

for n=1:length(xc)
    text(xc(n),yc(n)-2,zc(n), num2str(n))
end
for n=1:length(xl)
    text(xl(n),yl(n)-4,zl(n), num2str(n))
end
xlabel('X-axis')
ylabel('Y-axis')
zlabel('Z-axis')

grid, axis equal
APPENDIX G - ACHILLES TENDON MOMENT ARM REPEATABILITY AMONG TYPICALLY DEVELOPING CHILDREN

METHODS

Fifteen typically developing children, aged 4-12 years, with no history of musculoskeletal injuries or conditions were recruited and participated in this analysis. Ethics approval from both the Princess Margaret Hospital for Children Ethics Committee (2013085EP) and the University of Western Australia Human Research Ethics Committee (RA/4/1/6780) were obtained prior to commencement of the study. Informed consent was obtained from the parents/guardians on behalf of the participants.

Each participant underwent an MRI scan of the lower limb using a 1.5-T whole body Magnetic Resonance Unit (Signa, General Electric Medical Systems, Milwaukee) on a single occasion at Princess Margaret Hospital (PMH). T1 images of the dominant ankle in both the transverse and sagittal planes were taken, from the level of the middle of the tibia to the calcaneus. During the scan sequence, participants lay supine with the ankle held in passive plantar-flexion at a comfortable neutral angle. Standard patient positioning techniques including foam pads and straps were used to ensure that participants’ ankles did not move during the scan sequence. Images were collected using a repetition time of 572ms, echo time of 13ms, slice thickness of 3mm, and mean inter-slice gap of 0.3mm. A matrix size of 256x136mm was used for all scans, and the field of view (135-180mm) was varied to maximise in-plane resolution for each scan. The time taken to complete each scan sequence was approximately six minutes.

MR images were processed using Mimics®. The Achilles tendon was defined from seven digitized points from the scan closest to the midline of the joint. The first point was placed at the most proximal point of bony insertion, and the fifth point placed at the most distal appearance of muscle, with the second, third and fourth points distributed between. The 6th and 7th points were placed immediately proximal to the musculotendinous junction.

The talus was manually segmented and reconstructed as a 3D form. To this 3D form, a cylinder was manually fit to the talar dome. The orientation and radius of the cylinder was manually adjusted to subjectively ‘best-fit’ the curvature of each individual’s talus dome. The plantar-/dorsi-flexion axis of the talocrural was defined as the polar axis of the cylinder. All seven digitized points of the AT tendon as well as the and cylinder parameters were exported from Mimics into Matlab (R2015a, The Mathworks, Massachusetts, U.S.A) to calculate each participant’s 3D ATMA (Figure 1C). In Matlab the seven digitized points of the AT were modelled as a fifth-order polynomial curve. The AT moment arm was calculated as the shortest Euclidean distance between the polar axis of the cylinder and the polynomial defining the 3D orientation of the AT.
To test the intra- and inter-rater reliability of the ATMA estimates were calculated. The first researcher processed the scans and calculated the AT moment arms twice (one week apart between measurements), and a second researcher did this once. Intraclass correlations (ICC), Bland-Altman plots and limits of agreement (95% CI) were used. Bland-Altman plots were also used to compare the relative differences between the methods as well as the limits of agreement of their respective measurement errors (±2 SD).

RESULTS

For the intra- tester repeatability test, there was excellent within tester measurement agreement (ICC (ρ) = 0.987; α=0.001; 1-β = 99.9%). The Bland-Altman plots showed a mean measurement difference of -1.0 mm and a limit of agreement of ±1.8 mm. For the inter- tester repeatability test, there was excellent between tester measurement agreement (ICC (ρ) = 0.985 α=0.001; 1-β = 99.9%). The Bland-Altman plots showed a mean measurement difference of -1.7 mm and a limit of agreement of ±1.9 mm.

**Intra- tester Repeatability**

![Intra-tester Repeatability](figure1a.png)

**Inter- tester Repeatability**

![Inter-tester Repeatability](figure1b.png)

**Figure S1:** Intra- and inter tester repeatability of 3D in-vivo ATMA model. Bland-Altman plots and limits of agreement (dashed horizontal line), with ICC data imbedded.
APPENDIX H – VECTOR FIELD STATISTICS CODES

MATLAB CODE FOR TRANSFORMING DATA FROM EXCEL SPREADSHEET TO MAT FILE

% Script to format UWA Gait Data for SPM analysis
% Import from an Excel spreadsheet the gait data from a UWA gait analysis.
% All data will be reorganised suitable for SPM analysis and saved to a MAT file
% Written by Mark Robinson m.a.robinson@ljmu.ac.uk
% This code is supplied with warranty, explicit or implied.

% Requirements
% .xlsx file
% right data stacked under left
% Known number of variables, currently 13 @May 2017

%% Step 1 - Import data
clear,clc

[filename,pathname] = uigetfile("*.xlsx","Open .xlsx file");
pathfile = strcat(pathname,filename);
data = importdata(pathfile, '\t');

% align headers with data
data.textdata = data.textdata(2:end);

%% Remove NaNs from data & format array

% combine data for easier analysis (not needed)
% data2 = cat(1,data.data.LEFT, data.data.RIGHT);

% Identify nan
nan = (isnan(data.data(:,1)));

% Get row indexes for actual data (not nan)
ind = nan < 1;
ind = find(ind);
% Generate angle data variable ordered by trial
angles_tr = data.data(ind,:);

% Determine number of trials knowing there are 13 variables
ANGLES = 13; % important constant
TRIALS = length(angles_tr) / ANGLES;

% Separate into left and right data
ang_l = angles_tr(1:length(angles_tr)/2,:);
ang_r = angles_tr(length(angles_tr)/2+1:length(angles_tr),:);

%% Arrange data into variable order
ii = 1; % angle type
while ii <= ANGLES % num angles
    temp_ind = (ii:ANGLES:ANGLES*TRIALS/2); % index for each trial
    %left
    temp_l_ang = ang_l(temp_ind,:);
    ang_l_order{ii,1} = temp_l_ang; % rows are the diff angles
    %right
    temp_r_ang = ang_r(temp_ind,:);
    ang_r_order{ii,1} = temp_r_ang;

    clear temp*
    ii = ii + 1;
end

% Concatenate into 3d array
left_3d = cat(3,ang_l_order{:,1});
right_3d = cat(3,ang_r_order{:,1});

%% Step 3 - Write to mat file.
% save L and R vector variables to matfile
save(strcat(filename(1:length(filename)-5),'_LR_py.mat'),'left_3d','right_3d');
clear,clc
# Indep t-test for CP patient vs normal (L&R)
# Dataset - Normative data for the left and right sides
# This will output a PDF report and a csv file.

import os
import numpy as np
import scipy.io
import spm1d
import csv
from matplotlib import pyplot
from matplotlib.backends.backend_pdf import PdfPages

################ Z Curve Integration Function

def zCurveInt(spmi):
    # integration of the spmi.z curve
    zint = np.trapz(abs(spmi.z))
    return(zint);

###########
### Figure preparation:
fig_width_mm = 240
fig_height_mm = 190
mm2in         = 1/25.4
fig_width     = fig_width_mm*mm2in  # width in inches
fig_height    = fig_height_mm*mm2in  # height in inches
params = {
    'backend': 'ps',
    'axes.labelsize':10,
    'font.size':10,
    'legend.fontsize':10,
    'xtick.labelsize':10,
    'ytick.labelsize':10,
    'font.family':'times new roman',
    'lines.linewidth':0.5,
    'patch.linewidth':0.25,
    'figure.figsize': [fig_width, fig_height]
}
pylot.rcParams.update(params)

##########
#(0) Load matfiles and create variables:
dir1 = 'F:/Gait/Data Outputs/SPM/'  # specify data file path
fname0 = os.path.join(dir1, 'Norm_SPM_May17_v3_13_LR_py')  # Norm data, Input manually
L_norm, R_norm = scipy.io.loadmat(fname0)  # load the normative data

print(list(Z0.keys()))  # print the names of the matfile variables
L_norm, R_norm = Z0['left_3d'], Z0['right_3d']
# CP data
Z1 = scipy.io.loadmat(fname1) # load the patient data - select fname1, or fname2 etc
print list(Z1.keys()) # print the names of the matfile variables
L_cp, R_cp = Z1['left_3d'], Z1['right_3d']

# Combine L and R norm.
norms=[L_norm,R_norm]
norm = np.vstack(norms)

#####################################Normative vs CP data
Report###################################
###################################
# (1) Define constants
# Define axis index, variable labels, axis limits.
ax=[
"PelvisTilt",
"PelvisObliq",
"PelvisRot",
"HipFlexExt",
"HipAddAbd",
"HipIntExtRot",
"KneeFlexExt",
"KneeAddAbd",
"KneeIntExtRot",
"AnklePFlex",
"AnkleInvEv",
"AnkleAddAbd",
"FootProgression"]

ymax = [30,15,15,70,40,40,20,40,40,40,40,40]
ymin = [-30,-15,-15,-10,-40,-40,-20,-40,-20,-40,-40,-40]

# Manual definition of toe off
RTO = 60.8
LTO = 58.0

###################################
# Page 1 Figure - Kinematics CP L&R to Norm
pyplot.close('all')
fig1 = pyplot.figure(figsize=(8,12))
for ii in range(13):
    if ii < 12:
        ax0 = pyplot.subplot(5,3,ii+1)
    else:
        ax0 = pyplot.subplot(5,3,ii+3) # put foot prog. with transverse plane
        
        spml1.plot.plot_mean_sd(norm[:,:,ii],
        linecolor=(0.7,0.7,0.7),facecolor=(0.7,0.7,0.7), label='Normative')
        spml1.plot.plot_mean_sd(L_cp[:,:,ii],
        linecolor='r',facecolor=(1,0.7,0.7), edgecolor='r', label='Left')
        spml1.plot.plot_mean_sd(R_cp[:,:,ii],
        linecolor='b',facecolor=(0.7,0.7,1), edgecolor='b', label='Right')
    #ax2.text(50, 0.1, muscle_labels[i], ha='center')
    pyplot.xlim([0,100])
    pyplot.ylim([ymin[ii],ymax[ii]])
    pyplot.title(labels[ii])
    pyplot.xlabel('Gait Cycle (%)')
pyplot.ylabel('Angle ($^\circ$)')
pyplot.axvline(RTO, color='b')
pyplot.axvline(LTO, color='r')
if ii < 1:
    pyplot.legend(loc=8)

pyplot.tight_layout()
pyplot.show()

# (2) Run indep t-test L&R vs norm
alpha = 0.05
nTests = 13
p_critical = spm1d.util.p_critical_bonf(alpha, nTests)
t_inf_l = []
t_inf_r = []
tmaxl = []
tmaxr = []

for ii in range(nTests):
    t_l = spm1d.stats.ttest2(L_cp[:,:,ii],norm[:,:,ii],equal_var=False)
    ti_l= t_l.inference(p_critical,two_tailed=True)
    t_inf_l.append (ti_l)
    tmaxl.append(max(abs(ti_l.z)))
    t_r = spm1d.stats.ttest2(R_cp[:,:,ii],norm[:,:,ii],equal_var=False)
    ti_r= t_r.inference(p_critical,two_tailed=True)
    t_inf_r.append (ti_r)
    tmaxr.append(max(abs(ti_r.z)))

# Get max t-values across L&R for y-axis scaling
tmax = np.concatenate([tmaxl,tmaxr])
tmax1=max(tmax) #used in ylim

# Get L&R z curve integrals
z_int_l = []
z_int_r = []

for ii in range(nTests):
    zi_l = zCurveInt(t_inf_l[ii])
    z_int_l.append(zi_l)
    zi_r = zCurveInt(t_inf_r[ii])
    z_int_r.append(zi_r)

# Page 2 Plot t-tests L
ax=[]

#pyplot.close('all')
fig2 = pyplot.figure(fignsize=(8,12))
for ii in range(13):
    if ii < 12:
        ax0 = pyplot.subplot(5,3,ii+1)
    else:
        ax0 = pyplot.subplot(5,3,ii+3) # put foot prog. with transverse plane
    ax.append (ax0)
    t_inf_l[ii].plot(color='r',facecolor=(1,0.8,0.8)) # red for left
```python
pyplot.xlim([0,100])
pyplot.ylim(tmax1*1,tmax1)
pyplot.title(labels[ii])
pyplot.xlabel('Gait Cycle (%)')
pyplot.ylabel('SPM{t}',fontsize=12)

ax[1].text(50, tmax1+15, 'LEFT vs NORM', ha='center')

pyplot.tight_layout()
pyplot.show()

# Page 3 Plot t-tests R

fig3 = pyplot.figure(figsize=(8,12))
for ii in range(13):
    if ii < 12:
        ax0 = pyplot.subplot(5,3,ii+1)
    else:
        ax0 = pyplot.subplot(5,3,ii+3)  # put foot prog. with transverse plane
    ax.append (ax0)
    t_inf_r[ii].plot(color='b',facecolor=(0.8,0.8,1))  # blue for right
    pyplot.xlim([0,100])
    pyplot.ylim(tmax1*1,tmax1)
    pyplot.title(labels[ii])
    pyplot.xlabel('Gait Cycle (%)')
    pyplot.ylabel('SPM{t}',fontsize=12)

ax[1].text(50, tmax1+15, 'RIGHT vs NORM', ha='center')

pyplot.tight_layout()
pyplot.show()

#pyplot.close('all')

fig3 = pyplot.figure(figsize=(8,12))
for ii in range(13):
    if ii < 12:
        ax0 = pyplot.subplot(5,3,ii+1)
    else:
        ax0 = pyplot.subplot(5,3,ii+3)  # put foot prog. with transverse plane
    ax.append (ax0)
    t_inf_r[ii].plot(color='b',facecolor=(0.8,0.8,1))  # blue for right
    pyplot.xlim([0,100])
    pyplot.ylim(tmax1*1,tmax1)
    pyplot.title(labels[ii])
    pyplot.xlabel('Gait Cycle (%)')
    pyplot.ylabel('SPM{t}',fontsize=12)

ax[1].text(50, tmax1+15, 'RIGHT vs NORM', ha='center')

pyplot.tight_layout()
pyplot.show()

##########################################
# Vector t-test PAIRS ###################
### XY, XZ, YZ ######################
### Comparing L and Right separately to Norm ####
alpha = 0.05

T2i_l_pairs = []
T2i_r_pairs = []
T2i_l_pairs_max = []
T2i_r_pairs_max = []

pair1 = [0,0,1,3,3,4,6,6,7,9,9,10]
pair2 = [1,2,2,4,5,5,7,8,8,10,11,11]

# Output order (T2i_(l or r)_pairs = pelv xy, pelv xz, pelv yz, hip xy, hip xz, hip yz, knee xy, knee xz, knee yz, ank xy, ank xz, ank yz.
for ii in range(12):  # num pairs
    #left
    T2i_l_pairs = spm1d.stats.hotellings2(L_cp[:,:,[pair1[ii],pair2[ii]]],norm[:,:,[pair1[ii],pair2[ii]]]);
    T2i_lp = T2i_l_pairs.inference(alpha)
    T2i_l_pairs.append(T2i_lp)
    T2i_l_pairs_max.append(max(T2i_lp.z))

    #right
```
T2_r_pairs = spm1d.stats.hotellings2(R_cp[:,:,[pair1[ii],pair2[ii]]],norm[:,:,[pair1[ii],pair2[ii]]])
T2i_rp = T2_r_pairs.inference(alpha)
T2i_r_pairs.append(T2i_rp)
T2i_r_pairs_max.append(max(T2i_rp.z))

# Get max T2-values across L&R for y-axis scaling
T2i_pairs_max = np.concatenate([T2i_l_pairs_max,T2i_r_pairs_max])
T2i_pairs_max1 = max(T2i_pairs_max)  # used in ylim

# Get z Curve integrals
z_int2_l = []
z_int2_r = []

for ii in range(12):
    zi2_l = zCurveInt(T2i_l_pairs[ii])
    z_int2_l.append(zi2_l)
    zi2_r = zCurveInt(T2i_r_pairs[ii])
    z_int2_r.append(zi2_r)

# Plot vector pairs outputs
# Page 4
# Plot Vector t-tests L
ax = []
labels_pairs = ('Pelv xy', 'Pelv xz', 'Pelv yz', 'Hip xy', 'Hip xz',
                'Hip yz', 'Knee xy', 'Knee xz', 'Knee yz', 'Ank xy', 'Ank xz', 'Ank yz')

fig4 = pyplot.figure(figsize=(8,12))
for ii in range(12):
    ax0 = pyplot.subplot(4,3,ii+1)
    ax.append(ax0)
    T2i_l_pairs[ii].plot(color='r',facecolor=(1,0.8,0.8))
    pyplot.xlim([0,100])
    pyplot.ylim([0,T2i_pairs_max1])
    pyplot.title(labels_pairs[ii])
    pyplot.xlabel('Gait Cycle (%)')
    pyplot.ylabel('SPM$T^2$',fontsize=12)
    ax[1].text(50, T2i_pairs_max1*1.1, 'LEFT vs NORM Vector Pairs', ha='center')
pyplot.tight_layout()
pyplot.show()

# Plot vector pairs outputs
# Page 5
# Plot Vector t-tests R
ax = []

fig5 = pyplot.figure(figsize=(8,12))
for ii in range(12):
    ax0 = pyplot.subplot(4,3,ii+1)
    ax.append(ax0)
    T2i_r_pairs[ii].plot(color='b',facecolor=(0.8,0.8,1))
    pyplot.xlim([0,100])
    pyplot.ylim([0,T2i_pairs_max1])
    pyplot.title(labels_pairs[ii])
    pyplot.xlabel('Gait Cycle (%)')
    pyplot.ylabel('SPM$T^2$',fontsize=12)
import matplotlib.pyplot as pyplot

ax[1].text(50, T2i_pairs_max1*1.1, 'RIGHT vs NORM Vector Pairs', ha='center')
pyplot.tight_layout()
pyplot.show()

pyplot.close('all')

# Vector t-test XYZ

# SPM T2 analysis:
pelv_ind = [0,1,2]
hip_ind = [3,4,5]
knee_ind = [6,7,8]
ank_ind = [9,10,11]

joint_ind = [pelv_ind,hip_ind,knee_ind,ank_ind]

alpha = 0.05
T2i_l = []
T2i_r = []
T2i_l_max = []
T2i_r_max = []

for ii in range(4):
    #left
    T2_l = spm1d.stats.hotellings2(L_cp[:,:,joint_ind[ii]],norm[:,:,joint_ind[ii]])
    T2il = T2_l.inference(alpha)
    T2i_l.append(T2il)
    T2i_l_max.append(max(T2il.z))
    #right
    T2_r = spm1d.stats.hotellings2(R_cp[:,:,joint_ind[ii]],norm[:,:,joint_ind[ii]])
    T2ir = T2_r.inference(alpha)
    T2i_r.append(T2ir)
    T2i_r_max.append(max(T2ir.z))

# Combine outputs
out=[T2i_l,T2i_r]
T2i_both = np.vstack(out)

T2i_max= np.concatenate([T2i_l_max,T2i_r_max])
T2i_max1=max(T2i_max) #used in ylim

# Get z Curve integrals
z_int3_l = []
z_int3_r = []

for ii in range(4):
    zi3_l = zCurveInt(T2i_l[ii])
    z_int3_l.append(zi3_l)
    zi3_r = zCurveInt(T2i_r[ii])
    z_int3_r.append(zi3_r)

# order plots to allow left in col 1 right in col 2
order = [1,3,5,7,2,4,6,8]

#pyplot.close('all')
fig6 = pyplot.figure(figsize=(8,12))
for ii in range(8):
    ax0 = pyplot.subplot(4,2,order[ii])
    ax.append(ax0)
    if ii < 4:
        T2i_l[ii].plot(color='r',facecolor=(1,0.8,0.8))
        pyplot.ylim([0,T2i_max1])
    if ii >= 4:
        T2i_r[ii-4].plot(color='b',facecolor=(0.8,0.8,1))
        pyplot.ylim([0,T2i_max1])
        pyplot.xlim([0,100])
        pyplot.ylabel('SPM$T^2$')
        pyplot.title(vect_labels[ii])
        pyplot.xlabel('Gait Cycle (%)')
        pyplot.tight_layout()
pyplot.show()

# Indep t-test for CP patient vs patient baseline (L&R) time 0 (Ax0)
# Note the data to be compared to baseline has to be selected manually.
# Dataset - baseline data & follow up data for the left and right sides
# This will output a PDF report and a csv file.

import os
import numpy as np
import scipy.io
import spm1d
import csv
from matplotlib import pyplot
from matplotlib.backends.backend_pdf import PdfPages

# Indep t-test for CP patient vs patient baseline (L&R) time 0 (Ax0)
# Note the data to be compared to baseline has to be selected manually.
# Dataset - baseline data & follow up data for the left and right sides
# This will output a PDF report and a csv file.
def zCurveInt(spmi):  # integration of the spmi.z curve
    zint = np.trapz(abs(spmi.z))
    return(zint);

####################################################################
#(0) Load matfiles and create variables:
dir1 = 'F:\Gait\Data Outputs\SPM'  # specify data file path
fname0 = os.path.join(dir1, 'Norm_SPM_May17_v3_13_LR_py')  #
Normative data, Input manually
fname1 = os.path.join(dir1, 'CP5_Ax0_SPM_LR_py')  # Baseline
Input manually
fname2 = os.path.join(dir1, 'CP5_Ax1_SPM_LR_py')  # Week 4 Input
 manually
fname3 = os.path.join(dir1, 'CP5_Ax2_SPM_LR_py')  # Week 12 Input
 manually
fname4 = os.path.join(dir1, 'CP5_Ax3_SPM_LR_py')  # Week 24 Input
 manually
# Norm data
Z0 = scipy.io.loadmat(fname0)  # load matfile
L_norm, R_norm = Z0['left_3d'], Z0['right_3d']

# pre data
Z1 = scipy.io.loadmat(fname1)  # load matfile
L_cp_pre, R_cp_pre = Z1['left_3d'], Z1['right_3d']

# post data
Z2 = scipy.io.loadmat(fname4)  # SPECIFY NAME OF POST FILE HERE
e.g. "fname2" for week 4
L_cp_post1, R_cp_post1 = Z2['left_3d'], Z2['right_3d']

# Combine L and R norm.
norms=[L_norm,R_norm]
norm = np.vstack(norms)
# (1) Define constants

# Define axis index, variable labels, axis limits.
ax = []
labels = ['PelvisTilt', 'PelvisObliq', 'PelvisRot', 'HipFlexExt', 'HipAddAbd', 'HipIntExtRot', 'KneeFlexExt', 'KneeAddAbd', 'KneeIntExtRot', 'AnklePDFlex', 'AnkleInvEv', 'AnkleAddAbd', 'FootProgression']

ymax = [30, 15, 15, 70, 40, 40, 40, 40, 40, 40, 40, 40]
ymin = [-30, -15, -15, -10, -40, -40, -10, -20, -20, -40, -40, -40]

# Manual definition of toe off
RTO = 60.8
LTO = 58.0

# Page 1 Figure - Kinematics CP L&R Baseline to Norm
pyplot.close('all')
fig1 = pyplot.figure(figsize=(8, 12))
for ii in range(13):
    if ii < 12:
        ax0 = pyplot.subplot(5, 3, ii + 1)
    else:
        ax0 = pyplot.subplot(5, 3, ii + 3)
    spm1d.plot.plot_mean_sd(norm[:,:,ii], linecolor=(0.7, 0.7, 0.7), facecolor=(0.7, 0.7, 0.7), label='Normative')
    spm1d.plot.plot_mean_sd(L_cp_pre[:,:,ii], linecolor='r', facecolor=(1, 0.7, 0.7), edgecolor='r', label='Left Pre')
    if ii < 1:
        pyplot.legend(loc=8)
pyplot.ylim([ymin[ii], ymax[ii]])
pyplot.title(labels[ii])
pyplot.xlabel('Gait Cycle (%)')
pyplot.ylabel('Angle ($^\circ$)')
pyplot.axvline(RTO, color='b')
pyplot.axvline(LTO, color='r')
if ii < 1:
    pyplot.legend(loc=8)

pyplot.tight_layout()
pyplot.show()

# Page 1b New Figure - Kinematics CP L&R Post to Norm
pyplot.close('all')
fig1b = pyplot.figure(figsize=(8, 12))
for ii in range(13):
if ii < 12:
    ax0 = pyplot.subplot(5,3,ii+1)
else:
    ax0 = pyplot.subplot(5,3,ii+3)  # put foot prog. with transverse plane

# put foot prog. with transverse plane
spm1d.plot.plot_mean_sd(norm[:,:,ii],
                        linecolor=(0.7,0.7,0.7), facetcolor=(0.7,0.7,0.7), label='Normative')
spm1d.plot.plot_mean_sd(L_cp_post1[:,:,ii],
                        linecolor='r', facetcolr=(1,0.7,0.7), edgecolor='r', label='Left Post',
                        linestyle='--')
spm1d.plot.plot_mean_sd(R_cp_post1[:,:,ii],
                        linecolor='b', facetcolor=(0.7,0.7,1), edgecolor='b', label='Right Post',
                        linestyle='--')
ax2.text(50, 0.1, muscle_labels[i], ha='center')
pyplot.xlim([0,100])
pyplot.ylim([ymin[ii], ymax[ii]])
pyplot.title(labels[ii])
pyplot.xlabel('Gait Cycle (%)')
pyplot.ylabel('Angle ($^\circ$)')
pyplot.axvline(RTO, color='b')
pyplot.axvline(LTO, color='m')
if ii < 1:
    pyplot.legend(loc=8)
pyplot.tight_layout()
pyplot.show()
```python
# Page 3 Figure - Kinematics CP R Baseline to Post

fig3 = pyplot.figure(figsize=(8,12))
for ii in range(13):
    if ii < 12:
        ax0 = pyplot.subplot(5,3,ii+1)
    else:
        ax0 = pyplot.subplot(5,3,ii+3)  # put foot prog. with transverse plane

    spm1d.plot.plot_mean_sd(norm[:,:,ii], linecolor=(0.7,0.7,0.7), facecolor=(0.7,0.7,0.7), label='Normative')
    spm1d.plot.plot_mean_sd(R_cp_pre[:,:,ii], linecolor='b', facecolor=(0.7,0.7,1), edgecolor='b', label='Right Pre')
    spm1d.plot.plot_mean_sd(R_cp_post1[:,:,ii], linecolor='b', facecolor=(0.7,0.7,1), edgecolor='b', label='Right Post', linestyle='--')

    # put foot prog. with transverse plane
    if ii < 1:
        pyplot.legend(loc=8)

pyplot.tight_layout()
pyplot.show()

# (4) Run indep t-test L&R post vs Baseline

alpha      = 0.05
nTests     = 13
p_critical = spm1d.util.p_critical_bonf(alpha, nTests)

for ii in range(13):
    t_l = spm1d.stats.ttest2(L_cp_pre[:,:,ii],L_cp_post1[:,:,ii],equal_var=False)
    ti_l= t_l.inference(p_critical,two_tailed=True)
    t_inf_l.append (ti_l.z)
    tmaxl.append(max(abs(ti_l.z)))

    t_r = spm1d.stats.ttest2(R_cp_pre[:,:,ii],R_cp_post1[:,:,ii],equal_var=False)
    ti_r= t_r.inference(p_critical,two_tailed=True)
    t_inf_r.append (ti_r.z)
    tmaxr.append(max(abs(ti_r.z)))

# Get max t-values across L&R for y-axis scaling

# Get L&R z curve integrals
```

# Page 212
z_int_l = []
z_int_r = []

for ii in range(nTests):
    zi_l = zCurveInt(t_inf_l[ii])
    z_int_l.append(zi_l)
    zi_r = zCurveInt(t_inf_r[ii])
    z_int_r.append(zi_r)

# Page 4 Plot t-tests L post to Baseline
ax=[]

#pyplot.close('all')
fig4 = pyplot.figure(figsize=(8,12))
for ii in range(13):
    if ii < 12:
        ax0 = pyplot.subplot(5,3,ii+1)
    else:
        ax0 = pyplot.subplot(5,3,ii+3)
    # put foot prog. with transverse plane
    ax.append (ax0)
    t_inf_l[ii].plot(color='r',facecolor=(1,0.8,0.8)) # red for left
    pyplot.xlim([0,100])
    pyplot.ylim(tmax1-1,tmax1)
    pyplot.title(labels[ii])
    pyplot.xlabel('Gait Cycle (%)')
    pyplot.ylabel('SPM\{t\}',fontsize=12)

    ax[1].text(50, tmax1+40,'LEFT POST vs BASELINE', ha='center')

pyplot.tight_layout()
pyplot.show()

# Page 5 Plot t-tests R
ax=[]

#pyplot.close('all')
fig5 = pyplot.figure(figsize=(8,12))
for ii in range(13):
    if ii < 12:
        ax0 = pyplot.subplot(5,3,ii+1)
    else:
        ax0 = pyplot.subplot(5,3,ii+3) # put foot prog. with transverse plane
    ax.append (ax0)
    t_inf_r[ii].plot(color='b',facecolor=(0.8,0.8,1)) # blue for right
    pyplot.xlim([0,100])
    pyplot.ylim(tmax1-1,tmax1)
    pyplot.title(labels[ii])
    pyplot.xlabel('Gait Cycle (%)')
    pyplot.ylabel('SPM\{t\}',fontsize=12)

    ax[1].text(50, tmax1+40,'RIGHT POST vs BASELINE', ha='center')

pyplot.tight_layout()
pyplot.show()
#### XY, XZ, YZ #######
#### Comparing L and Right separately to Baseline #####
alpha = 0.05

T2i_l_pairs = []
T2i_r_pairs = []
T2i_l_pairs_max = []
T2i_r_pairs_max = []

pair1 = [0,0,1,3,3,4,6,6,7,9,9,10]
pair2 = [1,2,2,4,5,5,7,8,8,10,11,11]

# Output order (T2i_(l or r)_pairs = pelv xy, pelv xz, pelv yz, hip xy, hip xz, hip yz, knee xy, knee xz, knee yz, ank xy, ank xz, ank yz.
for ii in range(12): # num pairs
    # left
    T2i_l_pairs = spm1d.stats.hotellings2(L_cp_pre[:,:,[pair1[ii],pair2[ii]]],L_cp_post1[:,:,[pair1[ii],pair2[ii]]])
    T2i_l_pairs = T2i_l_pairs.inference(alpha)
    T2i_l_pairs.append(T2i_l_pairs)
    T2i_l_pairs_max.append(max(T2i_l_pairs.z))

    # right
    T2i_r_pairs = spm1d.stats.hotellings2(R_cp_pre[:,:,[pair1[ii],pair2[ii]]],R_cp_post1[:,:,[pair1[ii],pair2[ii]]])
    T2i_r_pairs = T2i_r_pairs.inference(alpha)
    T2i_r_pairs.append(T2i_r_pairs)
    T2i_r_pairs_max.append(max(T2i_r_pairs.z))

    # Get max T2-values across L&R for y-axis scaling
    T2i_pairs_max = np.concatenate([T2i_l_pairs_max,T2i_r_pairs_max])
    T2i_pairs_max1 = max(T2i_pairs_max) #used in ylim

    # Get z Curve integrals
    z_int2_l = []
z_int2_r = []
    for ii in range(12):
        zi2_l = zCurveInt(T2i_l_pairs[ii])
        z_int2_l.append(zi2_l)
        zi2_r = zCurveInt(T2i_r_pairs[ii])
        z_int2_r.append(zi2_r)

############################
# Plot vector pairs outputs
# Page 6

ax=[]
#pyplot.close('all')
fig6 = pyplot.figure(figsize=(8,12))
for ii in range(12):
    ax0 = pyplot.subplot(4,3,ii+1)
    ax.append(ax0)
    T2i_l_pairs[ii].plot(color='r',facecolor=(1,0.8,0.8))
    pyplot.xlim([0,100])
    pyplot.ylim([0,T2i_pairs_max1])
    pyplot.title(labels_pairs[ii])
```python
pyplot.xlabel('Gait Cycle (%)')
pyplot.ylabel('SPM{$T^2$}', fontsize=12)

ax[1].text(50, T2i_pairs_max1*1.1, 'LEFT PRE vs BASELINE Vector Pairs', ha='center')
pyplot.tight_layout()
pyplot.show()

# Page 7
# Plot t-tests R
ax=[]

for ii in range(12):
    ax0 = pyplot.subplot(4,3,ii+1)
    ax.append(ax0)
    T2i_r_pairs[ii].plot(color='b',facecolor=(0.8,0.8,1))
    pyplot.xlim([0,100])
    pyplot.ylim([0,T2i_pairs_max1])
    pyplot.title(labels_pairs[ii])
    pyplot.xlabel('Gait Cycle (%)')
    pyplot.ylabel('SPM{$T^2$}', fontsize=12)
    ax[1].text(50, T2i_pairs_max1*1.1, 'RIGHT PRE vs BASELINE Vector Pairs', ha='center')
    pyplot.tight_layout()
    pyplot.show()

joint_ind = [pelv_ind,hip_ind,knee_ind,ank_ind]

alpha = 0.05
T2i_l = []
T2i_r = []
T2i_l_max = []
T2i_r_max = []

for ii in range(4):
    T2_l = spm1d.stats.hotellings2(L_cp_pre[:,:,joint_ind[ii]],L_cp_post1[:,:,joint_ind[ii]])
    T2il = T2_l.inference(alpha)
    T2i_l.append(T2il)
    T2i_l_max.append(max(T2il.z))

    T2_r = spm1d.stats.hotellings2(R_cp_pre[:,:,joint_ind[ii]],R_cp_post1[:,:,joint_ind[ii]])
    T2ir = T2_r.inference(alpha)
    T2i_r.append(T2ir)
    T2i_r_max.append(max(T2ir.z))
```

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# Combine outputs
out=[T2i_l,T2i_r]
T2i_both = np.vstack(out)

# Get max T2-values across L&R for y-axis scaling
T2i_max= np.concatenate([T2i_l_max,T2i_r_max])
T2i_max1=max(T2i_max) #used in ylim

# Get z Curve integrals
z_int3_l = []
z_int3_r = []
for ii in range(4):
    zi3_l = zCurveInt(T2i_l[ii])
    z_int3_l.append(zi3_l)
    zi3_r = zCurveInt(T2i_r[ii])
    z_int3_r.append(zi3_r)

# order plots to allow left in col 1 right in col 2
order = [1,3,5,7,2,4,6,8]

######################################################################
#### SAVE FIGURES ######
with PdfPages('CP_Pre_vs_Post.pdf') as pdf:
    pdf.savefig(fig1)
    pdf.savefig(fig1b)
    pdf.savefig(fig2)
    pdf.savefig(fig3)
    pdf.savefig(fig4)
    pdf.savefig(fig5)
    pdf.savefig(fig6)
    pdf.savefig(fig7)
    pdf.savefig(fig8)
##### SAVE CLUSTER OUTPUT #####

gap = []
zint_out =
[labels, z_int_l, z_int_r, gap, labels_pairs, z_int2_l, z_int2_r, gap, vect_labels, z_int3_l, z_int3_r]

with open("cpprepost_zint.csv", "wb") as f:
    writer = csv.writer(f)
    writer.writerows(zint_out)