Exploring Muscle Structure, Function, and Gait Patterns in People with Distal Hereditary Motor Neuropathy: Natural History and the Effect of Rehabilitation Interventions

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PhD Thesis

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Declaration

I, Aljwhara Alangary, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Background Distal Hereditary Motor Neuropathy (DHMN) is a rare heterogenous inherited neuromuscular disorder. It is characterised by distal progressive weakness.

Objectives This thesis provides preliminary longitudinal data to describe the natural history of DHMN in terms of muscle structure, muscle strength, and gait parameters, also to investigate the effect of commonly used rehabilitation interventions.

Methods DHMN adult participants underwent the following measures: MRI scans of the foot, calf, and thigh muscles, isokinetic and isometric strength measures of the lower limb using dynamometer, 3D motion analysis to capture kinetic and kinematic data of walking gait. For direct comparison, matched health controls underwent the same measures. Measures were repeated after 6 and 12 months to explore the natural history of the disease. DHMN participants underwent additional gait analysis wearing bilateral carbon fibre ankle foot orthoses to explore the effect on gait. Eligible DHMN participants were prescribed a home based resistance training program, and the response to training was analysed by the same measures after 6 months of training.

Results The study identified significant progressive muscle atrophy and increased intramuscular fat accumulation at the calf in DHMN participants, with a notable decline in muscle strength over time and altered gait mechanics. The use of ankle-foot orthoses showed improvements in gait stability, while the resistance training program indicated potential benefits in maintaining muscle function, but adherence was a key challenge.

Conclusion The preliminary data from this study provide valuable insights into the natural history of DHMN, highlighting the progressive nature of muscle degeneration and functional decline. These findings offer useful guidance for health practitioners in managing DHMN and emphasize the need for targeted rehabilitation interventions to improve patient outcomes. Future research should focus on longer-term studies with larger cohorts to validate these findings and further explore effective management strategies.

Impact Statement

Distal hereditary motor neuropathy (DHMN) is one of the rarest inherited diseases. It causes gradual muscle weakness and wasting starting from the feet, lower leg, then the thigh. This weakness can negatively influence walking abilities in the affected people, having an impact on their activities of daily living and their quality of life. The causes of DHMN are still under investigation, and it is incurable. Despite lacking enough supporting evidence, rehabilitation options including exercises and orthotics are commonly used to improve walking in people with DHMN.

This study is the first to explore DHMN effect on muscle tissue over 12 months, to provide a detailed understanding on the pattern of muscle involvement and how this affects function in terms of muscle strength and walking. In addition, the effect of strengthening exercises and ankle foot orthoses, as a commonly prescribed rehabilitation options, were explored.

This thesis used the following: MRI scans, dynamometry, and 3D motion analysis, and identified muscle involvement patterns and alteration in walking gait in people with DHMN compared to controls. Changes over 12 months were best detectable by MRI scans. These findings can guide the diagnosis process clinically and inform future research on outcome measures suitable for use in DHMN research.

The outcomes from intervening with exercise and ankle foot orthoses can inform clinical decision making, and appropriate prescription, according to individual needs. It can also inform future exercises trials in designing an adherable exercise protocol.

The study design and findings were presented at a number of scientific events and discussed with experts in this field. The work took place in a single site in the UK, and dissemination of this study will allow future, multisite research collaboration in DHMN as a rare and under researched condition.

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List of Abbreviations

µm	Micrometres
3D	Three Dimensional
AD	Autosomal Dominant
AFO	Ankle Foot Orthoses
AR	Autosomal Recessive
ATP7A	Copper-Transporting Atpase 1
B0	Static main field
B1	Radiofrequency magnetic fields
BAG3	Bcl2-Associated Athanogene 3
BICD2	BICD cargo adaptor 2
BSCL2	Berardinellie-Seip Congenital Lipodystrophy Type 2
cm/s	centimetre per second
CMT	Charcot Marie Tooth
CMTESv2	CMT Examination Score Version 2
COM	Centre of mass
COQ7	Coenzyme Q7
CSA	Cross sectional area
DCTN1	P150 Subunit of Dynactin
DHMN	Distal Hereditary Motor Neuropathy
DYNC1H1	Cytoplasmic Dynein Heavy Chain 1
EMG	Electromyography
EVB	Embedded victor bases
F	Fat
FA	Feet adjacent
FF	Fat Fraction
FOV	Field of view
FPI-6	Foot Posture Index
FSE	Fast spin-echo
GARS	Glycyl-tRNA synthetase
GCS	Global coordinate system
GDI1	GDP dissociation inhibitor 1
GRE	Gradient-echo
GRF	Ground Reaction Force
HARS1	Histidyl-tRNA synthetase 1
HR	Heel rise
HRA	Health Research Authority
HSPB1	Heat-Shock Protein B1
HSPB3	Heat-Shock Protein B3
HSPB8	Heat-Shock Protein B8
Hz	Hertz
IBM	Inclusion body myositis
IC	Initial contact
IGHMBP2	Immunoglobulin M Binding Protein 2
iPat	Integrated Parallel Acquisition Techniques
ITE	Inter-echo time
J/kg	Joule Per Kilogram
m	meter

m/s	meter per second
MAX GET	Maximum Gravity Effect Torque
mm	millimetre
MMT	Manual Muscle Testing
mNCV	Motor Nerve Conduction Velocity
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
MTR	Magnetization transfer ratio
MUAP	Motor Unit Action Potentials
n	Number of cases
N	Newton
NEX	Number of excitations
NHNN	National Hospital for Neurology and Neurosurgery
Nm	Newton meter
Nm/Kg	Newton Meter per Kilogram
NMD	Neuromuscular diseases
OI	Opposite initial contact
OT	Opposite toe off
RNA	Ribonucleic Acid
ROI	Region of interest
ROM	Range Of Motion
s	second
SE	Spin-echo
SETX	Senataxin
SIGMAR1	Sigma Non-Opioid Intracellular Receptor 1
SORD	Sorbitol Dehydrogenase
STIR	Short-TI inversion recovery
T1	Longitudinal relaxation time
T2	Transverse relaxation time
TE	Echo Time
TO	Toe off
TR	Repetition Time
TRPV4	Transient Receptor Vallanoid 4 Gene
TRPV4	Transient receptor vallanoid 4 gene
TSE	Turbo-spin-echo
TV	Tibia vertical
UCL	University College London
UCLH	University College London Hospitals
UMN	Upper Motor Neuron
VAS	The Visual Analog Scale
VO2	Oxygen uptake
VRK1	VRK serine/threonine kinase 1
W	Water
W/kg	Watts Per Kilogram

Chapter 1: Introduction

Distal Hereditary Motor Neuropathies (DHMN), also known as distal spinal muscular atrophies, are a heterogeneous group of neural diseases that predominantly affect the lower motor neuron (Rossor et al., 2017, Beetz et al., 2012). This group of diseases is characterised by length dependent, slowly progressive weakness and atrophy of distal muscles in the upper and lower limbs (Greenbaum et al., 2020, Blumen et al., 2012). Distal Hereditary Motor Neuropathies (DHMN) lay under the Charcot-Marie-Tooth disease (CMT) umbrella due to the clinical and genetic features overlap. However, unlike CMT, sensory involvement and foot deformities are infrequent with DHMN. On neurophysiology, the sensory responses are usually preserved or mildly affected, while electromyography (EMG) shows signs of muscle denervation in the affected muscle groups (Barwick et al., 2012). It usually starts in the first two to four decades of life (Rossor et al., 2017) and symptoms are initially associated with poor motor functioning. People report poor performance in sports at school (Rossor et al., 2012b), and early signs of plantar flexor weakness including difficulties with standing balance and standing on toes (Rossor et al., 2017).

Distal Hereditary Motor Neuropathies (DHMN) is a clinically and genetically heterogeneous disorder associated with mutations in a number of genes (Sumner et al., 2013). The prevalence of DHMN is 2.14 affected individuals per 100,000 inhabitants (95% confidence interval 1.62–2.66) in the North of England

(Bansagi et al., 2017). Recent evolution in genetic testing improved diagnostic rates, and a genetic diagnosis is usually reached in 48.9% of DHMN cases (Record et al., 2024). These mutations affect cellular functions, for example: protein misfolding (HSPB1, HSPB8, BSCL2), RNA metabolism (IGHMBP2, SETX, GARS), axonal transport (HSPB1, DYNC1H1, DCTN1) and cation-channel dysfunction (ATP7A and TRPV4) (Rossor et al., 2012a).

Previous exploration of muscle function and structure in Charcot Marie Tooth disease (CMT) gives us the tools to understand more about the presentation and impact of DHMN. MRI imaging has revealed the type and extent of muscle atrophy in CMT and has been proven to be sensitive to change in CMT1A (Morrow et al., 2016). Muscle function has been explored in CMT type 1A using dynamometer and can be applied to DHMN (Morrow et al., 2016). So, there is potential to use MRI and dynamometer in the same way to also help us understand how muscles are affected in people with DHMN. Alongside muscle function, muscle performance during walking can be better understood using three-dimensional (3D) motion analysis (Lee et al., 2013). Qualitative and quantitative methods have been used to describe different gait patterns in similar neurological conditions (Ounpuu et al., 2013, Don et al., 2007, Newman et al., 2007). Three-dimensional (3D) motion analysis can be applied to DHMN to observe joint motion and force during walking.

Various rehabilitation interventions have been considered to improve gait and muscle function among people with peripheral neuropathies, including orthotics (Õunpuu et al., 2021, Dufek et al., 2014), and therapeutic exercises (Djordjevic et al., 2017, Burns et al., 2017). Previous studies, however, do not specifically focus on specific intervention outcomes for DHMN. As such, the current study seeks to consider observation and intervention outcomes for DHMN, specifically in the context of improving muscle function and gait for people living with this condition.

The current study will contribute to our understanding of the presentation and the natural history of the disease, providing information for health practitioners and clinical experts to guide management of DHMN. The outcomes of this research can potentially help improve patient care and outcomes in terms of mobility and management of muscle weakness. DHMN is a rarer condition than CMT and has a different pattern of involvement (Rossor et al., 2012b). This work has potential to ascertain if there is indeed a difference in muscle structure and progression rate. Also, it will investigate the effect of two common rehabilitation interventions. This will have implications for more focused disease specific rehabilitation in this type of neuropathy.

This PhD project provides an evaluation of the relationships between muscle structure, muscle function, and function within an activity for people with Distal Hereditary Motor Neuropathy. Over 12 months, muscle changes in DHMN were

observed in terms of structure and function using three observational methods; MRI; dynamometry; and 3D motion analysis. In addition, the effect of a 24-weeks (6 months) exercise program on muscle structure and function in DHMN was measured by the same observational methods. To address walking gait directly in DHMN, gait patterns were compared with and without carbon fibre ankle foot orthoses (AFO) using 3D motion analysis.

An overview of DHMN and published literature related to those measurement techniques and rehabilitation methods are reviewed in chapter 2. Chapter 3 provides the main objectives of the PhD research project. Methods used to fulfil those objectives including assessments, participants, and overall statistical methods are provided in chapter 4. Chapter 5 explores the Pattern of Involvement and deviation from "normal" in muscle structure and function in Distal Hereditary Motor Neuropathy (DHMN) . Chapter 6 explore the natural history of muscle structure and function in Distal Hereditary Motor Neuropathy (DHMN) over one year. Chapter 7 is dedicated to ascertaining relationships between intramuscular fat fraction and muscle volume (measured by MRI), isokinetic and isometric muscle strength (measured by dynamometry) and moments/power generation (measured by 3D motion analysis). Chapter 8 explore the effect of bilateral carbon fibre ankle foot orthoses (AFO) on the kinetics and kinematics data of gait of people with DHMN (measured by 3D motion analysis). Chapter 9 explore the effect of resistance training on muscle structure, function, and gait patterns.

Chapter 10 provides an overall discussion of the research project methods and outcomes. Chapter 11 concludes this thesis with a brief summary of the project and its findings and recommendations for future research. An illustration of the thesis structure and chapters content is shown in Figure 1.

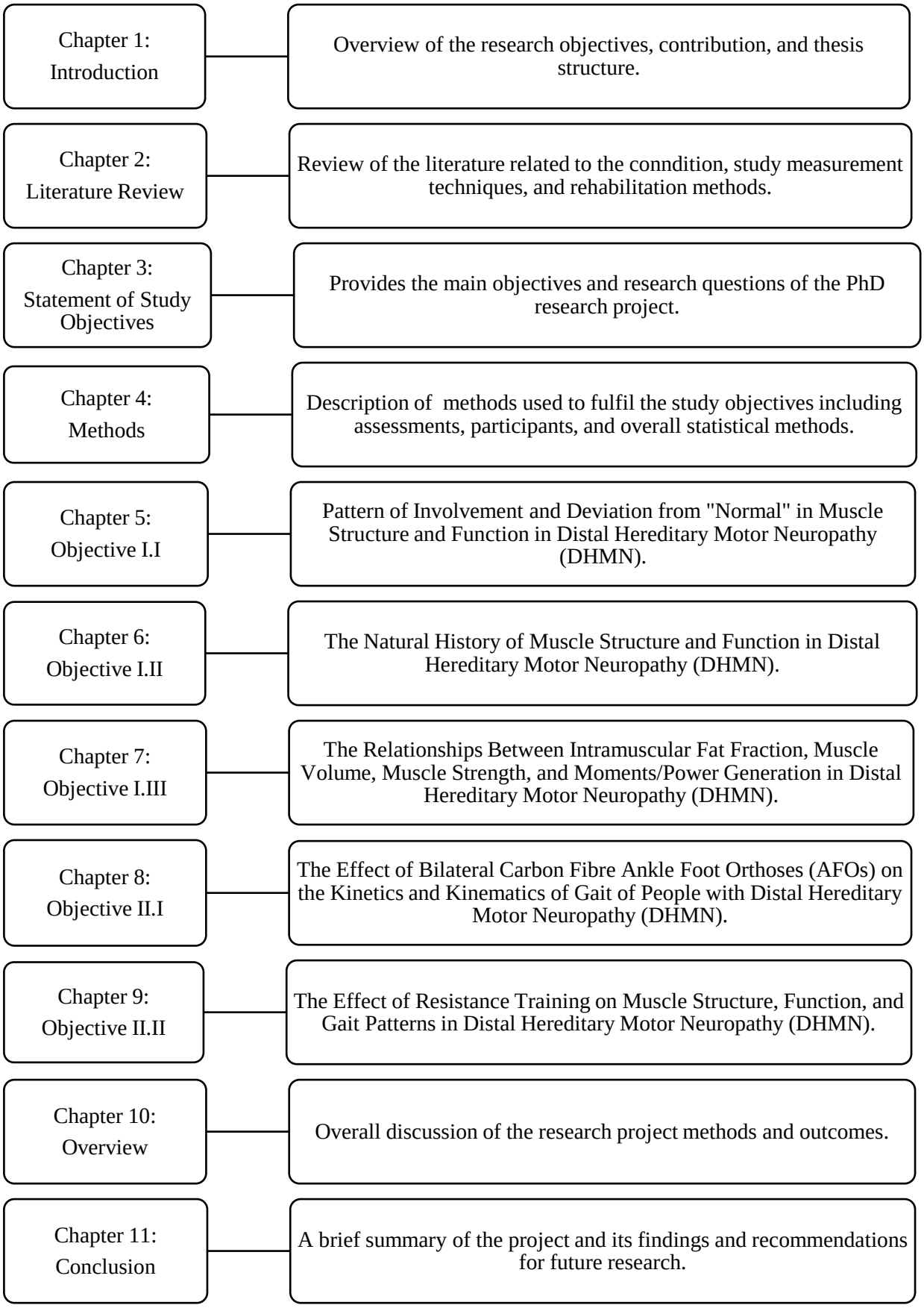


Figure 1: Thesis structure and chapters content.

Chapter 2: Literature Review

2.1. Distal Hereditary Motor Neuropathy (DHMN) Overview

Distal Hereditary Motor Neuropathies (DHMN) is a clinically and genetically heterogenous hereditary neuropathy characterized by predominant or exclusive motor involvement and degeneration of lower motor neurons (Kang et al., 2020, Rossor et al., 2017). Affected people usually present with slowly progressive, adult-onset distal muscle weakness and wasting without sensory involvement and infrequently with skeletal deformity due to its late onset (Wu et al., 2022, Tanabe et al., 2018). Although DHMN mainly affects lower motor neurons, the clinical and genetical heterogenicity overlaps with axonal forms of Charcot Marie Tooth disease type 2 (CMT2). With more than 30 genes identified, DHMN can be due to inheritance of autosomal dominant, autosomal recessive or X-linked gene mutations (Wu et al., 2022, Kang et al., 2020, Greenbaum et al., 2020, Rossor et al., 2017).

Neurophysiology and neuropathology of sensory nerves in DHMN are usually normal. Peripheral nerve biopsy does not show abnormalities in hereditary motor neuropathy patients, muscle biopsy shows mainly denervation changes and autopsy studies show atrophy of spinal roots, loss of anterior horn cells and chromatolysis of the motor neurons (Ishihara et al., 2020, Rossor et al., 2017, Kuhlenbäumer et al., 2005). Neurophysiological tests usually show normal motor nerve conduction velocity (mNCV) with reduced motor responses, indicating

axonal neuropathy. In some cases, mNCVs might be slightly reduced (35±60 m/s) due to the loss of large fibres. Sensory responses are usually normal (Ishihara et al., 2020, Kuhlenbäumer et al., 2005). Electromyography (EMG) reveals typical signs of denervation including high motor unit action potentials (MUAP), so called “neurogenic” potentials with an amplitude of over 10 mV, pathologic spontaneous activity, consisting of fibrillations, complex repetitive discharges, and positive sharp waves, are often observed which are most prominent in distal muscles (Ishihara et al., 2020, Kuhlenbäumer et al., 2005).

Distal Hereditary Motor Neuropathy (DHMN) has been classified into different subtypes (DHMN I – DHMN VII) (Harding, 1993). However, the more recent identification of genes causing DHMN has helped with the classification and differentiation from other neuropathies. Types and clinical features described in previous studies of DHMN are summarized in Table 1 (de Fuenmayor-Fernández de la Hoz et al., 2024, Jacquier et al., 2023, Pons et al., 2023, Wu et al., 2022, Frasquet et al., 2021, Liu et al., 2020, Ververis et al., 2019, Rossor et al., 2012a, Rossor et al., 2012b, Kuhlenbäumer et al., 2005).

DHMN type	Phenotype	Inheritance	Known gene	Age unable to walk	Life expectancy
DHMN I	Juvenile onset with distal wasting and weakness	AD	HSPB1, HSPB8, GARS, DYNC1H1	Rare	Normal
DHMN II	Adult onset with distal wasting and weakness	AD	HSPB1, HSPB8, BSCL2, HSPB3, BAG3	Rare	Normal
DHMN III	Slowly progressive wasting and weakness	AR	SORD	Rare	Normal
DHMN IV	Slowly progressive wasting and weakness with diaphragmatic paralysis	AR	Unknown	About 30 years	Unknown
DHMN V	Upper-limb predominance	AD	GARS BSCL2	Rare	Normal
DHMN VI	Spinal muscular atrophy with respiratory distress type 1	AR	IGHMBP2	Never	1 year
DHMN VII	Adult onset with vocal-cord paralysis	AD	DCTN1 TRPV4	Rare	Probably normal
X-linked	Distal onset wasting and weakness	X-linked	ATP7A	Rare	Probably normal
DHMN with UMN signs	DHMN and upper motor neuron signs	AD	SETX, BSCL2, COQ7	Rare	Probably normal
DHMN Jerash	DHMN and pyramidal signs originating in the Jerash region of Jordan	AR	SIGMAR1	Rare	Probably normal
Congenital distal spinal muscular atrophy	Distal weakness at birth and arthrogryposis	AD	TRPV4	Rare	Probably normal

AD= autosomal dominant, AR= autosomal recessive, ATP7A= copper-transporting ATPase 1, BAG3= Bcl2-associated athanogene 3, BSCL2= Berardinellie-Seip congenital lipodystrophy type 2, COQ7= Coenzyme Q7, DCTN1= P150 subunit of dynactin, DYNC1H1= cytoplasmic dynein heavy chain 1, GARS= glycyl-tRNA synthetase, HSPB1= heat-shock protein B1, HSPB3= heat-shock protein B3, HSPB8= heat-shock protein B8, IGHMBP2= immunoglobulin m binding protein 2, TRPV4= transient receptor vallanoid 4 gene, SORD= Sorbitol dehydrogenase, SIGMAR1= sigma non-opioid intracellular receptor 1.

Table 1: Types and clinical features of Distal Hereditary Motor Neuropathies (DHMN).

2.2. Muscle MRI in Hereditary Peripheral Neuropathy

Muscle tissue is adversely affected by denervation in the same way despite the underlying cause of neuropathy. Intramuscular fat accumulation is observed with chronic muscle denervation and has been used as an indirect outcome measure in neuropathies to diagnose and monitor the progression of the disease, as well as to identify patterns of muscles denervation in different neuropathies. MRI is one of the imaging methods widely used in the medical field that allows safe and frequent use without exposure to ionizing radiation.

2.2.1. Muscle MRI Physics

Signals from the nuclei of hydrogen atoms in water and lipid molecules in human body originate in response to a radiofrequency pulse applied during MRI scan. Magnetic resonance causes changes in the physical properties of those nuclei. Understanding variant signal magnitude detected, due to altered physical properties of proton density, resonant frequency, and relaxation time, allows different MRI sequences to be applied, and acquisition of images with source of contrasts to differentiate tissues (Grimm et al., 2018, McRobbie et al., 2017).

T1 weighted sequence gives an image generated from signals during the Repetition Time (TR); the amount of time in between excitations by radiofrequency pulse. Tissues with shorter T1 value, for example the fat or blood, appears brighter than tissues with longer T1 values for example bones or the lungs (table 2) (Grimm et al., 2018, McRobbie et al., 2017). When the tissue is affected

by a pathology the T1 value can be affected as well. Therefore, it can be expected that conditions associated with oedema, or an increased number of capillaries will appear darker compared to the surrounding healthy tissue in MRI images, whereas lesions containing a high amount of fat will appear brighter due to their higher signal intensities (Table 2) (McRobbie et al., 2017).

T2 weighted sequences give an image generated from signals during the Echo Time (TE); the time following the spin excitation by radiofrequency pulse. Tissues with longer T2 value, for example blood, appears brighter than tissues with shorter T2 values such as bones (Grimm et al., 2018, McRobbie et al., 2017). T2 scans are commonly used in pathology as the collection of abnormal fluid appear bright (increased signal) against the darker normal surrounding tissue. For example, the accumulation of intramuscular water (muscle oedema) can be used as a sign of active denervation in peripheral neuropathy (Table 2) (McRobbie et al., 2017).

Fat signal magnitudes are very high in most of the MRI sequences due to its short relaxation time (T) and appears bright in T1 and T2 weighted images. Fat signal suppression found to be useful for diagnostic purposes. Examples of fat-suppression techniques include frequency-selective saturation pulse sequences, inversion recovery, and hybrid chemical shift-based techniques, such as the Dixon technique (Grimm et al., 2018).

The most common fat suppression techniques are fat selective saturation and short-TI inversion recovery (STIR), both of which are based on the difference between the behaviour of water and that of fat in the MRI environment (Table 2). Since water and fat molecules have different resonant frequencies, this chemical shift allows the use of fat saturation where the saturation radiofrequency pulse has selective frequency centred on the main fat peak, meaning that the total signal will have a minimal fat contribution. Thus, the fat saturation technique can be applied to T1, T2, or proton density weighted images in spin-echo (SE), fast spin-echo (FSE), or gradient-echo (GRE) and is reliable for intravenous contrast-enhanced T1 imaging. However, it is prone to incomplete fat suppression, because of inhomogeneity of the calibrations of main field (B0) and radiofrequency (B1) magnetic fields, resulting in imperfect fat saturation (Grimm et al., 2018).

Tissue	**T1 weighted**	**T2 weighted**	**STIR**
Healthy muscle	Dark	Dark	Dark
Muscle oedema	Dark	Bright	Bright
Fat	Bright	Bright	Very Dark
Nerve	Dark	Dark	Dark
Injured nerve	Dark	Bright	Bright
Bone	Dark	Dark	Dark
Bone marrow	Bright	Bright	Dark

Table 2: Tissue appearance in T1, T2, and STIR MRI scans.

The Dixon technique, unlike other fat suppression features, allows the contribution of the fat signal to be suppressed in post-processing rather than during acquisition, as well as providing water and fat distribution maps (Lins et

al., 2020, Ma, 2008). Based on the chemical shift, the Dixon method requires knowledge of the calibrations of main field (B0) variations to properly separate water from fat signals. It allows four types of images to be obtained: fat-only, water-only, in-phase, and out-of-phase. Water-only images represent total fat suppression while fat-only images, visibly similar to T1-weighted images, allow the study of fat, and it is important to remember that in this case only the fat will have a high signal intensity (Lins et al., 2020, Ma, 2008). Fat Fraction (FF) can be derived from these water-only and fat-only images where FF = F/(W+F) (Chen et al., 2019), often expressed as a percentage: %FF = 100*F/(W+F). Increased muscular FF reflecting intramuscular fat accumulation which is a common effect of denervation in peripheral neuropathies and has been shown to be a valuable biomarker in neuromuscular diseases (Morrow et al., 2018, Morrow et al., 2016, O'Donnell et al., 2022, Esteller et al., 2023).

2.2.2. Intramuscular Fat Quantification

Different semi-quantification scales to classify intramuscular fat infiltration were used in the literature. Goutallier scale is one of the most common scales used with different conditions involving muscle degeneration. It has 5 stages to describe areas of high signals on T1-weighted images (Kim et al., 2019).

Modified Mercuri's scale is another example of semi-quantification scale. It also describe areas of high signals on T1-weighted images but using 6 grading scores (Mercuri et al., 2003).

However, classifications with limited number of grades do not consider the floor effect found in early stages of slowly progressive disease like hereditary peripheral neuropathy. Alternatively, the 3-point Dixon method was found to be a more sensitive outcome measure to quantify intramuscular fat infiltration. In a study by Kim et al. (2019), they evaluated the potential value of 3D multiple gradient echo Dixon-based MRI sequence as a tool for thigh intramuscular fat quantification in people with Charcot Marie Tooth disease (CMT). They performed a prospective comparison study including 18 CMT participants and 18 matched controls. MRI using 3D multiple gradient echo Dixon-based imaging was performed for each subject. Region of interest analyses were performed at the upper and lower third of both thighs. The two-sample t-test or Wilcoxon rank sum test was used for intergroup comparison of the mean muscle fat fraction. Intraclass correlation coefficients were used to evaluate the interobserver agreement and test–retest reproducibility. Semi- quantitative analysis using the Goutallier classification (Grades 0–4) was done on T1-weighted images in upper thigh muscles. For Goutallier Grade 0 muscles, comparison of the mean intramuscular fat fraction between volunteers and CMT patients was performed. The interobserver agreements were excellent for all measurements (intraclass correlation coefficients > 0.8). Mean muscle fat fractions were significantly higher in all the measured muscles of CMT participants ($P < 0.05$) except in the adductor magnus in the upper thigh ($P = 0.109$). Goutallier Grade 0 muscles of the CMT participants showed a significantly higher mean fat fraction compared

with that of the volunteers ($P < 0.05$). These finding suggest that the 3D multiple gradient echo Dixon-based MRI is a reproducible and sensitive technique which can reveal a significant difference in the fat fraction of thigh muscle, including muscles with Goutallier Grade 0 (Kim et al., 2019). These results came consistent with other studies aimed to explore the validity and responsiveness of fat fraction as a biomarker measured using Dixon-based quantitative MRI in Hereditary sensory neuropathy type 1 (Kugathasan et al., 2019), CMT1A, and inclusion body myositis (IBM) (Morrow et al., 2018, Morrow et al., 2016) which may imply that intramuscular fat measurement using a Dixon-based quantitative MRI sequence can be a more sensitive and objective tool for screening of muscular degeneration.

2.2.3. Muscle MRI As an Outcome Measure in Hereditary Peripheral Neuropathy

Muscle MRI correlation, as an outcome measure in peripheral neuropathy, with other validated measures for muscle strength and general function was explored. It has been shown that there is a significant correlation between MRI and manual muscle testing with DHMN and CMT (Del Porto et al., 2010). While manual muscle testing (MMT) lacks sensitivity and validity for mild weakness; higher MMT grade, MRI had the ability to detect subclinical muscle pathology (Del Porto et al., 2010). Morrow et al. (2016) investigated the sensitivity of MRI to detect changes in 20 patients with Charcot-Marie-Tooth disease 1A (CMT1A) and 20 patients with inclusion body myositis (IBM). The validity of the magnetic

resonance measures was supported by strong correlations with clinical functional measures, and the responsiveness to disease progression over 1 year was shown to be better with MRI than with the clinical functional tests. Even though Charcot Marie Tooth disease 1A progresses slowly, magnetic resonance measures detected substantial increases in disease pathological changes in 1 year. They also found that MRI-measured T2 and MTR are abnormal in these diseases even when fat fraction values are within normal limits (Morrow et al., 2016). Suggesting that MRI can detect early signs of active intramuscular denervation before fatty infiltration. In an earlier study using the same MRI protocol, they explored the dependence upon age, gender and weight (body mass index) (Morrow et al., 2014). They found that quantitative MRI measurements show small but significant inter-subject age and weight dependency. However, these demographically driven differences are smaller than the expected pathological changes in NMDs, and thus too small to pose a significant finding in longitudinal studies (Morrow et al., 2014).

The three-point Dixon technique is an MRI sequence that has been applied to skeletal muscles in neuropathy to quantify intramuscular fat accumulation in different events. This sequence has been found to be highly responsive as presented by Kim et al. (2019) and correlating with other validated outcome measures (Kim et al., 2019). The London MRC Centre for Neuromuscular Diseases has developed a quantitative muscle MRI protocol (using the three-point

Dixon technique) as a highly responsive biomarker for CMT1A in adult patients. Effort to validate its responsiveness has been undertaken by Morrow and colleagues (2018). The results of this study confirmed the reliability, validity, and responsiveness of the MRC centre MRI quantified calf muscle FF protocol as an outcome measure in CMT1A. Selection of study participants with increased baseline calf muscle FF provided a highly responsive biomarker in this patient group, suitable for utilization in multicentre international clinical trials (Morrow et al., 2018). The MRC centre MRI calf muscle fat fraction protocol has also been validated in hereditary sensory neuropathy type 1 (HSN1) (Kugathasan et al., 2019).

Patterns of intramuscular fat and muscle atrophy were also explored using MRI in more recent studies. Esteller et al. (2023) explored MRI imaging in a large cohort of DHMN (Esteller et al., 2023). They carried out a retrospective analysis of clinical, genetic, and muscle imaging data of 84 people with DHMN. The MRI examinations included T1-weighted and T2-weighted Short Tau Inversion Recovery (STIR-T2w) images. The extent of muscle fat infiltration was measured using the Mercuri score. They used hierarchical clustering to detect specific patterns of muscle involvement (Esteller et al., 2023). Although the cohort showed proximal lower limbs and distal upper limbs involvement, the most common phenotype was distal lower limb weakness. Among the 84 cases, only 38 (45.2%) with a causative pathogenic variant identified, most commonly in

HSPB1 in 12 cases (14.2%) (Esteller et al., 2023). Analysis of MRI images in general was symmetric in most cases (64/84 cases) and showed distal to proximal gradient of increasing fat replacement in all muscles of the lower legs while in the thigh, it was commonly seen in the vastus lateralis, intermedius, and the semimembranosus. They described muscle involvement in 4 different patterns shown in Table 3 (Esteller et al., 2023).

In another study by O'Donnell et al. (2022), they used T1-weighted and Short Tau Inversion Recovery (STIR) to qualitatively analyse a group of 6 people with SORD pathogen (5 DHMN, 1CMT2) (O'Donnell et al., 2022). Mean fat accumulation, atrophy and STIR grades were higher in calf than thigh muscles on all scans. However, some fat accumulation was seen in thigh muscles of all patients, most commonly distally within vastus lateralis and intermedius. There was calf muscle atrophy in all patients, and the overall pattern was of greater involvement of posterior and lateral compartments. STIR hyperintensity, representing active denervation, was present on all scans, most markedly in tibialis anterior (Table 3). Analysis showed a highly significant correlation between calf anterior compartment fat accumulation with ankle dorsiflexion MRC grade (r −0.827, p 0.001) (O'Donnell et al., 2022).

The findings of these studies (Table 3) (Esteller et al., 2023, O'Donnell et al., 2022) revealed detailed characteristics and patterns of muscle involvement using MRI in DHMN cases with known pathogen that can be used for diagnostic

purposes. They showed a predominant involvement of the posterior calf compartment, with lateral compartment in some cases. However, these studies are limited by its retrospective nature, lack of control group, and the reliability of the MRI analysis could be affected by the choice of qualitative Mercuri score and by multiple observers.

Study	Pattern (n)	Known Pathogen (n)	MRI Muscle involvement
(Esteller et al., 2023)	1 (9)	HSPB1(3), HSPB8(2), TRPV4 (1) , DCTN1 (1).	Severe involvement of all muscles on the lower leg.
	2 (6)	BICD2(3), DYNC1H1(3)	Severe fat replacement of the lower leg with preservation of the toe extensor muscles
	3 (12)	GARS (1), HARS1(1), VRK1(1)	Predominant involvement of the superficial posterior compartment of the legs.
	4 (11)	HARS1(1), DYNC1H1(1), GDI1(1)	More severe involvement of the peroneus group in comparison with the rest of the compartments
(O'Donnell et al., 2022)		SORD (5)	Greater involvement of posterior and lateral compartments of the lower leg with some fat accumulation in vastus lateralis and intermedius.
(n)= number of cases, BICD2= BICD cargo adaptor 2, DCTN1= P150 subunit of dynactin, DYNC1H1= cytoplasmic dynein heavy chain 1, GARS= glycyl-tRNA synthetase, GDI1= GDP dissociation inhibitor 1, HARS1= histidyl-tRNA synthetase 1, HSPB1= heat-shock protein B1, HSPB8= heat-shock protein B8, TRPV4= transient receptor vallanoid 4 gene, VRK1= VRK serine/threonine kinase 1, SORD= Sorbitol dehydrogenase.			

Table 3: Pattern of muscle involvement in DHMN cohort.

MRI studies in CMT revealed a different pattern of fatty infiltration. In a study aimed to quantitatively describe the MRI fat infiltration pattern of muscle degeneration in CMT1A disease and to look for correlations with clinical variables, Bas et al. (2020) assessed MRI fat fraction in lower-limb muscles of patients with CMT1A and healthy controls. In particular, 14 muscle

compartments were selected at leg and thigh levels and for proximal, distal, and medial slices. Muscle fat infiltration profile was determined quantitatively in each muscle compartment and along the entire volume of acquisition to determine a length-dependent gradient of fat infiltration (Bas et al., 2020). Clinical impairment was evaluated with muscle strength measurements and CMT Examination Score (CMTES). Based on quantitative MRI measurements combined with a dedicated segmentation method, muscle fat infiltration quantified in patients with CMT1A revealed a length dependent pattern of infiltration with the largest fat infiltration was quantified in the anterior and lateral compartments of the lower leg (17.7% and 21.8%, respectively, in comparison to controls). Muscle fat infiltration was correlated to main clinical variables including muscle strength measurements and CMTES (Bas et al., 2020). Despite the genetic overlap between CMT and DHMN, they exhibit distinct patterns of muscle involvement. Both conditions are length dependent with a predominant calf involvement, However, in CMT, the anterior compartment is more affected, while in DHMN, the posterior compartment is primarily involved.

Muscle oedema, the accumulation of water within the muscle as a sign of active denervation, can also be detected and quantified using MRI. Locher et al. (2020) assessed quantitative water T2 relaxometry for the early detection of neuromuscular diseases (NMD) in comparison to standard qualitative MRI imaging in a clinical setting (Locher et al., 2022). They retrospectively analysed

83 patients with suspected NMD who underwent MRI with a subsequent muscle biopsy between 2015 and 2019. Qualitative T1-weighted and fat suppressed T2-weighted images were graded to be either pathological or normal. Mean and median water T2 relaxation times were obtained from manually drawn region of interests in biopsied muscle from multi-echo sequence. Histopathologic pattern of corresponding muscle biopsies was used as a reference. Analysis in cases prior to late-stage fatty infiltration signal alternations in T1-weighted images showed that quantitative water T2 relaxometry had a significantly higher sensitivity in detecting muscle abnormalities than subjective grading of T2- weighted images. In 49 patients without late-stage changes, T2- weighted grading achieved a sensitivity of 56.4%, while mean and median water T2 had a sensitivity of 87.2% and 97.4% to detect early-stage neuromuscular diseases. Median water T2 ranged between 36 and 42 ms depending on histopathologic pattern (Locher et al., 2022). The author recommended that quantitative water T2 relaxometry should be considered complementary to subjectively rated fat-suppressed T2-weighted images in clinical practice to detect early changes in neuromuscular diseases (Locher et al., 2022). The ability to detect early changes by quantifying intramuscular water can be utilised in clinical trials to assess the safety and efficacy of therapies such as resistance exercises.

In summary, quantitative muscle MRI analysis is a valid and responsive tool in longitudinal studies to explore intramuscular fat using Dixon technique and to

explore muscle oedema using T2-relaxometry to identify early signs of active denervation. Qualitative MRI analysis to explore intramuscular fat using T1-weighted image and to explore muscle oedema using STIR imaging, was shown to be sufficient to describe the pattern of muscle involvement and can be used clinically for diagnostic purposes.

2.3. Dynamometry and Muscle Strength Assessment in Hereditary Peripheral Neuropathy

There are a number of techniques used to assess muscle strength. Some of these methods are relevant in the clinical setting and others are more applicable for research purposes. These include manual muscle testing using the MRC scale, hand-held dynamometry (with or without an immobilizing device), and isometric and isokinetic dynamometry.

The concept of isokinetic dynamometry was developed by Perrine and Hislop in 1967 (Hislop and Perrine, 1967). Since then, there has been an increase in its use for rehabilitation and research. Isokinetic dynamometers are considered the gold standard in evaluating dynamic muscular performance (Le-Ngoc and Jansse, 2012, Lund et al., 2005, Drouin et al., 2004, Kannus, 1994, Baltzopoulos and Brodie, 1989, Osternig, 1986). It is used for isometric and isokinetic strength measurements, allowing valid and reliable measurement of maximal voluntary muscle contraction (isometric), as well as maximal muscle contraction through a specific range of movement with controlled speed and position (isokinetic). Strength profiles are created to illustrate the relationship between instant torque and joint angle, allowing the identification of various characteristics including dynamic peak torque, the angle at which peak torque occurs, torque specific to certain angles, power, and the energy expended. These dynamic strength profiles also enable the identification of minor weaknesses within particular ranges of

motion (ROM) of a joint. These features made it useful clinically as it provides sufficient sensitivity to detect muscle strength progress during rehabilitation (Webber and Porter, 2010, Tiffreau et al., 2007, Andersen, 1996).

Additionally, the isokinetic dynamometer offers several benefits over alternative methods for assessing muscle strength. It eliminates the need for the assessor's strength, ensures consistent stabilization of the subject during tests, and allows simultaneous measurement of joint angle and strength (Le-Ngoc and Jansse, 2012, Martin et al., 2006, Lund et al., 2005, Harlaar et al., 1996). Disadvantages of these devices are their size and cost, which make them impractical for routine clinical examinations (Le-Ngoc and Jansse, 2012, Li et al., 2006, Mital et al., 1995).

One of the primary parameters measured using dynamometry is muscle torque. Torque is the moment of forces applied about an axis of rotation. The formula of torque is:

Torque (Nm) = Force (N) × Distance (m)

where “distance” indicates the perpendicular distance from the input of force to the centre of rotation (Dvir, 2004, Dvir, 2000, Perrin, 1994). In isokinetic testing, Peak torque is the maximum torque production during movement throughout the range of motion. Peak torque is an indicative of maximum muscular tension capability taking into account changes due to biomechanical leverage and the muscular length and tension relationship that occurs throughout the range of

motion. Torque can also be measured under isometric conditions against immobile lever of the dynamometer, which is an important indicator of explosive force production of the tested muscle or the ability of a muscle to exert maximal force promptly (Dvir, 2004, Dvir, 2000, Perrin, 1994).

Muscle force varies at different joint angles due to numerous biomechanical elements of the musculoskeletal system (Baltzopoulos and Brodie, 1989). In the isokinetic method, resistance in the dynamometer would be equal to the muscular capacity in numerous joint angles, providing efficient loading in the muscles at dynamic points (Baltzopoulos and Brodie, 1989). Moreover, isokinetic dynamometers in comparison to gravity-loaded systems do not store potential energy and therefore the return movement does not require eccentric contraction to control the return of the limb-lever arm system to the initial position (Baltzopoulos and Brodie, 1989, Thistle et al., 1967).

The reliability of isokinetic dynamometer has been shown to be high in healthy subjects (Webber and Porter, 2010), and in people affected with neuromuscular diseases even with significant weakness (≤ 3- on MRC scale) (Tiffreau et al., 2007). In the MRI study by Morrow et al. (2016), isokinetic dynamometer correlated with MRI measures in Charcot-Marie-Tooth disease type 1A (CMT1A) and inclusion body myositis (IBM), and was able to detect difference in strength over 12 months (Morrow et al., 2016).

In a further study, that also included people with CMT1A (n=33) aimed to determine correlations between the isokinetic muscular strength of knee flexors and knee extensors and walk parameters, People with CMT1A showed excellent correlation between isokinetic knee extension and walking speed, and moderate correlation between isometric knee flexion and walking speed (Reynaud et al., 2019). These correlations were higher in participants younger than 50 years old. The lack of correlation in older patients may be explained by other factors limiting the quality of walking in these patients such as degenerative phenomena (osteoarthritis, the natural evolution of neuropathy, or the static deformation of the feet) and orthopaedic surgical history of the feet and/or ankle, which is frequent in older patients and was not considered in the study design (Reynaud et al., 2019).

A trial explored the feasibility and effect of community-based aerobic exercise training for people with two of the most common neuromuscular diseases: Charcot-Marie-Tooth disease type 1A (CMT) and inclusion body myositis (IBM) (Wallace et al., 2019). Isokinetic dynamometer was used as a secondary outcome measures to evaluate the effect of aerobic exercises on muscle strength, in addition to peak oxygen uptake (VO2 peak) as a primary outcome measure. Both disease groups demonstrated improvements in VO2 peak, with a moderate effect size in the CMT participants (Cohen's d = 0.53) and a strong effect size in the IBM group (Cohen's d = 1.72). However, there was no major changes in the

isokinetic dynamometer (Wallace et al., 2019). There was a notable challenge when using the isokinetic dynamometer for very weak muscles in generating sufficient torque to trigger the motor. This may have affected the reliability of the dynamometry data (Wallace et al., 2019). Moreover, using VO2 peak is more suitable outcome measure in training trials targeting aerobic capacity, rather than using isokinetic dynamometer which is more suitable for measuring peak torque in muscle resistance training trials (Carter et al., 1995).

The reliability of isokinetic dynamometer in neuromuscular conditions has been explored, and the use of isokinetic dynamometer in CMT for longitudinal studies or for clinical trials as an outcome measure has been described. However, none of these studies explore DHMN specifically. When utilizing isokinetic dynamometers in clinical trials, several factors may influence the reliability of measurements, that need careful consideration. These include reduced range of joint motion, which may be associated with aging or orthopaedic surgeries, and the focus of the exercise program, with isokinetic dynamometry most relevant for training targeting muscle strength.

2.4. Gait and Walking in Hereditary Peripheral Neuropathy

Quantifying human gait has utility for research, diagnostic or clinical purposes. Subsequently, gait analysis is an important assessment tool that uses physical measurements and models, to identify functional capabilities, limitations, and gait pattern in different neuropathies with the purpose of evaluating the effectiveness of rehabilitation therapies.

2.4.1. Definitions and Terminology

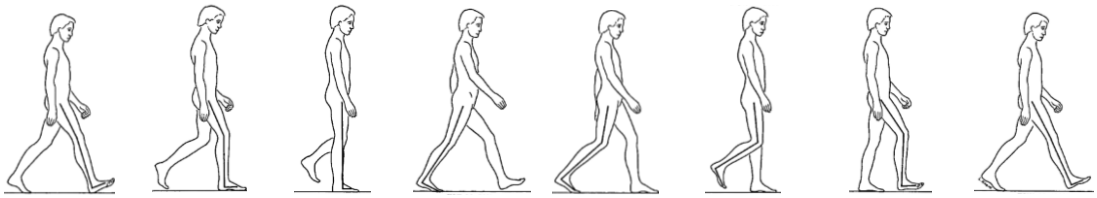
Locomotion can be described as the motion to move from one point to another (Rose and Gamble, 2006). Walking is one method of locomotion. In humans, bipedal gait uses cyclical motion to progress the body forward in a linear path. Forward progression occurs by placing one foot in front of the other repeatedly but never having both feet off the ground at the same time. While running could be considered faster walking with shorter gait cycle and never having both feet on the ground at the same time, the difference is there is a period where both feet are off the ground (flight phase) (Rose and Gamble, 2006, Whittle, 2007).

Angular motion of a joint that connects adjacent body segments is caused by force acting at a distance from the joint axis resulting in a torque or moment (Valero-Cuevas, 2016, Robertson DGE et al., 2014a, Knudson, 2007, Dvir, 2000). The direction of joint motion is determined by combining all moments acting on body segments. Angular moments during walking are caused by a combination of externally and internally generated forces and moments. External forces include

gravity force that acts through the centre of mass of the segment in downward direction, and ground reaction force that acts at the point where the body contacts the ground. Internally generated moments are mainly due to the actions of muscles (Valero-Cuevas, 2016, Robertson DGE et al., 2014a, Knudson, 2007, Dvir, 2000). The contributions of ligaments and surrounding soft tissues to the torque are minimal because their points of action are near the joint axis, and these structures are usually lax unless the joints are fully extended or flexed (Valero-Cuevas, 2016, Robertson DGE et al., 2014a, Knudson, 2007, Dvir, 2000).

2.4.2. The Gait Cycle

The gait cycle can be divided into two major phases, the stance phase, and the swing phase, which make up approximately 60% and 40% of the cycle, respectively. Phases, events, periods, and support during a full gait cycle are explained in Table 4 (Vu et al., 2018, Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).



% of cycle	0%	0-10%	10-30%	30-50%	50-60%	60-70%	70-85%	85-100%
Phase	Stance phase					Swing phase		
Event	Initial contact	Foot flat, Opposite toe off		Heel off, Opposite initial contact		Toe off	Tibia vertical, Feet adjacent	Initial contact (next cycle)
Period	Loading response	Mid-stance		Terminal stance	Pre-swing	Initial swing	Mid-swing	Terminal swing
Support	Double support	Single leg support			Double support	Single leg support		

Table 4: Normal gait cycle, Right side.

The sequential combination of the phases enables the limb to accomplish three basic tasks. These are weight acceptance, single limb support, and limb advancement. Weight acceptance initiates the stance phase and uses initial contact and loading response. Single limb support continues stance with the mid stance and terminal stance. Limb advancement begins in the final period of stance (pre-swing) and then continues through the three phases of swing (initial swing, mid-swing, and terminal swing). Table 5 is a summary of the objectives and joints positions in each gait period (Vu et al., 2018, Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

Period	Initial Contact	Loading Response	Mid Stance	Terminal Stance	Pre-Swing	Initial Swing	Mid Swing	Terminal Swing
Hip	Flexed to 30°	Flexed	Extending	Extending 15°-30°	Flexing	Flexing	Flexing to 30°	Flexed to 30°
Knee	Extended	Flexing 5°-10°	Extending but not to 0°	Extend, then Flex	Flexing 30°-40°	Flexing up to 65°	Extending	Extending
Ankle	Neutral	Plantarflexing to 20°	Dorsiflexing	15° Dorsiflexing to Neutral	Plantarflexing 20°-30°	Dorsiflexing to 0°	Dorsiflexing to 0°	Neutral
Objective	Begin Stance	Shock Absorption, Advance body over Heel Rocker	Advance body over stationary foot, ankle rocker	Advance body over forefoot rocker	Prepare for Swing, transfer load to contralateral limb	Clear foot and advance limb	Advance limb and clear foot	Advance limb

Table 5: Summary of the objectives and joints positions in each gait period.

2.4.3. Gait Mechanics and Locomotor Functions

2.4.3.1. Role of Major Joints and Muscles

Propulsion and Shock Absorption are the major functions of the joints and muscles during walking. Below is a summary of the main functions of the locomotion intersegmental joints and the muscles acting upon them during gait

cycle (Figure 2), emphasising the role of the ankle joint and muscles that are generally affected in peripheral neuropathy (Vaughan, 1999, Chambers and Sutherland, 2002).

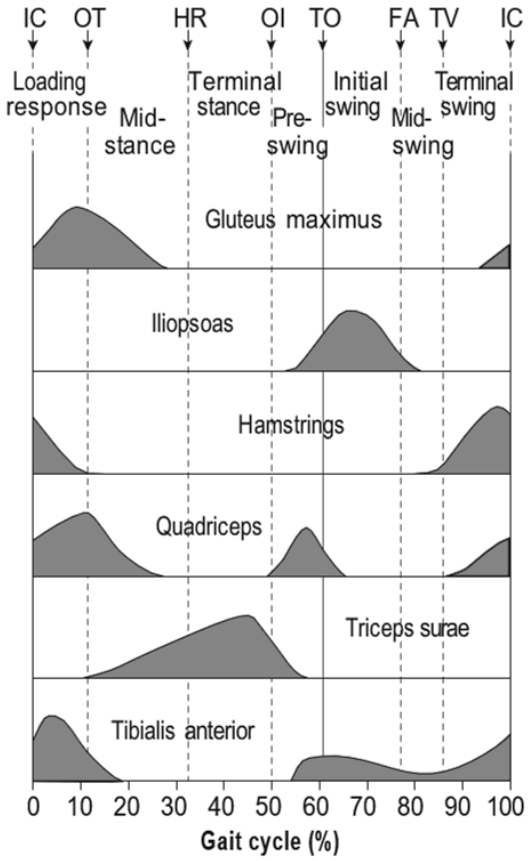


Figure 2: Typical activity of major muscle groups during the gait cycle.
IC = initial contact, OT = opposite toe off, HR = heel rise, OI = opposite initial contact, TO = toe off, FA = feet adjacent, TV = tibia vertical. Figure adapted from (Whittle, 2007).

Advancement of the body depends on stance limb mobility. As body weight is dropped onto the limb, the force is primarily directed toward the floor. Advancement of the body depends on redirecting some of this force in a manner that combines progression and stability. The essential element for progression over the stance limb is rocker action by the foot and ankle. Full ranges of

extension at the knee and hip are the other critical factors for progression (Whittle, 2007, Rose and Gamble, 2006, Perry and Burnfield, 1992).

Heel Rocker: Using the heel as the fulcrum, the foot rolls into plantar flexion as the stance phase begins. Limb progression is preserved as the Pretibial muscles decelerate the foot drop and draw the tibia forward. Quadriceps action extends the progression of the tibia initiated by the heel rocker to advancing the thigh (Brockett and Chapman, 2016, Whittle, 2007, Rose and Gamble, 2006, Perry and Burnfield, 1992).

Ankle Rocker: With the ankle as the fulcrum, the tibia (and whole limb) rolls forward in response to the body momentum. The rate of tibial progression is decelerated by the soleus muscle (Brockett and Chapman, 2016, Whittle, 2007, Rose and Gamble, 2006, Perry and Burnfield, 1992).

Forefoot Rocker: Tibial progression is continued over the forefoot rocker. Both gastrocnemius and soleus act vigorously to decelerate the rate of tibial advancement (Brockett and Chapman, 2016, Whittle, 2007, Rose and Gamble, 2006, Perry and Burnfield, 1992).

The knee Joint: Plays a vital role in shock absorption through flexion immediately after heel strike during the early stance phase and aids in propulsion by extending before toe-off in the late stance phase. In the swing phase, it flexes to allow foot clearance from the ground (Whittle, 2007, Rose and Gamble, 2006, Perry and Burnfield, 1992).

The hip Joint: Plays a critical role in providing stability and movement. During the stance phase, the hip extensors work to support the body, while during the swing phase, the hip flexors facilitate leg advancement (Whittle, 2007, Rose and Gamble, 2006, Perry and Burnfield, 1992).

2.4.3.2. Energy Conservation

Walking utilizes energy-saving mechanisms that reduce the metabolic cost of walking by storing and releasing energy such as the inverted pendulum effect, where the body vaults over the leg in stance phase, conserving energy through gravitational potential energy and kinetic energy conversion. Another mechanism is the elastic recoil of tendons. Tendons store energy during certain phases and release it to aid movement, reducing overall energy expenditure. For example, the calf store energy when it is stretched in terminal stance, and recoil in pre-swing to assist progressing the leg forward (Perry and Burnfield, 1992, Kuo and Donelan, 2010, Chambers and Sutherland, 2002).

2.4.3.3. Stance Stability and Balance

Functionally, the body is divided into two units, the passenger unit, which includes the head, neck, trunk and arms, and the locomotor unit, which include the two lower limbs and pelvis (Perry and Burnfield, 1992). Stability in the upright position is determined by the functional balance between the alignment of the body and muscle activity at each joint. The falling body weight, ligamentous tension, and muscular activity are forces that can act on the joints in

upright position (Perry and Burnfield, 1992). Standing stability is challenged by three anatomical features. First is the mass distribution between the passenger unit (70%) and the locomotor system (30%). Second is the multisegmented nature of the supporting limbs. The third factor is the contours of the lower limb joints. The centre of gravity is a hypothetical point around which the force of gravity appears to act, located approximately anterior to the second sacral vertebra and is the origin of the gravity line (Perry and Burnfield, 1992). The goal of static balance in standing is to keep the gravity line; a line falling from the centre of gravity down to the ground, within the base of support; or the distance between the centre of both heels sideways (Perry and Burnfield, 1992, Kuo and Donelan, 2010).

Balance during walking is maintained by minimum lateral and vertical displacement of the centre of gravity. It changes constantly with body or limbs movements. (Rose and Gamble, 2006). Two actions are essential to preserve balance over a single limb during walking, including lateral shift of the body mass and local muscular stabilization of the hip joint to keep the pelvis and trunk erect (Kuo and Donelan, 2010).

Central and peripheral nervous systems orchestrate the timing and strength of muscle contractions. The central pattern generators in the spinal cord produce basic rhythmic patterns, while the brain adjusts these patterns for dynamic

balance, obstacle avoidance, and direction changes (Winter, 2009, Stergiou, 2020).

The six optimizations for body adjustments used to minimize the excursions of the centre of gravity during walking are called the ‘determinants of gait’ by Saunders et al. (1953) and they are; pelvic rotation, pelvic obliquity, knee flexion in stance phase, ankle mechanism when the heel sticks out beyond the ankle joint and effectively lengthens the leg during the loading response, foot mechanism when the ankle moves from dorsiflexion into plantarflexion the forefoot lengthens the leg at the end of stance, and lateral displacement of body (Whittle, 2007, Stergiou, 2020, Kuo and Donelan, 2010, Chambers and Sutherland, 2002).

2.4.3.4. Biomechanical Adaptations and Variability

Biomechanical variability arises from individual differences in anatomy, age, and health status. For instance, children and elderly individuals have distinct gait patterns due to different levels of muscle strength, flexibility, and balance. People with disabilities or injuries may develop compensatory gait patterns, altering their stride length, cadence, or joint use to accommodate pain or reduced mobility (Chambers and Sutherland, 2002, Kuo and Donelan, 2010, Vaughan, 1999). Moreover, obesity showed an impact on gait kinetics and kinematics with reduced ankle angle and decreased plantar flexor moment and power generation at terminal stance (Capodaglio et al., 2021).

Understanding these detailed aspects of walking gait biomechanics helps in identifying gait abnormalities, designing effective rehabilitation strategies, and improving mobility aids such as prosthetics and orthotics. This knowledge is crucial for professionals in biomechanics, physical therapy, and related fields to enhance patient care and support mobility and health.

2.4.4. Methods of Gait Assessment

A comprehensive gait assessment usually includes the study of body movement and position in space (kinematics), and the study of forces, moments, and powers associated with this movement (Kinetics). In normal walking, there are five major attributes which are frequently lost in pathological gait: stability in stance, foot clearance in swing, pre-positioning of the foot for initial contact, adequate step length, and energy conservation (Chambers and Sutherland, 2002). Observing these five attributes and any deficits which might cause them provides a particularly good functional assessment of the walking patterns (Chambers and Sutherland, 2002, Kuo and Donelan, 2010). Gait can also be described in relation to time (Table 6) or linear parameters (Table 7) (Whittle, 2007, Perry and Burnfield, 1992).

Cadence	The number of steps in a standard time frame.
Cycle Time	The duration to complete a full gait cycle.
Speed	The distance covered by the whole body in a given time.

Table 6: Time parameters to describe the gait cycle.

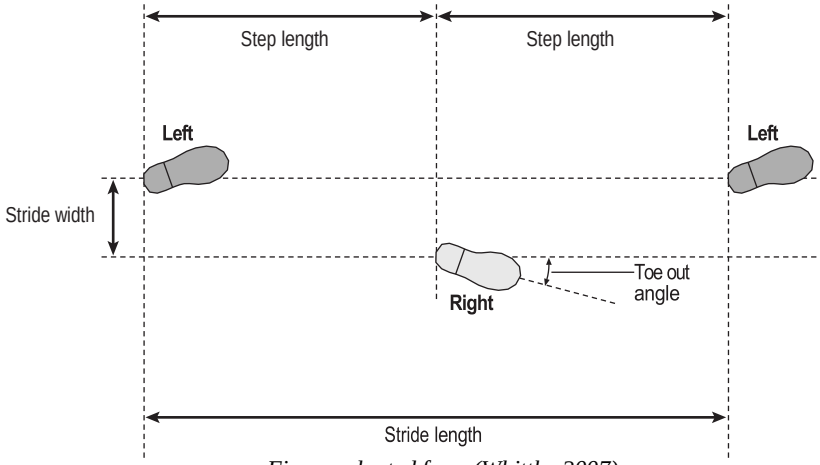


Figure adapted from (Whittle, 2007)

Step Length	The distance between the same point on each foot (usually the heel), during double limb support.
Stride Length	The distance travelled between two successive foot strikes of the same foot.
Walking Base (Stride width)	The side-to-side distance between the line of the two feet.
Toe Out Angle	The angle in degrees between the direction of progression and a reference line on the sole of the foot.

Table 7: Linear parameters to describe the foot placement on the ground during a full gait cycle.

There are a number of different assessment tools used widely for clinical and research purposes for kinematic analysis of gait. Kinematics describes the motion of joint angular displacement, velocity, and accelerations. Assessment of movement kinematics can be obtained by electro goniometers, video recording or a simple visual gait analysis. However, the data collected is two dimensional and subject to inaccuracies and bias (Winter, 2009, Stergiou, 2020).

Three-dimensional motion analysis systems digitally reconstruct the individual's body as a multisegmented system. After optoelectronic infrared markers are

placed at specific anatomic landmarks, their position is triangulated by cameras to calibrate the individual into the system. Construction of the coordinates and orientation of the rigid body segments allow calculation of joint angles of the proximal and distal segment, joint angular velocity, and joint acceleration. Measurements are collected for each joint in all three cardinal planes of motion (Whittle, 2007, Winter, 2009, Stergiou, 2020).

Kinetics reflect the cause of movement, and therefore the forces, power, and energy that affect the manner in which an individual moves. Ground reaction forces are measured using force plates on the ground to refer to the forces that act on the body throughout the stance phase. Analysis of the ground reaction force acting on the centre of gravity is typically broken down into vertical, mediolateral, and anteroposterior force plots (Whittle, 2007, Winter, 2009, Stergiou, 2020).

2.4.5. The Normal Gait in Three-Dimensional Motion Analysis

2.4.5.1. Kinematics

The description in this section is focused on the sagittal plane measurements as they are probably the most studied, best understood, and most accurately reproduced (Figure 3) (Rose and Gamble, 2006, Whittle, 2007).

Anterior/Posterior Pelvic Tilt: This aspect of the graph shows the forward and backward tilting motion of the pelvis. A normal walking pattern displays a rhythmic oscillation of anterior and posterior tilt, which contributes to leg

extension and absorption of impact during the stance phase. The pelvis is most horizontal (least amount of tilt) at foot-off and opposite foot-off, with maximum flexion occurring in mid- to late stance and terminal swing (Figure 3) (Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

Hip Flexion/Extension: Increase in hip flexion at initial contact at the beginning of the stance phase, followed by a gradual extension as the body moves over the supporting leg until opposite foot contact. As soon as the opposite foot strikes the ground, weight is transferred to the forward limb, and the behind leg begins to flex at the knee and hip, while pivoting on the forefoot to initiate swing phase. As the foot leaves the ground, the hip continues to flex throughout the swing phase. The hip extensor muscles decelerate the thigh and diminish the hip flexion in preparation for weight acceptance (Figure 3) (Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

Knee Flexion/Extension: The knee typically undergoes flexion immediately after heel strike to absorb impact and then extends during mid-stance as the ground reaction force line progresses anterior to the knee to support body weight. It should be noted that this passive extension cannot occur without the strong eccentric contraction of the plantar flexors restraining the shank from progressive forward rotation. During the swing phase, the knee flexes again to facilitate foot clearance from the ground. It reaches maximum flexion as the swinging foot passes the opposite limb. The knee joint is then rapidly extended nearly to full

extension just prior to foot strike (Figure 3) (Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

Ankle Plantar Flexion/Dorsiflexion: The most complex of the sagittal curves and can be broken down into four separate functional segments:

This first segment occurs between foot strike and opposite foot-off. The ankle is positioned at approximately neutral when the foot strikes with heel first. The position of the ground reaction force is posterior to the ankle centre causes plantar flexion until the foot is flat prior to opposite foot-off. This part of the ankle motion curve is also known as the first rocker (Figure 3) (Brockett and Chapman, 2016, Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

The second segment occurs during single limb stance and opposite limb swing. It is typically convex superiorly and reflects the body passing over the fixed flat foot (second rocker). At approximately 40% of the cycle, the heel begins to rise as the plantar flexors increase their force of contraction and act concentrically (third rocker) (Figure 3) (Brockett and Chapman, 2016, Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

The third segment continues with rapid plantar flexion to a maximum of 20 to 25 degrees just as the foot is lifted off the ground. The transfer of weight to the opposite limb occurs very rapidly, and the plantar flexion movement occurring after opposite foot strike is passive. This movement may be entirely due to gravity

and inertia or the passive tension in the plantar flexors (Figure 3) (Brockett and Chapman, 2016, Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

The fourth segment is rapid ankle dorsiflexion throughout the swing phase to ensure maximum foot clearance. The ankle is maintained in this neutral position by isometric contraction of the anterior compartment muscles until foot strike when these same muscles are again needed to eccentrically restrain the plantar flexion that repeats the first segment of the cycle (Figure 3) (Brockett and Chapman, 2016, Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

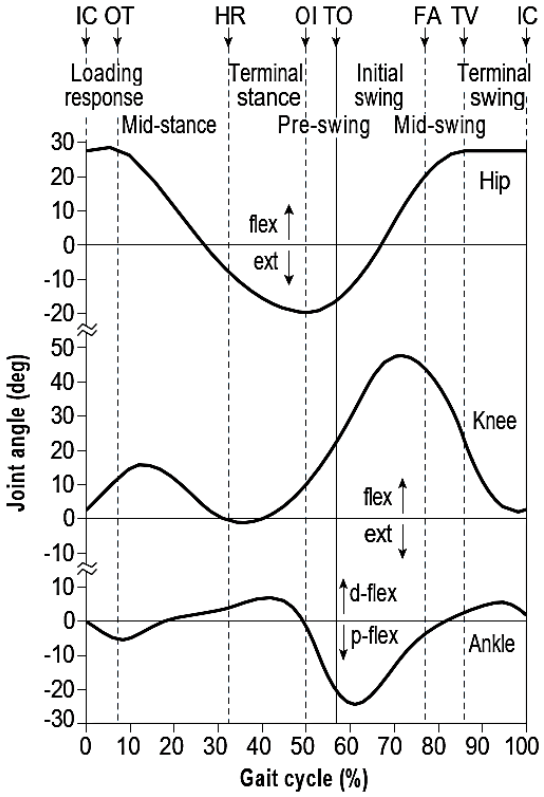


Figure 3: Sagittal plane joint angles (degrees) during a single gait cycle of right hip (flexion positive), knee (flexion positive) and ankle (dorsiflexion positive).

IC = initial contact, OT = opposite toe off, HR = heel rise, OI = opposite initial contact, TO = toe off, FA = feet adjacent, TV = tibia vertical. Figure adapted from (Whittle, 2007).

2.4.5.2. Kinetics

Ground Reaction Force (GRF)

Vertical Component: This is often the most significant part of the GRF and illustrates the forces acting perpendicular to the ground. It shows two main peaks during a walking cycle. The first peak corresponds to the initial contact and weight acceptance phase (heel strike), indicating the impact force. The second peak reflects the push-off phase (toe-off), demonstrating the force used to propel the body forward. The valley between these peaks represents the midstance phase, where the body's weight is fully supported by the limb (Figure 4) (Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

Anteroposterior Component: This component shows the forces acting in the forward and backward direction. Initially, there's a decelerating force as the foot makes contact with the ground and decelerates the body, followed by a propulsive force during the push-off phase, accelerating the body forward (Figure 4) (Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

Mediolateral Component: Representing the forces acting from side to side, this component is usually smaller compared to the vertical and anteroposterior forces. It illustrates the body's need to stabilize and balance during the gait cycle, with shifts in weight from one foot to the other (Figure 4) (Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

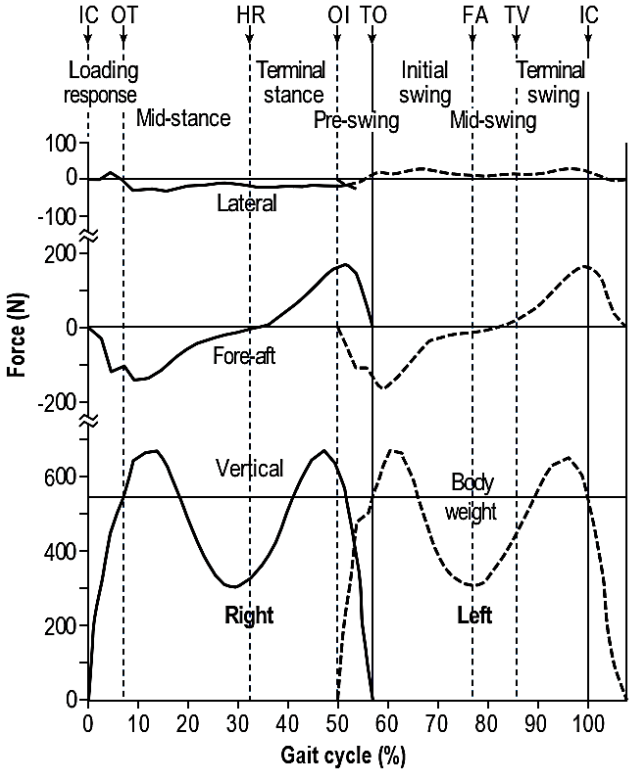


Figure 4: Ground reaction forces. Lateral, Anteroposterior (fore–aft), and vertical components of the ground reaction force, in newtons, for right foot (solid line) and left foot (dashed line).

IC = initial contact, OT = opposite toe off, HR = heel rise, OI = opposite initial contact, TO = toe off, FA = feet adjacent, TV = tibia vertical. Figure adapted from (Whittle, 2007).

Moment

Hip Flexion/Extension Moments: During the initial stance phase, an extension moment is observed as the hip works to support the body's weight and stabilize the pelvis. This shifts to a flexion moment as the leg moves forward during the swing phase, preparing for the next step (Figure 5) (Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

Knee Flexion/Extension Moments: An extension moment is noted in the early stance phase, aiding in supporting the body's weight and stabilizing the knee as it bears the load. As the gait cycle progresses, flexion moments might be observed, particularly as the foot prepares to leave the ground, facilitating the transition to

the swing phase (Figure 5) (Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

Ankle Plantar flexion/Dorsiflexion Moments: In the early stance phase, dorsiflexion moments prevail as the foot adjusts to ground contact, facilitating shock absorption. The transition to plantarflexion moments marks the push-off phase, where the foot exerts force against the ground to propel the body forward (Figure 5) (Brockett and Chapman, 2016, Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

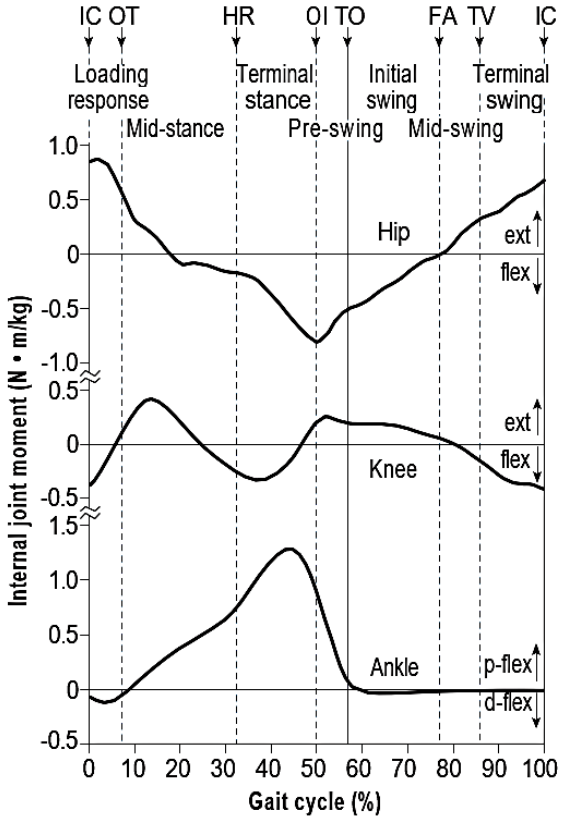


Figure 5: Sagittal plane internal joint moments (newton-meters per kilogram body mass) during a single gait cycle of right hip (extensor moment positive), knee (extensor moment positive) and ankle (plantar flexor moment positive).

IC = initial contact, OT = opposite toe off, HR = heel rise, OI = opposite initial contact, TO = toe off, FA = feet adjacent, TV = tibia vertical. Figure adapted from (Whittle, 2007).

Power

Hip Power Generation and Absorption: Power absorption mainly occur during the initial contact and early stance phase as the hip adjusts to load bearing, and power generation is mostly notable during the transition from stance to swing phase. These phases are indicative of the hip's role in stabilizing the body and propelling it forward (Figure 6) (Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

Knee Power Absorption and Generation: The knee absorbs power primarily during initial contact and early stance when the limb is loaded, and phases of power generation, notably in the latter part of the stance phase leading into the swing phase. These phases reflect the knee's role in damping the impact with the ground and then contributing to propulsion (Figure 6) (Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

Ankle Power Generation and Absorption: An initial phase of power absorption occurs during heel strike and early stance, where the muscles and tendons store energy as the foot makes contact with the ground. Followed by a significant phase of power generation during push-off, where the ankle muscles actively produce force to propel the body forward (Figure 6) (Brockett and Chapman, 2016, Whittle, 2007, Rose and Gamble, 2006, Vaughan, 1999, Perry and Burnfield, 1992).

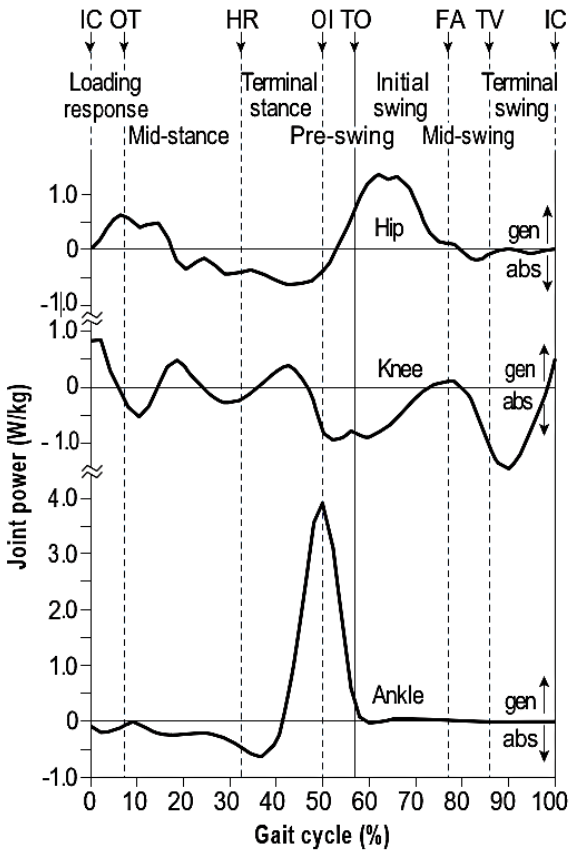


Figure 6: Sagittal plane joint powers (watts per kilogram body mass) during a single gait cycle of right hip, knee, and ankle. Power generation is positive, absorption is negative.

IC = initial contact, OT = opposite toe off, HR = heel rise, OI = opposite initial contact, TO = toe off, FA = feet adjacent, TV = tibia vertical. Figure adapted from (Whittle, 2007).

2.4.6. Altered Gait in Hereditary Peripheral Neuropathy

Early signs of gait alteration in hereditary peripheral neuropathy may start in childhood or adolescence as the weakness progresses slowly. The change in gait pattern is associated with muscle weakness due to denervation muscle atrophy (Wang et al., 2019, Hong et al., 2019). Other possible causes of altered gait could include limited joint range of motion, foot deformities, pain (Beckmann et al., 2015), sensory impairment (Nardone et al., 2014), and high body mass index (Hackett et al., 2019). Limited walking abilities could affect the activity level, energy consumption, and overall wellbeing (Menotti et al., 2011). Although the underlying genetic cause and clinical characteristics vary between affected people, their gait starts to deviate initially at the point when the symptoms around

the foot and ankle are present. Gait abnormalities in most DHMN cases are expected to start in late adulthood when the symptoms are progressed since the condition is a slowly progressive adult-onset disease (Forrester et al., 2020, Wang et al., 2019, Tanabe et al., 2018). Because of the limited existing literature in gait abnormalities in DHMN, herein a review of gait abnormalities in adult population affected by CMT, as this is a related condition and often overlaps with DHMN clinically and genetically (Kang et al., 2020, Forrester et al., 2020, Rossor et al., 2017).

Newman et al. (2007) was the first to use 3D motion analysis to observe gait in people with CMT. They analysed 16 CMT subjects aged 8–52 years old (11 with type I, 5 with type II) and 40 matched control subjects with no gait impairment or chronic health condition. The CMT group showed 15% slower gait and a combination of tight tendo-Achilles, footdrop, failure of plantar flexion and increased foot supination. They also presented with excessive internal rotation of the knee and/or tibia, knee hyperextension in stance phase, excessive external rotation at the hips and decreased hip adduction in stance. More proximal deviations may be adaptations to or consequences of the disrupted ankle and foot biomechanics. A direct relation to the neuropathy is also possible since sub-normal muscle power was observed in the proximal joints for most subjects on both manual testing and kinetic analysis (Newman et al., 2007). Nevertheless, due to the heterogeneity of the biomechanical impairments in that study they were

not able to identify a specific gait strategy as a compensatory mechanism. On the contrary, Don et al. (2007) considered functional differences within the CMT group and how they are related to different gait patterns. They evaluated a sample of 21 CMT subjects and 21 matched controls to describe the temporal, kinetic, kinematic, electromyographic and the mechanical energy expenditure of the body in terms of energy recovery and energy consumption in relation to the whole-body centre of mass (COM) in CMT subjects with foot drop and plantar flexion failure. According to Vinci and Perelli (2002), foot drop is the inability of the foot to dorsiflex up to 90° during the swing phase of gait making the limb functionally longer, and plantar flexor failure is the inability to stabilize the ankle joint in the sagittal plane while wearing a shoe with a 2cm heel, which is the most common heel height for ready-made footwear (Vinci and Perelli, 2002, Don et al., 2007). Don et al. (2007) classified the CMT group as having isolated foot drop (group 1) and combination of foot drop and plantar flexion failure (group 2). They observed two distinctive gait patterns when CMT group were subdivided into group 1 or 2. Group 1 showed a gait pattern with some characteristics of the 'steppage pattern'. The complex motor strategy adopted by group 1 assists foot clearance and preserves step length despite high energy consumption and slowed the swing velocity. Patients in Group 1 typically land with an excessively plantar-flexed ankle in loading response. Most of the individuals exhibit a flatfoot landing due to a lack of dorsiflexor angular impulse, attributed to the weakness of dorsiflexor muscles which fail to control foot landing properly. To compensate

for the weak dorsiflexor muscles and preserve body progression, patients increase passive ankle dorsiflexion in mid-stance by delaying plantar flexor muscle activation, as evidenced by electromyographic (EMG) data. This results in reduced plantar flexor angular impulse. There is an increase in hip extension and higher knee angular impulse during the stance phase. This coordination is necessary to maintain body progression and balance, necessitating additional effort from knee extensors, indicated by prolonged EMG activity in the vastus medialis and rectus femoris muscles. Despite delayed plantar flexor activation, an increased plantar flexor angular impulse is observed in push-off, which contributes to propulsion by engaging muscles less affected by the disease. Hip flexors also play a crucial role in propulsion, lifting the lower limb and enhancing hip flexion. During swing phase, patients exhibit foot drop, compensated by increased knee and hip flexion. This adaptation leads to a 'steppage' gait pattern, characterized by reduced gait speed due to increased swing phase duration, yet maintaining normal step length. Compared to healthy individuals, these patients exhibit increased energy consumption and recovery during gait, attributed to the mechanical effort required for their unique gait strategy, especially in hip flexor, knee extensor, and ankle plantar flexor muscles (Don et al., 2007). Group 2 displayed a ‘clumsy pattern’ characterised by very slow gait with reduced step length, a broader support area and great reduction in the cadence. This group of patients is showed a low energy consumption and greater energy recovery (Don et al., 2007). Similar to Group 1, patients in Group 2 land with an excessively

plantar flexed ankle, though to a lesser degree than group 1. They also exhibit a lack of dorsiflexion angular impulse during the Loading-Response. However, they show an increased hip extensor angular impulse and decreased knee flexion range of motion (ROM) and knee extensor angular impulse, suggesting reliance on hip extensors over knee extensors for load acceptance. In Mid-Stance, Group 2 display greater passive ankle dorsiflexion and decreased plantar flexor angular impulse compared to Group 1, indicative of a compensatory mechanism for flatfoot landing and a mechanical effect of plantar flexor failure. This failure also leads to decreased hip extension, aimed at maintaining anterior-posterior balance. In Push-Off, plantar flexor failure, characterised by decreased angular impulse and plantar flexion ROM, was partially compensated by increased knee extensor angular impulse. Unlike Group 1, hip flexor angular impulse does not increase, reflecting different compensation mechanisms during swinging. In Swing-Phase there was a reduction in hip and knee flexion, with increased hip abduction and pelvic elevation on the swinging side through prolonged Gluteus Medius activation. Despite similar degrees of foot drop as Group 1, Group 2 has a lower degree of flatfoot landing at initial contact, requiring less lower limb elevation to prevent tripping. Group 2 shows greater energy recovery and lower energy consumption compared to Group 1, attributed to broader muscle weakness requiring greater energy recovery and compensatory mechanisms possibly related to frontal plane movements (Don et al., 2007).

It has been suggested that hip flexors play a role in compensation for plantar flexors weakness to initiate the swing phase during walking (Ramdharry et al., 2009). This has been studied in a cohort of 18 subjects with CMT who were compared with 14 matched controls while they walked on a treadmill to a predetermined point of perceived effort. A significant reduction was observed in peak hip flexor velocity during walking and hip flexor maximal voluntary contraction. In a second session following selective fatigue of the hip flexors by performing isometric contractions, hip flexor velocity decreased immediately on walking, and walking duration was greatly reduced. This study suggests that hip flexors compensate for distal weakness and that fatigue in the hip flexors can limit walking duration (Ramdharry et al., 2009).

These findings highlight the importance of considering functional differences and the level of weakness in motion analysis studies in peripheral neuropathies to identify patterns of deviation and compensation accurately. Understanding compensatory mechanisms can inform future research in designing therapeutic exercises to target associated muscle in order to improve gait and function.

The effect of muscle strength on gait has been studied by Guillebastre et al. (2013). They examined the relationships between lower limb muscular weakness and postural and gait capacities of 26 CMT subjects and 19 matched controls. Barefoot gait and postural control were analysed using a walking mat and a force platform, respectively. Muscular strength of the plantar and dorsal ankle flexors

was assessed using the Medical Research Council scale (MRC). The CMT group was subcategorized into Group 1 and Group 2 according to their MRC score for plantar and dorsal ankle flexors muscles (up to 5 MRC and up to 3 MRC, respectively) (Guillebastre et al., 2013). Postural parameters correlated only with plantar flexor strength, whereas gait parameters correlated with both dorsiflexors and plantar flexors strength. In comparison to control, Group 2, the weaker group, showed less postural control along the medio-lateral and antero-posterior axes, whereas Group 1, the stronger group, showed less postural control along the antero-posterior axes only. Gait velocity, cadence, and step length were impaired in Group 2 more than Group 1 (Guillebastre et al., 2013). These findings highlight the role of plantar flexor muscle strength in postural control and the role of both dorsiflexor and plantar flexor muscle strength in maintaining gait performance in individuals with CMT. Reyn et. al (2019) looked specifically at correlations between the isokinetic muscular strength of knee flexors and knee extensors and walking parameters for 33 subjects with CMT1A. The isokinetic muscular strength of the knee was assessed on an isokinetic dynamometer (Cybex) and the gait was assessed by instrumented walkway analysis (GaitRite). Results showed a moderate correlation between walking speed and isokinetic muscular strength of knee extensors for the entire cohort and stronger correlations between walking speed and isokinetic muscular strength of knee extensors and knee flexors for patients younger than 50 years. This correlation highlights the importance of knee muscle strength in maintaining walking speed. Knee extensors are crucial for

controlling knee flexion during the loading response of walking. While knee flexors are involved in decelerating the leg during the swing phase and preparing for foot placement. However, the study findings showed no correlation with cadence or step/stride length (Reynaud et al., 2019). The lack of correlation between isokinetic knee muscles strength and walking cadence or stride length suggests that other factors beyond knee muscle strength are more influential in determining these specific gait parameters. This finding highlights the importance of comprehensive gait analysis in peripheral neuropathy, considering the contributions of muscle groups at the ankle and the hip levels.

Mildly affected people with CMT may not show altered gait while walking on a level surface with relatively low speed. Mild weakness can result in altered gait kinetics, kinematics, and economics during more complex ambulatory tasks. Lencioni et. al (2018) investigated biomechanics of step negotiation during walking. They compared gait during walking on level ground and steps ascending and steps descending on two-step stair of 21 CMT subjects with mild-to-moderate impairment and normalized ROM during swing and work produced at the ankle during push-off comparable to 31 healthy subjects. To assess muscle activity, each EMG profile was integrated over 100% of task duration and the activation percentage was computed in four phases that constituted the step negotiation tasks (Lencioni et al., 2018). The CMT group showed distal muscle hypoactivation with proximal adaptive motor strategies to overcome the challenge of stair

ascending and descending, even though the enrolled CMT subjects had no apparent level walking abnormalities. In both tasks they showed a dorsiflexion deficit and an increased hip flexion during the swing phase allowing for a safe clearance of the foot moving above the step edge. In addition, during ascending stance phase CMT subjects displayed less activation of vastus medialis and rectus femoris and a greater activation of hip extensor in comparison to healthy subjects. This pattern was associated to reduced moment and power at the knee joint and a considerable larger hip extensor moment and power production (Lencioni et al., 2018).

Mild impairment was found to have an effect on walking energy as well. Menotti et al. (2011) quantified the walking energy cost of a group of CMT1A subjects with low severity of walking impairment in comparison with matched controls. Oxygen uptake was measured in 8 CMT subjects and 8 healthy individuals when walking on a circuit for 5-min at their self-selected speeds (slow, comfortable, and fast). Both comfortable and fast speeds were lower in CMT group than in the control group, whereas walking energy cost per unit of distance was higher in patients than in the control group. CMT1A subjects, therefore, chose to walk slower but with higher metabolic cost compared to controls, despite no clinically evident walking impairment, which is likely due to altered walking patterns (Menotti et al., 2011).

These findings confirm the importance of comprehensive motion analysis in identifying coping strategies in individuals mildly affected by peripheral neuropathies. Cases with mild weakness may show early signs of adaptation in their energy expenditure or in ascending and descending stepping, which are more physically demanding tasks than walking on a level surface. Early detection of these signs can inform patient care and rehabilitation research, addressing these functional challenges effectively.

Besides muscle weakness, sensory loss is an important factor contributing to the challenges faced by CMT patients with walking balance. People with CMT fall frequently (Ramdharry et al., 2018). Afferent input loss was found to contribute to a delayed response to unexpected uneven surfaces while walking (Nardone et al., 2014, Van der Linden et al., 2009). However, DHMN is a condition of motor neuron loss and muscle weakness so the impact of sensory impairment will not be a significant feature in this cohort.

2.5. Current Management and Rehabilitation Methods in Hereditary Peripheral Neuropathy

Currently, there are no medical treatment for DHMN, and management is based on symptomatic treatments which include physiotherapy, surgery, and pain management. The effect of rehabilitative treatment in DHMN has not been systematically investigated.

Recently, advances in molecular genetics and molecular biology, and the development of various animal models of different neuropathies, have led to a better understanding of disease pathomechanisms. This knowledge represents a prerequisite for the development of future therapies. Although the efficacy of various molecules has been shown in vitro and in animal models, no significant positive effect has yet been showed in clinical trials in CMT and related disorders, however some clinical trials are still ongoing.

Rehabilitation is currently the only conservative symptomatic treatment available for this type of neuropathies. However, in cases with complex presentation and rigid deformities surgery found to be more beneficial to restore foot posture (Laurá et al., 2024). Rehabilitation includes different types of exercises, orthotics and devices prescribed by physiotherapists, orthotists, and occupational therapists. Interventions aim to address issues affecting locomotion and the function of daily activities of neuropathy patients including weakness, loss of balance, fatigue, and pain. Although rehabilitation does not affect the natural progression of the disease, it provides some improvement in functional status and walking stability (Dimitrova et al., 2016). Working in partnership with patients to include their preferences helps to optimize their rehabilitation program (Padua et al., 2014).

2.5.1. Ankle Foot Orthoses (AFOs)

Ankle foot orthoses (AFOs) are commonly prescribed for patients with peripheral neuropathy to support ankle and foot posture and to address foot drop. Different designs of AFOs are made from different materials; plastic, carbon fibre, or elastic fabric, working in different ways to support the ankle and foot. Size, weight, comfort, and acceptability can vary with different devices (Landfeldt et al., 2017, McCaughan et al., 2019). They give stability and support during walking (Phillips et al., 2011, McCaughan et al., 2019), can improve walking economy (Bean et al., 2001, Menotti et al., 2014b), and minimizes the load on the proximal muscles as that compensate for distal weakness (Ramdharry et al., 2012b). They are prescribed for people with CMT who are present with mild to moderate plantar flexors weakness and/or foot drop to assist the progression of the foot into swing phase and support the ankle during standing and stance phase in gait (Laurá et al., 2024).

However, AFOs may not always meet cosmetic or practical standards for the people expected to wear them (Phillips et al., 2011, McCaughan et al., 2019). Studies showed poor compliance with AFO use. Some people with CMT discard AFOs because they highlight their disability, if they are not essential for daily walking and/or are uncomfortable (Vinci and Gargiulo, 2008). Studies also indicated that CMT patients with more severe symptoms tend to use AFOs more often to improve function (Ramdharry et al., 2012b).

Recent studies evaluated the user experience of AFO as a commonly prescribed orthotics for individuals with CMT (Bertini et al., 2024, Zuccarino et al., 2021). They used an online based survey with questions related to the device and the service provided. Bertini et al. (2024) included responses from 266 CMT participants. Overall, 70% of subjects were prescribed lower limb orthoses, but 19% did not use them. AFOs was prescribed for 23% of subjects including Codivilla spring (41%), Toe- off (28%) and Peromed (24%) as the most frequently used, followed by Dyna- ankle (6%) and Foot- up (1%) (Bertini et al., 2024). Frequency of abandonment was 31% for AFOs after a mean time of use of 6.3±9.1 years. Complications were more frequently related to AFOs including skin reddening, moderate to severe pain, foot ulcerations, calluses, and emotional distress which contributed to AFO abandonments (Bertini et al., 2024). Interestingly, distal weakness and CMT severity influenced AFO acceptance. Users with moderate to severe weakness (MRC ≤3) and high disease burden (CMTES>8) reported overall higher tolerability, compliance, and perceived benefit than those with mild or no weakness (MRC ≥4-) and low disease burden (CMTES ≤8) (Bertini et al., 2024). Zuccarino et al (2021) reported a high rate of satisfaction in a 314 CMT participant, Ratings of AFO showed that greater than 50% of individuals responded in the affirmative (Strongly Agree or Agree) to most questions. However, over a third of individuals provided negative responses to multiple questions regarding their AFOs. This included 42% who indicated they dislike the appearance of their AFO, 32% who experienced discomfort, 35%

who experienced abrasions or irritations, and 36% who experienced pain with AFO use (Zuccarino et al., 2021). Both studies showed a high satisfactory rate, however they identified clear areas of improvements regarding prescription and AFO designs. A rational, patient oriented and multidisciplinary approach to orthoses prescription must be encouraged to ensure appropriate prescription. Improvement in AFO's material and customization techniques including 3D printing have potentials in improving overall satisfaction and minimize discomfort and emotional distress related to function or appearance.

Carbon fibre AFOs showed a high satisfactory rate (89%) in neuromuscular diseases population (Mnatsakanian et al., 2017). Functionally, the carbon fibre material allows the orthoses to store energy and release at pre-swing and therefore benefit patients with plantarflexion weakness (Zou et al., 2014, Bartonek et al., 2007). The effect of custom-made carbon fibre AFOs on gait has been studied by Dufek et al. (2014). They compared walking with and without AFOs in 8 CMT patients. Participants walked faster (89.4 ± 13.3 vs 115.6 ± 18.0 cm/s), with longer steps and greater frequency in the braced versus unbraced condition. As velocity increased, maximum joint moments during loading response shifted from the hip joint to the ankle and knee joints. During propulsion, the hip joint moment dominated, and most subjects loaded the brace tibial shell with an average load of 54.6% at the highest velocity and 16.6% with the lowest velocity. Energy storage in the brace averaged 9.6 ± 6.6 J/kg (Dufek et al., 2014). These

results may imply that ankle weakness can be compensated by the recoil action of the carbon fibre AFO instead of using proximal muscles at the knee and hip as described by Ramdharry et al. (2012,2009).

Customization using 3D printing was explored in children with CMT (Wojciechowski et al., 2022). They included 14 CMT children and comprehensively assessed their gait with 4 different conditions including traditional AFOs, 3D printed replica AFOs (same design as traditional AFOs), 3D printed redesigned AFOs, and a shoes only control condition. The 3D printed replica had the same effect on gait as the traditional AFO. However, the redesigned 3D printed AFO significantly lighter (mean −35.2, SD 13.3%), and normalized maximum ankle dorsiflexor moment in loading response compared to shoes only and traditional AFOs (Wojciechowski et al., 2022). The study showed overall improvement functionally with redesigned 3D printed AFOs, however, CMT is known for its variability and analysing the effect of AFO would be more useful if the study participants were sub-grouped according to their functional variations. Similar to the study by Õunpuu et al. (2021), they explored the effect of 4 types of AFO on gait in CMT children. Due to the gait variability between cases, the cohort was sub-grouped for analysis into 2 groups. Group A included patients who had increased peak dorsiflexion in terminal stance or plantar flexion failure, and group B included patient who had increased equinus in mid-swing or foot drop (Õunpuu et al., 2021). Across the cohort there was a

significant improvement in most of the gait parameters, spatiotemporal, kinematics and kinetics. But when looking at gait based sub-grouping, only group B, who had foot drop, showed a significant improvement in ankle dorsiflexion angle, ankle moments and power. Improvement was in parameter where a sufficient dorsiflexion angle is needed. children with foot drop, without AFOs, showed compensations including increased peak hip power generation at toe off and increased hip flexion in swing to clear the foot. But these compensations were no longer needed when ankle angle in swing was corrected. With the AFO there was a reduction in the peak hip power generation at toe off and mean peak hip flexion in swing.(Õunpuu et al., 2021). On the other hand, in group A, who had plantar flexion failure, the only change in stance was a decrease in peak ankle power generation. This indicates that AFOs reduce the ankle plantar flexion abilities at toe-off. In this group, the main gait disfunction was plantar flexion failure which caused increased ankle dorsiflexion with wight bearing, delayed heal raise, and decrease in ankle moment and power in terminal stance (Õunpuu et al., 2021). The AFO design and material used did not allow enough plantar flexion range of motion and it was not flexible enough to store energy with weight bearing, therefore, inhibited the use of the existing plantar flexor strength at toe-off (Õunpuu et al., 2021). With mild to moderate plantar flexors weakness, less stiff and less restrictive AFO would be more appropriate. Carbon fibre spring splints, for example, can increase ankle power generation in pre-swing. The carbon fibre material allows the orthoses to store energy and release on push off

and therefore benefit patients with plantarflexion weakness (Bartonek et al., 2007).

In another study by Van Der Wilk et al. (2018), they introduced adjustable mechanical hinge AFOs to optimize ankle range of motion and AFO stiffness needed throughout the gait cycle (Van Der Wilk et al., 2018). However, it was applied on one case of flaccid paralysis and not specifically designed for CMT cases that could be presented with limited range of motion and foot deformities.

In the long term, AFOs combined with exercises can optimize a patient's function. Bensoussan et al. (2016) reported a 10-year follow-up case where bracing with orthopaedic shoes along with physical therapy twice a week was effective in treating pain, improving the gait, and enhancing the walking distance (>500 m) without assistive device in a 55 year old woman with Charcot-Marie-Tooth disease. Moreover, during the 10 years of follow-up, the physical examination parameters had stabilized since 2001; falls, sprains and walking distance had improved as compared to 2000; pain had alleviated since 2001 and gait parameters had improved up to 2007 and stabilized between 2007 and 2011 (Bensoussan et al., 2016). However, the results of this study cannot be generalized as it presents a single case, and the level of therapy received may not be transferrable to other health systems.

2.5.2. Therapeutic Exercise

Therapeutic exercise is one of the few available treatments for peripheral neuropathies (Montes and Garber, 2017). Exercise has the potential to improve balance, fatigue, physical performance, psychological condition and therefore the quality of life (Roberts-Clarke et al., 2016b, Vita et al., 2016, Ramdharry et al., 2012c, Burns et al., 2017). However, the long term effect and safety of exercises on peripheral neuropathy remains unclear (Sman et al., 2015). Currently, there is a paucity of evidence-based exercise protocols for peripheral neuropathy and designing exercise plans depends mainly on the therapist experience (Djordjevic et al., 2017, Montes and Garber, 2017, Chetlin et al., 2004a, Chetlin et al., 2004b). Different types of exercises can include strengthening, aerobic, and stretching exercises. Combinations of these types have also been proposed (Maggi et al., 2011).

As muscle weakness is the major clinical manifestation of CMT, studies in the literature mainly explored strengthening exercises as a treatment. A study by Djordjevic and colleagues (2017) aimed to examine differences in strength among exercising and non-exercising CMT individuals. This was the first study that explored the association between self-directed full body exercises and muscle strength. Two hundred and ninety seven participants with CMT who exercised were significantly stronger in elbow flexion and dorsiflexion than those who did not exercise. They suggested that self-directed exercise may be a convenient,

sustainable, and effective method of improving strength and decreasing disability in this population (Djordjevic et al., 2017). However, due to its retrospective nature, the study lacked a standardized measurement or definition of exercise. It also lacked longitudinal strength measurements.

An exercise trial by Chetlin and colleagues (2004) explored the effect of a 3 month home based resistance training program combined with creatine supplement (group 1) or with placebo (group 2) in 20 CMT participants. Among both groups, results showed that strength and function improved with increased type I muscle fibre diameter (48.2 ± 14.2 μm vs. 55.4 ± 14.8 μm) in response to resistive exercises (Chetlin et al., 2004b, Smith et al., 2006). Comparison between groups showed changes in myosin heavy chain composition in the creatine group (group 1) which was associated with improvement in function (faster chair rise-times). However, difference in muscle strength between groups was not detected (Chetlin et al., 2004b, Smith et al., 2006). Nine of the 20 participants agreed to undergo a follow up assessment after 20 to 34 months after the completion of the program, and only 3 of the 9 participants reported continuing the resistance exercises program. Findings showed that both those who continued training and those who discontinued training lost strength, but functional ability was lost only in those who discontinued training (Chetlin et al., 2010). This trial provided a protocol for exercises prescription which can inform clinical trials and exercise progression (Chetlin et al., 2004a). However, they failed to ascertain the effect of

creatine supplement on muscle strength possibly due to the lack of appropriate muscle strength measurement as their protocol evaluated strength only at the strongest angle, where moment arms of effort were maximized (90°). In exercise protocols with low to medium intensity, it is possible that patients experienced improvement in strength at the weaker angles, therefore, measuring isometric strength in different angles throughout the range or using isokinetic testing might detect strength gain (Knapik et al., 1983, Marginson and Eston, 2001, Yang et al., 2014, Ha and Han, 2017).

Considering the role of the proximal muscles in compensatory gait patterns, as discussed in section 2.4.6, it has been hypothesized that targeting proximal muscles in strengthening exercises has a potential in improving gait. This hypothesis was explored by a pilot study in CMT (Ramdharry et al., 2014). They investigated the effect of a 16-week home-based programme of resistance training on hip flexor muscle strength in 26 people with CMT. Results showed no negative effects and a high level of adherence (93%). Despite a small change in strength on the left side, no changes were observed in walking speed and endurance measures. Variability of cases, the exercises protocol, and the outcome measures used may contributed to the lack of more significant improvement as the authors discussed (Ramdharry et al., 2014). Moreover, the condition of the foot and ankle, and the appropriate foot and ankle support, was not considered in the assessment or treatment protocol. Vinci (2001) suggests that if distal

alterations causing postural changes in stance are not first corrected, proximal strengthening may not be effective (Vinci, 2001).

Proximal exercises, however, were found to be more helpful in improving balance in CMT. Individualized multi-sensory balance training combined with proximal lower limb and trunk strength training showed moderate improvements in postural stability in CMT1A people (Dudziec et al., 2024). Sensory impairment and foot deformity are commonly present in CMT which may contribute to balance impairments in this cohort more than DHMN.

Overwork weakness has been a concern in therapeutic exercises for people with CMT. Studies that have investigated the role of overwork weakness in CMT have typically compared the muscle strength of dominant with nondominant sides of the body, mainly because overwork weakness occurs in muscles that are used more frequently. However, these studies gave contradictory and inconclusive results (Roberts-Clarke et al., 2016a). Studies using manual muscle testing as an outcome measure, which is somewhat subjective and dependent on the strength of the examiner, are unlikely to be sensitive enough to detect smaller differences in strength between dominant and nondominant sides (Piscosquito et al., 2014). Using hand dynamometry to measure grip and pinch strength might be a more objective measure of muscle strength. However, the fact that grip and pinch strength is generated not only by the intrinsic hand muscles, but also by relatively unaffected forearm muscles that can undergo hypertrophy with overuse as in

healthy subjects (Prada et al., 2018). It has been argued that as normal control showed stronger dominant side, an equal strength between the dominant and non-dominant sides in CMT could possibly be a sign of overwork weakness (Vinci et al., 2009).

A study by Burns et al. (2017) explored the safety and efficacy of 6 months progressive resistance exercise using adjustable exercise cuffs for foot dorsiflexion weakness in children with Charcot-Marie-Tooth disease (Burns et al., 2017). They randomly assigned 60 children to receive progressive resistance exercise (n=30) or sham training (n=30). The primary efficacy outcome was the isometric dorsiflexion strength assessed by hand-held dynamometry. The primary safety outcome was MRI analysis to quantify muscle volume, intramuscular fat fraction, and any sign of acute denervation. The difference between the two groups was measured at baseline, 6, 12, and 24 months (Burns et al., 2017). Analysis showed no immediate differences in strength between groups at 6 months, however, there was an overall gain of 5% in dorsiflexion strength after 24 months in the resistance exercise group compared with a deterioration of 23% in the sham group (Burns et al., 2017). Moreover, there was no effect on muscle volume, intramuscular fat fraction, and no signs of acute denervation or overwork on MRI (Burns et al., 2017). These finding suggest that resistance training has an effect in preserving muscle tissue over time safely rather than improving muscle strength by increasing muscle volume. MRI signal intensity as an indicator for

acute denervation was utilized to assess safety of the exercise programme used in this study (Burns et al., 2017), which supports the accuracy of the study results as discussed in section 2.2.3. The gain in muscle strength in the resistance exercises group without change in the MRI muscle volume supports the hypothesis that strength gain induced by resistance training can be due to neural enhancements rather than changes in the contractile components of skeletal muscle. Resistance training enhance corticospinal excitability, improving the transmission of neural signals from the brain to muscles. This results in better motor unit recruitment and increased firing rates leading to greater force production. (Herda, 2022, Deschenes and Kraemer, 2002).

Impaired walking capacity and aerobic function due to muscle weakness is often reported in people with CMT. Interval exercises cycling training (El Mhandi et al., 2011, El Mhandi et al., 2008) and anti-gravity treadmill training (Knak et al., 2017) showed improvement in cardiorespiratory capacities and ambulatory functions in small CMT cohort (8 and 5 participants respectively). However, these studies were undertaken in hospital environments and supervised by health professionals. It has been suggested that transitioning exercise from hospitals, as an intervention, to community gyms, as a leisure activity, could facilitate physical activity and encourage self-management (Wallace et al., 2019). This hypothesis was tested in 23 people with CMT1A and 17 people with Inclusion Body Myositis (IBM), they were included in a 12-week aerobic training program using exercise

bicycles compared to a control period. The training occurred 3 times per week in community gyms local to the participants. Support was available from trained gym staff and a research physiotherapist (Wallace et al., 2019). The study findings provided evidence that aerobic training programs increase aerobic capacity for people with CMT1A and IBM. They showed that supervised aerobic training in community leisure facilities is feasible and safe. However, there was a low rate of continuation following the study related to time, expenses, and self-confidence as reported by study participants (Wallace et al., 2019). Therefore, developing an approach to self-management support can improve adherence to physical activity, help overcome challenges, and improve the quality of life in people with neuromuscular conditions.

People with CMT carry out a low level of activity in comparison to healthy controls, which correlated with muscle strength in adults and children (Menotti et al., 2014a). Physical activity and quality of life are influenced by the CMT severity as well as mental status. The mental domain of SF-36 (the Short Form 36 Health Status Survey for assessment of quality of life) strongly correlate with the CMT exam score ($r = 0.75$, $p = 0.03$) (Roberts-Clarke et al., 2016b). This raises the possibility that the negative effects of CMT on quality of life were more related to how the individual coped with the condition psychologically, than with the specific clinical realities of how severely they were affected. People with CMT reported that fatigue has a major impact on physical activity (Ramdharry et

al., 2012c). A later exploratory study revealed that personal factors such as fatigue, poor balance, muscle weakness, and pain were important barriers for physical activity (Anens et al., 2015). While self-efficacy for physical activity, activity-related factors, and assistive devices were facilitators of physical activity (Anens et al., 2015). Using guidance, modelling, and feedback to increase self-efficacy can be utilized for behavioural modification. Health systems and professionals need to be aware of the barriers to physical activity, such as fatigue, to support patients with CMT disease to overcome these barriers, and designing interventions to successfully support engagement in physical activity need to be multi-level to achieve meaningful change, e.g. education and supported self-management programs (Buscemi et al., 2023).

Chapter 3: Study Objectives

Based on the gaps in knowledge explored in chapter 2, I am presenting in Figure 7 the main objectives for the thesis and associated research questions stemming from each objective.

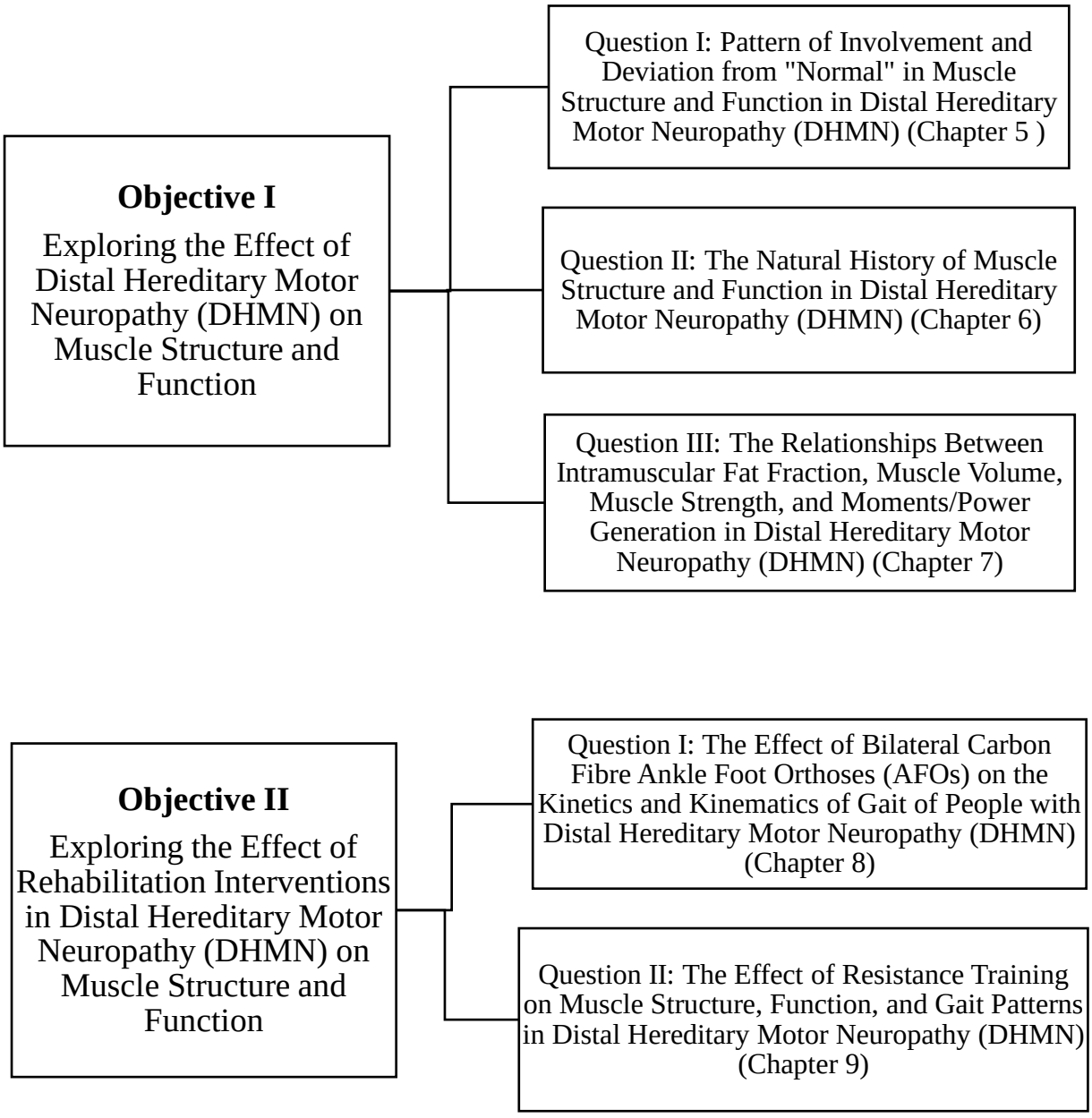


Figure 7: Flowchart outlining the thesis objectives and research questions.

Chapter 4: Methods

This thesis discusses five questions each using similar methods, all of which are described in this chapter. Any methodological details specific to one or another study are indicated in Figure 8.

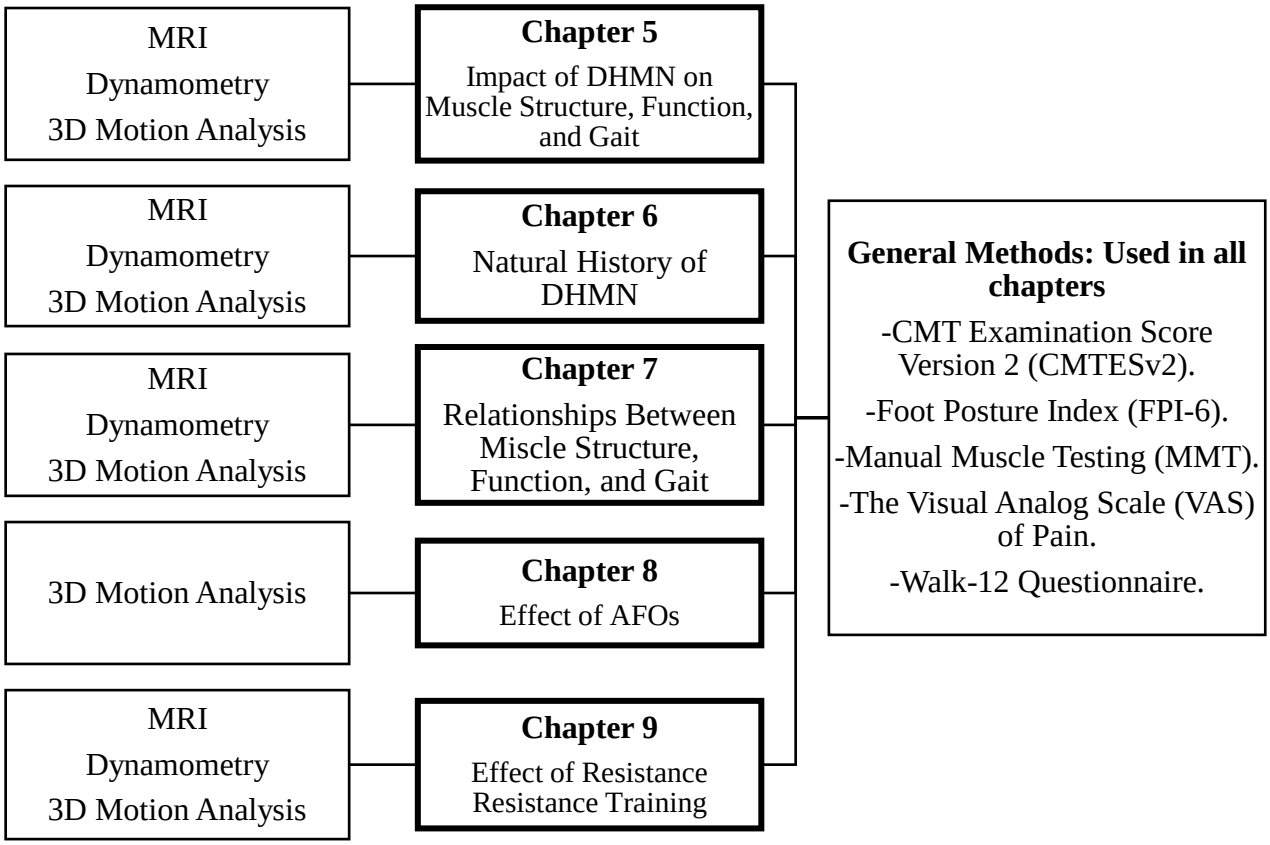


Figure 8: Summary of the measurements' methods used in each chapter. AFOs, Ankle Foot Orthoses.

4.1. MRI Methods

4.1.1. MRI Protocol

The MRI protocol was primarily developed by the Neuromuscular MRI team from the Queen Square Centre for Neuromuscular Diseases, UCL: Dr Stephen Wastling, Dr Jasper Morrow, and Professor John Thornton. This protocol has been used in a number of exploratory and natural history studies in neuromuscular diseases (Morrow et al., 2016, Kugathasan et al., 2019, Morrow et al., 2018), found to correlate with validated clinical measurements (CMTES) and Dynamometry (Morrow et al., 2016), and have a minimal association with age, gender, and body wight (body mass index) (Morrow et al., 2014)

4.1.2. Hardware

Imaging was performed with a whole-body MR PrismaFit system (Siemens Healthineers) operating at 3 Tesla (T) in body-coil transmit mode with a 36-channel radio frequency (RF) receive coil designed to image the legs (PS matrix coil), which includes a dedicated leg rest to support both calf. A 16-channel foot coil was used to scan one foot. The subjects were imaged feet-first and supine, with thigh and calf imaged separately.

4.1.3. Positioning

A scout-image-based slice prescription was used. The right knee joint space was identified on a coronal scout image and an axial slice through the knee joint space. The main imaging sections was then prescribed such that the central slice was a

fixed distance of 20 cm above, and 15 cm below the right knee joint space for thigh and calf, respectively (Fischmann et al., 2014).

4.1.4. Choice of Anatomical Coverage

DHMN is a length-dependent neuropathy, and the pathology is expected to start distally, progressing proximally over time (Rossor et al., 2012b). Lower limb muscles including thigh, calf, and foot were chosen as the region for study as they are a key site of pathology in DHMN and weakness in these areas is a key cause of gait alteration in this patient group. Lower limb imaging has practical advantages since both limbs may be imaged simultaneously, which reduces the scan time (compared to scanning the arms for example). Unlike calf and thigh, both feet cannot be scanned simultaneously hence a single foot was scanned at all visits. Data from a single foot is adequate for the analysis considering the symmetrical pathological nature of peripheral neuropathies (Rossor et al., 2012b).

Both limbs were scanned within the field of view (FOV). Axial-slice matrices and FOV is 256x128 and 41x200mm for thigh and calf level images, except for FF acquisitions where matrices were 448x224. A single foot was scanned with the minimal FOV (>12mm).

4.1.5. MRI Sequences

4.1.5.1. Primary Sequence

Dixon fat fraction measurement

A 3D 3-point Dixon acquisition was performed for a single foot, and both calf and thigh from the upper thigh to ankles. This provides accurate measures of muscle cross sectional area and percentage intramuscular fat accumulation.

Three 3D gradient-echo acquisitions were performed with parameters TE1/TE2/TE3=3.45/4.60/5.75ms, TR=23 ms, flip angle= 5^{o}, bandwidth 450 Hz/pixel, NEX=1, 96 contiguous 5mm slices, 448x224 matrix (160x160 for foot), iPat=2. Phase unwrapping is performed using PRELUDE (FSL, FMRIB, Oxford) (Smith et al., 2004) and after fat (F) and water (W) image decomposition (Glover and Schneider, 1991), FF calculated as FF = 100% x F/(F+W). The TE1=3.45ms image is used for the ROI placement.

4.1.5.2. Secondary Sequences

T1 weighted imaging

Standard T1-weighted images were acquired for one foot and both calf to allow qualitative assessment of intramuscular fat accumulation. Images were acquired with a turbo-spin-echo readout prior to commencing the quantitative protocol (TR/TE=700/8.1ms, 10 slices, 10mm thickness, 10mm slice gap, 256x128

matrix, iPat acceleration of 2, 444 Hz/pixel bandwidth (BW), TSE factor=3, refocusing flip angle (fa) 120º, NEX=1, acquisition time (TA) = 63s).

Short tau inversion recovery imaging (STIR)

STIR imaging was performed for the single foot, both calf, as a qualitative measure of acute muscle denervation or oedema.

For the calf, STIR was acquired with a (TR/TE/Inversion Time = 5500/57/220ms, NEX=1, flip angle 180°, parallel imaging factor =2) imaging performed with (10x10mm slices, 10mm gap, 256x128 matrix).

For the foot, STIR was acquired with a (TR/TE/Inversion Time = 5700/64/220ms, NEX=2, flip angle 180°, parallel imaging factor =2) imaging performed with (30x6mm slices, 1.5mm gap, 192x144 matrix).

T2-Relaxometry (T_{2m})

Multi-echo T2-relaxometry was performed of a single foot and through both calf. This was analysed to quantify the T2 relaxation time of the water within the muscle, as a marker of acute denervation or muscle injury. Multi-echo turbo spin echo with 22 echoes (TE 10 to 220 ms in 10 ms steps), TR=3500 ms, 1 average, flip angle 180°, 128x128 matrix, iPat 2. T_{2m} was calculated as a multi-component slice profile-corrected EPG model [$s(TE) = (1 - ff_a) \cdot sEPG(B_1f, T_{2m}, \alpha, \sigma_N, TE) + ff_a \cdot [\, 0.33 \cdot sEPG(B_1f, T_2=40ms, \alpha, \sigma_N, TE) + 0.67 \cdot sEPG(B_1f, T_2=198ms, \alpha, \sigma_N, TE)]$ was fitted pixel-wise to the data using maximum likelihood estimation

(MLE) in a custom-written MATLAB tool, to estimate T_{2m}, the B_1 field error factor (B_1f) , apparent fat fraction (ff_a), overall amplitude (α) and Rician noise SD (σ_N). The fixed 2-component fat signal model parameters were determined in a preliminary calibration as mean values estimated from 4 subcutaneous fat ROIs in 8 representative subjects. The TE1=3.45ms image is used for the ROI placement.

4.1.6. Qualitative Image Analysis

4.1.6.1. Slice Selection

The axial slices were identified at 15cm distal to the right knee joint space for calf scan (Fischmann et al., 2014). Coronal foot slice was identified using the first tarsal metatarsal joint from sagittal view.

4.1.6.2 Qualitative Image Grading

Time and group blinded qualitative analysis of apparent fatty infiltration on the T1 weighted images was performed by grading using the 6-point Mercuri scale (Mercuri et al., 2003) (0=normal,1=mild fatty streaks, 2a=early confluence, 2b=fatty infiltration 30-60%, 3=fatty infiltration >60%, 4=complete fat replacement). For STIR hyperintensity a three point scale (0=none, 1=mild, 2=marked) was used to grade the intensity and a four point scale (0=none, 1=< 30%, 2=30%-60%, 3=>60%) was used to grade the extent. Repeat scans were graded without reference to baseline scans. The same muscles were assessed as in the quantitative analysis below.

4.1.7. Quantitative Image Analysis

4.1.7.1. Slice Selection

The axial slices were identified at 20cm proximal and 15cm distal to the right knee joint space for thigh and calf scan, respectively (Fischmann et al., 2014). Coronal foot slice was identified using the first tarsal metatarsal joint from sagittal view.

4.1.7.2. Region of Interest Definition

I undertook the established training programme for defining regions of interest (ROI) on lower limb muscles and assessed as competent in this before starting analysis in this dataset (Morrow et al., 2024). ROI was defined for each subject on the single slice as described above, at mid-thigh, mid-calf, and mid-foot level of an unprocessed Dixon acquisition (TE=3.45ms) using ITK-SNAP software (Yushkevich et al., 2006). ROIs were defined to encompass the entire muscle cross-sectional area to the fascia (Table 8).

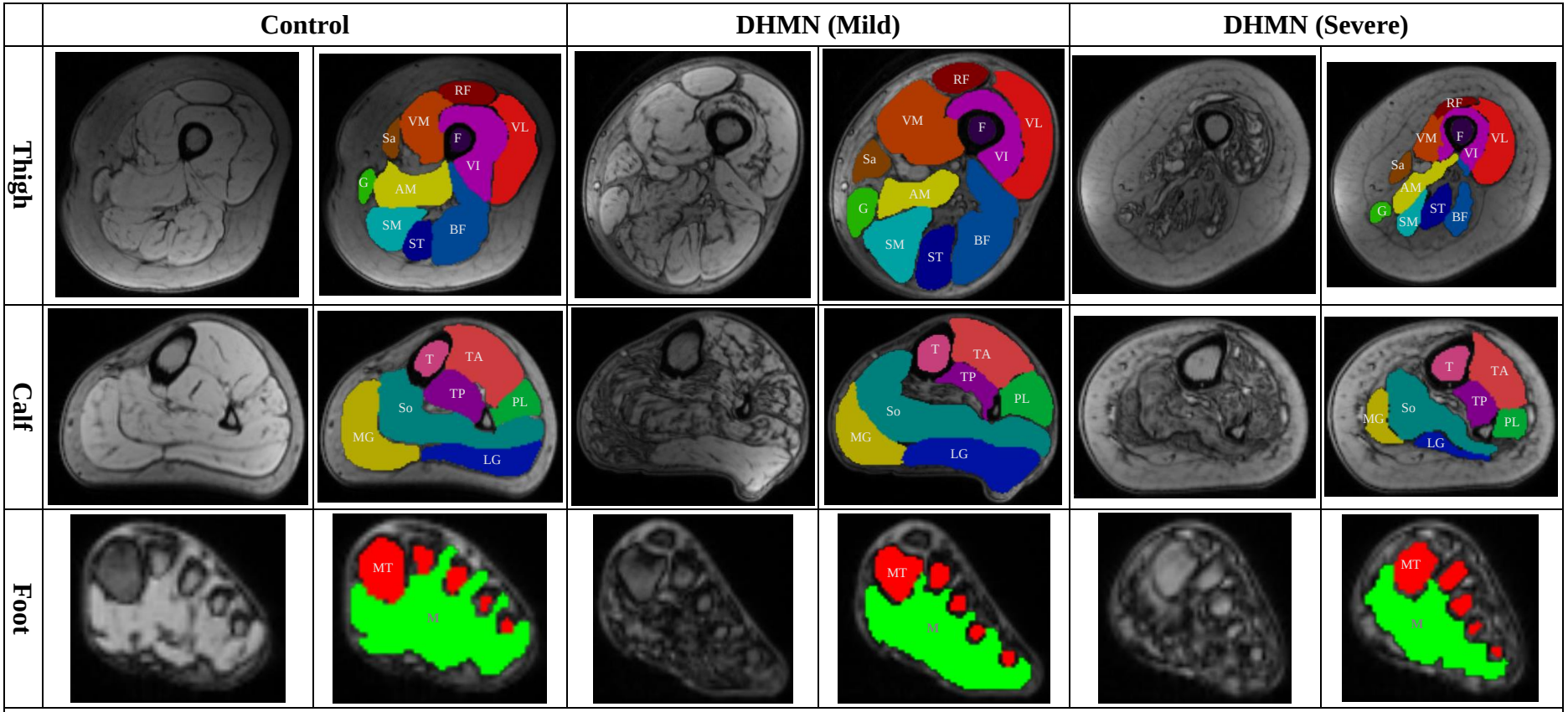
Left and right limb ROIs were defined for the rectus femoris, vastus lateralis, vastus intermedius, vastus medialis, semimembranosus, semitendinosus, biceps femoris, adductor magnus, sartorius, gracilis, tibialis anterior, peroneus longus, lateral gastrocnemius, medial gastrocnemius, soleus, tibialis posterior, and foot muscles (Table 8).

4.1.7.3. Transfer of Region of Interest to Maps

The ROIs were transferred to the co-registered parameter maps (FF, T2) and quantitative parameters extracted. All generated maps were inspected visually for correct ROI placement and presence of artefact. ROI values originating from areas of gross artefact were excluded from the analysis.

4.1.7.4. Data Analysis

For each muscle custom written software extract mean fat fraction (FF), cross-sectional area (CSA), and muscle water (T_{2m}) from whole ROI. After extraction, all data was cross-checked for outliers and errors identified were corrected. In addition to individual muscle values, summary measures for each parameter were created for all muscles at thigh level, calf levels, and foot level separately. Total CSA and the remaining muscle area were also calculated. Longitudinal changes were quantified on a muscle-by-muscle, parameter-by-parameter basis, and combined as detailed above to create separate all-muscle summary variables at thigh, calf, and foot levels.



RF= Rectus Femoris, VL= Vastus Lateralis, VM= Vastus Medialis, VI= Vastus Intermedius, Sa= Sartorius, G= Gracilis, AM= Adductor Magnus, SM= Semimembranosus, ST= Semitendinosus, BF= Biceps Femoris, TA= Tibialis Anterior Group, MG= Medial Head Of Gastrocnemius, So= Soleus, TP= Tibialis Posterior, PL= Peroneus Longus, LG= Lateral Head Of Gastrocnemius, M= Foot Muscles, F= Femur, T= Tibia. MT= Metatarsals.

Table 8: Sample axial images of unprocessed Dixon sequence (echo time=3·45 ms) with overlaid muscle regions of interest of the left side for the same image in a healthy control, mild DHMN case, and severe DHMN case.

4.2. Dynamometry Methods

4.2.1. Hardware

The HUMAC NORM Testing & Rehabilitation System (CSMi, Massachusetts, USA) (Figure 8) was used to explore muscle function. Its components and capabilities are designed to conduct isometric, isotonic, isokinetic, and passive range of motion tests for all major muscle groups. Key components of the HUMAC NORM Dynamometer system include:

Dynamometer Unit: The core of the system equipped is with a torque sensor to measure force across various movements and exercises.

Computer and Software: The system comes with the HUMAC application program (CSMi, Massachusetts, USA) to control the dynamometer, collect data, and analyse performance. The software provides real-time feedback, detailed reports, and allows for customization of testing protocols.

Adjustable Chair or Bench: This ensures that the participant can be positioned correctly for a wide range of tests, accommodating different exercises and body sizes.

Attachments and Accessories: Various grips, straps, and attachments allow for a broad spectrum of muscle testing and rehabilitative exercises, targeting specific muscle groups accurately.

Safety Features: Includes emergency stop mechanisms and adjustable limits on range of motion to ensure the safety of users during testing and rehabilitation sessions.

Figure 9: HUMAC NORM Testing & Rehabilitation System (CSMi, Massachusetts, USA).

4.2.2. Parameters Measured

4.2.2.1. Isometric Torque

The NORM system measures isometric Peak torque (Newton-Meter) while the participant is exerting maximum force against a stationary arm at a specific joint angle.

4.2.2.2. Isokinetic Torque

The NORM system measures isokinetic torque (Newton-Meter) instantaneously every half degree in the range of motion. Peak torque is the maximum torque production during movement throughout the range of motion.

4.2.2.3. Gravity Effect Torque

When testing with the NORM System, the gravity effect torque of the limb and the input adapter was determined; the computer can then correct for the effect of gravity. This is called Maximum Gravity Effect Torque (MAX GET). The computer uses this value and multiplies it by the cosine of the angle at each point in the participant's range of motion. This is the amount of torque that is being contributed or taken away due to the weight of the limb and input accessory. For example, during knee extension, when gravity resists the motion, the appropriate gravity effect torque value is added, and the reported torque will be calculated as:

*Reported Torque = Measured Torque + (MaxGET * Cosine (Angle))*

During knee flexion, when gravity assists the patient, the appropriate gravity effect torque value is subtracted, and the reported torque will be calculated as:

*Reported Torque = Measured Torque - (MaxGET * Cosine (Angle))*

4.2.3. Dynamometry Protocol

Patients and controls had detailed lower limb dynamometry on a HUMAC NORM dynamometer (CSMi, Massachusetts, USA). Dynamometry was

performed according to Table 9. Hip extension, hip flexion, knee extension, knee flexion, ankle dorsiflexion and ankle plantarflexion were assessed bilaterally using both isometric and isokinetic protocols and the maximum torque in Newton-Meter (Nm) was recorded for analysis. Isometric assessments consisted of three attempts following a practice run of 3 seconds duration with 10 seconds interval of which the best attempt was selected. For the isokinetic assessments, following a practice run and 10 seconds interval, three successive movements through full range were performed and the highest value obtained was selected. The machine setup was recorded at first visit using the included software, which was then retrieved to allow identical set-up on repeat testing. Isometric and Isokinetic data were exported as a report in PDF format. This protocol has been used in previous research in similar neuromuscular conditions (Wallace et al., 2019) (Morrow et al., 2016).

Joint	**Muscle Function**	**Movement**	**Angle(°)/Speed(s)**
Hip	Isometric	Extension	45°
		Flexion	45°
	Isokinetic	Extension/ Flexion	60°/60s
Knee	Isometric	Extension	45°
		Extension	90°
		Flexion	45°
		Flexion	90°
	Isokinetic	Extension/Flexion	60°/60s
		Extension/Flexion	120°/120s
Ankle	Isometric	Plantarflexion	10°
		Dorsiflexion	10°
		Dorsiflexion	30°
	Isokinetic	Plantarflexion/Dorsiflexion	60°/60s

Table 9: Dynamometry protocol.

4.3. Three-Dimensional Motion Analysis

4.3.1. Kinematics

To describe 3D kinematics, the rigid link segment model is used. In such method, the lower part of the body is modelled as a rigid chain of segments interconnected by joints, assuming that the length of each defined segment is a constant distance between the centre of the proximal and distal joint and the centre of mass for each segment is located in the middle of the mass. In gait analysis, segments including foot, leg, thigh, and pelvic are used. These segments are connected with ankle, knee, and hip joints respectively (Robertson DGE et al., 2014b). The model measures Euler rotations (Figure 10) of segments in relation to the pelvis.

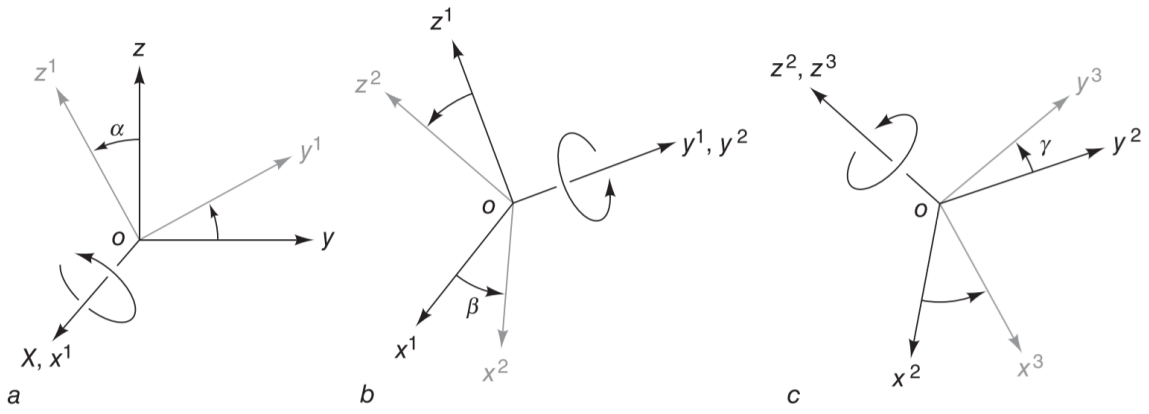


Figure 10: Euler angles are quantified as ordered rotations about 3 axes and represent joint motion displacements in the anatomical body planes.
Euler sequence XYZ of rotations first about (a) the X-axis of the stationary coordinate system (α); then about (b) the new y^1-axis (β); and finally, about (c) the z^2-axis (γ). Figure adapted from (Robertson DGE et al., 2014).

4.3.1.1. Segmental Angle Kinematics

The model measures the amount of Euler displacement of a segment in relation to the local coordinate system during gait. A local coordinate system or embedded vector bases (EVB) is consisted of three orthogonal axis (Table 10, Figure 11) and defined for each segment using the joint centre and markers including virtual and actual markers. The centre of each joint, and relevant virtual markers was automatically computed using markers placed on specific anatomical points and measurements using a calliper. Markers extended away from the body, on the pelvic frame, thigh wand, and tibial wand, were used to improve visibility and to capture segmental rotation around the uz axis at the local coordinate system (Table 10, Figure 11) (CharnwoodDynamics, 2004, Robertson DGE et al., 2014a).

EVB	Principal axis	2nd axis	3rd axis
Foot	Line connecting the heel and toe markers that is offset by 1/2 inter-malleolar distance (u_x)	Line running from the heel marker to ankle marker and orthogonal to the principal axis (u_z)	Orthogonal to 1st and 2nd axes (u_y)
Shank	Ankle joint centre to knee joint centre (u_z)	Tibial wand orientation orthogonal to principal axis (u_x)	Orthogonal to 1st and 2nd axes (u_y)
Thigh	Knee joint centre to hip joint centre (u_z)	Thigh wand orientation orthogonal to principal axis (u_x)	Orthogonal to 1st and 2nd axes (u_y)
Pelvis	Line between right and left ASIS markers (u_y)	Line connecting mid PSIS to mid ASIS and orthogonal to the principal axis (u_x)	Orthogonal to 1st and 2nd axes (u_z)

Table 10: Derivation of embedded vector basis (EVBs) for each segment.

u; unit vector, x; axis x, y; axis y, z; axis z.

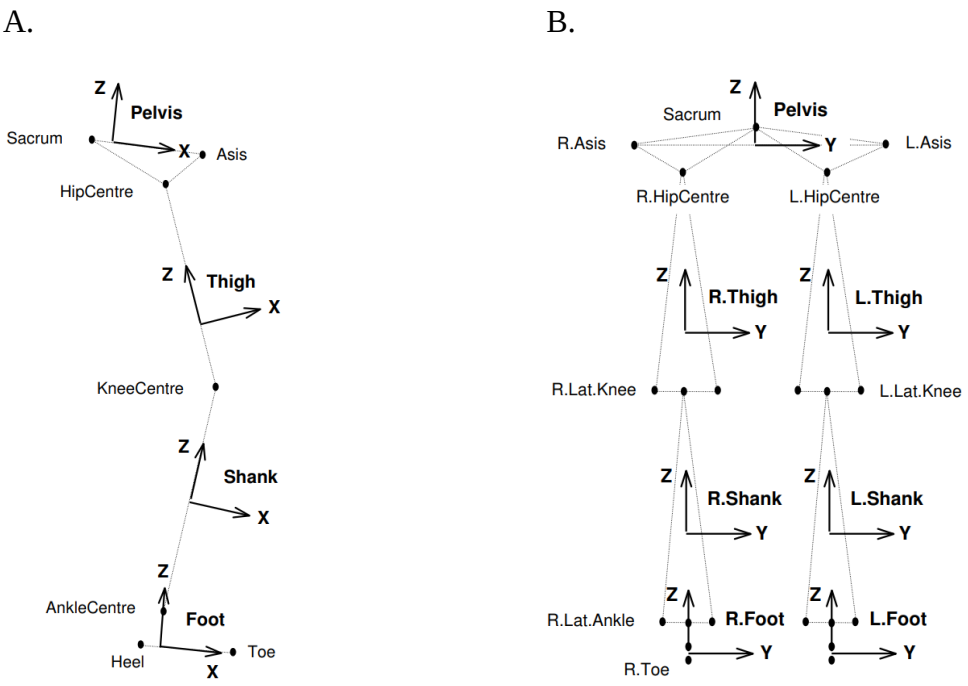


Figure 11:Segment Embedded Coordinates in sagittal (A) and frontal (B) view. Adapted from CODAmotion ODIN user guide.

4.3.1.2. Inter-Segmental (Joint) Angle

The intersegmental joint angle is the Euler angle between two segments on either side of the joint. The rotation of the distal segment about the proximal segment occurred in the following sequence: Z (internal/external axial rotation) then X (medio-lateral bending) then Y (flexion/ extension). Distal segmental orientation (distal EVB) was described in relation to the proximal segment (proximal EVB) except pelvis and foot segments were described according to the global coordinate system (GCS) (Table 11). Once the angles were known, the time derivatives were calculated to give angular velocity and acceleration (CharnwoodDynamics, 2004).

Joint Angle	Distal Segment	Proximal Segment
Hip Joint Angle	Thigh EVB	Pelvis EVB
Knee Joint Angle	Shank EVB	Thigh EVB
Ankle Joint Angle	Foot EVB	Shank EVB
Pelvic Rotation	Pelvis EVB	GCS
Foot Rotation	GCS	Foot EVB

Table 11: Relation of distal segment Euler angles to a reference frame.
EVB; Embedded vector basis, GCS; global coordinate system.

4.3.2. Kinetics

Inverse dynamics is a biomechanical approach where Equations of motion are used to calculate Joint forces, moments, and power from Segmental inertial characteristics, Kinematics (positions, velocities, accelerations), and External forces (ground reaction force) (Robertson DGE et al., 2014c).

Kinematics data are measured as described in the previous section. External forces (ground reaction force) are measured using force plates impeded in the walkway. Segmental inertial characteristics are estimated from anthropometric data based on the subjects' height, weight, and gender as they are important in calculating the segment length, segment mass, and centre of mass. To simplify inertial properties, each segment is assumed to have fixed and uniform distribution of mass around a longitudinal axis connecting the joint centres. Once kinematics, external force, and inertial characteristics are known, automatically computed scripts are used to solve equation of motions and identify forces, moments, and power around the lower limb joints (CharnwoodDynamics, 2004). The net moment or torque at the major joints during walking, is the sum of all the active moments generated by muscle contraction, and passive moments generated

by soft tissue, ligaments, and bone to bone force. Joint power represents the rate of change of energy. Regions of positive power represent power generation through concentric muscle activity. And regions of negative power represent power absorption through eccentric muscle action.

4.3.3. Lab Settings and Equipment

4.3.3.1. Hardware

The CODA motion CX-1 3-D Motion tracking system (Coda cx-1; Charnwood Dynamics, Leicestershire, UK) was used for calculations of 3-dimensional segmental gait analysis. The measurement unit contains three pre-aligned solid-state cameras which track the position of a number of active markers (infra-red LEDs) in real-time with 200Hz sampling rate. The interfacing for synchronous analogue and digital data acquisition enables measuring the ground reaction force and torque using two force plates impeded in the walk walkway (AMTI's AccuGait, Watertown, MA, USA).

4.3.3.2. Marker Placement

The Helen Hayes model with 24 markers for the lower body was used as suggested by the CODA motion system company. The different position of wands, marker drive boxes, and markers are described on Figure 12 (CODAmotion, 2016).

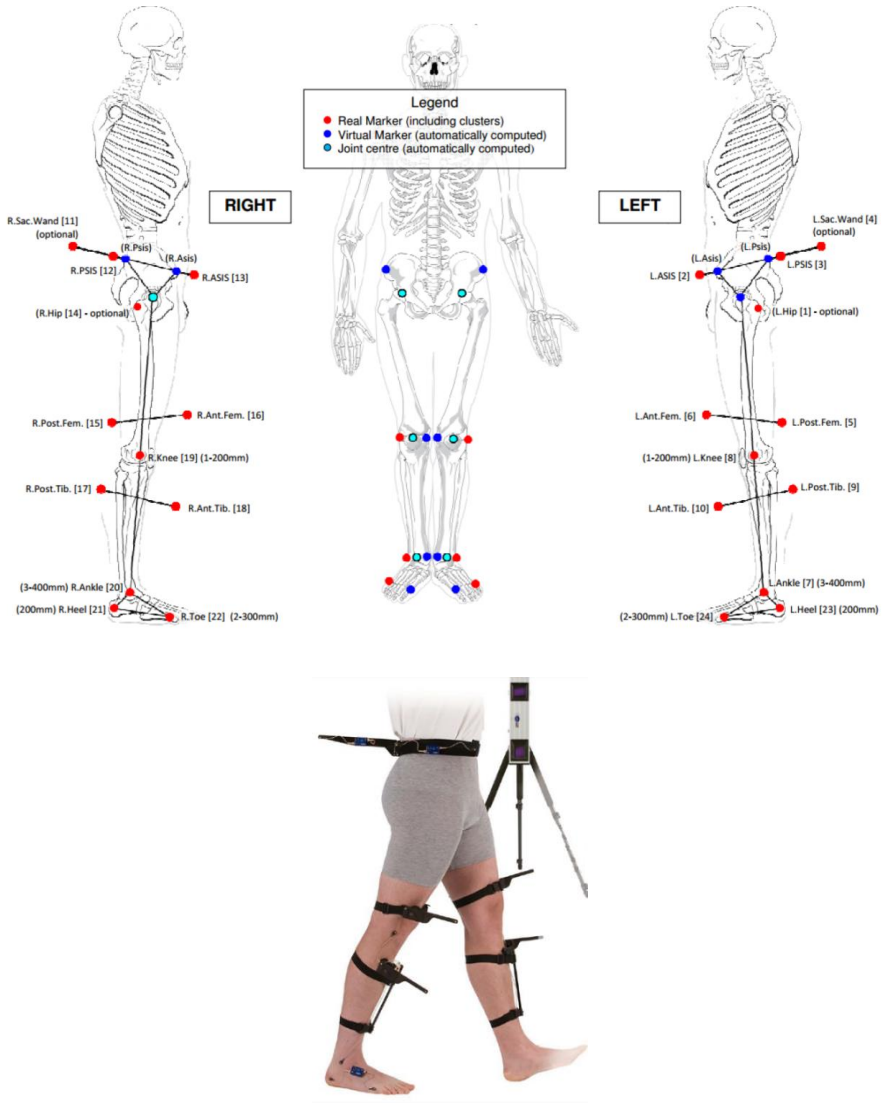


Figure 12: Helen Hayes model for marker placement. Adapted from CODAmotion ODIN user guide.

4.3.4. Gait Parameters and Analysis

ODIN software (Codamotion Ltd., Rothley, UK) was used to analyse and export reports of 3 gait cycles of left and right side for each participant each visit. A single gait cycle was identified from the heel marker acceleration traces and vertical forces. After exporting to Microsoft Excel, the three cycles were averaged and the data from the left and right legs were averaged. Exported variables are listed in Table 12.

Horizontal Angle (°)	Pelvic z Maximum (Rotation forwards)
	Pelvic z Minimum (Rotation backwords)
Coronal Angle (°)	Pelvic x Maximum (Obliquity, lateral raise)
	Pelvic x Minimum (Obliquity, lateral drop)
Sagittal Angle (°)	Pelvic y Maximum (Anterior tilt)
	Pelvic y Minimum (Posterior tilt)
	Hip y Maximum (Flexion)
	Hip y Minimum (Extension)
	Knee y Maximum (Flexion)
	Knee y Minimum (Extension)
	Ankle y Maximum (Dorsiflexion)
	Ankle y Minimum (Plantarflexion)
Sagittal Moments (Nm/Kg)	Hip Maximum (Extension)
	Hip Minimum (Flexion)
	Knee Maximum (Extension)
	Knee Minimum (Flexion)
	Ankle Maximum (Plantarflexion)
	Ankle Minimum (Dorsiflexion)
Sagittal Power (W/kg)	Hip Maximum (generation in swing phase)
	Hip Minimum (absorption in stance phase)
	Hip Maximum (generation in stance phase)
	Knee Maximum (generation in stance phase)
	Knee Maximum (generation in swing phase)
	Knee Minimum (absorption in stance phase)
	Ankle Maximum (generation in stance phase)
	Ankle Minimum (absorption in stance phase)
	Ankle Maximum (generation in swing phase)
Spatiotemporal	Speed (m/s)
	Stride Length (m)
	Stride Time (s)
	Strides / Minute
	Step Length (m)
	Step Time (s)
	Steps / Minute
	Percent Stance (%)
	Single Support (s)
	Double Support (s)
	Opposite Toe Off %
	Opposite Foot Contact (%)

Table 12: Gait parameters used for primary and secondary analysis.

4.4. Clinical Assessment

Along with primary measures, clinical assessment was undertaken each visit for descriptive purposes of the cohort and to correlate with the primary measurements, including CMT Examination Score version 2 (CMTESv2), Foot Posture Index (FPI-6), Manual Muscle Testing (MMT), The Visual Analog Scale (VAS) of Pain, and Walk-12 Questionnaire.

4.4.1. CMT Examination Score Version 2 (CMTESv2)

The Charcot-Marie-Tooth neuropathy score (CMTNS) is a reliable and validated composite scale of nine assessments including symptoms (three items), signs (four items), and neurophysiology (two items) (Shy et al., 2005). CMT Examination Score (CMTES) is a sub score of the CMTNS, calculated by the sum of the symptoms plus the signs. It is designed to measure length-dependent motor and sensory impairment in genetic neuropathies (Shy et al., 2005). Each assessment is scored on a 0 to 4 point scale, reflecting severity of impairment. In version 2, CMTNS was modified to reduce floor and ceiling effects and to standardize patient assessment, aiming to improve its sensitivity for detecting change over time and the effect of an intervention (Murphy et al., 2011). CMTNS2 is a reliable scale in patients with CMT, with high inter- and intra-rater reliability for its clinical symptoms and signs components (Murphy et al., 2011).

4.4.2. Foot Posture Index (FPI-6)

The Foot Posture Index-6 (FPI-6) is a simple and reliable quantification tool to assess static foot alignment in all three planes (Redmond et al., 2006, Keenan et al., 2007). The Foot Posture Index comprises six individual parameters, to which a score from −2 to +2 can be allocated. Negative numbers indicate supination, and positive numbers indicate pronation. The total result defines the foot posture and ranges between −12 and +12 (Redmond et al., 2006).

4.4.3. Manual Muscle Testing (MMT)

Muscle strength was assessed using a modified Medical Research Council Scale (MRC) with 5: normal strength, 5-: barely detectable weakness, 4+: gravity and moderate to maximal resistance, 4: Gravity and moderate resistance, 4-: Gravity and minimal resistance, 3: Full range of motion against gravity only, 2: Movement when gravity is eliminated, 1: Flicker of movement seen or felt, 0: No movement (O'Brien, 2023).

4.4.4. The Visual Analog Scale (VAS) of Pain

The Visual Analog Scale (VAS) is a validated, subjective measure of a person's pain intensity. It is a simple and commonly used tool in clinical and research settings (Delgado et al., 2018). The VAS is typically a horizontal line, 10 centimetres in length, anchored by two verbal descriptors, one for each end. "No pain" at 0 and "worst imaginable pain" at 10. Patients mark on the line the point

that they feel represents their perception of their current state of pain (Huskisson, 1974, Delgado et al., 2018).

4.4.5. Walk-12 Questionnaire

Patient reported outcome measure designed to assess walking ability and impairment in individuals, particularly those with peripheral neuropathy. The questionnaire evaluates three key aspects of walking: pain severity, distance, and speed. It consists of 12 items that ask respondents to rate their difficulty in walking various distances, walking speeds, and the degree of pain or discomfort experienced while walking. A higher score indicates a greater limitation in perceived walking ability (Graham and Hughes, 2006).

4.5. Participants

The disease investigated was DHMN. Our target was to include 20 people living with DHMN and 20 matched controls. The key inclusion and exclusion criteria are in Table 13. However, due to the restrictions resulting from the COVID-19 pandemic, we were only able to recruit a total of 12 DHMN participants and 9 matched controls.

Recruitment of participants living with DHMN was through the appropriate NHS consultant responsible for their care at the National Hospital for Neurology and Neurosurgery in London. Candidate participants with DHMN were informed about the study primarily when they attended a routine clinical appointment (remotely or in person) and their interest in the trial was assessed.

Healthy controls participants were recruited among relatives, spouses, and carers of the participating patients. controls were also recruited through staff in UCL departments and at the National Hospital for Neurology and Neurosurgery or their family members.

Written informed consent was obtained from each participant prior to participation in the trial, following adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the trial.

	DHMN	**Control**
Inclusion	1. Adult patients aged 18 or above. 2. Clinical diagnosis and electrophysiological features of DHMN. 3. Participant is able to give informed consent and has signed the informed consent form. 4. Patients who are able to complete calf and thigh dynamometry and 3D gait analysis. 5. Patients who have no contraindication to MRI scanning. 6. Participant for the exercises trial, at least one of the major muscle groups of the ankle, dorsiflexors or plantar flexors, score over 4/5 on the MRC scale.	1. Adult aged 18 or above. 2. Participants who have no known neuromuscular disease. 3. Participant is able to give informed consent and has signed the informed consent form. 4. Participants who are able to complete calf and thigh dynamometry and 3D gait analysis. 5. Participants who have no contraindication to MRI scanning.
Exclusion	1. Known neuromuscular disorder other than DHMN. 2. Lower limbs surgery planned during the study period or performed within the preceding 12 months. 3. Bilateral ankle arthrodesis. 4. The participant has a contraindication for MRI scan. 5. Females who are pregnant, planning pregnancy or breastfeeding.	1. The participant has a contraindication for MRI. 2. Participants who cannot complete calf and thigh dynamometry and 3D gait analysis. 3. Participant has a history, signs or symptoms of a neuromuscular disorder. 4. Females who are pregnant, planning pregnancy or breastfeeding.

Table 13: Inclusion and exclusion criteria.

4.6. Ethical Framework

This study was reviewed and given favourable opinion by the London – Camden and Kings Cross Research Ethics Committee and the Health Research Authority (HRA). People from Charcot Marie Tooth UK charity was involved in reviewing the participants information sheet.

4.7. Trial Design and Procedure

The study was three main visits. In each visit, study measurements were collected as explained in previous sections. Visit activity for each participant are illustrated in Table 14.

	Visit I (Baseline)			**Visit II (6 Months)**	**Visit III (12 Months)**
Activity	A) MRI, HUMAC, 3D Gait Analysis	B) 3D Gait Analysis: -Shoes - Carbon fibre AFOs	C) Test eligibility and recruit for the exercises trial	MRI, HUMAC, 3D Gait Analysis	MRI, HUMAC, 3D Gait Analysis
Subjects	DHMN, Controls	DHMN	DHMN	DHMN-Exercise, DHMN-No exercise	DHMN-Exercise, DHMN-No exercise
Objectives	Comparison to identify deviations	Effect of carbon fibre AFOs on walking	Randomise DHMN participants into: -Group A:Exercises -Group B: No exercise	Effect of resistance training on muscle.	-Explore natural history. -Explore muscles structure and function after Exercises washout period

Table 14: Visit activity for each participant.

4.8. Overall Statistical Considerations

4.8.1. Sample

Rather than a formal sample size calculation, convenience sampling was undertaken as one of the objectives of this study was initial exploration to

calculate the effect size to conduct a sample size calculation for a larger trial. Recruiting a large sample was challenging due to the low prevalence of the disease. Distal Hereditary Motor Neuropathies (DHMN) affects 2.14 individuals per 100,000 (Bansagi et al., 2017). The sample was recruited from the National Hospital for Neurology and Neurosurgery since it is a specialist hospital accepting referrals of neurological inherited diseases from most of the country. However, convenience sampling is often criticized for its lack of representativeness and higher risk of sampling bias, potentially limiting the generalizability of the findings to the broader population (Etikan et al., 2016).

4.8.2. Statistical Analysis

Demographic data (age, gender, weight, height) were collected. These measures are expressed as means (for continuous data) or medians (categorical data). Comparison was done using unpaired t-tests for continuous data, and Mann Whitney U tests for nominal or categorical data.

4.8.3. Difference Between Right and Left Sides

Due to the symmetrical nature of the condition, the decision was made to analyse the primary and secondary parameters for each objective using the average between right and left sides. Moreover, averaging right and left sides could eliminate type 1 error caused by multiple comparisons if both sides analysed separately. To ascertain symmetry in our sample, difference between right and left side in the primary parameters was tested using unpaired T.Test for normally

distributed data and Mann-Whitney test for non-normally distributed data. The test showed no significant difference in all primary parameters as shown in appendix I.

4.8.4. Data Normality Test

An assessment of the normality of data is a prerequisite for many statistical tests because normal data is an underlying assumption in parametric testing. Since the data collected for this study was from a small sample, less than 50 participants, the Shapiro-Wilk test was used to assess normality. Where the data was normal, parametric tests were used if the Significance value of the Shapiro-Wilk Test was greater than 0.05. If below 0.05, the data significantly deviates from a normal distribution and non-parametric tests were used for analysis.

4.8.5. Strategies to Minimise Statistical Errors

Multiple comparisons have higher probability of type I (false positive) error, defined as a statistically significant difference by chance when there is no real finding. To reduce the chance of type I error, first, the number of comparisons was decreased by using the average between right and left, rather than separately for each side as explained in section 4.8.3. The second approach used to account for multiple comparisons was adjusting the alpha value using modified Bonferroni procedure. The classical Bonferroni correction is considered too conservative as the correction does not account for dependency among the data (Simes, 1986). In addition, there is also the potential to increase type II (false

negative) error as the test shows no statistically significant difference when there is. A modified Bonferroni procedure was used as it is less conservative and based on the ordered P-values of individual tests which decreases type II error probability (Simes, 1986). The procedure is demonstrated in appendix I.

Chapter 5: Pattern of Involvement and Deviation from "Normal" in Muscle Structure and Function in Distal Hereditary Motor Neuropathy (DHMN)

5.1. Introduction

To address the first objective for this study, which is to explore the effect of Distal Hereditary Motor Neuropathy (DHMN) on muscle structure and function, we need to establish an understanding of the morphological differences and functional alterations associated with the condition. Previous MRI studies described DHMN groups with a predominant involvement of the lower leg, mainly in the posterior and lateral compartments. Which was associated with distal lower limb weakness (Esteller et al., 2023, O'Donnell et al., 2022). Other studies in CMT described gait alterations relevant to distal weakness as foot drop and/or plantarflexes failure with the possibility of proximal compensation (Don et al., 2007, Newman et al., 2007, Ramdharry et al., 2009). However, gait in DHMN has not been described before. This study is aimed to identify patterns of muscle involvement and walking gait deviations in people with DHMN in comparison to healthy controls. Based on previous findings in CMT, we expect our DHMN cohort to show:

- Higher rates of fatty infiltration and signs of active denervation distally more proximally, and in the posterior compartment more than anterior compartment of the calf.

- Predominant distal weakness more than proximal, and in plantar flexors more than dorsiflexors.
- Decreased ankle plantarflexion angle, moment, and power generation at pre-swing.
- Decreased ankle dorsiflexion angle in swing phase.
- Increased hip flexion angle, moment, and power generation at pre-swing and swing phase.
- Slow gait with shorter stride length.

5.2. Methods

Participants with DHMN were recruited to undergo clinical assessment, an MRI scan, isokinetic and isometric dynamometry of the lower limb, and 3D motion analysis to capture kinetic and kinematic data of walking gait as described in chapter 4. For direct comparison of gait deviations and muscle involvement, age and gender matched healthy controls were recruited to undergo the same measurements.

5.2.1. Data Analysis

To identify pattern of involvement and deviations from normal, comparisons of DHMN and control parameters of MRI, isokinetic/isometric dynamometry, and motion analysis using unpaired t-test, if both data sets were normally distributed or Mann-Whitney U test, if they are not normally distributed. A modified Bonferroni procedure was used to account for multiple comparisons. However, due to the large number of comparisons and small sample, it was expected that few of the group differences will show significant. Therefore, it was decided to look for non-significant trends in the data using $p<0.05$ as a guide.

5.3. Results

5.3.1. Subjects and Clinical Assessment

Twelve DHMN participants and 9 age and gender matched healthy controls were recruited. There were no significant differences observed in the groups matching criteria. One DHMN female participant used walking sticks. Two male DHMN participants with HSPB1genetic diagnosis did not complete all measurements, one aged 53 completed MRI scan only, and one aged 41 completed all assessment except for the MRI scan (appendix VII). Assessment showed limitation in plantarflexion and dorsiflexion passive and active range of motion. Comparison between DHMN and control groups showed significant difference in manual muscle testing in dorsiflexion and plantarflexion strength (P=0.0001). A summary of the subjects' demographics and clinical assessment is presented in Table 15.

Demographics and clinical assessment	**DHMN**	**CONTROL**	**P**
Numbers	12	9	-
Gender (M/F)	(7/5)	(4/5)	-
Age; mean years; range	56 (41/75)	50 (29/76)	-
Differences in groups matching criteria (M/F)	-	-	(0.47/0.23)
Genetic diagnosis (HSPB1/unknown)	(8/4)	-	-
CMTES; mean (SD)	6.2 (3.6)	-	-
Foot Posture Index-6 (Pronated/Normal/Supinated)	(1/8/1)	-	-
Fall Frequency (weekly/Monthly/Yearly)	(1/1/2)	-	-
Walk-12 Questionnaire; mean (SD)	36 (12.4)	-	-
Range of Motion	**DHMN**	**CONTROL**	**P**
Dorsiflexion; count (Limited/Normal)	(12/0)	(0/9)	-
Plantarflexion; count (Limited/Normal)	(11/1)	(0/9)	-
Manual Muscle Testing	**DHMN**	**CONTROL**	**P**
Hip Flexion; median (IQR)	5 (5-4+)	5 (5-5)	0.0827
Hip Extension; median (IQR)	5 (5-4-)	5 (5-5)	0.0824
Knee Flexion; median (IQR)	5 (5-4-)	5 (5-5)	0.0824
Knee Extension; median (IQR)	5 (5-5)	5 (5-5)	0.1674
Dorsiflexion; median (IQR)	3+ (4-1+)	5 (5-5)	**0.0001***
Plantarflexion; median (IQR)	4 (4+-3)	5 (5-5)	**0.0001***

M= Male, F= Female, HSPB1= Heat-shock 27-KD Protein 1, SD= Standard Deviation, CMTES= Charcot-Marie-Tooth Disease Examination Score, IQR= interquartile range, *= significant after modified Bonferroni correction.

Table 15: Summary of demographics and clinical assessment of DHMN and control groups

5.3.2. Pattern of Muscle Structural Involvement on MRI

Three point Dixon images were processed to generate fat fraction (FF) maps. Region of interest at the thigh, calf, and foot levels were applied to the FF maps to quantify the cross sectional area, intramuscular fat fraction, and the remaining muscle area.

5.3.2.1. Muscle Cross Sectional Area

Quantitative analysis showed no significant difference in muscle cross sectional area between DHMN and control group, neither at the thigh, calf, or foot level (Table 16).

Region of interest mm²	**DHMN** mean (SD)	**CONTROL** mean (SD)	**Mean diff**	**P***
Total Thigh	21877.7(7024.5)	20184.2(5093.4)	-1693.53	0.55
Knee Extension	10748.8(3283)	10053.7(2985.9)	-695.07	0.60
Rectus Femoris	474.9(187.6)	436.5(160.9)	-38.40	0.63
Vastus Intermedius	1601.5(524.3)	1778.3(505.1)	176.75	0.46
Vastus Lateralis	1824.5(467.7)	1687.7(468.8)	-136.78	0.26
Vastus Medialis	1473.5(616.7)	1124.4(490.7)	-349.11	0.29
Knee Flexion	7975.2(2956.7)	6959.8(2407.9)	-1015.39	0.41
Semimembranosus	894.5(381.9)	838.2(437.4)	-56.26	0.46
Semitendinosus	791.6(350.7)	657.1(264.6)	-134.53	0.36
Biceps Femoris	1478.7(566.6)	1312.9(440.6)	-165.75	0.48
Sartorius	374.2(132.3)	326.5(112.7)	-47.65	0.40
Gracilis	448.7(190.4)	345.2(128.1)	-103.50	0.18
Adductor Magnus	1576.9(721.1)	1585.3(604.5)	8.46	0.98
Total Calf	10565.6(3102.8)	11572.9(1904.7)	1007.28	0.41
Dorsiflexion	2005.7(783.7)	2063.5(261.7)	57.83	0.84
Tibialis Anterior	1002.9(391.8)	1031.8(130.9)	28.92	0.84
Peroneus Longus	475.0(144.1)	524.1(135.8)	49.11	0.45
Plantarflexion	6838.8(2286.0)	7610.5(1434.4)	771.62	0.39
Lateral Gastrocnemius	665.1(439.5)	603.1(133.5)	-62.01	0.82
Medial Gastrocnemius	800.5(358.1)	1046.0(301.0)	245.49	0.12
Soleus	1953.9(607.1)	2156.2(374.0)	202.33	0.40
Tibialis Posterior	385.6(166.5)	425.4(120.6)	39.80	0.56
Foot	485.4(123.6)	434.9(82.7)	-50.46	0.31
SD= Standard Deviation, mm²= square millimetres, diff= difference, *= significance level with modified Bonferroni correction.				

Table 16: Muscle cross sectional area at the thigh, calf, and foot level of DHMN and control groups

5.3.2.2. Quantitative Analysis of Intramuscular Fat Fraction

Muscle fat fraction analysis showed significant differences at the foot (P<0.0001), and the total calf level (P=0.0007) (Table 17) (Figure 13). At the thigh level, however, significant difference was in the Biceps Femoris muscle only (P= 0.0459) (Table 17).

Region of interest %	**DHMN** mean (SD)	**CONTROL** mean (SD)	**Mean diff**	**P**
Total Thigh	12.4(12.6)	5(1.8)	-7.4	0.13
Knee Extension	11.4(13.4)	4.5(1.8)	-7	0.23
Rectus Femoris	9.2(9.3)	7.1(2.8)	-2.1	0.55
Vastus Intermedius	12.5(14.4)	3.5(1.7)	-8.9	0.07
Vastus Lateralis	13.0(16.5)	4.6(1.9)	-8.3	0.37
Vastus Medialis	8.5(8.3)	4.8(2.1)	-3.7	0.41
Knee Flexion	13.6(12.5)	6.3(2.4)	-7.3	0.08
Semimembranosus	14.3(13.0)	6.2(2.9)	-8.2	0.06
Semitendinosus	12.4(14.9)	5.2(2.2)	-7.2	0.23
Biceps Femoris	15.7(13.5)	5.9(2.1)	-9.8	**0.0459***
Sartorius	16.4(17.1)	9.9(4.6)	-6.5	0.88
Gracilis	8.7(9.8)	7.0(3.6)	-1.7	0.88
Adductor Magnus	14.5(18.2)	4.3(1.3)	-10.6	0.03
Total Calf	32.2(21)	3.2(1)	-28.9	**0.0007***
Dorsiflexion	18.(18.8)	2.7(0.60)	-35.2	**0.0031***
Tibialis Anterior	18.0(18)	2.3(0)	-15.8	**0.0031***
Peroneus Longus	29.8(28)	4.5(1)	-25.4	**0.0145***
Plantarflexion	38.7(23)	3.5(1.3)	-15.8	**0.0002***
Lateral Gastrocnemius	28.6(28)	4.1(1)	-24.5	**0.0002***
Medial Gastrocnemius	41.0(22)	3.6(1)	-37.4	**0.0001***
Soleus	42.2(24)	3.2(1)	-39	**0.0001***
Tibialis Posterior	22.9(22)	2.1(0)	-20.8	**<0.0001***
Foot	49(12.3)	6.2(2.2)	-42.7	**<0.0001***
SD= Standard Deviation, diff= difference, *= significant with modified Bonferroni correction.				

Table 17: Muscle Fat Fraction at the thigh, calf, and foot level of DHMN and control groups

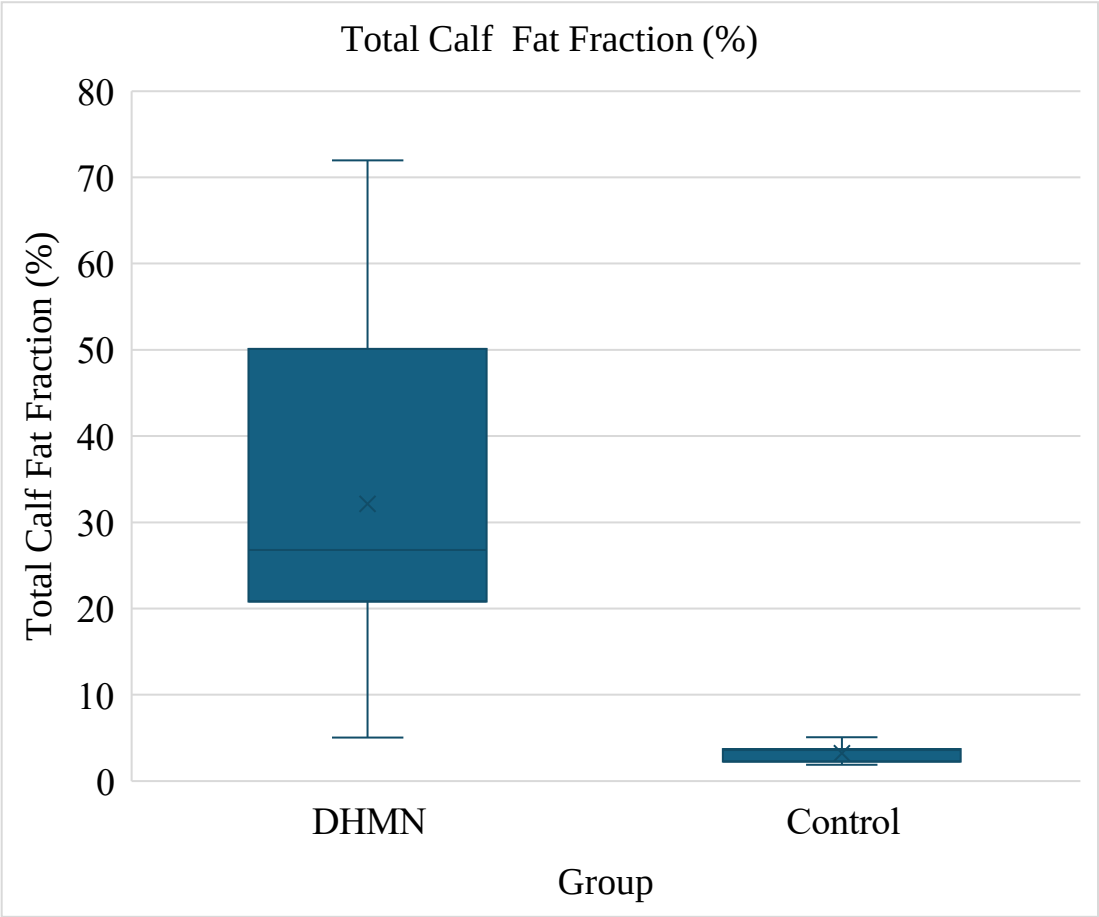


Figure 13: Box and whisker plot of the total calf fat fraction (%) in DHMN and control group.

5.3.2.3. Remaining Muscle Area

Analysis of the remaining muscle area showed significant difference at the foot (P=0.0038) and the calf level (P=0.0126). The difference at the calf level caused by a significant reduction of muscle area in the total plantar flexors (P=0.0082). in particular the Medial Gastrocnemius (P=0.0057), and Soleus (P=0.0029) muscles (Table 18).

Region of interest mm²	**DHMN** mean (SD)	**CONTROL** mean (SD)	**Mean diff**	**P**
Total Thigh	19735.1(7540.5)	19199.7(5048.8)	-535.5	0.82
Knee Extension	9804.6(3766.4)	9622.4(2968.9)	-182.2	0.88
Rectus Femoris	439.7(197.7)	407.4(159.6)	-32.2	0.94
Vastus Intermedius	1447.1(600.3)	1716.9(496.5)	269.8	0.30
Vastus Lateralis	1631.3(592.4)	1612.9(470.1)	-18.4	0.77
Vastus Medialis	1384.2(632.6)	1073.5(482.4)	-310.8	0.46
Knee Flexion	7139.7(3045.3)	6545.6(2401.6)	-594.2	0.50
Semimembranosus	797.9(376.6)	791.7(434.3)	-6.2	0.66
Semitendinosus	722.3(354.5)	623.2(256.1)	-99.1	0.49
Biceps Femoris	1302.6(606.1)	1239.9(435.9)	-62.7	0.80
Sartorius	326.3(150.9)	295.1(109.2)	-31.2	0.77
Gracilis	420.8(192.0)	323.1(128.2)	-97.6	0.21
Adductor Magnus	1395.4(702.3)	1515.9(576.1)	120.5	0.68
Total Calf	7454.9(3677.1)	11201.5(1860.1)	3746.6	**0.0126***
Dorsiflexion	1744.3(910.6)	2016.8(254.1)	272.5	0.40
Tibialis Anterior	872.1(455.3)	1008.4(127.1)	136.3	0.40
Peroneus Longus	337.0(165.7)	500.7(131.3)	163.8	0.027†
Plantarflexion	4420.2(2661.5)	7350.2(1413.9)	2930.0	**0.0082***
Lateral Gastrocnemius	529.1(478.6)	578.9(130.9)	49.8	0.46
Medial Gastrocnemius	503.8(371.1)	1009.3(295.8)	505.5	**0.0057***
Soleus	1177.2(718.1)	2086.9(364.3)	909.8	**0.0029***
Tibialis Posterior	308.2(183.6)	416.5(117.9)	108.3	0.14
Foot	256.8(113.7)	408.7(84.2)	152	**0.0038***

SD= Standard Deviation, mm²= square millimetres, † = significant with P<0.05, diff= difference, *= significant with modified Bonferroni correction.

Table 18: Remaining Muscle Area at the thigh, calf, and foot level of DHMN and control groups

5.3.2.4. Qualitative Analysis of Intramuscular Fat

Qualitative analysis of intramuscular fat using Modified Mercuri's Scale showed consistent results as the intramuscular fat quantification at the calf and foot level (Table 19) (Figure 14). The difference was significant at the foot (P<0.0001) and all calf muscles (P=0.0003-0.0034).

Region of interest	DHMN median (IQR)	CONTROL median (IQR)	P
Tibialis Anterior	2.5 (2.0 to 3.375)	1 (1.0 to 1.0)	**0.0034***
Tibialis Posterior	3(2.0 to 4.875)	1 (1.0 to 1.0)	**0.0034***
Peroneus Longus	3.5(2.0 to 4.875)	1 (1.0 to 1.0)	**0.0035***
Medial Gastrocnemius	4 (3.125 to 5.0)	1 (1.0 to 1.0)	**0.0003***
Lateral Gastrocnemius	3 (2.0 to 5.0)	1 (1.0 to 1.0)	**0.0012***
Soleus	4.5 (3.0 to 5.375)	1 (1.0 to 1.0)	**0.0003***
Foot	6 (6.0 to 6.0)	1 (1.0 to 1.0)	**<0.0001***

Modified Mercuri's Scale: 1=Stage 0= Normal appearance; 2=Stage 1= Early Moth-eaten appearance, with scattered small areas of increased signal; 3=Stage 2a= Late Moth-eaten appearance, with numerous discrete areas of increased signal with beginning confluence, comprising less than 30% of the volume of the individual muscle; 4=Stage 2b= Late Moth-eaten appearance, with numerous discrete areas of increased signal with beginning confluence, comprising 30 – 60% of the volume of the individual muscle; 5=Stage 3= Washed-out appearance, fuzzy appearance due to confluent areas of increased signal; 6=Stage 4= End stage appearance, muscle replaced increased density connective tissue and fat, with only A rim of fascia and neurovascular structures distinguishable, IQR= interquartile range, *= significant with modified Bonferroni correction.

Table 19: MRI Muscle Fat qualitative score using Modified Mercuri's Scale at the calf and foot level of DHMN and control groups

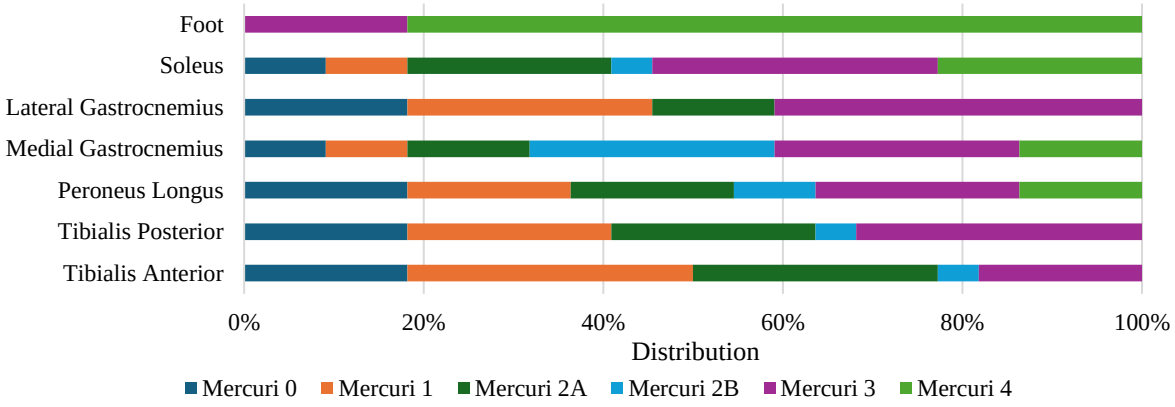


Figure 14:Pattern of fat infiltration using Modified Mercuri's Scale at the calf and foot level of DHMN group.

5.3.2.5. Quantitative Analysis of Intramuscular Oedema

Region of interest was applied to the T2 map at the calf and foot level to quantify intramuscular oedema as a sign of active denervation. Analysis showed significant differences between DHMN and control group at the calf (P=0.0028) and foot (P<0.0001) (Table 20).

Region of interest ms	**DHMN** mean (SD)	**CONTROL** mean (SD)	**Mean diff**	**P**
Total Calf	38.52(7.09)	30.07(1.83)	-8.45	**0.0028***
Dorsiflexion	37.66(7.08)	30.02(2.32)	-7.64	**0.0063***
Tibialis Anterior	37.66(7.08)	30.02(2.32)	-7.64	**0.0063***
Peroneus Longus	38.73(8.58)	30.41(1.97)	-8.32	**0.0109***
Plantarflexion	39.03(7.50)	30.09(1.76)	-8.93	**0.0001***
Lateral Gastrocnemius	38.61(8.86)	30.62(1.85)	-7.98	**0.0165***
Medial Gastrocnemius	39.81(8.10)	30.28(2.40)	-9.53	**0.0032***
Soleus	38.52(7.34)	29.92(1.50)	-8.60	**<0.0001***
Tibialis Posterior	37.40(6.74)	29.59(1.71)	-7.81	**0.0034***
Foot	45.61(5.35)	30.38(2.37)	-15.23	**<0.0001***
SD= Standard Deviation, ms= millisecond, diff= difference, *= significant with modified Bonferroni correction.				

Table 20: MRI Muscle water quantitative analysis using T2 mapping at the calf and foot level of DHMN and control groups

5.3.2.6. Qualitative Analysis of Intramuscular Oedema

Qualitative analysis of short tau inversion recovery imaging (STIR) hyperintensity showed consistent results as the quantitative T2 mapping analysis. There was a significant difference at the foot (P=0.0001) and all calf muscles (P=0.0002-0.0074) (Table 21).

Region of interest	**DHMN** median (IQR)		**CONTROL** median (IQR)		P
	Intensity	**Extent**	**Intensity**	**Extent**	
Tibialis Anterior	1.5(1.5 to 2)	2.5 (2.5 to 3)	0 (0 to 0)	0 (0 to 0)	**0.0002***
Tibialis Posterior	1(1.6 to 1.4)	2 (2.6 to 3)	0 (0 to 0)	0 (0 to 0)	**0.0008***
Peroneus Longus	1(1.6 to 1.4)	1.5 (1.5 to 2.7)	0 (0 to 0)	0 (0 to 0)	**0.0003***
Medial Gastrocnemius	1(1. to 1.4)	2 (2 to 2.4)	0.5 (0.5 to 1)	0.5 (0.5 to 1)	**0.0074***
Lateral Gastrocnemius	1(1. to 1.500)	1 (1 to 3)	0 (0 to 1)	0(0 to 1)	**0.0046***
Soleus	1.5(1.5 to 1.9)	1.5 (1.5 to 2.9)	0 (0 to 0)	0 (0 to 0)	**0.0002***
Foot	2(2 to 2)	2 (2 to 2)	0 (0 to 0)	0 (0 to 0)	**0.0001***
Intensity: 0= none, 1=mild, 2=marked, Extent: 0= none, 1= < 30%, 2=30%-60%, 3= >60%, IQR= interquartile range, *= significant with modified Bonferroni correction.					

Table 21: MRI Muscle water qualitative analysis using STIR image at the calf and foot level of DHMN and control groups

5.3.3. Difference in Muscles Isokinetic and Isometric Strength

Dynamometric muscle strength was reduced in distal muscle groups compared with their matched controls (Table 22). Differences were significant only in isometric dorsiflexion 10° (P=0.0134), isometric dorsiflexion 30° (P=0.0121), and isokinetic plantarflexion 60°/60° (P=0.0062). However, no difference was seen in isokinetic dorsiflexion (P=0.0729) (Table 22).

Isometric Dynamometry Nm	**DHMN** mean (SD)	**CONTROL** mean (SD)	**Mean diff**	**P**
Hip Extension 45°	137.2(68.5)	142.9(56.1)	5.66	0.84
Hip Flexion 45°	107.7(47.5)	99.6(34.4)	-8.13	0.88
Knee Extension 45°	108.4(59.4)	114.6(59.9)	6.20	1.00
Knee Extension 90°	110.2(58.2)	116.3(49.9)	6.15	0.81
Knee Flexion 45°	60.4(36.7)	65.9(32.4)	5.54	0.73
Knee Flexion 90°	40.0(25.7)	46.6(20.8)	6.61	0.54
Ankle Plantarflexion 10°	27.3(18.7)	45.9(5.7)	18.62	0.0102†
Ankle Dorsiflexion 10°	14.0(17.2)	27.9(5.6)	13.93	**0.0134***
Ankle Dorsiflexion 30°	18.8(17.3)	33.1(9.0)	14.28	**0.0121***
Isokinetic Dynamometry Nm	**DHMN** mean (SD)	**CONTROL** mean (SD)	**Mean diff**	**P**
Hip Extension 60°/60s	113.5(71.6)	105.8(70.2)	-7.72	0.81
Hip Flexion 60°/60s	103.3(51.5)	95.4(36.5)	-7.83	0.71
Knee Extension 60°/60s	84.1(52.3)	89.9(49.4)	5.85	0.80
Knee Flexion 60°/60s	42.3(27.2)	51.4(40.7)	9.13	0.82
Knee Extension 120°/120s	60.8(44.6)	74.7(55.9)	13.90	0.54
Knee Flexion 120°/120s	32.9(24.4)	41.6(33.3)	8.70	0.51
Ankle Plantarflexion 60°/60°	16.8(16.7)	42.2(24.0)	25.35	**0.0062***
Ankle Dorsiflexion 60°/60°	14.7(8.2)	21.6(7.8)	6.88	0.07
SD= Standard Deviation, Nm= Newton meter, † = significant with P<0.05, diff= difference, *= significant after modified Bonferroni correction.				

Table 22: Isometric and isokinetic dynamometry of DHMN and control groups

5.3.4. Deviations From Normal in Walking Gait

5.3.4.1. Spatiotemporal Analysis

Three dimensional gait analysis showed significant difference in parameter related to time rather than distance (Table 23). DHMN group walked with slower speed (P=0.0322) with more stride time (P=0.0279) and step time (P=0.0279), and therefore, less strides and steps per minute (P=0.0279). DHMN group spent more time in single and double support (P=0.032, 0.0015, respectively) which contributed to a higher stance phase rates (P=0.0002) and delayed opposite toe off (P=0.003) (Table 23).

Gait Spatiotemporal Parameters	**DHMN** mean (SD)	**CONTROL** mean (SD)	**Mean diff**	**P**
Speed; m/s	0.98(0.29)	1.23(0.14)	0.24	**0.0322***
Stride Length; m	1.18(0.26)	1.37(0.15)	0.19	0.07
Stride Time; s	1.23(0.16)	1.12(0.08)	-0.11	**0.0279***
Strides Per Minute	49.48(5.08)	53.87(3.79)	4.39	**0.0279***
Step Length; m	0.59(0.13)	0.69(0.07)	0.10	0.07
Step Time; s	0.62(0.08)	0.56(0.04)	-0.06	**0.0279***
Steps Per Minute	98.96(10.17)	107.74(7.58)	8.78	**0.0279***
Percent Stance	0.64(0.02)	0.59(0.03)	-0.05	**0.0002***
Single Support Time; s	0.61(0.05)	0.56(0.04)	-0.05	**0.032***
Double Support Time; s	0.17(0.08)	0.10(0.03)	-0.08	**0.0015***
Percent Opposite Toe Off	13.90(4.51)	8.84(2.74)	-5.06	**0.003***
Percent Opposite Foot Contact	49.65(0.80)	48.48(3.52)	-1.16	0.45
SD= Standard Deviation, m= meters, s= seconds, diff= difference, *= significant with modified Bonferroni correction.				

Table 23: Gait spatiotemporal parameters of DHMN and control groups

5.3.4.2. Kinematics

People with DHMN walked with significantly higher dorsiflexion angle (P=0.0435) and lower plantar flexion angle (P=0.0259) than healthy controls. Higher dorsiflexion angle occurred in stance phase and lower plantar flexion angle at pre-swing to early swing (Figure 15). Significantly higher hip flexion angle (P=0.0076) was also observed in swing and lower hip extension angle in stance (P=0.0413) (Table 24) (Figure 15).

Gait Kinematics	**DHMN** mean (SD)	**CONTROL** mean (SD)	**Mean diff**	**P**
Pelvis X Max; °	4.49(1.32)	4.35(1.63)	-0.14	0.84
Pelvis X Min; °	-4.79(1.74)	-4.39(1.30)	0.40	0.58
Pelvis Y Max; °	15.82(5.84)	10.29(7.59)	-5.53	0.09
Pelvis Y Min; °	10.63(4.51)	5.42(8.29)	-5.22	0.10
Pelvis Z Max; °	6.75(2.55)	5.25(1.93)	-1.50	0.17
Pelvis Z Min; °	-6.96(2.98)	-5.52(1.93)	1.44	0.23
Hip Max; °	47.33(5.70)	36.93(11.78)	-10.39	**0.0076***
Hip Min; °	3.32(4.26)	-4.82(10.80)	-8.14	**0.0413***
Knee Max; °	74.00(4.74)	69.66(6.50)	-4.34	0.07
Knee Min; °	6.51(5.06)	4.48(7.81)	-2.03	0.36
Ankle Max; °	25.07(5.81)	23.42(20.73)	-1.66	**0.0435***
Ankle Min; °	-10.39(4.42)	-15.53(4.76)	-5.14	**0.0259***
SD= Standard Deviation, diff= difference, *= significant with modified Bonferroni correction= Pelvic z Maximum= Rotation forwards, Pelvic z Minimum= Rotation backwords, Pelvic x Maximum, lateral raise, Pelvic x Minimum= lateral drop, Pelvic y Maximum= Anterior tilt, Pelvic y Minimum = Posterior tilt, Hip y Maximum= Flexion, Hip y Minimum= Extension, Knee y Maximum= Flexion, Knee y Minimum= Extension, Ankle y Maximum= Dorsiflexion, Ankle y Minimum= Plantarflexion.				

Table 24: Gait kinematics parameters of DHMN and control groups

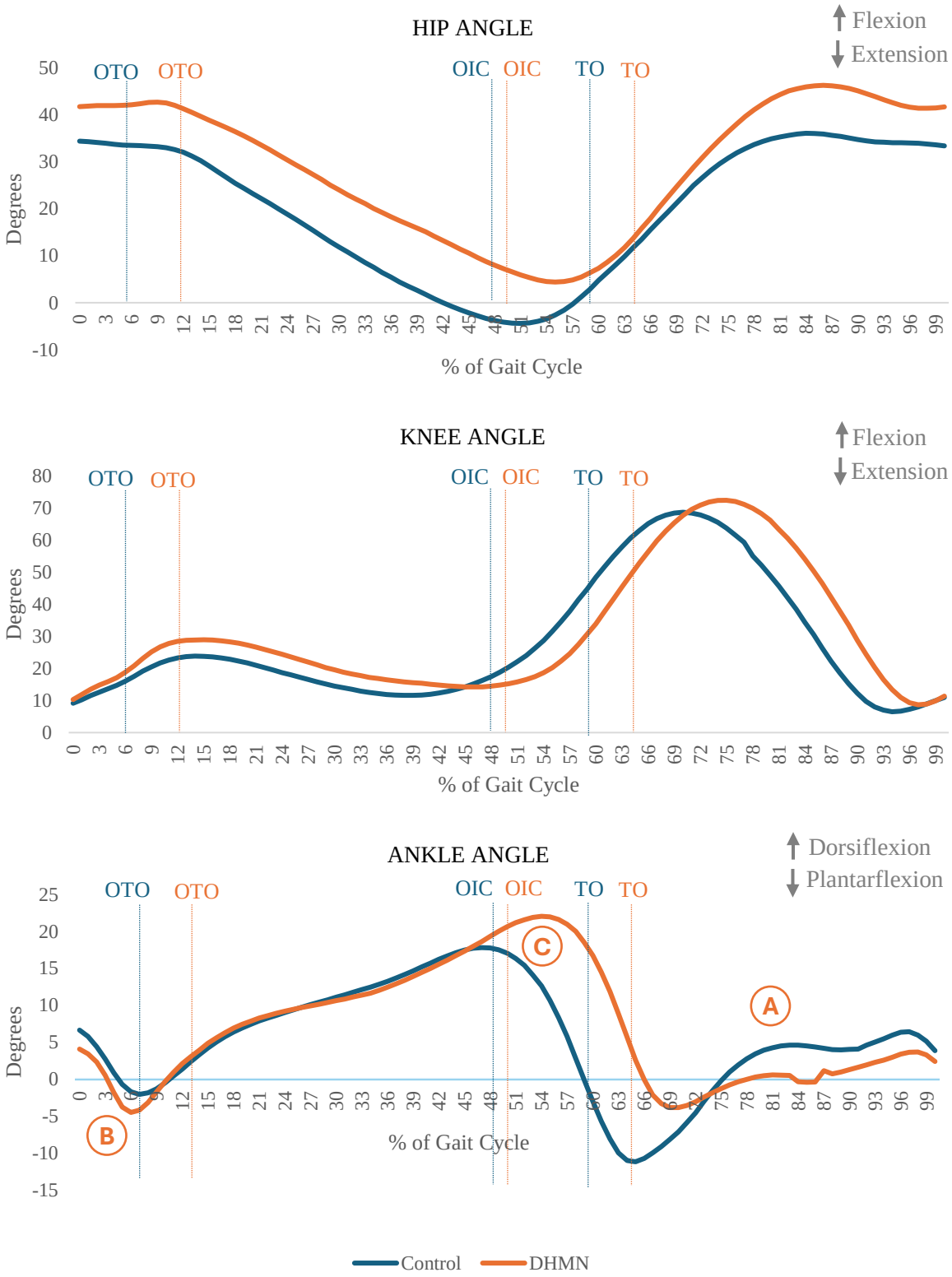


Figure 15:Grand average angular displacement in the sagittal plane over one gait cycle for the ankle, knee, and hip (average of left and right sides). Comparison of people with DHMN and controls.

OTO, Opposite side toe off; OIC, Opposite side initial contact; TO, Toe off; A, B, foot drop; C, plantar flexion failure.

5.3.4.3. Kinetics

People with DHMN produce smaller ankle moments and power generation during walking. Ankle plantar flexion moment at terminal stance was significantly lower than controls (P=0.0002) and significantly lower ankle power generation was observed at the same period (P=0.0011). There was also a significant reduction in ankle power generation in swing phase (p=0.0279) (Table 25) (Figure 16,Figure 17).

Gait Kinetics	DHMN mean (SD)	CONTROL mean (SD)	Mean diff	P
Hip Moment Y Max; Nm/Kg	1.49(0.56)	1.19(0.30)	-0.31	0.1614
Hip Moment Y Min; Nm/Kg	-0.49(0.17)	-0.51(0.11)	-0.01	0.8656
Knee Moment Y Max; Nm/Kg	0.69(0.32)	0.59(0.27)	-0.10	0.4945
Knee Moment Y Min; Nm/Kg	-0.68(0.29)	-0.50(0.09)	0.19	0.0837
Ankle Moment Y Max; Nm/Kg	1.03(0.26)	1.51(0.26)	0.48	**0.0002***
Ankle Moment Y Min; Nm/Kg	-0.08(0.07)	-0.12(0.05)	-0.04	0.0535
Hip Power Max During Swing; W/kg	1.02(0.32)	1.10(0.36)	0.08	0.6201
Hip Power Min During Stance; W/kg	-0.84(0.65)	-0.71(0.32)	0.14	0.5793
Hip Power Max During Stance; W/kg	1.84(0.75)	1.52(0.53)	-0.32	0.3054
Knee Power Max During Swing; W/kg	0.36(0.13)	0.45(0.20)	0.09	0.2779
Knee Power Min During Stance; W/kg	-1.53(0.91)	-1.26(0.84)	0.27	0.5168
Knee Power Max During Stance; W/kg	1.67(1.33)	1.03(0.50)	-0.63	0.198
Ankle Power Max During Swing; W/kg	0.04(0.02)	0.63(0.88)	0.59	**0.0279***
Ankle Power Min During Stance; W/kg	-1.16(0.38)	-1.23(0.50)	-0.07	0.7338
Ankle Power Max During Stance; W/kg	1.03(0.43)	2.15(0.77)	1.11	**0.0011***

SD= Standard Deviation, Nm/Kg= Newton Meter per Kilogram, W/kg= Watts Per Kilogram, diff= difference, *= significant with modified Bonferroni correction= Hip Moments Maximum= Extension, Hip Moments Minimum= Flexion, Knee Moments Maximum= Extension, Knee Moments Minimum= Flexion, Ankle Moments Maximum= Plantarflexion, Ankle Moments Minimum= Dorsiflexion, Hip Power Maximum = generation in swing phase, Hip Power Minimum= absorption in stance phase, Hip Power Maximum= generation in stance phase, Knee Power Maximum= generation in stance phase, Knee Power Maximum= generation in swing phase, Knee Power Minimum = absorption in stance phase, Ankle Power Maximum= generation in stance phase, Ankle Power Minimum= absorption in stance phase, Ankle Power Maximum= generation in swing phase.

Table 25: Gait kinetics parameters of DHMN and control groups

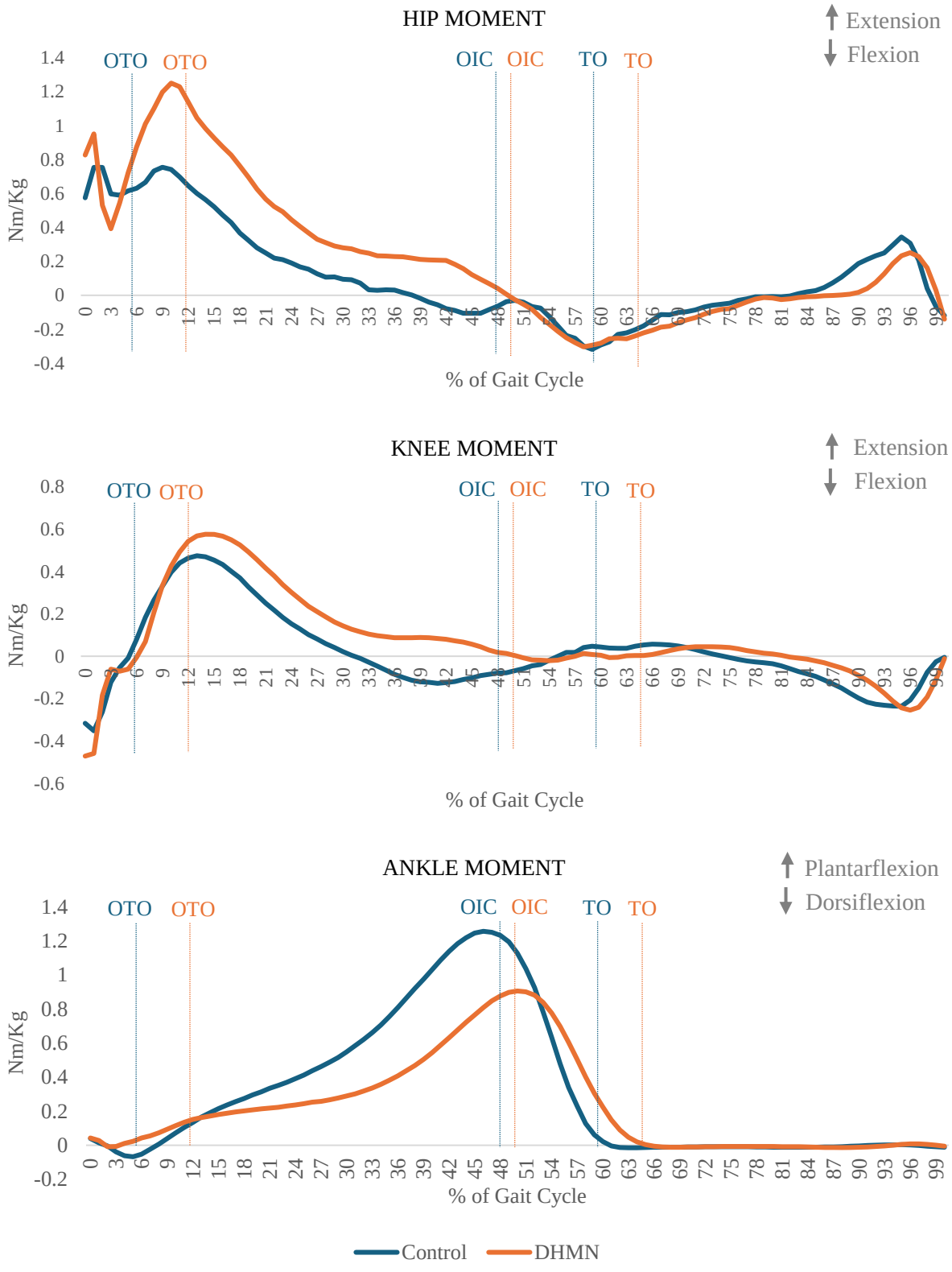


Figure 16:Grand average joint moments in the sagittal plane over one gait cycle for the ankle, knee and hip (average of left and right sides).

Comparison of people with DHMN and controls. OTO, Opposite side tore off; OIC, Opposite side initial contact; TO, Toe off.

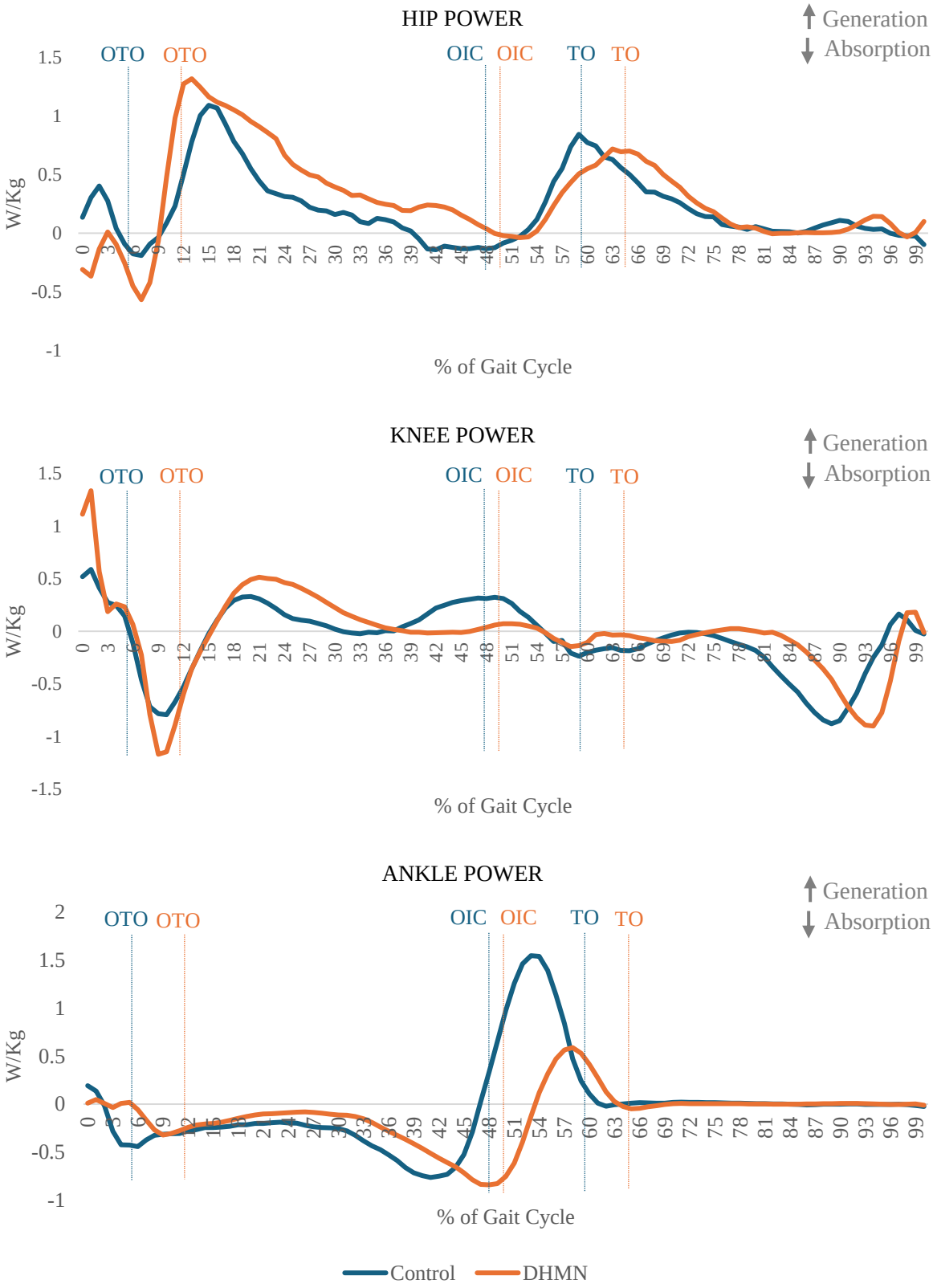


Figure 17:Grand average joint powers in the sagittal plane over one gait cycle for the ankle, knee and hip (average of left and right sides).

Comparison of people with DHMN and controls. OTO, Opposite side tore off; OIC, Opposite side initial contact; TO, Toe off.

5.4. Discussion

5.4.1. Pattern of Muscle Involvement

This study described the pattern of muscle involvement in DHMN cohort primarily using dynamometry and quantitative MRI analysis of muscle fat fraction and oedema. MRI parameters confirmed the study hypothesis. Results showed higher rates of fatty infiltration and signs of active denervation distally, in the foot and calf, more than proximally, at the thigh level, and in the posterior compartment more than anterior compartment of the calf. Results were consistent in both quantitative and qualitative analysis. Interestingly, the remaining muscle tissue variable was significantly lower in plantar flexors, in the Medial Gastrocnemius and Soleus specifically. This difference between DHMN and control was not seen in dorsiflexors or proximal muscle groups.

Proximally, Biceps femoris muscle is the only muscle that showed significantly higher fat fraction rate. However, this muscle involvement was not reflected on other MRI parameters or muscle dynamometry. Biceps femoris might be a candidate muscle to describe the pattern of involvement for future longitudinal studies and can be supported by observing the levels of active denervation measured using T2 mapping which, unfortunately, was not done in this study to shorten the scanning time.

As hypothesised, the cohort showed predominant distal weakness more than proximal, and in plantar flexors more than dorsiflexors. These findings are

consistent with the pattern of involvement shown in MRI. Isokinetic dynamometry showed weakness in plantar flexors. However, isometric dynamometry showed dorsiflexion weakness that was not reflected in isokinetic testing. Suggesting that the reduced isometric strength observed for ankle dorsiflexion may be partly due to the angle chosen for assessment combined with reduced range of ankle dorsiflexion. Moreover, the difference in isometric plantar flexors torque was not significant after modified Bonferroni correction, unlike isokinetic plantar flexion. It was noted that participants tend to engage their upper body to push downwards into the dynamometer footplate which might contributed to the overall torque measured. Changing the testing position from supine to seated might eliminate this factor. Beside positioning and test application, the sample size was small with a high standard deviation due to high variability in strength within the cohort. A larger sample size will allow us to see true differences.

Regardless of the genetic diagnosis, our DHMN cohort showed similar patterns of muscle involvement as previously reported samples (Esteller et al., 2023, O'Donnell et al., 2022). However, if we consider that most of our cohort are with HSPB1 genetic diagnosis (7/12), Esteller et al.(2023) reported a different pattern of involvement in their HSPB1 cohort (2 participants). They showed Severe involvement of all muscles on the lower leg (Esteller et al., 2023). Although the number of participants is limited in our study, it still presents the largest DHMN

cohort with most of them genetically diagnosed, and the first to utilise quantitative MRI analysis to identify the pattern of involvement in this particular group.

5.4.2. Deviation from Normal in Walking Gait

The DHMN group took more time to complete a gait cycle over an equivalent distance compared to the control group. The increase in step and stride time led to the decreased number of steps and strides per minute, and therefore, a slower walking speed.

A delay in opposite toe off contributed to the increase of the double support time and therefore a longer stance phase. Delayed opposite toe off and no difference on opposite foot contact, led to increased double support time in stance phase, and shorter single support time. Therefore, any increase in the single support time is most likely to be relevant to the swing phase which is commonly affected by foot drop in peripheral neuropathy.

Kinematic data showed less ankle dorsiflexion angle in swing phase (Figure 15 , A, B) that could relate to the increased hip flexion angle observed, and decreased ankle dorsiflexors power generation. This pattern suggests the presence of foot drop combined with compensatory strategy using hip flexion to improve foot clearance of the swinging side. This pattern has been seen in CMT cohorts (Ramdharry et al., 2009, Don et al., 2007, Vinci and Perelli, 2002).

In stance phase, kinematic variables showed reduced ankle control with increased passive dorsiflexion angle in terminal stance and decreased ankle plantar flexion in pre-swing (Figure 15, C). Kinetic analysis showed a decrease in plantar flexion moment and power generation in terminal stance, which confirms the presence of plantar flexion failure. This pattern has been seen in CMT cohorts (Don et al., 2007, Vinci and Perelli, 2002).

The differences between the healthy controls and people with DHMN when walking could partly be explained by their differences in walking speed, as gait kinetic and kinematic parameters are decreased at slower speeds and increased at faster speeds (Fukuchi et al., 2019). This highlights the importance of taking into account the effects of walking speed when comparing gait data of pathological individuals with normal or control individuals. Future studies involving such type of comparisons must control for the effects of different gait speeds, for example by having walking speed as a covariate in the analysis or getting the control group to walk at matched speeds.

Although MRI studies showed different patterns of muscle involvement between CMT and DHMN (Esteller et al., 2023, Bas et al., 2020), 3D motion analysis showed more similar patterns. Plantar flexion failure and foot drop with proximal compensation are patterns described previously in CMT cohorts with predominant distal muscle weakness (Ramdharry et al., 2009, Don et al., 2007, Vinci and Perelli, 2002). These studies included mixed types of CMT and severe

cases with plantar flexion weakness, which explain the presence of the plantar flexion failure pattern. Unlike CMT, the DHMN cohort of the current study did not show significant dorsiflexors involvement, however, gait analysis showed pattern of foot drop, suggesting that even with mild dorsiflexors weakness, foot clearance of the swinging limb can be affected.

The findings of the current study suggest that although 3D motion analysis is a valuable tool in describing function and detecting deviation from normal, quantitative MRI is more sensitive to differences between neuropathy groups to describe muscular involvement.

5.5. Summary

The current study described pattern of involvement and deviation from normal in muscle structure and function. The study findings are summarised in Table 26.

<table>
<tr><th colspan="10">DHMN Pattern in Muscle Involvement and Gait</th></tr>
<tr><td>Muscle Structure</td><td colspan="9">Higher rates of fatty infiltration and signs of active denervation distally at the foot and calf more than proximally, and in the posterior compartment (Plantar flexors) more than anterior compartment (Dorsiflexors) of the calf.</td></tr>
<tr><td>Muscle Function</td><td colspan="5">Plantar flexion (PF) weakness affected the stability of the ankle joint in stance phase</td><td colspan="4">Dorsiflexion (DF) weakness affected foot clearance in swing phase</td></tr>
<tr><td>% of cycle</td><td>0%</td><td>0-10%</td><td>10-30%</td><td>30-50%</td><td>50-60%</td><td>60-70%</td><td>70-85%</td><td>85-100%</td></tr>
<tr><td>Phase</td><td colspan="5">Stance phase</td><td colspan="4">Swing phase</td></tr>
<tr><td rowspan="2">Spatio-Temporal Variables</td><td colspan="3">Increased double support time due to delayed OTO</td><td colspan="2">Increased stance time due to increased step and stride length</td><td colspan="4">Increased single support time</td></tr>
<tr><td colspan="9">Slow gait due to the events timing</td></tr>
<tr><td>Kinematics Variables</td><td colspan="4">Increased ankle passive dorsiflexion</td><td>Decreased ankle plantar flexion</td><td colspan="4">Decreased ankle dorsiflexion and increased hip flexion</td></tr>
<tr><td>Kinetics Variables</td><td colspan="5">Decreased plantar flexion moment and power</td><td colspan="4">Decreased ankle power generation</td></tr>
<tr><td>Gait Pattern</td><td colspan="5">Plantar flexion failure</td><td colspan="4">Foot drop</td></tr>
<tr><th colspan="10">The Normal Gait Cycle</th></tr>
<tr><td>% of cycle</td><td>0%</td><td>0-10%</td><td>10-30%</td><td>30-50%</td><td>50-60%</td><td>60-70%</td><td>70-85%</td><td>85-100%</td></tr>
<tr><td>Phase</td><td colspan="5">Stance phase</td><td colspan="4">Swing phase</td></tr>
<tr><td>Event</td><td>Initial contact</td><td colspan="2">Foot flat, Opposite toe off (OTO)</td><td colspan="2">Heel off, Opposite initial contact</td><td>Toe off</td><td colspan="2">Tibia vertical, Feet adjacent</td><td>Initial contact (next cycle)</td></tr>
<tr><td>Period</td><td>Loading response</td><td colspan="2">Mid-stance</td><td colspan="2">Terminal stance</td><td>Pre-swing</td><td>Initial swing</td><td>Mid-swing</td><td>Terminal swing</td></tr>
<tr><td>Support</td><td>Double support</td><td colspan="3">Single leg support</td><td>Double support</td><td colspan="4">Single leg support</td></tr>
</table>

Table 26: Summary of pattern in gait and muscle involvement in DHMN

Chapter 6: The Natural History of Muscle Structure and Function in Distal Hereditary Motor Neuropathy (DHMN)

6.1. Introduction

Distal Hereditary Motor Neuropathy (DHMN) is a slowly progressive condition that is incurable. There is a need to understand the condition trajectory in order to improve patients' outcomes in managing symptoms and prevent complications. Until therapeutic options are available for clinical trials, there is a need to confirm the responsiveness of outcome measures in DHMN for potential use in future investigation of effect.

Previous natural history studies in CMT showed longitudinal change at the calf level and high responsiveness of quantitative muscle fat fraction MRI over one year (Morrow et al., 2016). Quantitative MRI, to measure intramuscular oedema, was sensitive enough to detect subclinical muscle abnormality as well (Locher et al., 2022, Morrow et al., 2016).

The current longitudinal study is the first to explore the progression of DHMN over one year and to assess the responsiveness of the quantitative methods used (MRI, dynamometry, and 3D motion analysis) in order to determine predictors of change in muscle structure, function, and gait patterns. Based on the findings in chapter 5 and previous studies in CMT we expect our DHMN cohort to show:

- Significant change in MRI quantitative parameters in the distal lower limb muscles, alongside deterioration in isokinetic strength, plus moment and power generation during gait.
- Responsiveness in quantitative MRI parameters at the calf level will be higher in comparison to the thigh level and other methods of quantification, dynamometry and motion analysis.

6.2. Methods

Eight DHMN participants were recruited in the natural history group to undergo an MRI scan, isokinetic and isometric dynamometry of the lower limbs, and 3D motion analysis to capture kinetic and kinematic data of walking gait as described in chapter 4. The same measurements were repeated after 6 months and 12 months to observe the natural history of the disease.

6.2.1. Data Analysis

To explore the natural history of muscle structure and function in Distal Hereditary Motor Neuropathy (DHMN) over one year, baseline, 6 months, and 12 months follow up values were compared on a muscle-by-muscle, parameter-by-parameter basis using repeated measures analysis of variances (ANOVA). A post hoc analysis compared the different measurement to each other using paired t-test. If data sets were not normally distributed, a Friedman test was used. If the Friedman test was positive ($P<0.05$), the different measurement points were compared for the post hoc analysis using a paired Wilcoxson- Signed rank test. Modified Bonferroni correction for multiple comparisons was applied for P-values and confidence intervals (Simes, 1986). Where the data type was ordinal, such as manual muscle testing and qualitative MRI scores, the Friedman test was used.

To ascertain the sensitivity of the MRI, Dynamometry, and 3D motion analysis variables, a standardised response mean (SRM) was calculated as the ratio of

mean change to standard deviation of change. SRM was categorised by magnitude according to Cohen's suggestion: < 0.2 minimal responsiveness; 0.2-0.5 small responsiveness; 0.5-0.8 moderate responsiveness; >0.8 large responsiveness. This statistical method has been used in previous research to examine the sensitivity of MRI and dynamometry longitudinally in slowly progressive in conditions (Morrow, et al., 2016).

6.3. Results

6.3.1. Subjects and Clinical Assessment

Eight DHMN participants were recruited. They underwent MRI scan, Dynamometry, and 3D motion analysis at baseline, 6 months, and 12 months. One DHMN female participant completed only baseline and 6 months measurements before she withdrew from the study (appendix VII), therefore, the last observation at 6 months was carried forward to 12 months for analysis purposes. There were no significant changes in clinical assessment over 12 month in terms of disease severity (CMTES), foot posture, ankle ROM, falls, and perceived walking ability (walk-12). A summary of the subjects' demographics and clinical assessment over 12 months is presented in Table 27.

Demographics and clinical assessment	**BASELINE**	**6 MONTHS**	**12 MONTHS**
Numbers	8	8	7
Gender (M/F)	(3/5)	(3/5)	(3/5)
Age; mean years; range	57 (42/75)	57 (42/75)	57 (42/75)
Genetic diagnosis (HSPB1/unknown)	(6/2)	(6/2)	(6/2)
CMTES; mean (SD)	7.25(3.23)	6.62(3.31)	6.87(3.5)
Foot Posture Index-6 (Pronated/Normal/Supinated)	(1/6/1)	(1/3/4)	(1/3/4)
Fall Frequency (weekly/Monthly/Yearly)	(1/1/2)	(1/1/2)	(1/1/2)
Walk-12; mean (SD)	36(12)	35(13)	36(13)
Range of Motion	**BASELINE**	**6 MONTHS**	**12 MONTHS**
Dorsiflexion; count (Limited/Normal)	(8/0)	(8/0)	(8/0)
Plantarflexion; count (Limited/Normal)	(8/0)	(8/0)	(8/0)
M= Male, F= Female, HSPB1= Heat-shock 27-KD Protein 1, SD= Standard Deviation, CMTES= Charcot-Marie-Tooth Disease Examination Score, *= significant after modified Bonferroni correction.			

Table 27: : Summary of demographics and clinical assessment of DHMN at baseline, 6 months, and 12 months.

6.3.2. Longitudinal Change in Intramuscular Fat on MRI

Quantitative analysis of MRI muscle fat fraction showed significant longitudinal difference at the calf level (P=0.003) in most of the calf muscles except the medial and lateral gastrocnemius. No change was shown at the thigh and foot (Table 28) (Figure 18). Qualitative Modified Mercuri's Scale analysis showed significant longitudinal difference at the foot (P<0.00001) and calf muscles (P=0.00781-0.03725) except the lateral gastrocnemius (Table 29) (Figure 18). The Friedman test was calculated manually to confirm the results (appendix II). Calculating the test statistics ($\chi 2$) yield a value of 0 and P-value =1. Thus, the null hypothesis is accepted and there is no difference between measurements.

Region of interest %	**BASELINE** mean (SD)	**6 MONTHS** mean (SD)	**12 MONTHS** mean (SD)	**F**	**P**
Total Thigh	15.95(13.22)	16.58(15.22)	17.15(15.18)	2.28	0.14
Knee Extension	14.83(14.34)	15.26(15.21)	15.41(15.56)	0.67	0.53
Rectus Femoris	11.45(10.04)	11.23(10.45)	11.21(10.18)	0.21	0.81
Vastus Intermedius	16.43(15.12)	16.77(15.37)	17.18(15.50)	0.60	0.56
Vastus Lateralis	16.85(18.01)	17.21(18.45)	16.94(18.97)	0.89	0.43
Vastus Medialis	10.81(8.66)	11.78(11.76)	12.18(12.04)	0.09	0.92
Knee Flexion	17.40(12.74)	18.91(16.35)	19.14(16.07)	3.03	0.08
Semimembranosus	18.19(13.38)	19.28(15.91)	20.00(15.23)	1.75	0.21
Semitendinosus	16.18(16.09)	19.64(22.23)	20.45(22.50)	2.09	0.16
Biceps Femoris	20.30(12.98)	20.85(15.11)	21.59(14.78)	1.41	0.28
Sartorius	20.40(18.70)	21.41(19.57)	21.23(21.12)	0.03	0.97
Gracilis	10.72(11.02)	12.04(12.85)	11.37(13.18)	0.92	0.42
Adductor Magnus	18.37(20.25)	19.13(20.30)	19.53(21.05)	0.37	0.37
Total Calf	36.61(22.35)	39.17(22.87)	39.83(22.49)	13.27	**0.003***
Dorsiflexion	23.89(19.01)	25.83(19.60)	26.57(19.84)	13.42	**0.00056***
Tibialis Anterior	23.89(19.01)	25.83(19.60)	26.57(19.84)	12.50	**0.001***
Peroneus Longus	39.30(27.19)	40.41(27.26)	41.61(26.19)	7.05	**0.009***
Plantarflexion	40.16(23.61)	43.50(24.70)	44.04(24.11)	9.53	**0.009***
Lateral Gastrocnemius	32.88(31.04)	34.50(30.33)	35.71(30.63)	3.27	0.07

Medial Gastrocnemius	41.46(22.89)	43.44(21.59)	43.89(22.15)	1.91	0.20
Soleus	43.22(23.70)	47.67(25.74)	47.74(24.53)	8.57	**0.013***
Tibialis Posterior	30.03(22.23)	33.82(22.12)	34.05(22.18)	9.31	**0.008***
Foot	54.99(7.12)	56.00(7.86)	57.49(7.25)	3.05	0.09
N=8, SD= Standard Deviation, *= significant (P<0.05)= post-hoc test showed difference between baseline and 6 months.					

Table 28: Longitudinal MRI mean muscle fat fraction of DHMN and repeated measures analysis results.

Region of interest	**BASELINE** median (IQR)	**6 MONTHS** median (IQR)	**12 MONTHS** median (IQR)	**P**
Tibialis Anterior	2.75(2.5 to 4.25)	2.75(2.5 to 5)	2.75(2.5 to 5)	**0.02602***
Tibialis Posterior	3.75(2.75 to 5)	3.75(2.75 to 5.75)	3.75(2.75 to 5.75)	**0.02602***
Peroneus Longus	4.25(3.25 to 5.25)	4.25(3.25 to 6)	4.25(3.25 to 6)	**0.03725***
Medial Gastrocnemius	4(3.25 to 5)	4(3.25 to 6)	4(3.25 to 6)	**0.00781***
Lateral Gastrocnemius	2.5(2 to 5)	2.5(2 to 6)	2.5(2 to 6)	0.4
Soleus	4.75(3 to 5.25)	4.75(3 to 6)	4.75(3 to 6)	**0.00781***
Foot	6(6 to 6)	6(6 to 6)	6(6 to 6)	**<0.00001***
N=8, Modified Mercuri's Scale: 1=Stage 0= Normal appearance, 2=Stage 1= Early Moth-eaten appearance= with scattered small areas of increased signal, 3=Stage 2a= Late Moth-eaten appearance= with numerous discrete areas of increased signal with beginning confluence= comprising less than 30% of the volume of the individual muscle, 4=Stage 2b= Late Moth-eaten appearance= with numerous discrete areas of increased signal with beginning confluence= comprising 30 – 60% of the volume of the individual muscle, 5=Stage 3= Washed-out appearance= fuzzy appearance due to confluent areas of increased signal, 6=Stage 4= End stage appearance= muscle replaced increased density connective tissue and fat= with only A rim of fascia and neurovascular structures distinguishable, IQR= interquartile range, *= significant (P<0.05)= post-hoc test showed difference between baseline and 6 months.				

Table 29: Longitudinal MRI median muscle fat qualitative score using Modified Mercuri's Scale of DHMN and repeated measures analysis results.

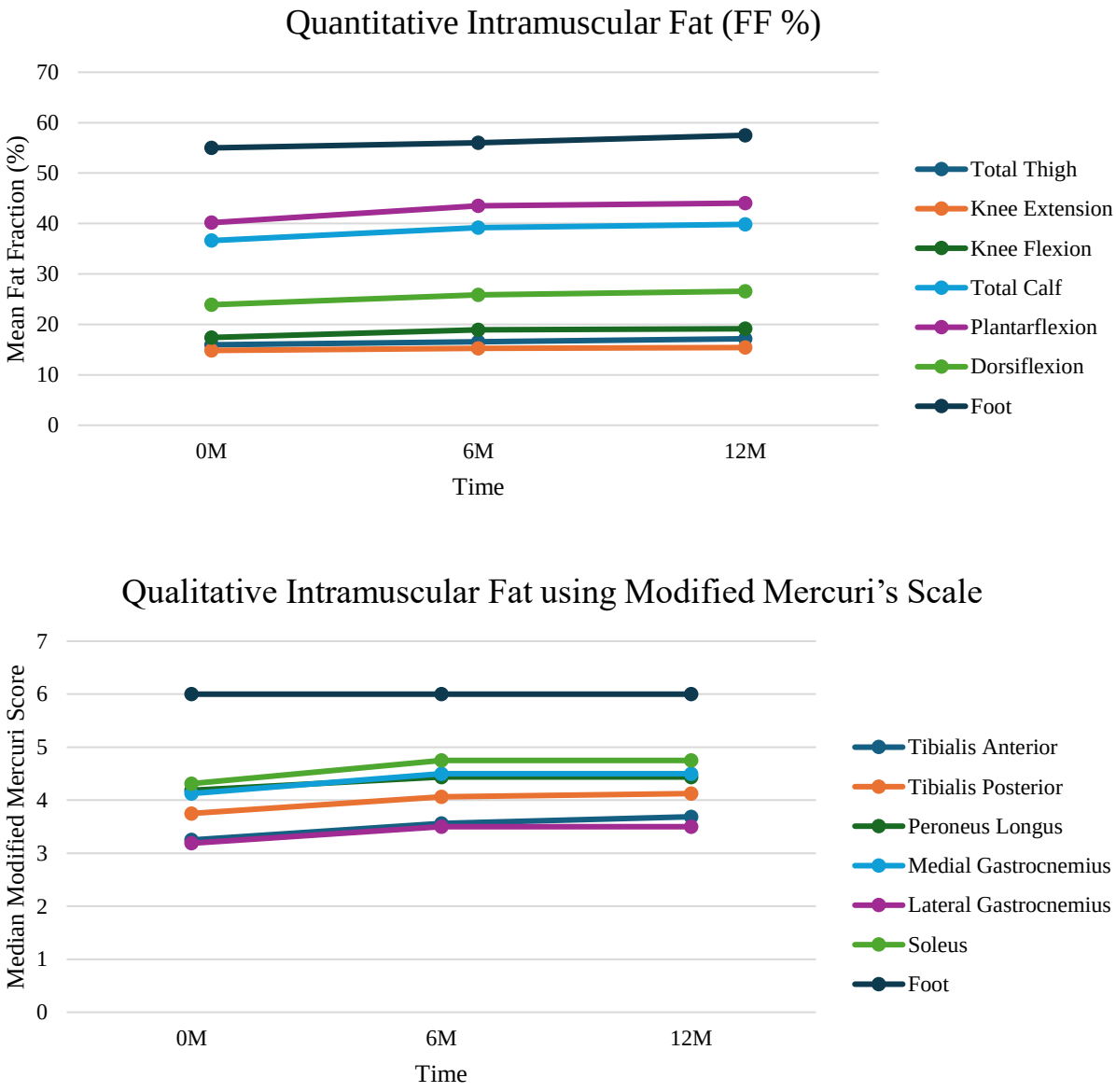


Figure 18: Longitudinal change of intramuscular fat in DHMN.

N=8, M= months.

6.3.3. Longitudinal Change in Muscle Area on MRI

Analysis of the cross sectional area did not show any change at all levels (Table 30). However, when the remaining muscle area was calculated significant longitudinal difference was seen at the gracilis muscle (P=0.046) and soleus muscle (P=0.015) which affected the total plantar flexion change over one year (P=0.048) (Table 31).

Region of interest mm²	**BASELINE** mean (SD)	**6 MONTHS** mean (SD)	**12 MONTHS** mean (SD)	**F**	**P**
Total Thigh	10009.8(3716.7)	9967.8(3544.5)	10066.9(3586.5)	0.46	0.63
Knee Extension	4758.2(1487.3)	4686.4(1431.1)	4718.3(1495.4)	0.59	0.52
Rectus Femoris	437.7(173.8)	426.6(175.9)	430.4(187.7)	1.21	0.33
Vastus Intermedius	1416.2(428.5)	1381.8(394.4)	1414.7(423.0)	0.99	0.40
Vastus Lateralis	1630.7(383.3)	1606.2(372.3)	1623.3(405.3)	0.47	0.57
Vastus Medialis	1273.5(601.6)	1271.8(601.3)	1249.9(594.1)	0.97	0.40
Knee Flexion	3691.6(1658.6)	3785.4(1649.2)	3772.0(1553.1)	1.41	0.28
Semimembranosus	820.3(426.4)	849.1(445.6)	812.3(402.6)	1.29	0.30
Semitendinosus	765.4(405.7)	805.3(401.1)	792.8(339.2)	0.90	0.43
Biceps Femoris	1306.5(577.2)	1330.6(570.0)	1341.1(566.2)	2.63	0.11
Sartorius	350.8(146.7)	352.2(141.7)	364.1(152.9)	0.58	0.53
Gracilis	448.6(224.7)	448.2(225.4)	461.6(235.0)	2.84	0.09
Adductor Magnus	1560.0(830.4)	1496.0(724.2)	1576.6(796.2)	2.00	0.19
Total Calf	4925.0(1636.0)	4813.6(1802.5)	4819.5(1860.3)	0.82	0.43
Dorsiflexion	828.6(277.4)	825.4(293.9)	830.6(301.8)	0.09	0.92
Tibialis Anterior	828.6(277.4)	825.4(293.9)	830.6(301.8)	0.09	0.92
Peroneus Longus	454.3(165.8)	461.8(192.1)	448.3(183.1)	0.38	0.69
Plantarflexion	3327.5(1235.4)	3199.9(1346.8)	3230.6(1387.3)	1.72	0.23
Lateral Gastrocnemius	663.3(512.5)	612.5(497.6)	635.5(489.1)	1.23	0.32
Medial Gastrocnemius	784.9(355.2)	761.2(385.4)	772.9(382.7)	0.24	0.69
Soleus	1879.3(656.5)	1826.1(693.9)	1822.3(703.8)	2.47	0.14
Tibialis Posterior	314.5(133.2)	326.5(125.4)	309.9(140.4)	0.31	0.64
Foot	465.9(127.7)	446.6(116.4)	442.5(102.8)	1.06	0.35
N=8, SD= Standard Deviation, mm²= square millimetres.					

Table 30: Longitudinal MRI muscle cross sectional area of DHMN and repeated measures analysis results.

Region of interest mm²	**BASELINE** mean (SD)	**6 MONTHS** mean (SD)	**12 MONTHS** mean (SD)	**F**	**P**
Total Thigh	17364.0(7525.7)	17204.2(7504.1)	17227.3(7442.8)	0.66	0.46
Knee Extension	8337.7(3319.3)	8251.5(3342.8)	8206.7(3344.1)	1.46	0.27
Rectus Femoris	395.8(185.3)	389.4(191.0)	390.5(197.7)	0.75	0.48
Vastus Intermedius	1217.6(473.5)	1190.6(448.7)	1200.3(461.6)	1.57	0.24
Vastus Lateralis	1387.7(493.2)	1370.4(500.6)	1371.8(497.2)	0.59	0.50
Vastus Medialis	1167.7(602.2)	1175.4(624.7)	1140.8(607.5)	2.87	0.09
Knee Flexion	6360.4(3268.6)	6446.6(3406.6)	6384.5(3174.1)	0.29	0.68
Semimembranosus	704.5(405.1)	725.0(440.9)	689.6(380.8)	0.83	0.41
Semitendinosus	677.0(401.9)	682.7(419.5)	669.7(379.7)	0.25	0.78
Biceps Femoris	1088.4(575.1)	1105.3(583.4)	1101.0(568.7)	0.59	0.57
Sartorius	294.2(165.2)	299.3(173.6)	308.3(177.1)	0.77	0.43
Gracilis	416.0(226.9)	410.9(226.9)	432.2(238.6)	3.86	**0.046***
Adductor Magnus	1332.9(795.5)	1253.1(705.5)	1329.1(800.1)	1.97	0.18
Total Calf	6469.0(3538.9)	6159.4(3559.1)	6110.2(3644.6)	3.78	0.08
Dorsiflexion	1322.9(616.8)	1293.1(631.0)	1288.4(645.1)	1.15	0.35
Tibialis Anterior	661.5(308.4)	646.5(315.5)	644.2(322.5)	1.15	0.35
Peroneus Longus	273.6(147.7)	272.7(156.4)	262.9(154.6)	1.07	0.37
Plantarflexion	4166.3(2608.4)	3879.6(2605.5)	3879.8(2644.0)	4.98	**0.048***
Lateral Gastrocnemius	512.2(553.5)	486.0(520.1)	486.3(521.6)	1.72	0.21
Medial Gastrocnemius	476.6(335.1)	455.2(339.5)	459.7(336.1)	1.02	0.36
Soleus	1094.3(642.4)	998.5(652.2)	993.9(638.1)	8.32	**0.015***
Tibialis Posterior	216.3(109.1)	220.7(125.0)	208.1(126.8)	0.32	0.63
Foot	215.9(89.6)	201.0(80.1)	193.0(74.7)	2.00	0.19
N=8, SD= Standard Deviation, mm²= square millimetres,*= significant (P<0.05).					

Table 31: Longitudinal MRI remaining muscle area of DHMN and repeated measures analysis results.

6.3.4. Longitudinal Change in Intramuscular Water on MRI

Quantitative analysis of intramuscular water on MRI showed significant longitudinal difference only at the tibialis posterior muscle (P=0.03725). No change was shown at the rest of the calf muscles and foot (Table 32). Qualitative analysis showed significant longitudinal difference only at the medial gastrocnemius muscle (P=0.01912). No change was shown at the rest of the calf muscles and foot (Table 33).

Region of interest ms	BASELINE mean (SD)	6 MONTHS mean (SD)	12 MONTHS mean (SD)	F	P
Total Calf	40.76(7.0)	38.94(7.2)	41.54(6.3)	2.59	0.11
Dorsiflexion	40.11(6.8)	39.51(6.5)	42.09(6.7)	3.66	0.05
Tibialis Anterior	40.11(6.8)	39.51(6.5)	42.09(6.7)	3.66	0.05
Peroneus Longus	41.50(8.5)	37.85(5.3)	41.27(6.3)	2.03	0.17
Plantarflexion	41.12(7.8)	39.07(8.2)	41.47(6.8)	1.72	0.21
Lateral Gastrocnemius	41.22(8.5)	37.58(7.9)	40.86(6.3)	2.16	0.15
Medial Gastrocnemius	42.25(8.0)	39.09(5.2)	41.11(4.1)	0.58	0.57
Soleus	40.36(7.8)	38.97(9.9)	41.27(8.5)	0.26	0.77
Tibialis Posterior	39.48(6.7)	39.59(9.2)	41.73(7.2)	4.20	**0.03725***
Foot	46.63(5.9)	46.79(7.3)	50.55(4.1)	2.44	0.15
N=8, SD= Standard Deviation, ms= millisecond, *= significant (P<0.05)= post-hoc test showed difference in 12 months in comparison to baseline and 6 months.					

Table 32: Longitudinal quantitative T_{2m} MRI muscle water of DHMN and repeated measures analysis results.

Region of interest	BASELINE median (IQR)		6 MONTHS median (IQR)		12 MONTHS median (IQR)		P
	Intensity	Extent	Intensity	Extent	Intensity	Extent	
Tibialis Anterior	2(1.5-2)	3(2.5-)	2(1.75-2)	3(2-3)	2(2-2)	3(2 to 3)	0.55
Tibialis Posterior	1(1-1.75)	2.75(1.75-3)	1.25(0.5-1.75)	2.25(0.5-3)	1.25(1-2)	2.25(1.25-3)	0.76
Peroneus Longus	1(1-1.5)	1.75(1-3)	1(0.75-1.5)	1.75(1-2.5)	1.25(0.75-1.5)	1.75(1- 2.75)	0.72
Medial Gastrocnemius	1(1-1.25)	2(1.25-2.5)	1(1-1.5)	2(1.25-2)	1.5(1-1.75)	2.25(1.75-2.75)	**0.01912***
Lateral Gastrocnemius	1(1-1.5)	1.5(1-3)	1.5(1-1.5)	2(1-3)	1.5(1-1.5)	2.25(1.5- 3)	0.16
Soleus	1.25(1- 1.5)	2.5(1.25- 3)	1.5(1-1.75)	2(1-2.75)	1.25(1-1.75)	2(1.25- 2.5)	0.53
Foot	1(1 to 2)	1.25(1 to 2)	1(1 to 2)	1.75(1-2.5)	1(0.75-1.5)	1.25(1-2.5)	0.61
N=8, Intensity: 0= none, 1=mild, 2=marked, Extent: 0= none, 1= < 30%, 2=30%-60%, 3= >60%, IQR= interquartile range, *= significant (P<0.05)= post-hoc test showed difference in 12 months in comparison to baseline and 6 months.							

Table 33: Longitudinal qualitative STIR MRI muscle water of DHMN and repeated measures analysis results.

6.3.5. Longitudinal Change in Strength Measurements

Friedman repeated measures analysis showed significant longitudinal difference only in the plantar flexion strength (P=0.046), measured using manual muscle testing (Table 34) (Figure 19). Post-hoc testing showed a decrease in median strength between baseline and 6 months. Repeated measures ANOVA showed longitudinal differences in muscle strength measured using dynamometry for isometric dorsiflexion and isokinetic plantar flexion (Table 35) (Figure 19). Post-

hoc testing showed differences were between baseline and 6 months, with an increase in mean ankle strength.

Manual Muscle Testing	**BASELINE** median (IQR)	**6 MONTHS** median (IQR)	**12 MONTHS** median (IQR)	**P**
Hip Flexion	5 (4 to 5)	5 (4 to 5)	5 (4 to 5)	0.13
Hip Extension	5 (3+ to 5)	5 (4- to 5)	5 (4- to 5)	0.39
Knee Flexion	5 (4- to 5)	5 (5 to 5)	5 (5 to 5)	0.13
Knee Extension	5 (4+ to 5)	5 (5 to 5)	5 (5 to 5)	0.39
Dorsiflexion	3- (1+ to 4)	2+ (1 to 4+)	2+ (1 to 4+)	0.84
Plantar flexion	4 (2 to 4+)	3+ (2- to 4+)	3+ (2- to 4+)	**0.04606***
N=8, IQR= interquartile range, *= significant (P<0.05)= post-hoc test showed difference between baseline and 6 months.				

Table 34:Longitudinal manual muscle testing of DHMN and repeated measures analysis results.

Isometric Dynamometry Nm	**BASELINE** mean (SD)	**6 MONTHS** mean (SD)	**12 MONTHS** mean (SD)	**F**	**P**
Hip Extension 45°	126.2(68.9)	122.8(67.5)	107.1(59.7)	1.85	0.20
Hip Flexion 45°	93.9(46.9)	98.8(50.2)	99.7(44.3)	0.66	0.53
Knee Extension 45°	86.1(44.5)	88.9(47.6)	80.6(42.3)	1.82	0.20
Knee Extension 90°	86.9(43.3)	85.4(43.8)	77.9(44.2)	1.90	0.20
Knee Flexion 45°	48.9(30.7)	50.8(33.2)	46.4(29.2)	0.66	0.47
Knee Flexion 90°	31.3(22.7)	37.6(23.6)	33.3(20.0)	1.52	0.25
Ankle Plantar flexion 10°	25.8(20.8)	28.6(23.8)	25.4(19.1)	0.42	0.67
Ankle Dorsiflexion 10°	5.6(6.5)	9.8(8.9)	9.1(6.5)	4.57	**0.03***
Ankle Dorsiflexion 30°	10.8(8.6)	17.8(12.1)	18.4(10.3)	6.70	**0.009***
Isokinetic Dynamometry Nm	**BASELINE** mean (SD)	**6 MONTHS** mean (SD)	**12 MONTHS** mean (SD)	**F**	**P**
Hip Extension 60°/60s	96.5(70.2)	101.6(71.6)	99.2(95.6)	0.39	0.69
Hip Flexion 60°/60s	86.1(45.0)	100.5(53.8)	103.9(95.5)	3.88	0.07
Knee Extension 60°/60s	63.4(39.1)	67.6(39.5)	64.7(95.7)	0.73	0.46
Knee Flexion 60°/60s	34.1(23.8)	36.9(26.2)	35.3(95.0)	0.61	0.56
Knee Extension 120°/120s	46.9(31.9)	52.0(31.3)	47.4(95.1)	1.68	0.23
Knee Flexion 120°/120s	25.4(18.3)	29.4(22.6)	26.2(95.3)	1.05	0.38
Ankle Plantar flexion 60°/60°	11.0(7.4)	18.9(13.8)	15.6(95.5)	5.78	**0.031***
Ankle Dorsiflexion 60°/60°	11.8(7.0)	17.4(10.6)	20.4(95.7)	3.53	0.06
N=8, SD= Standard Deviation, Nm= Newton meter, *= significant (P<0.05)= post-hoc test showed difference between baseline and 6 months.					

Table 35: Longitudinal dynamometry of DHMN and repeated measures analysis results.

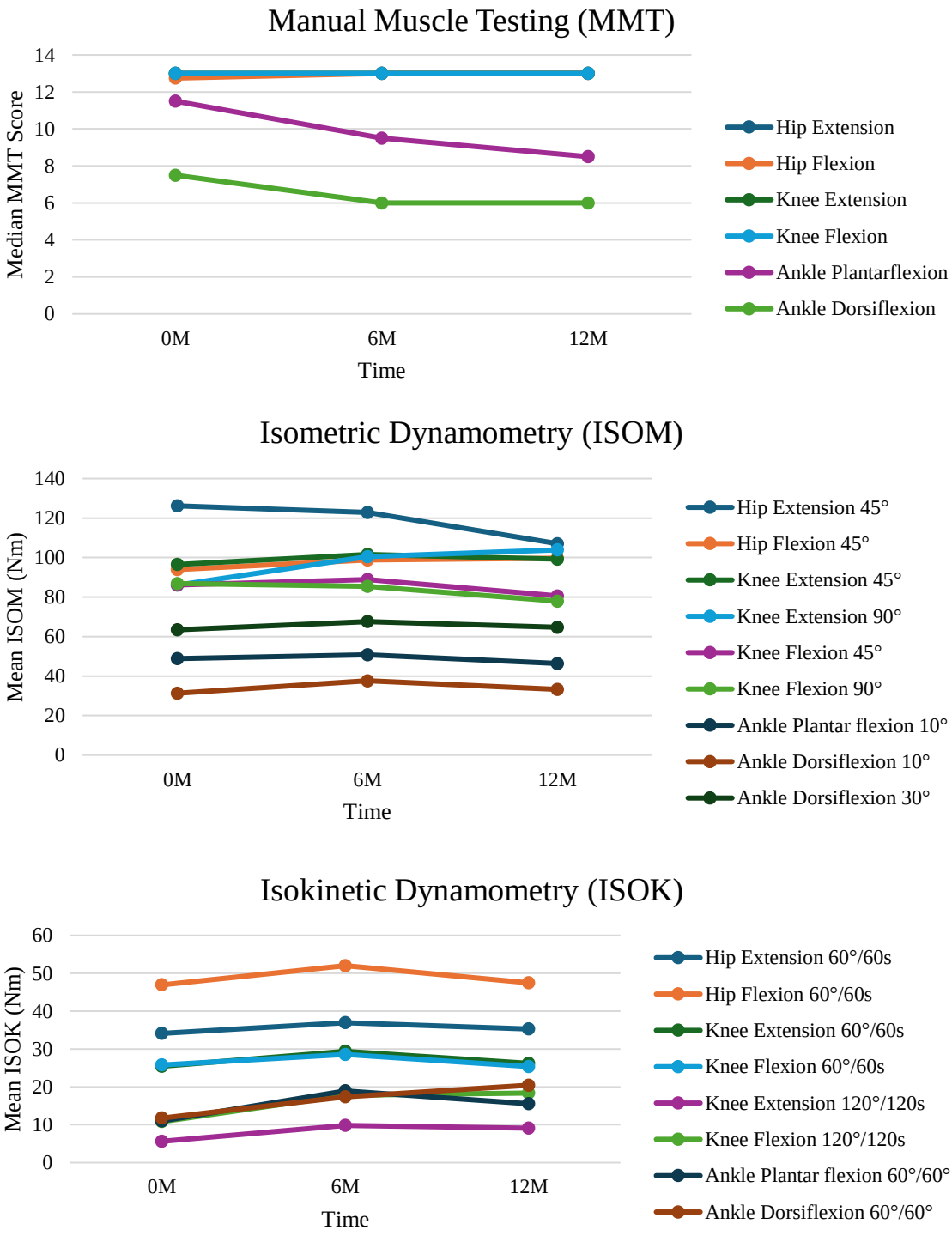


Figure 19: Longitudinal change of muscle strength in DHMN.

N=8, M= months, Nm= Newton meter. Hip and knee on MMT graph are at the same level of strength.

6.3.6. Longitudinal Change in Gait Parameters

Longitudinal analysis of 3D gait parameters, spatiotemporal (Table 36), kinematics (Table 37) (Figure 20), and kinetics (Table 38) (Figure 21, Figure 22), did not show significant change across the three measurement points. The only parameter that showed significant difference on Friedman test was the minimum hip moment (P= 0.00461). Post-hoc test showed difference in baseline in comparison to 6 months and 12 months. From baseline to 6 months, analysis showed an increase in hip flexor moment, however, the 12 months measurement showed a decrease in hip flexor moment.

Gait Spatiotemporal	**BASELINE** mean (SD)	**6 MONTHS** mean (SD)	**12 MONTHS** mean (SD)	**F**	**P**
Speed; m/s	0.90(0.25)	0.95(0.23)	0.90(0.27)	1.91	0.18
Stride Length; m	1.10(0.22)	1.12(0.19)	1.10(0.21)	1.19	0.33
Stride Time; s	1.25(0.18)	1.21(0.14)	1.26(0.17)	1.47	0.26
Strides Per Minute	48.79(5.47)	50.14(5.25)	48.5(6.27)	1.93	0.20
Step Length; m	0.55(0.11)	0.56(0.10)	0.55(0.10)	1.18	0.34
Step Time; s	0.63(0.09)	0.61(0.07)	0.63(0.09)	1.47	0.26
Steps Per Minute	97.58(10.93)	100.27(10.50)	97.1(12.55)	1.93	0.20
Percent Stance	0.64(0.02)	0.64(0.02)	0.65(0.02)	1.52	0.25
Single Support Time; s	0.61(0.05)	0.61(0.07)	0.64(0.10)	0.58	0.57
Double Support Time; s	0.19(0.08)	0.17(0.04)	0.17(0.04)	1.00	0.39
Percent Opposite Toe Off	15.09(4.16)	13.99(2.29)	13.5(1.78)	0.39	0.69
Percent Opposite Foot Contact	49.48(0.69)	49.96(0.67)	50.0(0.78)	2.75	0.10
N=8, SD= Standard Deviation, m= meters, s= seconds.					

Table 36: Longitudinal spatiotemporal parameters of DHMN and repeated measures analysis results.

Gait Kinematics	**BASELINE** mean (SD)	**6 MONTHS** mean (SD)	**12 MONTHS** mean (SD)	**F**	**P**
Pelvis X Max; °	4.08(0.85)	3.89(1.11)	3.94(0.79)	0.65	0.54
Pelvis X Min; °	-4.26(1.16)	-3.88(1.22)	-4.3(0.82)	0.26	0.77
Pelvis Y Max; °	15.75(5.97)	14.99(4.72)	16.58(3.58)	0.37	0.70
Pelvis Y Min; °	10.72(4.80)	9.80(3.87)	11.5(3.11)	0.48	0.63
Pelvis Z Max; °	7.04(2.78)	7.60(3.67)	7.23(4.49)	0.29	0.75
Pelvis Z Min; °	-7.33(3.15)	-7.11(3.84)	-7.8(3.76)	0.03	0.97

Hip Max; °	46.81(6.01)	44.49(5.48)	47.8(3.15)	0.20	0.82
Hip Min; °	4.18(4.38)	0.61(5.84)	3.18(3.54)	1.38	0.28
Knee Max; °	73.24(4.97)	73.94(3.33)	73.8(3.26)	0.12	0.82
Knee Min; °	6.47(5.35)	5.47(5.78)	5.41(8.09)	0.22	0.70
Ankle Max; °	24.09(6.15)	24.52(4.90)	24.0(4.55)	0.07	0.84
Ankle Min; °	-11.69(3.86)	-12.35(6.03)	-13.(5.28)	0.64	0.49
N=8, SD= Standard Deviation= Pelvic z Maximum= Rotation forwards, Pelvic z Minimum= Rotation backwords, Pelvic x Maximum, lateral raise, Pelvic x Minimum= lateral drop, Pelvic y Maximum= Anterior tilt, Pelvic y Minimum = Posterior tilt, Hip y Maximum= Flexion, Hip y Minimum= Extension, Knee y Maximum= Flexion, Knee y Minimum= Extension, Ankle y Maximum= Dorsiflexion, Ankle y Minimum= Plantarflexion.					

Table 37: Longitudinal kinematics parameters of DHMN and repeated measures analysis results.

Gait Kinetics	**BASELINE** mean (SD)	**6 MONTHS** mean (SD)	**12 MONTHS** mean (SD)	**F**	**P**
Hip Moment Y Max; Nm/Kg	1.29(0.41)	1.28(0.48)	1.28(0.61)	0.00	0.97
Hip Moment Y Min; Nm/Kg	-0.45(0.16)	-0.81(0.84)	-0.5(0.26)	8.10	**0.00461***
Knee Moment Y Max; Nm/Kg	0.58(0.26)	1.06(1.34)	0.73(0.32)	1.47	0.26
Knee Moment Y Min; Nm/Kg	-0.60(0.26)	-0.62(0.30)	-0.6(0.35)	0.37	0.70
Ankle Moment Y Max; Nm/Kg	0.97(0.26)	0.86(0.42)	1.00(0.26)	0.58	0.49
Ankle Moment Y Min; Nm/Kg	-0.05(0.04)	-0.47(1.22)	-0.0(0.05)	0.86	0.45
Hip Power Max During Swing; W/kg	0.90(0.20)	0.97(0.27)	0.95(0.33)	0.61	0.56
Hip Power Min During Stance; W/kg	-0.74(0.67)	-1.44(1.56)	-0.9(0.85)	0.65	0.54
Hip Power Max During Stance; W/kg	1.61(0.63)	1.80(0.80)	1.84(1.05)	0.51	0.56
Knee Power Max During Swing; W/kg	0.31(0.06)	0.41(0.33)	0.34(0.11)	0.26	0.77
Knee Power Min During Stance; W/kg	-1.33(0.91)	-2.77(4.22)	-1.8(1.11)	2.92	0.09
Knee Power Max During Stance; W/kg	1.14(0.66)	1.64(1.24)	1.41(1.10)	1.40	0.28
Ankle Power Max During Swing; W/kg	0.04(0.02)	0.06(0.04)	0.09(0.10)	1.72	0.21
Ankle Power Min During Stance; W/kg	-1.07(0.36)	-1.58(1.45)	-1.1(0.35)	0.86	0.45
Ankle Power Max During Stance; W/kg	1.02(0.46)	2.11(2.72)	1.11(0.35)	1.47	0.26
N=8, SD= Standard Deviation, Nm/Kg= Newton Meter per Kilogram, W/kg= Watts Per Kilogram, *= significant (P<0.05)= post-hoc test showed difference in baseline in comparison to 6 months and 12 months= Hip Moments Maximum= Extension, Hip Moments Minimum= Flexion, Knee Moments Maximum= Extension, Knee Moments Minimum= Flexion, Ankle Moments Maximum= Plantarflexion, Ankle Moments Minimum= Dorsiflexion, Hip Power Maximum = generation in swing phase, Hip Power Minimum= absorption in stance phase, Hip Power Maximum= generation in stance phase, Knee Power Maximum= generation in stance phase, Knee Power Maximum= generation in swing phase, Knee Power Minimum = absorption in stance phase, Ankle Power Maximum= generation in stance phase, Ankle Power Minimum= absorption in stance phase, Ankle Power Maximum= generation in swing phase.					

Table 38: Longitudinal kinetics parameters of DHMN and repeated measures analysis results.

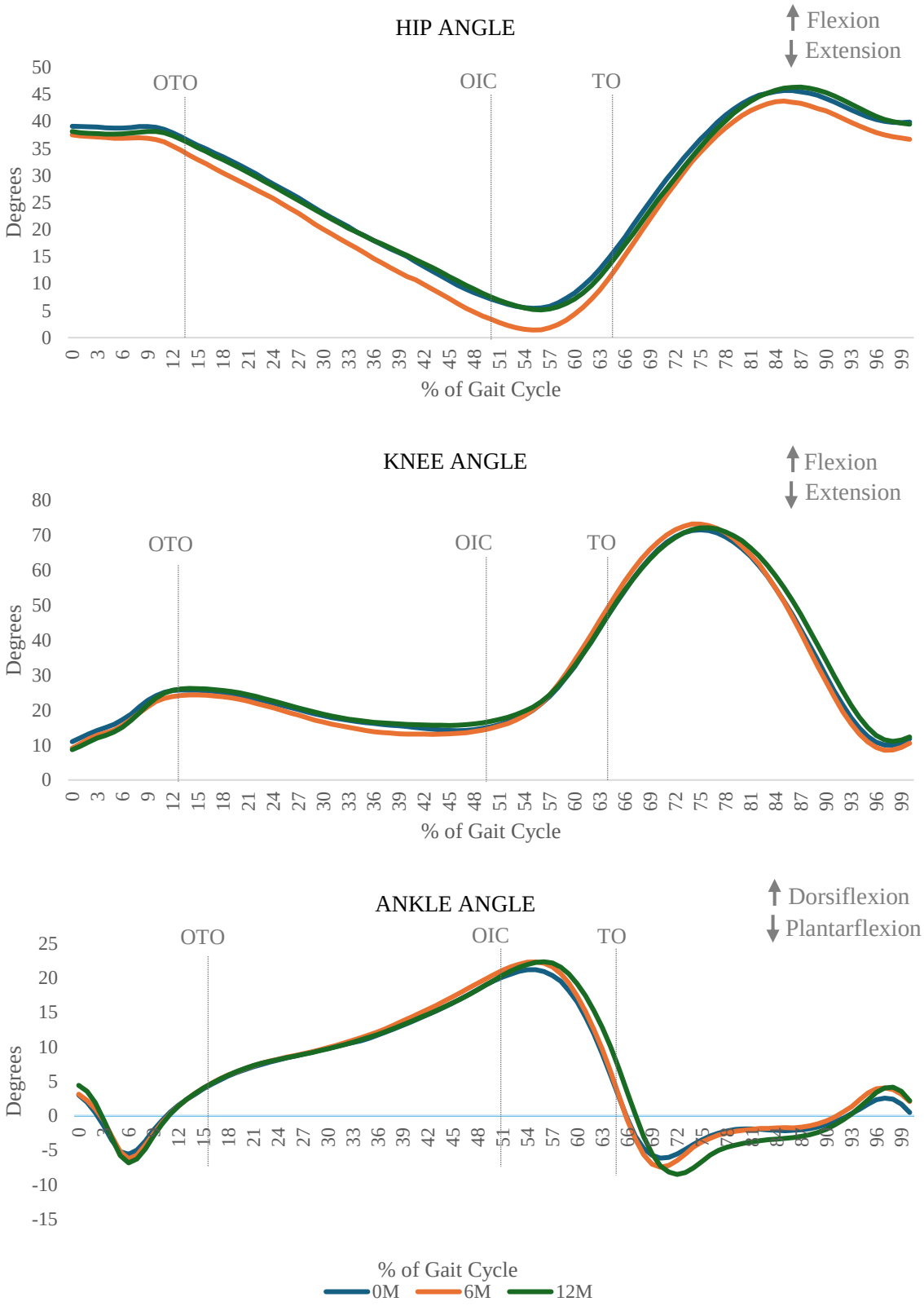


Figure 20:Grand average angular displacement in the sagittal plane over one gait cycle for the ankle, knee, and hip (average of left and right sides). Comparison of longitudinal measurements of people with DHMN.

OTO, Opposite side tore off; OIC, Opposite side initial contact; TO, Toe off, N=8, M= months.

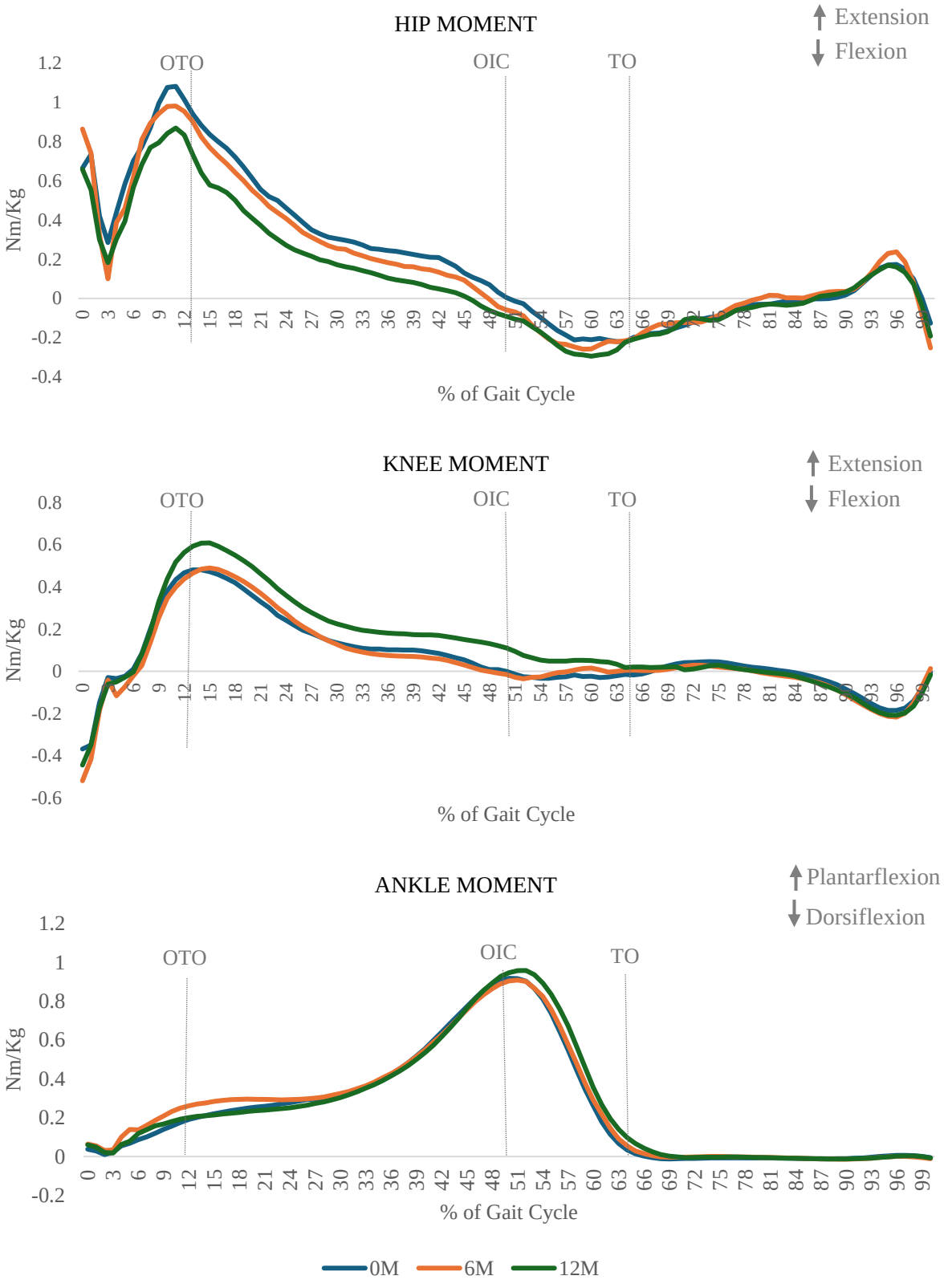


Figure 21: Grand average joint moments in the sagittal plane over one gait cycle for the ankle, knee and hip (average of left and right sides). Comparison of longitudinal measurements of people with DHMN.

OTO, Opposite side tore off; OIC, Opposite side initial contact; TO, Toe off, N=8, M= months.

Figure 22:Grand average joint powers in the sagittal plane over one gait cycle for the ankle, knee and hip (average of left and right sides). Comparison of longitudinal measurements of people with DHMN.

OTO, Opposite side tore off; OIC, Opposite side initial contact; TO, Toe off, N=8, M= months.

6.3.7. Responsiveness Over 12 Months

Standardise response mean (SRM) was calculated as the ratio of mean change to standard deviation of change to measure the responsiveness of the outcome measures used (Table 39). Overall, in parameters with significant longitudinal difference, they were responsive distally more than proximally (Table 39). Calf muscles MRI fat fraction was the most responsive with SRM ranging from 1.1 to 1.78 (Table 39). Calf remaining muscle area and muscle water T_{2m} showed large responsiveness as well with SRM ranging from -1.08 to 1.06 (Table 39). Moderate responsiveness was shown in Gracilis muscle remaining muscle area (SRM= 0.78) and hip flexors moment (SRM= -0.72) (Table 39).

Muscle Fat Fraction (%)	**BASELINE** mean (SD)	**12 MONTHS** mean (SD)	**Difference** mean (SD)	**P**	**SRM**
Total Calf	36.61(22.35)	39.83(22.49)	3.22(2.18)	0.003	1.48
Dorsiflexion	23.89(19.01)	26.57(19.84)	2.68(1.68)	0.001	1.60
Tibialis Anterior	23.89(19.01)	26.57(19.84)	2.68(1.68)	0.001	1.60
Peroneus Longus	39.30(27.19)	41.61(26.19)	2.31(1.47)	0.009	1.57
Plantarflexion	40.16(23.61)	44.04(24.11)	3.88(3.24)	0.009	1.20
Soleus	43.22(23.70)	47.74(24.53)	4.52(4.12)	0.013	1.10
Tibialis Posterior	30.03(22.23)	34.05(22.18)	4.02(2.26)	0.008	1.78
Remaining Muscle Area (mm^2)	**BASELINE** mean (SD)	**12 MONTHS** mean (SD)	**Difference** mean (SD)	**P**	**SRM**
Gracilis	416.0(226.9)	432.2(238.6)	16.14(20.6)	0.05	0.78
Plantarflexion	4166.3(2608.4)	3879.8(2644)	-286.6(306.3)	0.05	-0.94
Soleus	1094.3(642.4)	993.9(638.1)	-100.44(93)	0.02	-1.08
Muscle Water T_{2m} (ms)	**BASELINE** mean (SD)	**12 MONTHS** mean (SD)	**Difference** mean (SD)	**P**	**SRM**
Tibialis Posterior	39.48(6.7)	41.73(7.2)	2.25(2.1)	0.04	1.06
Gait Kinetics (Nm/Kg)	**BASELINE** mean (SD)	**12 MONTHS** mean (SD)	**Difference** mean (SD)	**P**	**SRM**
Hip Moment Y Min	-0.45(0.16)	-0.5(0.26)	-0.11(0.2)	0.005	-0.72

N=8, SRM= Standardise response mean, SD= standard deviation, mm^2= square millimetres, ms= millisecond, Nm/Kg= Newton Meter per Kilogram, SRM < 0.2 minimal responsiveness, SRM =0.2-0.5 small responsiveness, SRM =0.5-0.8 moderate responsiveness, SRM >0.8 large responsiveness.

Table 39: Responsiveness of the study outcome measures with significant difference over 12 months

6.4. Discussion

This study explored the longitudinal changes in a DHMN cohort primarily using MRI, dynamometry, and 3D motion analysis. Over 12 months, quantitative and qualitative MRI parameters were used to assess the effect of DHMN on muscle fatty infiltration, muscle oedema, and muscle cross sectional area. Manual muscle testing and dynamometry were used to assess the effect on muscle strength. Three-D motion analysis was used to understand functional deterioration in walking gait kinematics, kinetics, and spatiotemporal parameters.

To summarise, MRI fat fraction at the calf level was the most sensitive and responsive measure to change. Results showed significant deterioration with an increase in fatty infiltration over 12 months. This deterioration was not detected using qualitative measures. Analysis T1w image, using Modified Mercuri's Scale, did not show change over time. While cross sectional area did not change over 12 months, calculation of the actual remaining muscle area showed deterioration in the Soleus muscle which affected the overall plantar flexors muscle area. These findings are consistent with the established knowledge regarding the quantitative fat fraction in peripheral neuropathy from previous CMT studies (Morrow et al., 2016), and regarding plantar flexors as a targeted group of muscles in DHMN (Esteller et al., 2023, O'Donnell et al., 2023).

Qualitative analysis results were consistent with the quantitative analysis and the expected manifestation of peripheral neuropathy, however, the significant

difference shown at the foot level could be a type I error since the pairwise analysis did not show difference across measurement time points. The Friedman test was calculated manually to confirm the results (appendix II). Calculating the test statistics (χ^2) yield a value of 0 and P-value =1. Thus, the null hypothesis is accepted and there is no difference between measurements. This confirms that there should be no significant result when all values are the same and indicates that in such scenarios the test statistic calculation might leads to an invalid result (appendix II).

Signs of active denervation were shown in the posterior compartment of the lower leg superficially (Medial Gastrocnemius) and deeply (Tibialis posterior) in the qualitative (STIR) and quantitative (T_{2m}) analysis of muscle oedema, respectively. Qualitative Medial Gastrocnemius hyperintensity was not associated with a significant increase in fat fraction levels as in Tibialis posterior. Moreover, quantitative assessment is generally superior to qualitative visual inspection, as quantitative methods are observer-independent and can report changes on a continuous scale versus an ordinal scale for visual inspection (Willis et al., 2013, Dahlqvist et al., 2020).

Deterioration in muscle strength over 12 months was detectable using manual muscle testing. Although this method has been critiqued (Escolar et al., 2001), it showed change in strength over time that was not seen in dynamometry where there is distal weakness and limitation of range of motion that limit the testing

ability and alter ideal positioning and movement isolation. Besides the learning effect, it was noted that participants tend to engage their upper body to pull upwards and push downwards into the dynamometer footplate which might contributed to the overall torque measured and result in the increase in Ankle isokinetic and isometric measurements.

Earlier research highlighted the challenges of using dynamometry in the presence of significant weakness and limited joint mobility (Guillebastre et al., 2013, Reynaud et al., 2019) . In contrast, proximal testing of hip and knee movement where joints are not affected by the neuropathy weakness and limited range of motion, dynamometry was able to detect change over 12 months. Although repeated measure analysis was not powerful enough to show significant difference over one year due to the small sample size and subject variability, the standardised response mean showed high responsiveness in isokinetic hip flexion. However, a larger longitudinal study that include a DHMN group and a control group would help to understand the proximal changes overtime.

Proximal muscles often compensate for distal weaknesses in peripheral neuropathies (Ramdharry et al., 2009). Minimum hip moment represents the activation of hip flexors at pre-swing which is expected to increase as a compensatory strategy for plantar flexors weakness as discussed in chapter 3. The proximal changes including an increase in the Gracilis muscle area and an increase in hip flexion moment shows a possible pattern of proximal

compensation in DHMN. The Gracilis is a long, slender muscle located in the inner thigh. It is one of the muscles in the adductor group, yet it aids in hip and knee flexion (Neumann, 2010, Goldberg et al., 2004). Biomechanical studies showed that maximum knee flexion velocity in swing phase is influenced by the activation of Gracilis and plantar flexors muscles at pre-swing (Goldberg et al., 2004, Anderson et al., 2004, Arnold et al., 2005, Akalan et al., 2017). Therefore, with plantar flexors reduced moments and power generation, the Gracilis is expected to compensate to facilitate knee flexion at swing phase besides its secondary role in assessing hip flexion. This repeated overuse mechanism can cause muscle hypertrophy. However, this hypothesis is based on MRI and kinetic data from a small and variable sample of DHMN. The change in hip moment need to be treated with some caution, however, it is increased at 6 months and decreased at 12 months. This fluctuation would not be expected in a slowly progressive condition. The finding could be a type I error, more likely in this situation of a small sample with high between subject variability. Future research with a larger sample and electromyography studies in addition to MRI and 3d motion analysis can confirm this hypothesis.

6.5. Summary

The current study explored the natural history of DHMN over one year (Table 40), and the responsiveness of the measures used. Among measurements, distal parameters were more responsive than proximal parameters, and MRI fat fraction of the calf was the most responsive.

	Fat Fraction	Muscle Oedema	Muscle Area	Muscle Strength	Gait
Proximal	○	○	↑ Gracilis muscle area	○	↑ Hip flexors moment
Calf/ Distal	↑	○	↓	○	○
Dorsiflexion	↑	○	○	○	○
Tibialis Anterior	↑	○	○	○	○
Peroneus Longus	↑	○	○	○	○
Plantarflexion	↑	○	○	↓	○
Lateral Gastrocnemius	○	○	○	○	○
Medial Gastrocnemius	○	○	○	○	○
Soleus	↑	○	↓	○	○
Tibialis Posterior	↑	↑	○	○	○
Foot	○	○	○	○	○
○, No change; ↑, Increase; ↓, Decrease					

Table 40: Summary of longitudinal changes in DHMN cohort

Chapter 7: The Relationships Between Intramuscular Fat Fraction, Muscle Volume, Muscle Strength, and Moments/Power Generation in Distal Hereditary Motor Neuropathy (DHMN)

7.1. Introduction

In the previous chapters we established an idea of the clinical presentation of DHMN, and we identified a pattern of involvement showing weakness and fatty infiltration predominantly in the distal muscles. Results also showed trends of change in gait parameters at the ankle, knee, and hip level. Previous studies in CMT showed that intramuscular fat fraction correlate cross sectionally as well as longitudinally with strength and disease specific measurements. Understanding the relationship between measurements will inform future research and design of clinical trials. For example, understanding the relation between muscle strength and gait parameters can inform intervention trials in designing strengthening exercises programmes to improve walking.

This study aimed to ascertain relationships between intramuscular fat fraction, muscle volume, isokinetic and isometric muscle strength, and kinetics of gait. Based on the finding in the previous chapters, relationships were expected to be found between:

- Fat fraction with strength, moment, and power generation during gait, for distal lower limb muscles.

- Quantitative and qualitative measurements of intramuscular fat and water, and muscles strength measurements.
- Clinical severity measured with fat fraction, strength, and gait parameters.

7.2. Methods

Parameters associated directly with muscle performance were used primary to explore the relationships. Relations between the rest of the parameters and correlations between quantitative and qualitative measurements were explored cross sectionally. For cross sectional correlation analysis, baseline measurements from 10 DHMN participants recruited for study 1 (chapter 5) who completed MRI, dynamometry, and 3D motion analysis are used.

7.2.1. Data Analysis

To explore relationships between parameters, Pearson correlation was used, if the data set was normally distributed, or Spearman rank correlation, where the data set was not normally distributed. Correlation can be positive or negative and categorised by magnitude (Mukaka, 2012): 0.0-0.29, Minimal/Negligible; 0.30-0.49, Low; 0.50-0.69, Moderate; 0.70-0.89, High; 0.90-1.0, Very High.

It is worth noting that gait parameters with constant, absolute negative signs, such as minimum moments (dorsiflexion, knee and hip flexion), and minimum power (absorption), were interpreted by what they present and not by the absolute negative value. The negative value was used to differentiate the graphs, e.g. the muscle group, as in moment graphs, or power absorption or generation, in power graphs. For example, a value of -2.0 minimum power presents a higher power absorption levels than a value of -1.0 minimum power although -1.0 absolute value is more than -2.0. This could affect the correlation direction if not accounted

for when comparing against parameters with absolute constant positive value such as isometric strength. Therefore, minimum moment and minimum power variables were included in the analysis using their absolute value of the magnitude without the (-) sign. the Parameters for correlations test were paired according to Table 41.

Primary Comparisons	**Distal**	Baseline measurements of: • Calf MRI quantitative intramuscular FF and the calculated remaining muscle area. • Ankle isokinetic and isometric dynamometry. • Ankle moment and power generation during gait.
	Proximal	Baseline measurements of: • Thigh MRI quantitative intramuscular FF and the calculated remaining muscle area • Knee isokinetic and isometric dynamometry. • Knee moment and power generation during gait.
Secondary Comparisons	**Cross Sectional at Baseline**	• Between MRI quantitative measurements (FF, RMA, CSA,T_{2m}). • Between MRI FF and other clinical measurements (CMTES,Walk-12,FPI-6). • Between quantitative and qualitative measurements. • Between strength and spatiotemporal gait parameters.

Table 41: Primary and secondary correlation tests.

FF, fat fraction; RMA, remaining muscle area; CSA, cross sectional area; T_{2m}, quantitative intramuscular water; CMTES, CMT examination score; FPI, foot posture index.

7.3. Results

7.3.1. Subjects and Clinical Assessment

Data from 10 DHMN participants who underwent MRI scan, Dynamometry, and 3D motion analysis at baseline was used for cross sectional correlation analysis (appendix VII). A summary of the subjects' demographics and clinical assessment is presented in Table 42.

Demographics and clinical assessment	**BASELINE**
Numbers	10
Gender (M/F)	(5/5)
Age; mean years; range	57 (42/75)
Genetic diagnosis (HSPB1/unknown)	(7/4)
CMTES; mean (SD)	6.2(3.6)
Foot Posture Index-6 (Pronated/Normal/Supinated)	(1/8/1)
Fall Frequency (weekly/Monthly/Yearly)	(1/1/2)
Walk-12; mean (SD)	36(12.4)
Range of Motion	**BASELINE**
Dorsiflexion; count, (Limited/Normal)	(11/0)
Plantarflexion; count, (Limited/Normal)	(10/1)
M= Male, F= Female, HSPB1= Heat-shock 27-KD Protein 1, SD= Standard Deviation, CMTES= Charcot-Marie-Tooth Disease Examination Score, *= significant after modified Bonferroni correction.	

Table 42:Summary of demographics and clinical assessment of DHMN participants at baseline and 12 months

Correlation analysis was completed using Pearson correlation or Spearman rank correlation. Significant positive and negative correlations are reported below by muscle group; ankle plantar flexors, ankle dorsiflexors, knee extensors, and knee flexors. Correlograms showing the correlation and significance level for all parameters comparisons are shown in appendix III.

7.3.2. Relationships Between MRI Parameters

Plantar Flexors

Between MRI parameters, correlation analysis showed a significant high negative correlation between plantar flexion muscle fat fraction and the remaining muscle area (-0.791, P=0.0065) (Figure 23). The relationship was significant and highly positive between the remaining muscle area and cross sectional area (0.843, P=0.0022) (Figure 24) (Appendix III, Table 78). Quantitative calf intramuscular water measured using (T_{2m}) showed a moderate correlation with plantar flexion muscle fat fraction, however, it was not significant (0.58, P=0.0790) (Table 43).

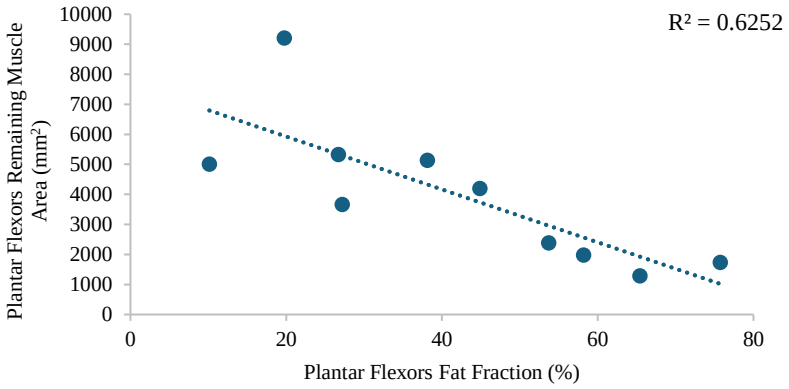


Figure 23: Correlation between plantar flexors fat fraction (%) and remaining muscle area (mm^2).

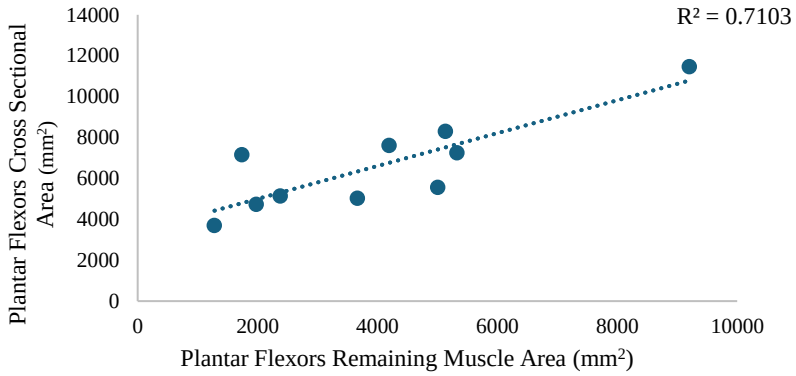


Figure 24: Correlation between plantar flexors remaining muscle area (mm^2) and cross sectional area (mm^2).

Dorsiflexors

Between MRI parameters, correlation analysis showed a significant high negative correlation between dorsiflexors muscle fat fraction with the remaining muscle area (-0.867, P=0.0012) and moderate negative with the cross sectional area (-0.673, P=0.0330) (Figure 25). The relationship was significant and very highly positive between the remaining muscle area and cross sectional area (0.981, P<0.0001) (Figure 26) (Appendix III, Table 79). Quantitative calf intramuscular water measured using T_{2m} showed a highly significant, positive correlation with dorsiflexors fat fraction (0.865, P=0.0012) (Table 43).

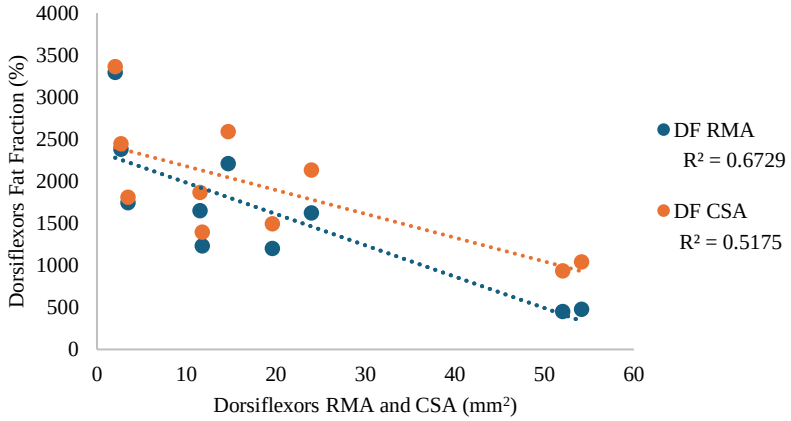


Figure 25: Correlation between dorsiflexors (DF) fat fraction (%) and remaining muscle area (RMA) and cross sectional area (CSA) (mm^2).

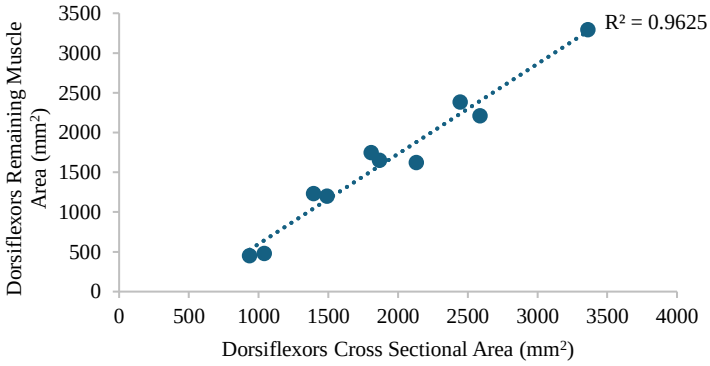


Figure 26: Correlation between dorsiflexors remaining muscle area (RMA) and cross sectional area (CSA) (mm^2).

Total Calf

Quantitative calf intramuscular water measured using (T_{2m}) showed a highly significant, positive correlation with the total calf fat fraction at baseline (0.736, P=0.0153) (Table 43) (Figure 27).

	Calf T_{2m}	Plantar flexion T_{2m}	Dorsiflexion T_{2m}	Foot T_{2m}
Calf FF	0.736 P=0.0153 n=10			
Plantar flexion FF		0.58 P=0.0790 n=10		
Dorsiflexion FF			0.865 P=0.0012 n=10	
Foot FF				0.535 P=0.1112 n=10

1 0 -1

Table 43: Correlation between baseline fat fraction (FF) and baseline T_{2m}.

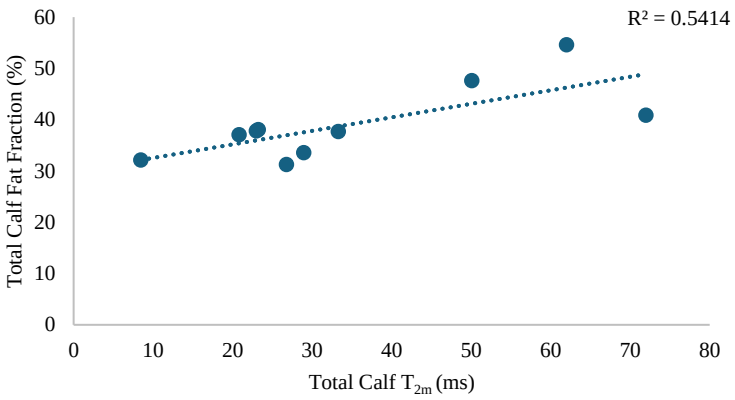


Figure 27: Correlation between total calf fat fraction (%) and T_{2m} intramuscular water (ms).

Knee Extensors

Between MRI parameters, correlation analysis showed a significant high negative correlation between knee extensors muscle fat fraction and the remaining muscle area (-0.812, P=0.0044) (Figure 28) and a significant moderate negative correlation with the cross sectional area (-0.695, P=0.0257) (Figure 28). The relationship was significant and very highly positive between the remaining muscle area and cross sectional area (0.977, P<0.0001) (Figure 29) (Appendix III, Table 80).

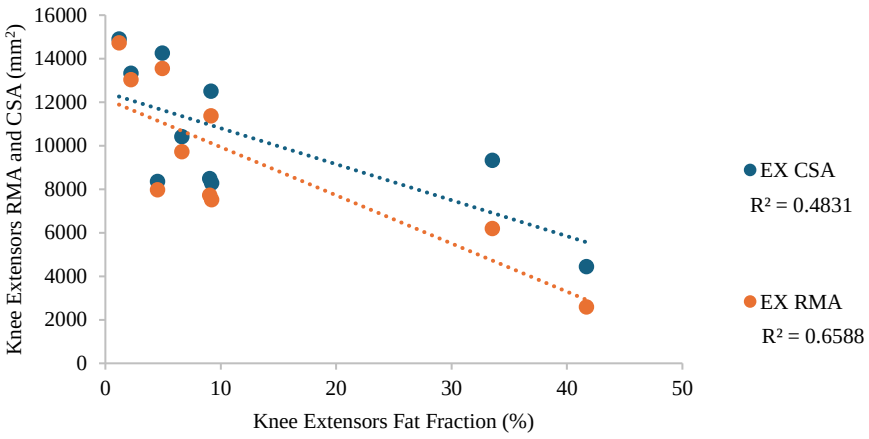


Figure 28: Correlation between knee extensors (EX) fat fraction (%) and remaining muscle area (RMA) and cross sectional area (CSA) (mm^2).

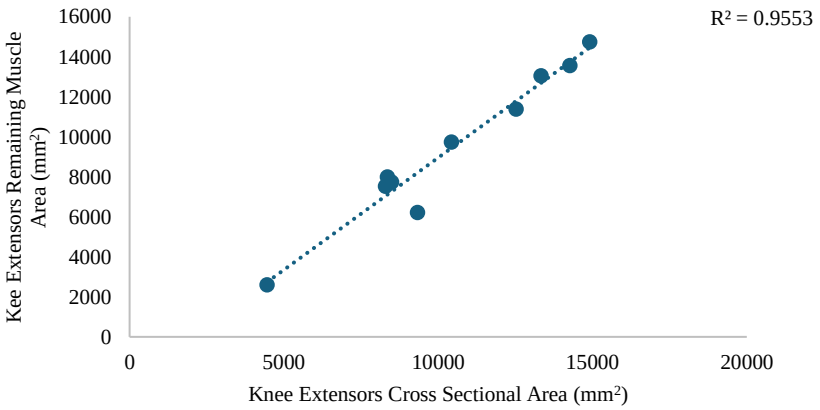


Figure 29: Correlation between knee extensors (EX) remaining muscle area (RMA) and cross sectional area (CSA) (mm^2).

Knee Flexors

Correlations between MRI parameters showed a significant high negative relationship between knee flexors muscle fat fraction with the remaining muscle area (-0.798, P=0.0056) (Figure 30) and cross sectional area (-0.735, P=0.0155) (Figure 30). the correlation was significant and very high positive between the remaining muscle area and cross sectional area (0.99, P<0.0001) (Figure 31) (Appendix III, Table 81).

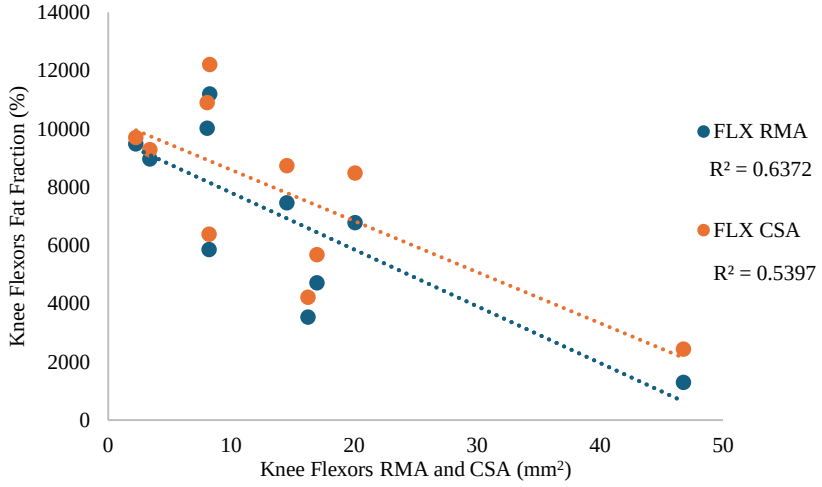


Figure 30: Correlation between knee flexors (FLX) fat fraction (%) and remaining muscle area (RMA) and cross sectional area (CSA) (mm^2).

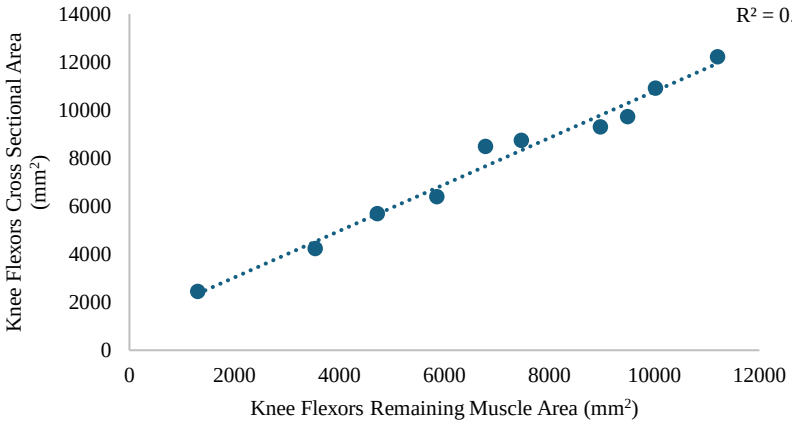


Figure 31: Correlation between knee flexors (FLX) remaining muscle area (RMA) and cross sectional area (CSA) (mm^2).

Relationship Between Quantitative and Qualitative MRI Measurements

Intramuscular fat measured qualitatively using modified Mercuri scale correlated significantly and positively with quantitative fat fraction. High correlations were observed with the calf (0.806, P=0.0048) (Table 44) (Figure 32) and the plantar flexors (0.85, P=0.0019) (Table 44) (Figure 33). A very high correlation was seen with the dorsiflexors (0.914, P=0.0002) (Table 44) (Figure 34).

	Calf	Plantar flexion	Dorsiflexion	Foot
Calf FF	0.806 P=0.0048 n=10			
Plantar flexion FF		0.85 P=0.0019 n=10		
Dorsiflexion FF			0.914 P=0.0002 n=10	
Foot FF				0.406 P=0.2441 n=10

1 0 -1

Table 44: Correlation between baseline fat fraction (FF) and Mercuri score.

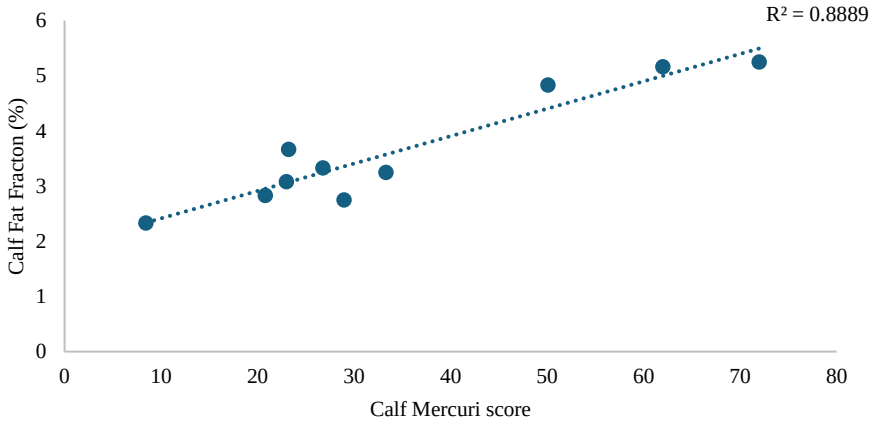


Figure 32: Correlation between calf fat fraction (%) and Mercuri score.

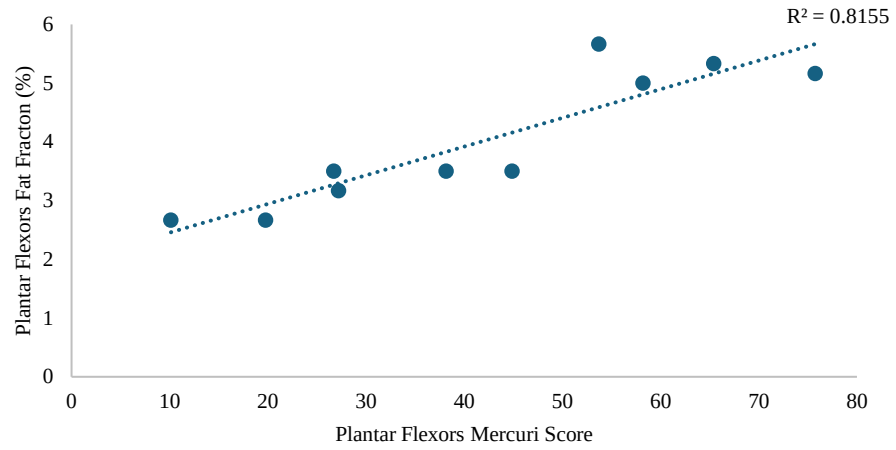


Figure 33: Correlation between plantar flexors fat fraction (%) and Mercuri score.

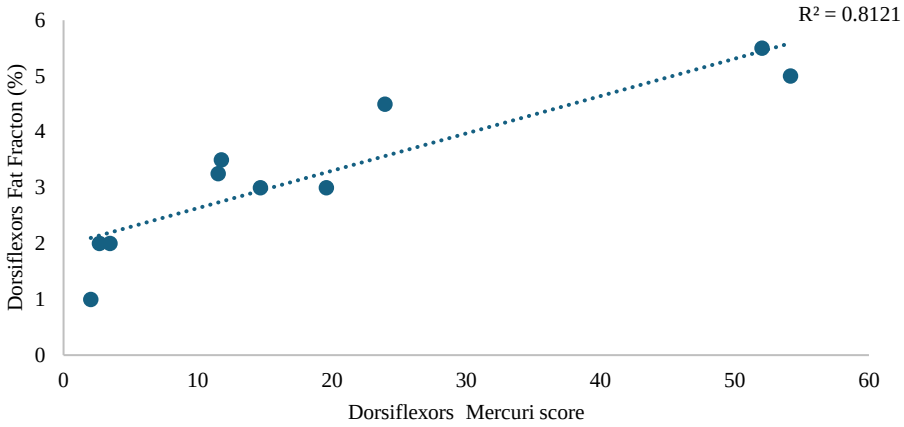


Figure 34: Correlation between dorsiflexors fat fraction (%) and Mercuri score.

Intramuscular water measured qualitatively using STIR hyperintensity score showed over all negligible correlation with quantitative T_{2m}, except at the dorsiflexors, which showed a significant moderate positive correlation (0.646, P=0.0437) (Table 45) (Figure 35).

	Calf	Plantar flexion	Dorsiflexion	Foot
Calf T_{2m}	0.177 P=0.6240 n=10			
Plantar flexion T_{2m}		0.073 P=0.8422 n=10		
Dorsiflexion T_{2m}			0.646 P=0.0437 n=10	
Foot T_{2m}				-0.319 P=0.3686 n=10

1 0 -1

Table 45: Correlation between baseline intramuscular water T_{2m} and STIR hyperintensity score.

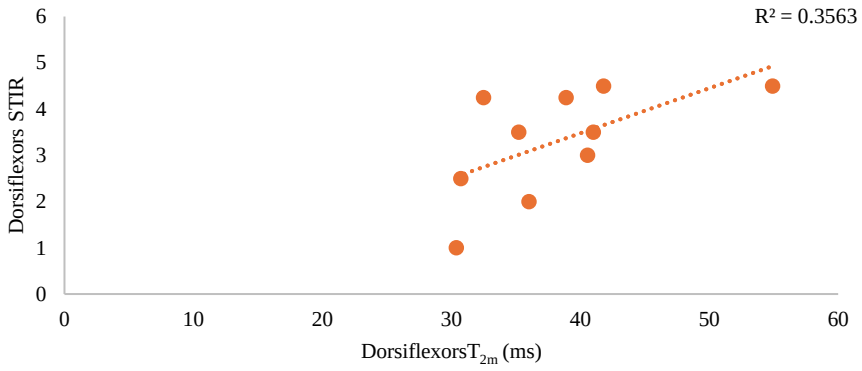


Figure 35: Correlation between dorsiflexors STIR score and T_{2m} intramuscular water (ms).

7.3.3. Relationships Between MRI and Dynamometry Parameters

Plantar Flexors

Plantar flexion isometric dynamometry showed a significant high positive correlations with MRI plantar flexors remaining muscle area (0.754, P=0.0118) (Figure 36) and cross sectional area (0.827, P=0.0031) (Figure 36) (Appendix III, Table 78).

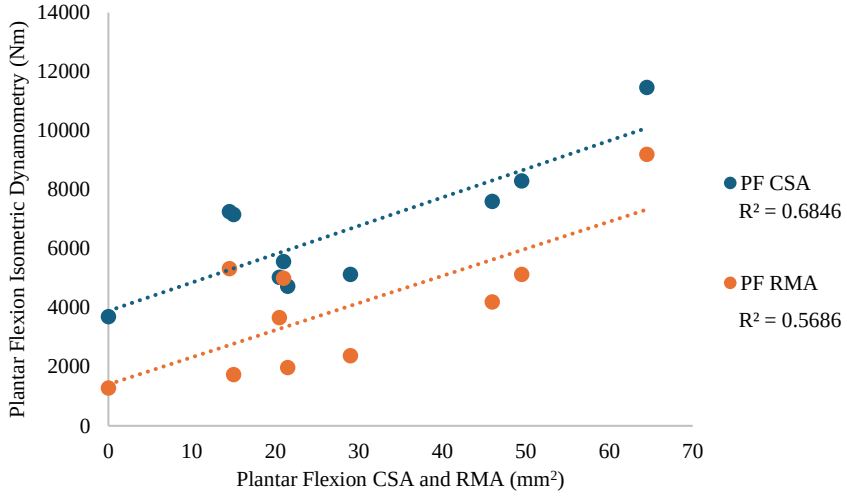


Figure 36: Correlation between Plantar flexion (PF) isometric dynamometry (Nm) and remaining muscle area (RMA) and cross sectional area (CSA) (mm^2).

Dorsiflexors

Dorsiflexor fat fraction showed a significant high negative correlation with dorsiflexion isometric dynamometry (-0.867, P=0.0012) (Figure 37) (Appendix III, Table 79).

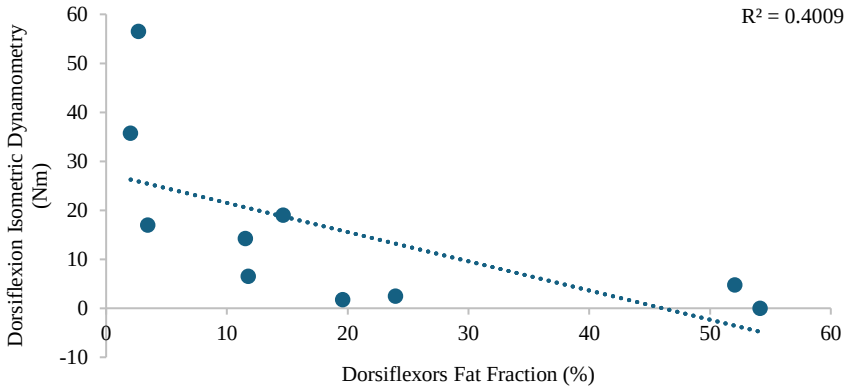


Figure 37: Correlation between dorsiflexion isometric dynamometry (Nm) and fat fraction (%).

Dorsiflexion isometric dynamometry showed a significant high positive correlation with the MRI dorsiflexors remaining muscle area (0.891, P=0.0005) (Figure 38) and with the cross sectional area (0.703, P=0.0235) (Figure 38) (Appendix III, Table 79).

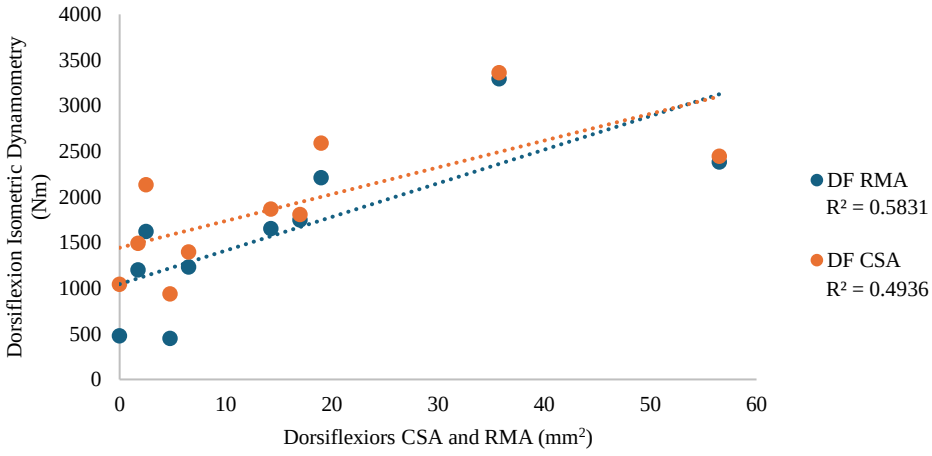


Figure 38: Correlation between dorsiflexion (DF) isometric dynamometry (Nm) and remaining muscle area (RMA) and cross sectional area (CSA) (mm^2).

Knee Extensors

Knee extensor cross sectional area showed a significant high correlation with isometric dynamometry (0.855, P=0.0016) (Figure 39) and with isokinetic dynamometry (0.785, P=0.0071) (Figure 39). Similarly, knee extensor remaining muscle area showed a significant, high correlation with isometric dynamometry (0.844, P=0.0021) (Figure 40) and with isokinetic dynamometry (0.761, P=0.0105) (Figure 40) (Appendix III, Table 80).

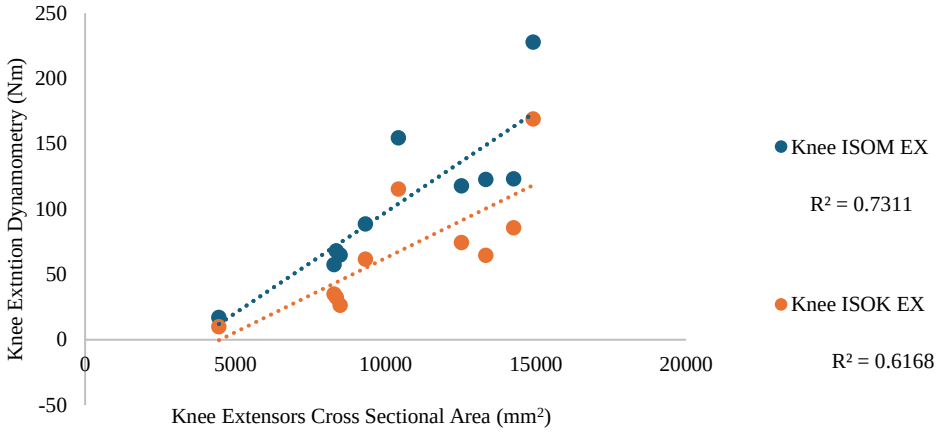


Figure 39: Correlation between knee extensors (EX) cross sectional area (CSA)(mm^2) and isometric (ISOM) and isokinetic (ISOK) dynamometry (Nm).

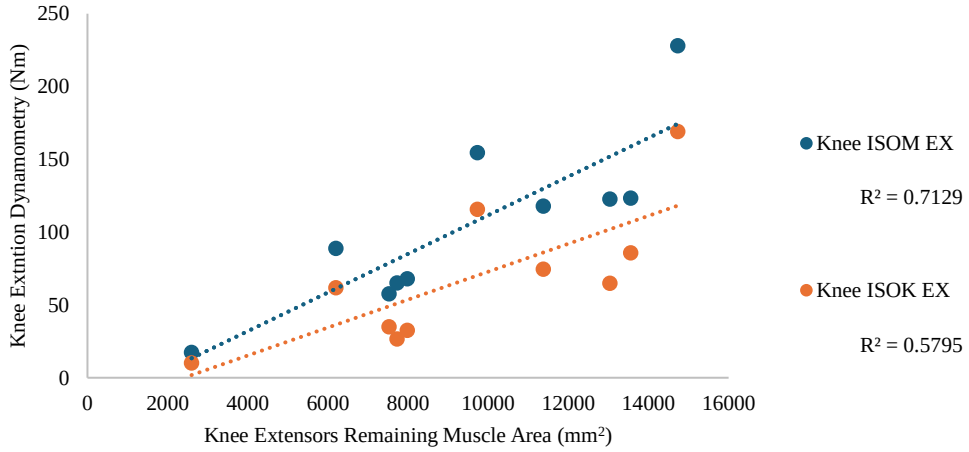


Figure 40: Correlation between knee extensors (EX) remaining muscle area (RMA)(mm^2) and isometric (ISOM) and isokinetic (ISOK) dynamometry (Nm).

Knee Flexors

Knee flexor isometric dynamometry showed a significant very high positive correlation with the remaining muscle area (0.918, P=0.0002) (Figure 41) and a significant high positive correlation with the cross sectional area (0.897, P=0.0004) (Figure 41). A strong negative correlation was observed with knee flexors fat fraction was significant and negatively high (-0.71, P=0.0213) (Figure 42). Knee flexor isokinetic dynamometry showed a significant high positive correlation with flexors remaining muscle area (0.831, P=0.0029) (Figure 43) and cross sectional area (0.806, P=0.0049) (Figure 43). However, correlation with knee flexors fat fraction was significant and negatively moderate (-0.649, P=0.0421) (Figure 44) (Appendix III, Table 81).

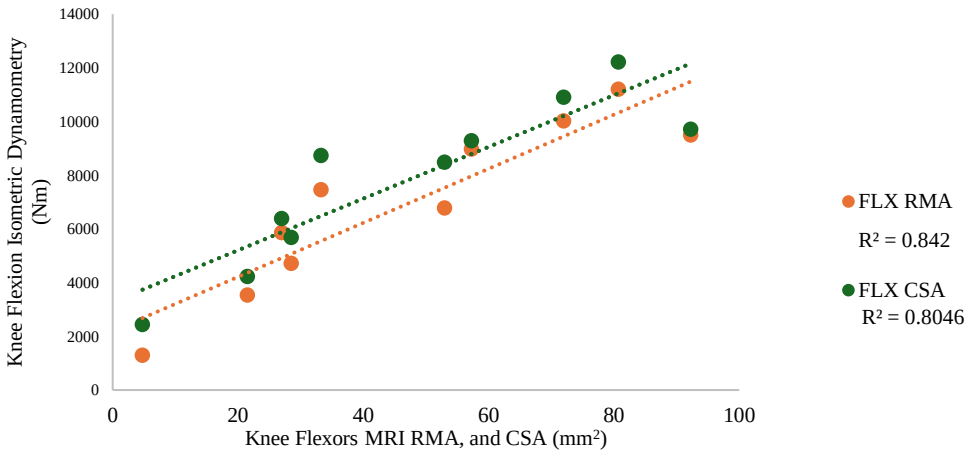


Figure 41: Correlation between knee flexion (FLX) isometric dynamometry (Nm) and remaining muscle area (RMA) (mm^2), and cross sectional area (CSA) (mm^2).

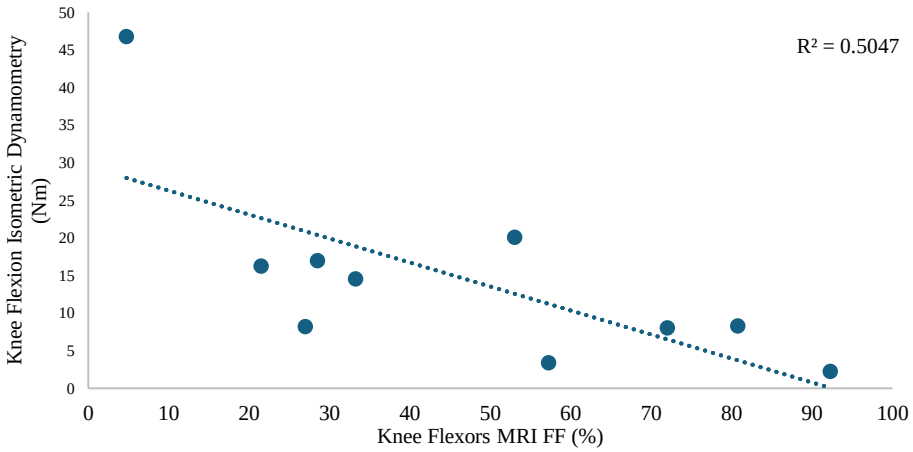


Figure 42: Correlation between knee flexion (FLX) isometric dynamometry (Nm) and fat fraction (FF)(%).

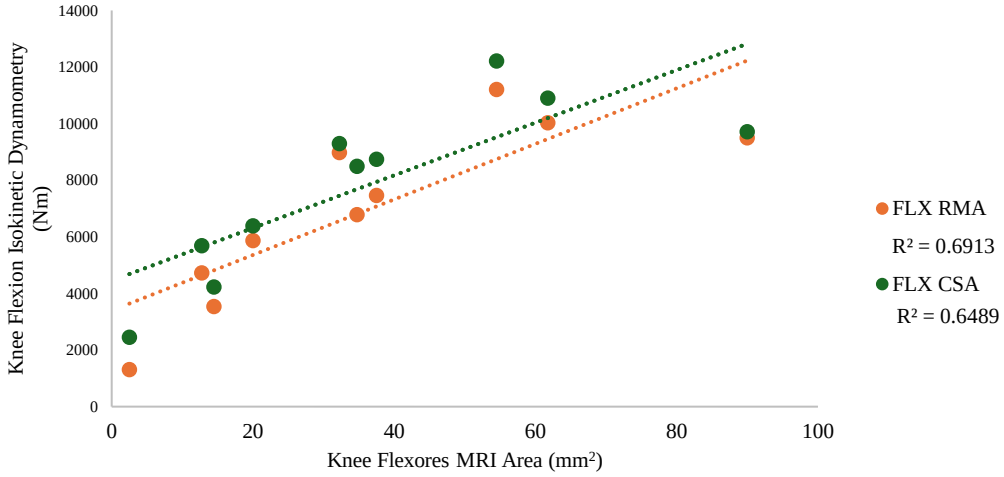


Figure 43: Correlation between knee flexion (FLX) isokinetic dynamometry (Nm) and remaining muscle area (RMA) (mm^2), and cross sectional area (CSA) (mm^2).

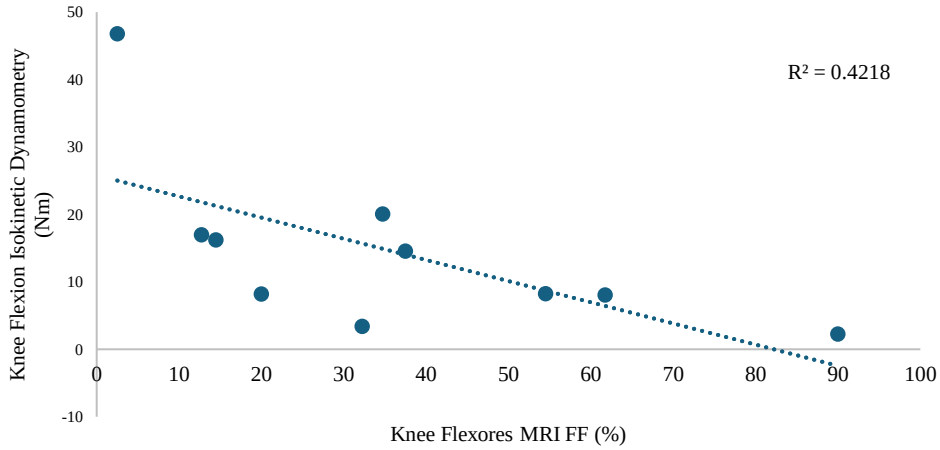


Figure 44: Correlation between knee flexion (FLX) isokinetic dynamometry (Nm) and fat fraction (FF)(%).

7.3.4. Relationships Between MRI and Gait Parameters

Dorsiflexors

Dorsiflexors fat fraction showed a significant moderate negative correlation with ankle power generation (maximum power) during swing (-0.673, P=0.0330) (Figure 45) (Appendix III, Table 79).

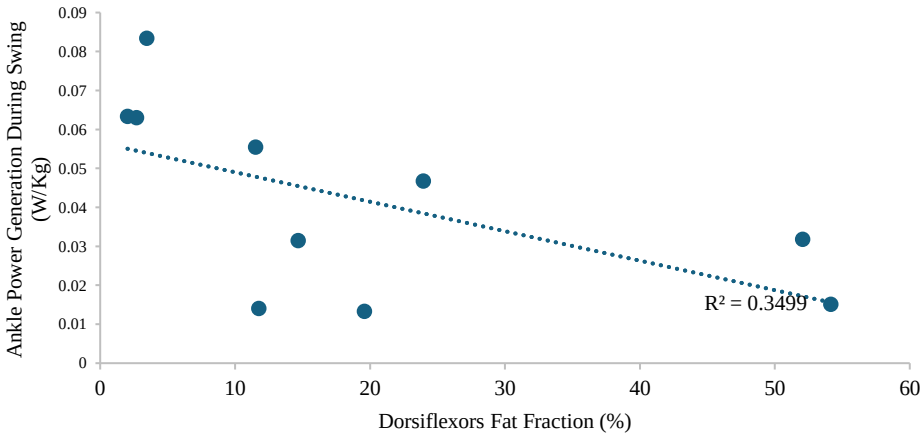


Figure 45: Correlation between dorsiflexors fat fraction (%) and ankle power generation during swing (W/Kg).

Knee Extensors

Knee extensor cross sectional area showed a significant positive high correlation with the knee extensor (maximum) moment during stance (0.876, P=0.0009) (Figure 46), a significant positive moderate correlation with knee power generation (maximum) during stance (0.659, P=0.0384) (Figure 46), and a significant high positive correlation with knee power absorption (minimum) during stance (0.865, P=0.0012) (Figure 46). Similarly, knee extensor remaining muscle area showed a significant positive high correlation with knee extensors (maximum) moment during stance (0.818, P=0.0038) (Figure 47), a significant

positive moderate correlation with knee power generation (maximum) during stance (0.657, P=0.0390) (Figure 47), and a significant high positive correlation with knee power absorption (minimum) during stance (-0.754, P=0.0117) (Figure 47) (Appendix III, Table 80).

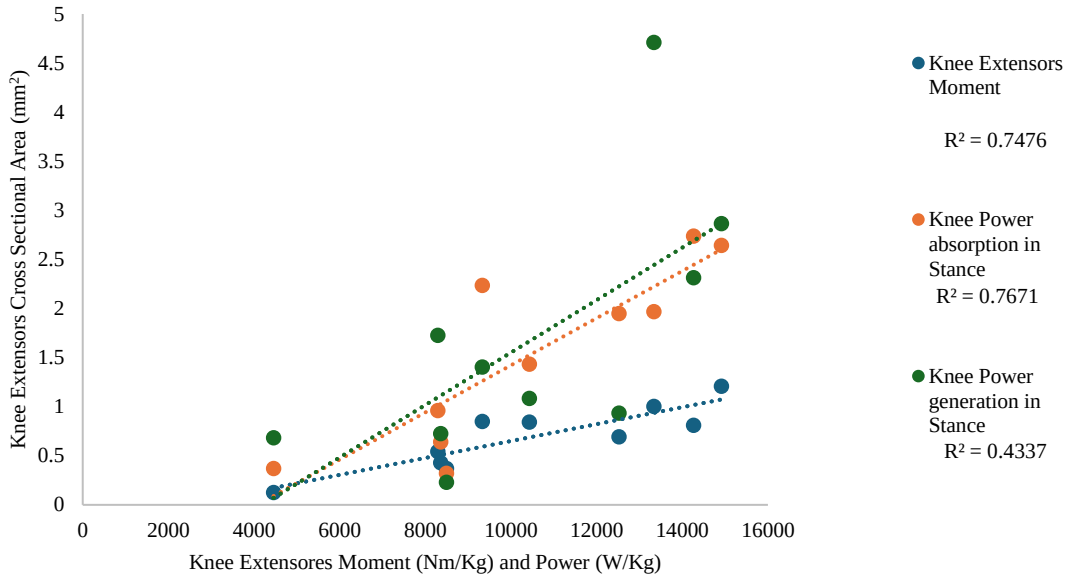


Figure 46: Correlation between knee extensor cross sectional area (mm^2) and knee extensor moment (Nm/Kg)and knee power generation and absorption (W/Kg).

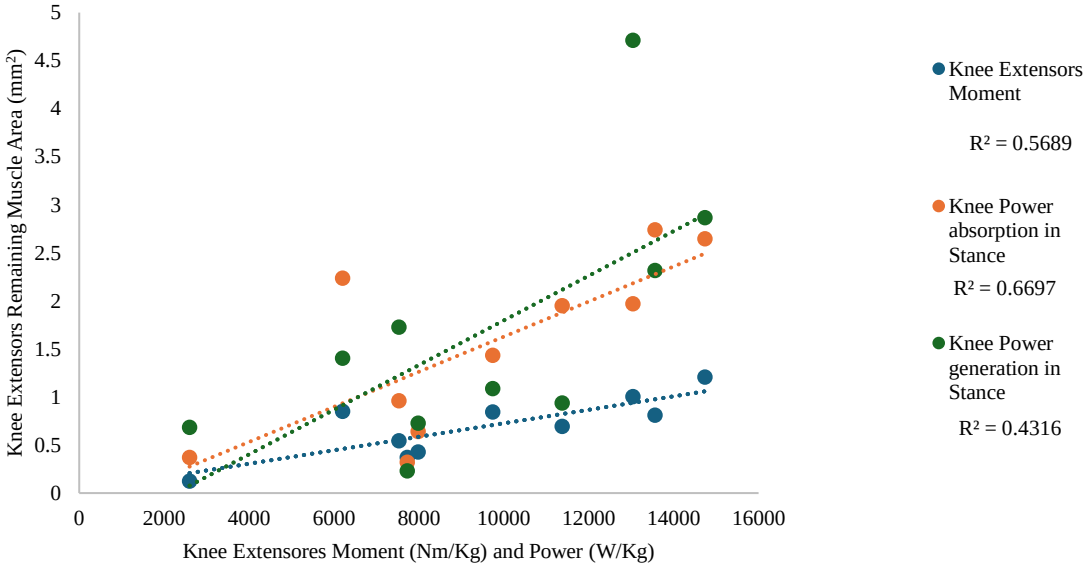


Figure 47: Correlation between knee extensor remaining muscle area (mm^2) and knee extensor moment (Nm/Kg)and knee power generation and absorption (W/Kg).

Knee Flexors

Knee power absorption (minimum) during stance showed a strong, significant positive correlation with knee flexor remaining muscle area (0.818, P=0.0039) (Figure 48) and cross sectional area (0.829, P=0.0031) (Figure 48). Knee extensors moment (maximum) in stance showed a strong, significant, positive correlation with knee flexor remaining muscle area (0.846, P=0.0021) (Figure 49) and cross sectional area (0.809, P=0.0046) (Figure 49). Knee flexors fat fraction showed a strong significant, negative correlation with knee extensor moment (maximum) in stance (-0.783, P=0.0074) (Figure 50) (Appendix III, Table 81).

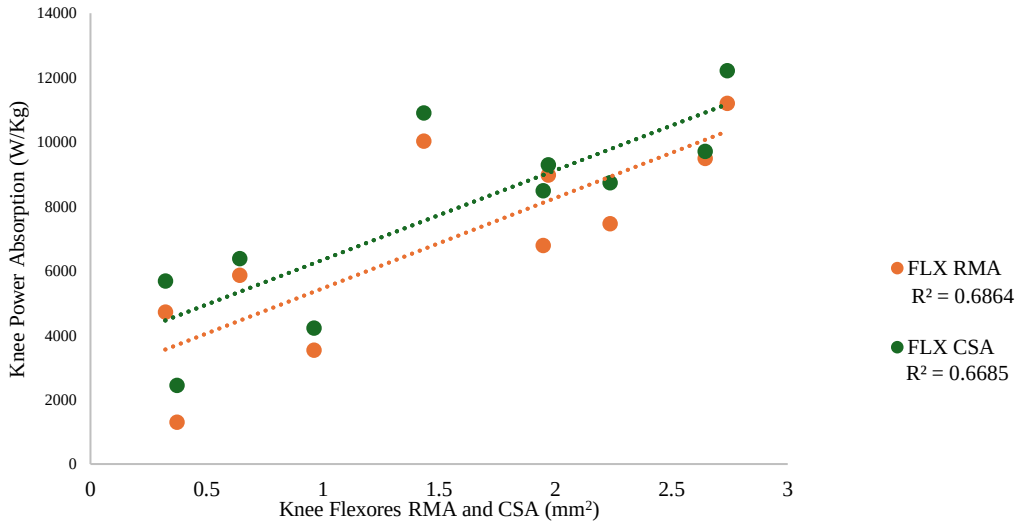


Figure 48: Correlation between knee power absorption (W/Kg) and knee flexors (FLX) cross sectional area (CSA) (mm^2), and remaining muscle area (RMA)(mm^2).

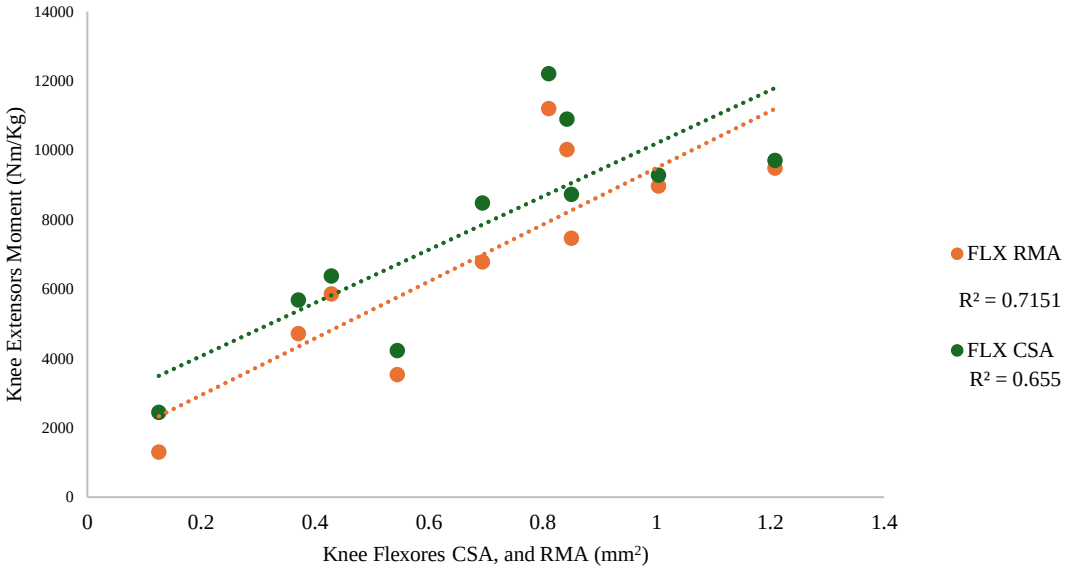


Figure 49: Correlation between knee extensors moment (Nm/Kg) and knee flexors (FLX) cross sectional area (CSA)(mm^2), and remaining muscle area (RMA)(mm^2).

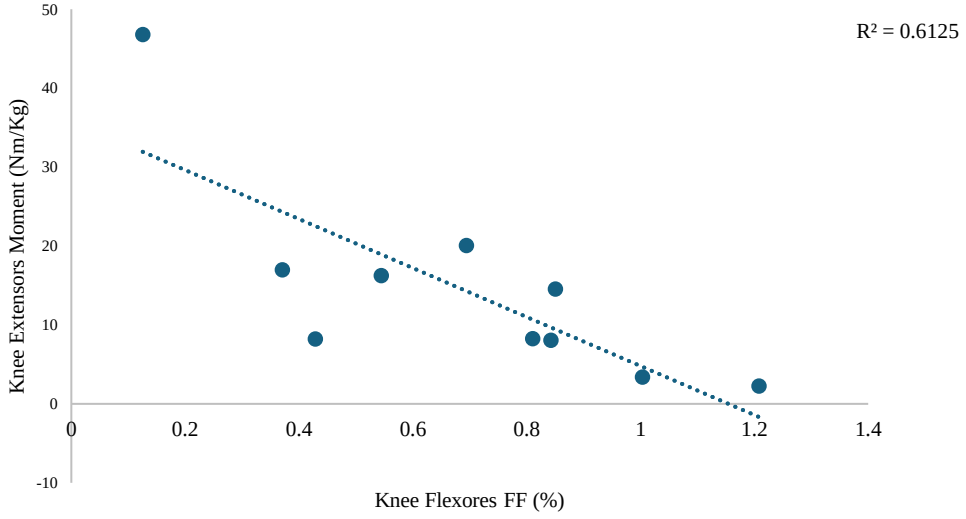


Figure 50: Correlation between knee extensors moment (Nm/Kg) and knee flexors (FLX) fat fraction (%).

7.3.5. Relationships Between MRI Parameters and Clinical Measurements

The Charcot Marie Tooth Examination Score (CMTES) showed significant positive correlations with MRI fat fraction. At the total thigh level, the correlation was high (0.891, P=0.0005) (Table 46) (Figure 51), with high correlation with knee flexors (0.89, P=0.0006), and moderate with knee extensors (0.741, P=0.0143). At the total calf level, the correlation was moderate (0.648, P=0.0427) (Table 46) (Figure 51), with ankle plantar flexors showing a low correlation (0.432, P=0.2128), and ankle dorsiflexors a strong correlation (0.782, P=0.0076) (Table 46).

CMTES	0.891 P=0.0005 n=10	0.741 P=0.0143 n=10	0.89 P=0.0006 n=10	0.648 P=0.0427 n=10	0.432 P=0.2128 n=10	0.782 P=0.0076 n=10	0.537 P=0.1092 n=10
WALK-12	0.412 P=0.2365 n=10	0.145 P=0.6890 n=10	0.538 P=0.1085 n=10	0.128 P=0.7251 n=10	0.081 P=0.8230 n=10	0.231 P=0.5201 n=10	0.185 P=0.6083 n=10
FPI-6	-0.232 P=0.5183 n=10	-0.204 P=0.5717 n=10	-0.176 P=0.6267 n=10	-0.396 P=0.2579 n=10	-0.507 P=0.1346 n=10	-0.272 P=0.4473 n=10	0.063 P=0.8638 n=10
	Thigh FF	**Knee extension FF**	**Knee flexion FF**	**Calf FF**	**Plantar flexion FF**	**Dorsiflexion FF**	**Foot FF**

1 0 -1

Table 46: Correlation between baseline clinical measurements and baseline fat fraction.

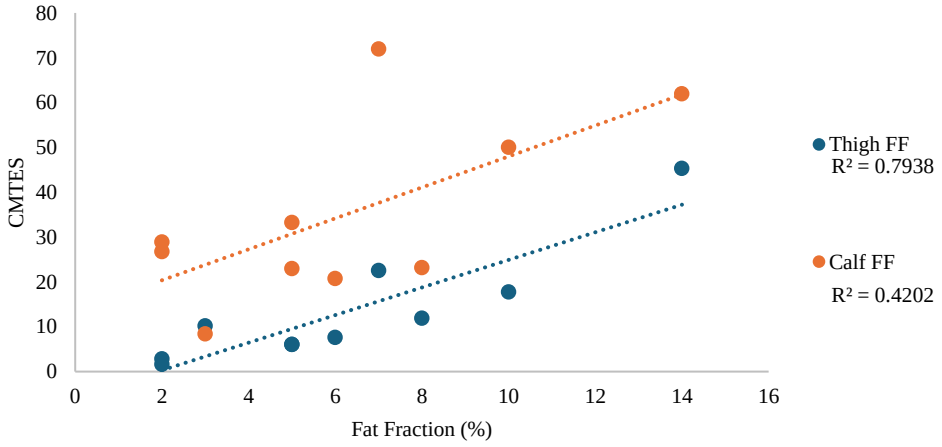


Figure 51: Correlation between the Charcot Marie Tooth Examination Score (CMTES) and fat fraction (FF) (%) at the thigh and calf level.

7.3.6. Relationships Between Dynamometry and Gait Parameters

Plantar Flexors

Plantar flexion isokinetic dynamometry showed a significant high positive correlations with gait plantar flexion moments (maximum) in stance (0.778, P=0.0080) (Figure 52) (Appendix III, Table 78).

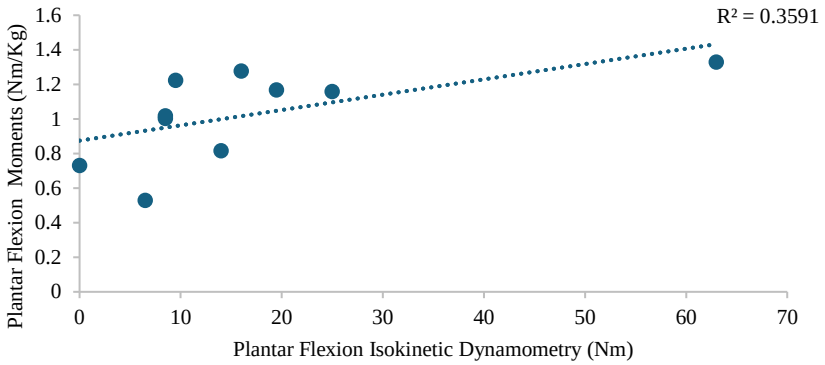


Figure 52: Correlation between plantar flexion moments (Nm/Kg)and plantar flexion isokinetic dynamometry (Nm).

Dorsiflexors

Dorsiflexion isometric dynamometry showed a significant moderate positive correlations with gait plantar flexion moments (maximum) in stance (0.666, P=0.0354) (Figure 53) (Appendix III, Table 79).

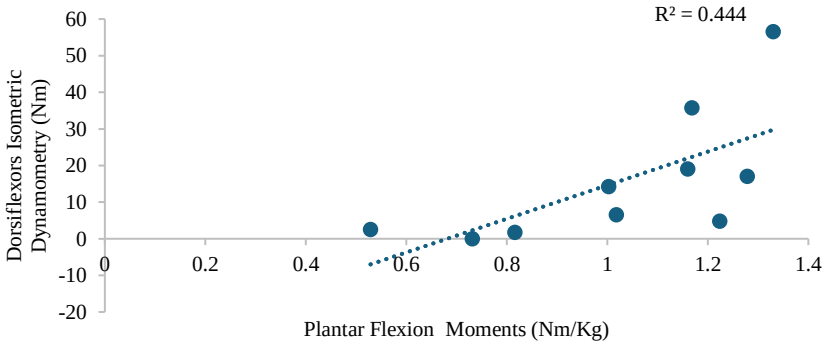


Figure 53: Correlation between dorsiflexors isometric dynamometry (Nm) and plantar flexion moments (Nm/Kg).

Knee Extensors

Knee extensors moment (maximum) in stance showed a significant very high positive correlation with isometric dynamometry (0.902, P=0.0004) (Figure 54) and a significant high positive correlation with isokinetic dynamometry (0.867, P=0.0012) (Figure 54). Knee power absorption (minimum) in stance showed a significant high positive correlation with isometric dynamometry (0.752, P=0.0121) (Figure 55) and with isokinetic dynamometry (0.754, P=0.0118) (Figure 55) (Appendix III, Table 80).

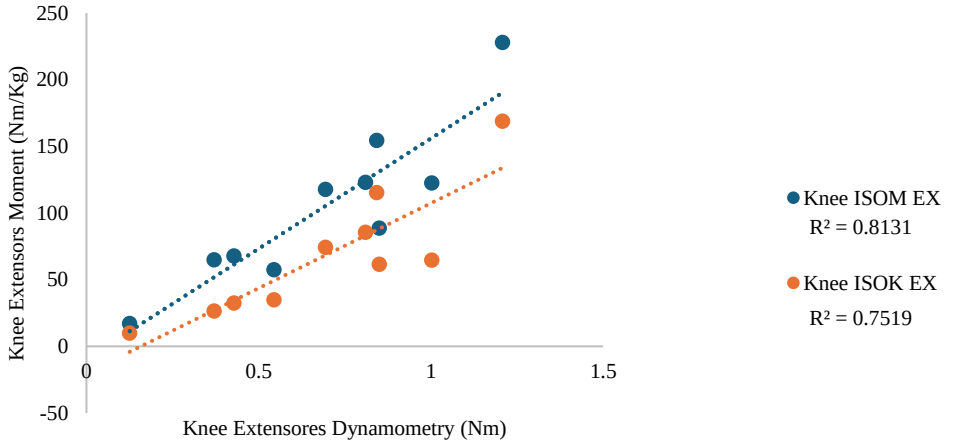


Figure 54: Correlation between knee extensors (EX) moment (Nm/Kg) and isometric (ISOM) and isokinetic (ISOK) dynamometry (Nm).

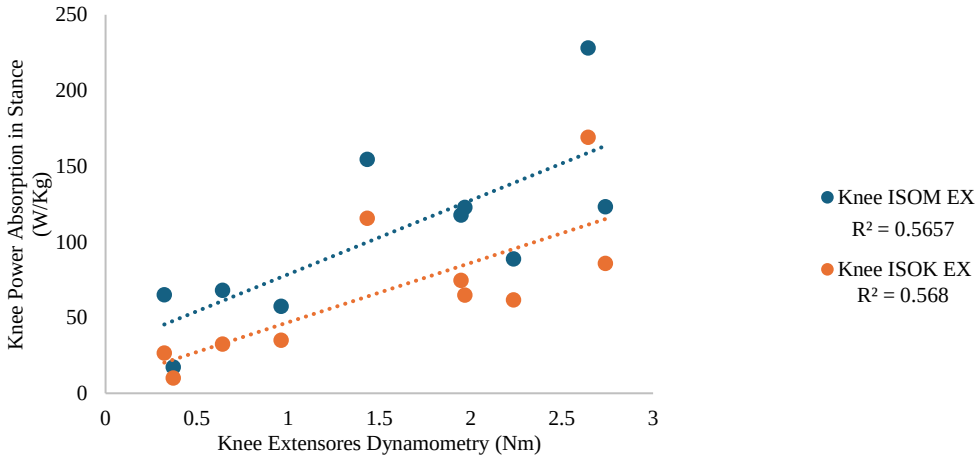


Figure 55: Correlation between knee power absorption in stance (W/Kg) and knee extensors (EX) isometric (ISOM) and isokinetic (ISOK) dynamometry (Nm).

Knee Flexors

Knee power absorption (minimum) in stance showed a significant high positive correlation with knee flexors isometric dynamometry (0.81, P=0.0045) (Figure 56) and with knee flexors isokinetic dynamometry (0.792, P=0.0064) (Figure 56). Knee extensors moment (maximum) in stance showed a significant high positive correlation with knee flexors isometric dynamometry (0.867, P=0.0012) (Figure 57) and knee flexors isokinetic dynamometry (0.856, P=0.0016) (Figure 57) (Appendix III, Table 81).

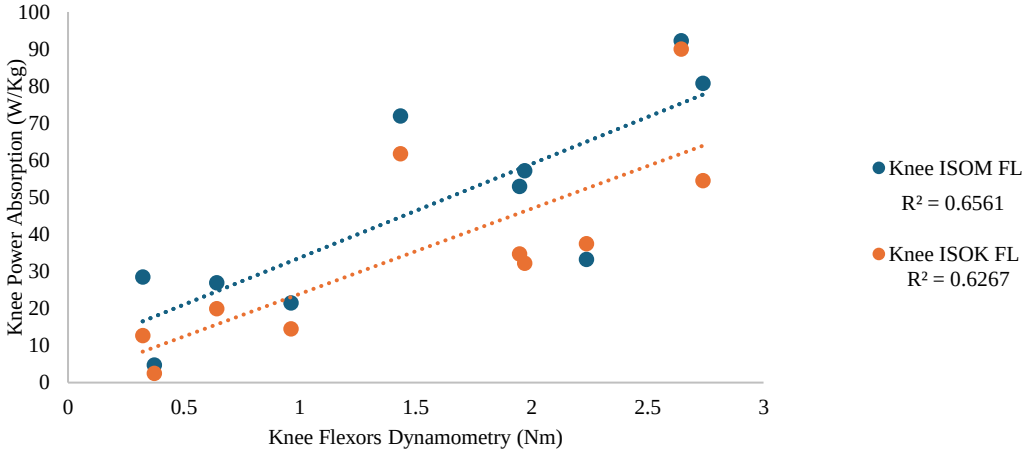


Figure 56: Correlation between knee power absorption (W/Kg) and knee flexors (FL) isometric (ISOM) and isokinetic (ISOK) dynamometry (Nm).

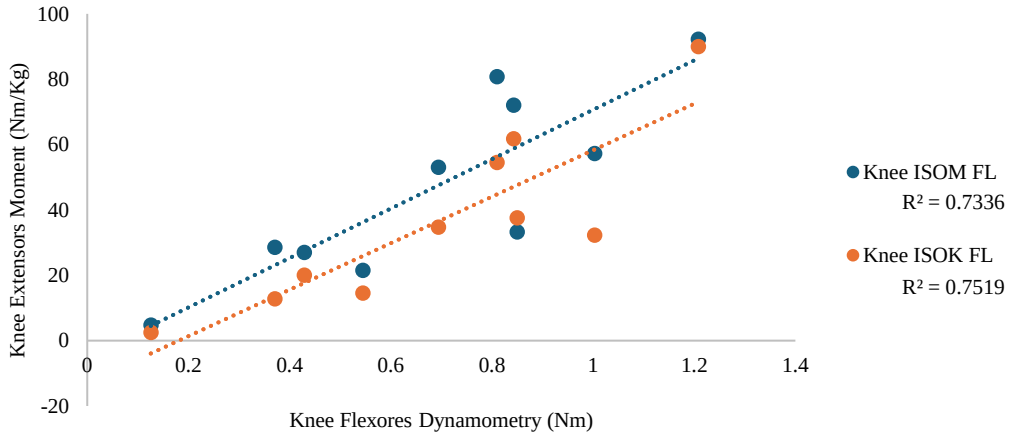


Figure 57: Correlation between knee extensors moment (Nm/Kg) and knee flexors (FL) isometric (ISOM) and isokinetic (ISOK) dynamometry (Nm).

Relationships Between Dynamometry and Gait Spatiotemporal Parameters

Isometric and isokinetic dynamometry correlated with speed, step length, and stride length significantly and positively. Proximally, correlations were high to moderate (0.765 - 0.647, P= 0.0433 - 0.0100) (appendix III, Table 83). Distally, isometric dorsiflexion correlated highly with speed (0.794, P=0.0061) (Figure 58), step length (0.818, P=0.0038) (Figure 58), and stride length (0.818, P=0.0038) (Figure 58). Speed correlated moderately with isometric plantar flexion (0.645, P=0.0439) and isokinetic plantar flexion (0.65, P=0.0417) (Appendix III, Table 83).

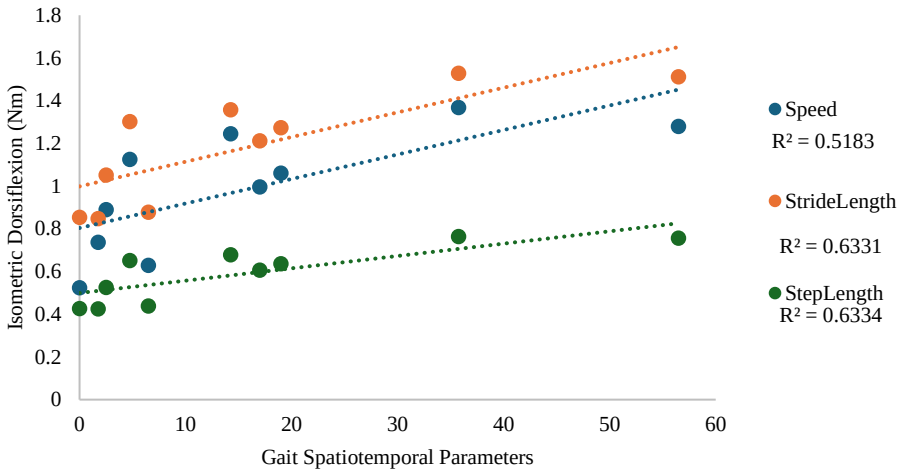


Figure 58: Correlation between isometric dorsiflexion (Nm) and gait spatiotemporal parameters.

7.3.7. Relationships Between Dynamometry Parameters and Clinical Measurements

The Charcot Marie Tooth Examination Score (CMTES) showed significant negative correlations with dynamometry parameters (Table 47) (Figure 59). Negative correlations were observed with isometric dorsiflexion (-0.781, P=0.0077), isokinetic plantar flexion (-0.817, P=0.0039), and isokinetic dorsiflexion (-0.825, P=0.0033). Correlation was moderately negative between walk-12 questionnaire and isokinetic plantar flexion (-0.634, P=0.0491).

	Isometric						Isokinetic					
	Hip Flexion	**Hip Extension**	**Knee Flexion**	**Knee Extension**	**Plantar flexion**	**Dorsiflexion**	**Hip Flexion**	**Hip Extension**	**Knee Flexion**	**Knee Extension**	**Plantar flexion**	**Dorsiflexion**
FPI-6	0.128 P=0.7235 n=10	0.095 P=0.7948 n=10	0.06 P=0.8687 n=10	0.187 P=0.6058 n=10	0.24 P=0.5049 n=10	0.243 P=0.4984 n=10	0.247 P=0.4923 n=10	0.258 P=0.4713 n=10	0.222 P=0.5372 n=10	0.268 P=0.4536 n=10	0.47 P=0.1710 n=10	0.335 P=0.3436 n=10
WALK-	-0.348 P=0.3246 n=10	-0.537 P=0.1091 n=10	-0.354 P=0.3155 n=10	-0.377 P=0.2824 n=10	-0.416 P=0.2320 n=10	-0.571 P=0.0850 n=10	-0.43 P=0.2147 n=10	-0.525 P=0.1188 n=10	-0.444 P=0.1987 n=10	-0.413 P=0.2352 n=10	-0.634 P=0.0491 n=10	-0.42 P=0.2270 n=10
CMTES	-0.511 P=0.1307 n=10	-0.323 P=0.3622 n=10	-0.542 P=0.1054 n=10	-0.572 P=0.0842 n=10	-0.545 P=0.1030 n=10	-0.781 P=0.0077 n=10	-0.553 P=0.0976 n=10	-0.451 P=0.1904 n=10	-0.509 P=0.1330 n=10	-0.497 P=0.1435 n=10	-0.817 P=0.0039 n=10	-0.825 P=0.0033 n=10

1 0 -1

Table 47: Correlation between baseline clinical measurements and baseline dynamometry parameters.

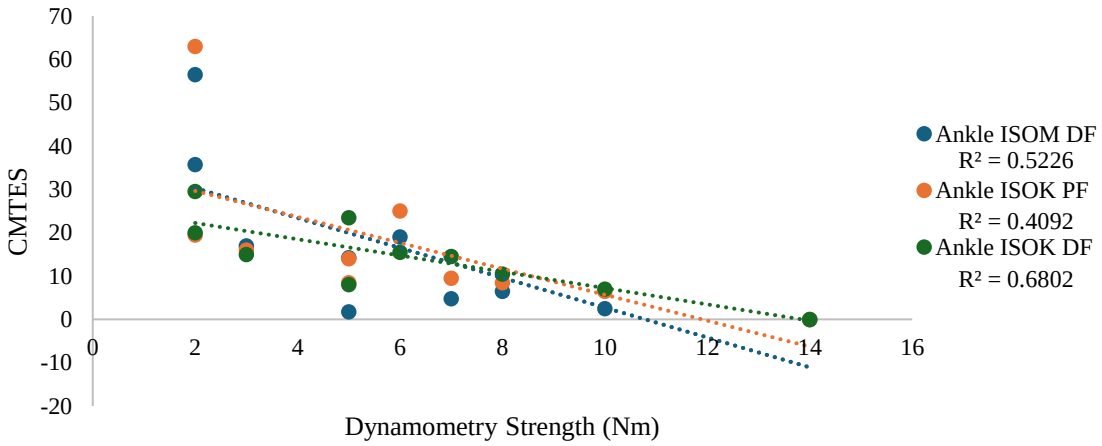


Figure 59: Correlation between the Charcot Marie Tooth Examination Score (CMTES) and dynamometry parameters.

Relationship Between Quantitative and Qualitative Strength Measurements

Muscle strength measured using manual muscle testing showed significant high positive correlation with isometric dynamometry in hip extension (0.764, P=0.0101), knee flexion (0.742, P=0.0141), and ankle dorsiflexion (0.75, P=0.0125) (Table 48). Manual muscle testing showed significant high to moderate positive correlation with isokinetic dynamometry in hip extension (0.809, P=0.0046), knee flexion (0.809, P=0.0046), and knee extension (0.696, P=0.0253) (Table 49).

	Hip Flexion	Hip Extension	Knee Flexion	Knee Extension	Plantarflexion	Dorsiflexion
Hip Flexion	0.529 P=0.1155 n=10					
Hip Extension		0.764 P=0.0101 n=10				
Knee Flexion			0.742 P=0.0141 n=10			
Knee Extension				0.609 P=0.0615 n=10		
Ankle Plantar Flexion					0.483 P=0.1573 n=10	
Ankle Dorsiflexion						0.75 P=0.0125 n=10

1 0 -1

Table 48: Correlation between isometric dynamometry (X) and manual muscle testing (Y).

	Hip Flexion	Hip Extension	Knee Flexion	Knee Extension	Plantarflexion	Dorsiflexion
Hip Flexion	0.619 P=0.0564 n=10					
Hip Extension		0.809 P=0.0046 n=10				
Knee Flexion			0.809 P=0.0046 n=10			
Knee Extension				0.696 P=0.0253 n=10		
Ankle Plantar Flexion					0.481 P=0.1589 n=10	
Ankle Dorsiflexion						0.372 P=0.2899 n=10

1 0 -1

Table 49: Correlation between isokinetic dynamometry (X) and manual muscle testing (Y).

7.3.8. Relationships Between Gait Parameters and Clinical Measurements

The Charcot Marie Tooth Examination Score (CMTES) showed significant correlations with spatiotemporal gait parameters. Negative correlations were observed with speed (-0.763, P=0.0103), step length (-0.71, P=0.0214), and stride length (-0.71, P=0.0214) (Table 50) (Figure 60). Correlations were moderately positive with stride time (0.561, P=0.0916), and step time (0.561, P=0.0916) (Table 50) (Figure 60). The Walk-12 questionnaire showed significant negative correlations with spatiotemporal gait parameters; highly correlated with speed (-0.773, P=0.0088), step length (-0.772, P=0.0089), and stride length (-0.772, P=0.0089) (Table 50) (Figure 61).

	Speed	Stride Length	Step Length	Stride Time	Step Time	Steps Per Minute	Strides Per Minute
CMTES	-0.763 P=0.0103 n=10	-0.71 P=0.0214 n=10	-0.71 P=0.0214 n=10	0.561 P=0.0916 n=10	0.561 P=0.0916 n=10	-0.47 P=0.1710 n=10	-0.47 P=0.1710 n=10
WALK-12	-0.773 P=0.0088 n=10	-0.772 P=0.0089 n=10	-0.772 P=0.0089 n=10	0.399 P=0.2536 n=10	0.399 P=0.2536 n=10	-0.337 P=0.3403 n=10	-0.337 P=0.3403 n=10
FPI-6	0.135 P=0.7104 n=10	0.148 P=0.6830 n=10	0.148 P=0.6827 n=10	0.134 P=0.7126 n=10	0.134 P=0.7126 n=10	-0.164 P=0.6505 n=10	-0.164 P=0.6505 n=10

1 0 -1

Table 50: Correlation between baseline clinical measurements and baseline spatiotemporal parameters.

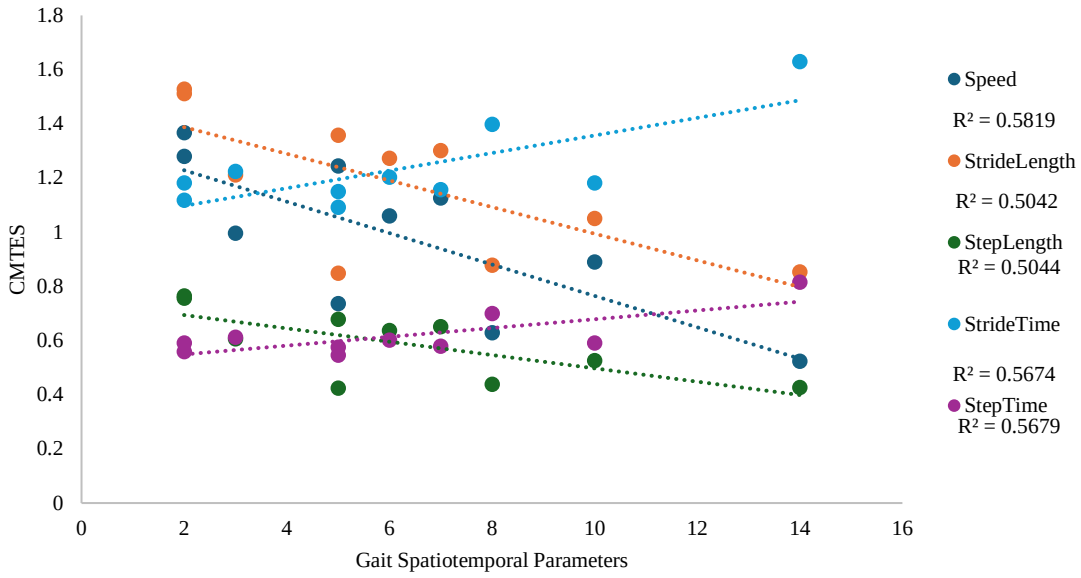


Figure 60: Correlation between the Charcot Marie Tooth Examination Score (CMTES) and gait spatiotemporal parameters.

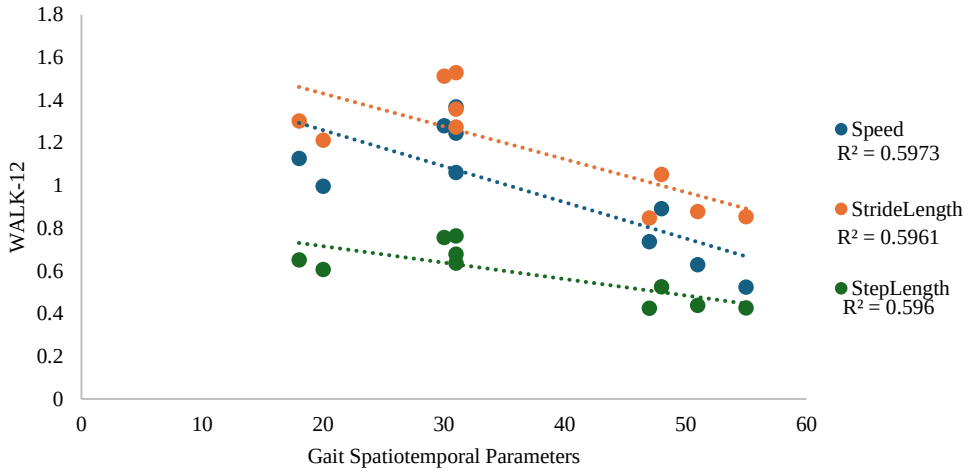


Figure 61: Correlation between Walk-12 questionnaire and gait spatiotemporal parameters.

7.4. Discussion

This study is aimed to explore relations between measurements at baseline. To the best of my knowledge, this study is the first to explore relations between MRI, dynamometry, and gait parameters in DHMN. Primarily to ascertain the effect of the neuropathy on muscle performance and function. Since the calf fat fraction was the most responsive in this cohort (chapter 6), it was used to guide the correlation analysis.

7.4.1. Relationships Between Intramuscular Fat Fraction, Muscle Volume, Isokinetic and Isometric Muscle Strength, and Kinetics of Gait

At the distal and proximal levels, muscle fat fraction correlated negatively with the remaining muscle area and cross sectional area. This relation is expected as the increase in the intramuscular fat and progressive muscle atrophy are symptoms of peripheral motor neuropathy. And since the remaining muscle area is part of the total cross sectional area, they showed positive high correlations at all levels.

At the proximal level, relationships were observed between MRI, dynamometry, and kinetics parameters, with knee extensors and flexors. These muscle groups are relatively less affected than the distal muscles. With increased intramuscular fat fraction, there was a decrease in muscular capacity; decreased isometric and isokinetic strength, and a decrease in moment and power generation and absorption during gait. on the contrary, muscle capacities were increased in

relation to higher muscle area which make MRI, dynamometry, and gait kinetics parameters candidate outcome measures in clinical trials that aim to target to muscle volume and performance.

At the distal level, where muscles are more affected by the neuropathy, as shown in chapter 5, relations were seen between MRI and isometric dynamometry. With greater muscle area in the plantar flexors and dorsiflexors, there was higher isometric strength, as there is more contractile tissue to generate force. On the other hand, with increased intramuscular fat fraction there was lower isometric strength as shown in dorsiflexion. We expect to see a deterioration in muscle strength as the neuropathy advances. Similar relationships were shown in people with hereditary peripheral neuropathies (O'Donnell et al., 2023, Morrow et al., 2016).

For the plantar flexors, relationships were observed between isokinetic strength and plantar flexors moments in gait. In stance phase, the greater the strength in plantar flexors, the more capable the muscle group to produce moments to control the ankle rocker movement in mid stance to generate a propulsive action in pre-swing (Whittle, 2007, Brockett and Chapman, 2016).

For the dorsiflexors, relationships were observed between fat fraction and power generation in swing phase. With more intramuscular fat fraction, there was a decrease in ankle power generation in swing phase. Power is generated in swing mostly from the dorsiflexors to assess in foot clearance (Whittle, 2007).

Therefore, identifying gait deviations and the underlying muscle involvement is informative for rehabilitation assessment and treatment. For instance, the effect of rehabilitation, including therapeutic exercises and orthotics, that focuses on the plantar flexors as a targeted group of muscles in DHMN can be assessed using plantar flexors moments in stance phase. And when the targeted muscle group is dorsiflexion, power is generation in swing will be more useful.

However, due to the high variability within a small sample, some significant correlations identified in this study may lack functional significance. For instance, the relationship between dorsiflexor strength and plantar flexor moments. Future research with a larger sample size and a control group is recommended to confirm these relationships.

7.4.2. Correlations with Clinical Measures

Clinical measurements showed correlations with MRI, dynamometry, and gait spatiotemporal parameters. With higher disease severity measured by the Charcot Marie Tooth Examination Score (CMTES), there was higher intramuscular fat fraction in the knee flexors and ankle dorsiflexors. Also, with higher severity and worse mobility measured by the CMTES and Walk-12 questionnaire, there was weaker ankle plantar flexor and dorsiflexor, slower speed, and shorter step length and stride length. Moreover, speed , step length, and stride length showed positive correlations with dorsiflexion isometric strength more than plantar flexion isometric strength. The neuropathy affected the speed and spatial parameters but

not the temporal, suggesting that they walk with slower gait, not because of the timing , but because of shorter strides. This may be due to the knee flexor and ankle dorsiflexor weakness that contributes to problems with foot clearance in swing phase.

7.4.3. Quantitative and Qualitative Measurements

Qualitative and quantitative measurements used in this study showed a range of results. MRI intramuscular fat showed high correlations at the calf level between the Mercuri scale and fat fraction, but intramuscular water correlations were mostly negligible. This can be explained by the lack of sensitivity in the hyperintensity scale with limited numbers of scores, 3 levels for intensity and 3 levels for extent. Isometric and isokinetic dynamometry showed mostly low correlations with manual muscle testing. This could be explained by challenges with ankle dynamometry testing, where there is limited joint range of motion. Proximal variables at the knee and hip showed higher correlations where there are fewer joint restrictions. It is worth noting that for the dorsiflexors, very high to high correlations were observed between quantitative and qualitative intramuscular fat, intramuscular water, and strength measurements. The dorsiflexors may be a candidate for future clinical trials in developing outcome measures in peripheral neuropathy.

7.5. Summary

The current study explored the relationships between MRI, dynamometry, and 3D motion analysis measures in DHMN. The study findings are summarised in Table 51.

	Positive correlations	Negative correlation
PF	RMA vs. CSA ISOM vs. RMA SOM vs. CSA ISOK vs. gait PF moments	FF vs. RMA
DF	RMA vs. CSA ISOM vs. RMA ISOM vs. gait PF moments ISOM DF vs. speed ISOM DF vs. step length ISOM DF vs. stride length	FF vs. RMA FF vs. CSA FF vs. ISOM FF vs. ankle power generation during swing
Calf	T_{2m} vs. FF	-
Knee EX	RMA vs. CSA CSA vs. ISOM CSA vs. ISOK CSA vs. knee extensor moment. RMA vs. knee extensor moment. ISOM & ISOK vs. Knee extensor moment. ISOM & ISOK vs. Knee power absorption.	FF vs. RMA FF vs. CSA
Knee FLX	RMA vs. CSA ISOM vs. RMA CSA vs. ISOM RMA & CSA vs. Knee power absorption	FF vs. RMA FF vs. CSA FF vs. ISOM
CMTES	CMTES vs. FF in thigh CMTES vs. FF in calf	CMTES vs. speed CMTES vs. ISOM CMTES vs. ISOK
MMT	MMT vs. ISOM in hip extension	-
PF= Plantar Flexors, DF= Dorsiflexors, EX= Knee Extension, FLX= Knee Flexion, RMA= Remaining Muscle Area, CSA= Cross Sectional Area, ISOM= Isometric Dynamometry, ISOK= Isokinetic Dynamometry, FF= Fat Fraction, CMTES= Charcot Marie Tooth Examination Score, MMT= Manual Muscle Testing.		

Table 51:Summary of positive and negative correlation between study parameters.

Chapter 8: The Effect of Bilateral Carbon Fibre Ankle Foot Orthoses (AFOs) on the Kinetics and Kinematics of Gait of People with Distal Hereditary Motor Neuropathy (DHMN)

8.1. Introduction

Ankle foot orthoses (AFO) are medical devices usually prescribed for people presenting with foot drop, and commonly issued to people with neuropathy (Landfeldt et al., 2017, McCaughan et al., 2019). Studies showed low adherence in using AFO, as users are concerned that they highlight their disability(Bertini et al., 2024), but they have been shown to help with ankle control (Phillips et al., 2011), and compensate for distal weakness(Ramdharry et al., 2012a).

Different materials used for ankle foot orthoses (AFOs) include plastic, carbon fibre, and elastic fabric. Each material supports the ankle and foot in different ways and varies in size, weight, comfort, and acceptability (Landfeldt et al., 2017, McCaughan et al., 2019). Carbon fibre is particularly notable for its high satisfaction rate among users with neuromuscular diseases(Mnatsakanian et al., 2017). This material is thought to assist walking by allowing the orthoses to store energy and release it at pre-swing, which benefits patients with plantar flexion weakness(Zou et al., 2014, Bartonek et al., 2007). In a study by Dufek et al. (2014), custom-made carbon fibre AFOs were found to improve walking speed, step length, and frequency in patients with Charcot-Marie-Tooth disease. The carbon fibre AFOs also helped shift the maximum joint moments during loading

from the hip to the ankle and knee joints, reducing the reliance on proximal muscles. This energy storage and release mechanism, averaging 9.6 J/kg, allows for better propulsion and compensates for ankle weakness (Dufek et al., 2014).

In this study we are aiming to explore the effect of carbon fibre AFO on gait kinetics and kinematics in our DHMN cohort using 3D motion analysis. The hypothesis is that the carbon fibre AFO material can store energy in stance phase and release it at toe off to compensate for ankle muscle weakness. Also, to explore any assist of foot clearance in swing phase.

8.2. Methods

To explore the effect of bilateral carbon fibre ankle foot orthoses on the kinetics and kinematics of gait of people with DHMN, 10 DHMN participants were recruited to undergo isokinetic and isometric dynamometry of the lower limbs and 3D motion analysis to capture spatiotemporal, kinetic, and kinematic data of walking gait, as described in chapter 4. There were two conditions: wearing shoes only, and wearing bilateral Matrix Max carbon fibre AFOs (Trulife, UK) (Figure 62). “Shoes only” was the control condition. The order of wearing or not wearing the AFOs was randomised to account for learning and/or fatigue effects. Small, medium, and large size AFOs were used to ensure fit. In cases where the participant’s footwear was unsuitable, lace up shoes were provided to wear.

Figure 62: Matrix Max Carbon fibre Ankle foot Orthosis (Trulife, UK)

8.2.1. Data analysis

To explore the effect of bilateral carbon fibre ankle foot orthoses (AFO) on the kinetics and kinematics of gait of people with DHMN, gait variables were compared with and without AFOs using a paired t-test, if all data sets were normally distributed, or a paired Wilcoxson- Signed rank test, if at least one of the data sets were not normally distributed. A modified Bonferroni procedure was used to account for multiple comparisons. However, due to the large number of comparisons, it was anticipated that with small sample, significant differences may be few. Therefore, it was decided to also explore trends in the data using $p<0.05$ as a guide.

To measure the effect size, Hedge's G was calculated as the difference between the two means divided by the pooled standard deviation, with a correction for small sample bias. The effect size was categorised by magnitude according to Cohen's suggestion: < 0.2 minimal effect; 0.2-0.5 small effect; 0.5-0.8 moderate effect; >0.8 large effect.

8.3. Results

8.3.1. Subjects and Clinical Assessment

Ten DHMN participants were recruited. Participants were 6 male and 4 female ranging in age from 41 to 75. Clinical assessment showed distal muscle weakness and limitation in plantarflexion and dorsiflexion active and passive range of motion. A summary of the subjects' demographics and clinical assessment is presented in Table 52 and Table 53.

Demographics and clinical assessment	
Numbers	10
Gender (M/F)	(6/4)
Age; mean years; range	57.5 (41/75)
Genetic diagnosis (HSPB1/unknown)	(6/4)
CMTES; mean (SD)	5.2(2.6)
Foot Posture Index-6 (Pronated/Normal/Supinated)	(1/8/1)
Fall Frequency (weekly/Monthly/Yearly)	(1/1/2)
Walk-12; mean (SD)	34(10.72)
Range of Motion	
Dorsiflexion; count (Limited/Normal)	(10/0)
Plantarflexion; count (Limited/Normal)	(9/1)
M= Male, F= Female, HSPB1= Heat-shock 27-KD Protein 1, SD= Standard Deviation, CMTES= Charcot-Marie-Tooth Disease Examination Score, *= significant after modified Bonferroni correction.	

Table 52: Summary of demographics and clinical assessment of DHMN participants.

Manual Muscle Testing	
Hip Flexion; mode; (range)	5 (5-3)
Hip Extension; mode; (range)	5 (5-3)
Knee Flexion; mode; (range)	5 (5-3)
Knee Extension; mode; (range)	5 (5-4)
Dorsiflexion; mode; (range)	4+ (5-0)
Plantarflexion; mode; (range)	4+ (4+-0)
Isometric Dynamometry	
Hip Extension 45° ; mean (SD), Nm	144.6(67.4)
Hip Flexion 45°; mean (SD), Nm	114.25(44.5)
Knee Extension 45°; mean (SD), Nm	117.55(53.9)
Knee Extension 90°; mean (SD), Nm	119.45(52.0)

Knee Flexion 45°; mean (SD), Nm	65.5(34.3)
Knee Flexion 90°; mean (SD), Nm	44(23.2)
Ankle Plantar flexion 10°; mean (SD), Nm	30(17.2)
Ankle Dorsiflexion 10°; mean (SD), Nm	15.35(17.4)
Ankle Dorsiflexion 30°; mean (SD), Nm	20.65(17)
Isokinetic Dynamometry	
Hip Extension 60°/60s; mean (SD), Nm	122.8(68.1)
Hip Flexion 60°/60s; mean (SD), Nm	110.35(48.4)
Knee Extension 60°/60s; mean (SD), Nm	91.35(48.9)
Knee Flexion 60°/60s; mean (SD), Nm	46.4(24.9)
Knee Extension 120°/120s; mean (SD), Nm	66.05(43.3)
Knee Flexion 120°/120s; mean (SD), Nm	35.85(23.6)
Ankle Plantar flexion 60°/60°; mean (SD), Nm	18.5(16.6)
Ankle Dorsiflexion 60°/60°; mean (SD), Nm	16.2(7)
SD= Standard Deviation, Nm= Newton meter	

Table 53: Strength assessment of DHMN participants.

8.3.2.The Effect of Carbon Fibre Ankle Foot Orthoses

There were significant differences when walking with AFOs. Analysis showed a decrease in ankle plantar flexion angle (P=0.002) (Table 55) and an increase in knee flexion moment (P=0.0384) (Table 56). However, when using P<0.05 significance level, cut off trends were observed in proximal and distal kinematics and spatiotemporal parameters (Table 54,Table 55,Table 56) (Figure 63,Figure 64,Figure 65). Walking with AFOs showed increase in hip extension angle (P=0.0273), decrease in knee flexion angle (P=0.0283), decrease in ankle dorsiflexion angle (P=0.0115), increase in speed (P=0.0486), increase in stride length (P=0.0127) and step length (P=0.0128), decrease in double support time (P=0.0173) and percent opposite toe off (P=0.0255) (Table 54,Table 55,Table 56) (Figure 63,Figure 64,Figure 65). Hedge's G showed the effect size to be the

largest in ankle angles with (-0.94) in dorsiflexion and (1.352) in plantar flexion (Table 54,Table 55,Table 56).

Gait Spatiotemporal	**NO AFO** mean (SD)	**AFO** mean (SD)	**Diff** mean (SD)	**P**	**Hedge's G**	**95% CI**
Speed; m/s	1.07(0.23)	1.16(0.23)	0.1(0.1)	0.0486†	0.38	-0.53 to 1.29
Stride Length; m	1.25(0.24)	1.35(0.23)	0.1(0.1)	0.0127†	0.40	-0.51 to 1.31
Stride Time; s	1.18(0.06)	1.18(0.10)	0.0(0.1)	0.8326	-0.05	-0.95 to 0.85
Strides Per Minute	51.05(2.52)	51.40(4.08)	0.4(2.7)	0.6852	0.10	-0.80 to 1.0
Step Length; m	0.62(0.12)	0.67(0.12)	0.0(0.0)	0.0128†	0.40	-0.51 to 1.31
Step Time; s	0.59(0.03)	0.59(0.05)	0.0(0.0)	0.8244	-0.05	-0.95 to 0.85
Steps Per Minute	102.09(5.04)	102.80(8.16)	0.7(5.3)	0.6852	0.10	-0.80 to 1.0
Percent Stance	0.63(0.02)	0.63(0.02)	0.0(0.0)	0.184	-0.39	-1.3 to 0.52
Single Support Time; s	0.60(0.04)	0.61(0.06)	0.0(0.0)	0.3553	0.25	-0.65 to 1.16
Double Support Time; s	0.15(0.03)	0.13(0.04)	0.0(0.0)	0.0173†	-0.64	-1.57 to 0.28
Percent Opposite Toe Off	12.83(2.40)	10.67(3.59)	-2.2(2.6)	0.0255†	-0.68	-1.61 to 0.25
Percent Opposite Foot Contact	49.85(0.77)	49.52(0.97)	-0.3(1.5)	0.4989	-0.36	-1.27 to 0.55
Diff= difference, SD= Standard Deviation, m= meters, s= seconds, † = significant with P<0.05, Hedge's G= < 0.2 minimal effect, 0.2-0.5 small effect, 0.5-0.8 moderate effect, >0.8 large effect.						

Table 54: Spatiotemporal data, difference, and effect size of walking with and without AFOs in DHMN.

Gait Kinematics	**NO AFO** mean (SD)	**AFO** mean (SD)	**Diff** mean (SD)	**P**	**Hedge's G**	**95% CI**
Pelvis X Max; °	4.83(1.58)	5.26(2.63)	0.4(1.6)	0.40	0.19	-0.7 to 1.09
Pelvis X Min; °	-5.16(1.81)	-4.72(1.53)	0.4(1.0)	0.21	0.25	-0.65 to 1.16
Pelvis Y Max; °	15.22(5.06)	14.94(3.42)	-0.3(3.7)	0.82	-0.06	-0.96 to 0.84
Pelvis Y Min; °	10.36(4.16)	10.16(2.96)	-0.2(3.0)	0.83	-0.06	-0.95 to 0.85
Pelvis Z Max; °	6.40(2.00)	7.52(2.81)	1.1(2.2)	0.15	0.44	-0.47 to 1.35
Pelvis Z Min; °	-6.32(2.13)	-5.79(4.12)	0.5(4.0)	0.63	0.15	-0.75 to 1.06
Hip Max; °	47.27(5.61)	44.69(5.62)	-2.6(6.0)	0.21	-0.44	-1.35 to 0.47
Hip Min; °	1.97(2.54)	-0.87(4.82)	-2.8(4.3)	0.0273†	-0.71	-1.64 to 0.22
Knee Max; °	75.09(2.82)	72.82(3.52)	-2.3(2.8)	0.0283†	-0.68	-1.61 to 0.25
Knee Min; °	6.77(4.44)	1.25(17.77)	-5.5(16.0)	0.49	-0.41	-1.32 to 0.5
Ankle Max; °	26.18(4.54)	21.73(4.54)	-4.5(4.5)	0.0115†	-0.94	-1.9 to 0.012
Ankle Min; °	-9.82(4.67)	-3.80(3.80)	6.0(5.2)	**0.002***	1.35	0.35 to 2.36
Diff= difference, SD= Standard Deviation, † = significant with P<0.05, *= significant with modified Bonferroni correction= Hedge's G= < 0.2 minimal effect, 0.2-0.5 small effect, 0.5-0.8 moderate effect, >0.8 large effect, Pelvic z Maximum= Rotation forwards, Pelvic z Minimum= Rotation backwords, Pelvic x Maximum, lateral raise, Pelvic x Minimum= lateral drop, Pelvic y Maximum= Anterior tilt, Pelvic y Minimum = Posterior tilt, Hip y Maximum= Flexion, Hip y Minimum= Extension, Knee y Maximum= Flexion, Knee y Minimum= Extension, Ankle y Maximum= Dorsiflexion, Ankle y Minimum= Plantarflexion.						

Table 55: Gait kinematic data, difference, and effect size of walking with and without AFOs in DHMN.

Gait Kinetics	NO AFO mean (SD)	AFO mean (SD)	Diff mean (SD)	P	Hedge's G	95% CI
Hip Moment Y Max; Nm/Kg	1.66(0.60)	1.77(0.58)	0.1(0.4)	0.38	0.19	-0.71 to 1.090
Hip Moment Y Min; Nm/Kg	-0.53(0.16)	-0.65(0.43)	-0.1(0.4)	0.92	-0.35	-1.25 to 0.560
Knee Moment Y Max; Nm/Kg	0.82(0.35)	0.82(0.33)	0.0(0.1)	0.85	-0.02	-0.92 to 0.881
Knee Moment Y Min; Nm/Kg	-0.75(0.35)	-0.87(0.34)	-0.1(0.2)	**0.0384***	-0.35	-1.25 to 0.560
Ankle Moment Y Max; Nm/Kg	1.04(0.27)	1.10(0.24)	0.1(0.1)	0.15	0.25	-0.65 to 1.158
Ankle Moment Y Min; Nm/Kg	-0.07(0.07)	-0.07(0.04)	0.0(0.1)	0.38	0.00	-0.90 to 0.897
Hip Power Max During Swing; W/kg	1.15(0.33)	1.06(0.32)	-0.1(0.3)	0.43	-0.26	-1.17 to 0.64
Hip Power Min During Stance; W/kg	-1.18(1.00)	-1.87(2.36)	-0.7(2.4)	0.63	-0.37	-1.27 to 0.54
Hip Power Max During Stance; W/kg	2.06(0.72)	2.42(1.77)	0.4(1.4)	0.85	0.26	-0.64 to 1.16
Knee Power Max During Swing; W/kg	0.43(0.22)	0.46(0.27)	0.0(0.2)	0.56	0.12	-0.78 to 1.02
Knee Power Min During Stance; W/kg	-1.85(1.00)	-1.96(1.06)	-0.1(0.9)	0.70	-0.10	-1.002 to 0.8
Knee Power Max During Stance; W/kg	2.24(1.91)	2.96(3.00)	0.7(1.9)	0.38	0.27	-0.63 to 1.18
Ankle Power Max During Swing; W/kg	0.04(0.02)	0.07(0.08)	0.0(0.1)	0.18	0.49	-0.42 to 1.41
Ankle Power Min During Stance; W/kg	-1.20(0.33)	-1.10(0.39)	0.1(0.3)	0.31	0.29	-0.62 to 1.19
Ankle Power Max During Stance; W/kg	1.09(0.40)	0.84(0.45)	-0.3(0.5)	0.11	-0.57	-1.49 to 0.35

Diff= difference, SD= Standard Deviation, Nm/Kg= Newton Meter per Kilogram, W/kg= Watts Per Kilogram, *= significant with modified Bonferroni correction= Hedge's G= < 0.2 minimal effect, 0.2-0.5 small effect, 0.5-0.8 moderate effect, >0.8 large effect= Hip Moments Maximum= Extension, Hip Moments Minimum= Flexion, Knee Moments Maximum= Extension, Knee Moments Minimum= Flexion, Ankle Moments Maximum= Plantarflexion, Ankle Moments Minimum= Dorsiflexion, Hip Power Maximum = generation in swing phase, Hip Power Minimum= absorption in stance phase, Hip Power Maximum= generation in stance phase, Knee Power Maximum= generation in stance phase, Knee Power Maximum= generation in swing phase, Knee Power Minimum = absorption in stance phase, Ankle Power Maximum= generation in stance phase, Ankle Power Minimum= absorption in stance phase, Ankle Power Maximum= generation in swing phase.

Table 56:Gait kinetics data, difference, and effect size of walking with and without AFOs in DHMN.

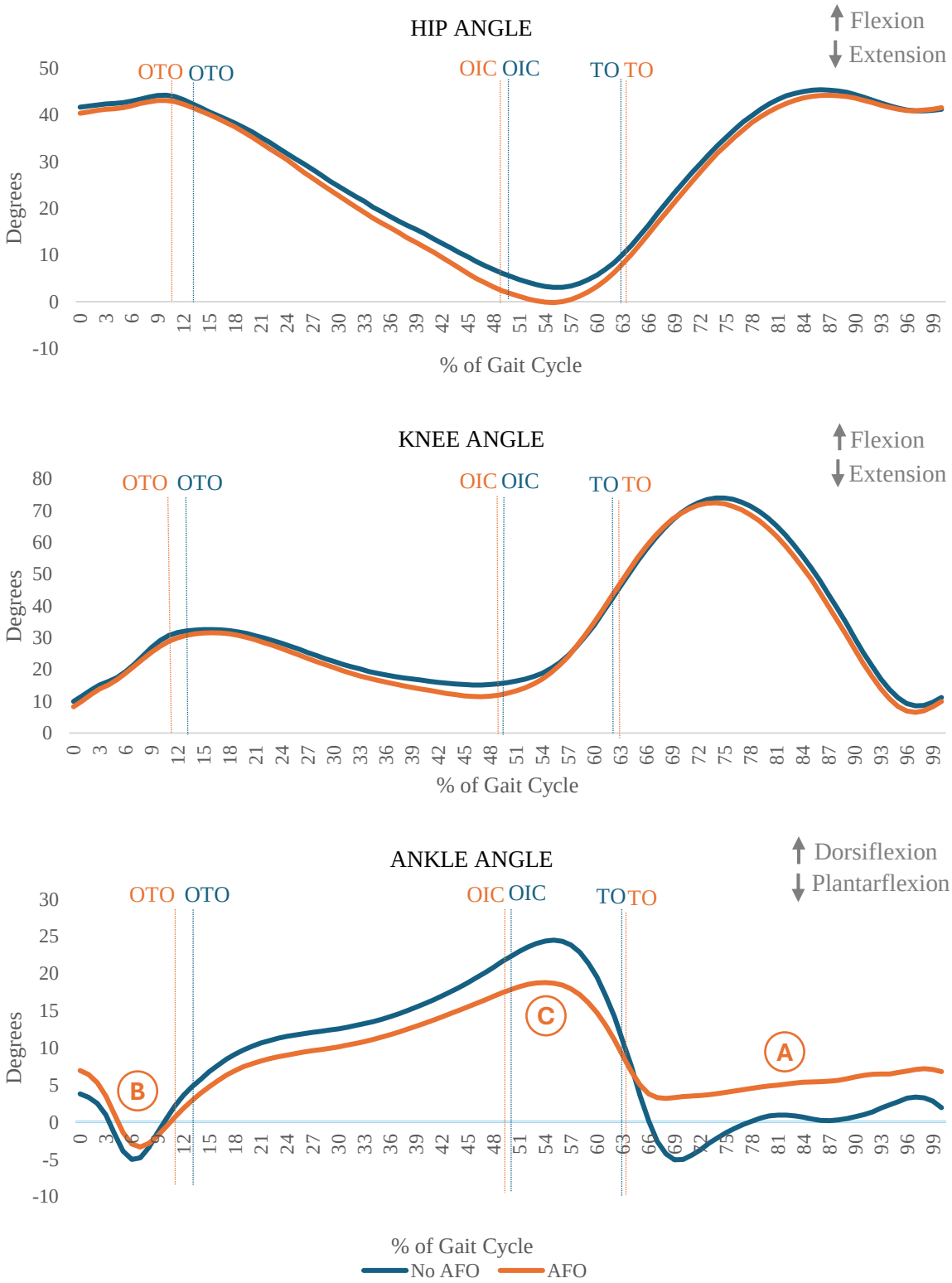


Figure 63:Grand average angular displacement in the sagittal plane over one gait cycle for the ankle, knee, and hip (average of left and right sides). Comparison of people with DHMN walking with and without AFO.

OTO, Opposite side tore off; OIC, Opposite side initial contact; TO, Toe off; A, improve foot clearance; B,; C, improve ankle support, , N=10.

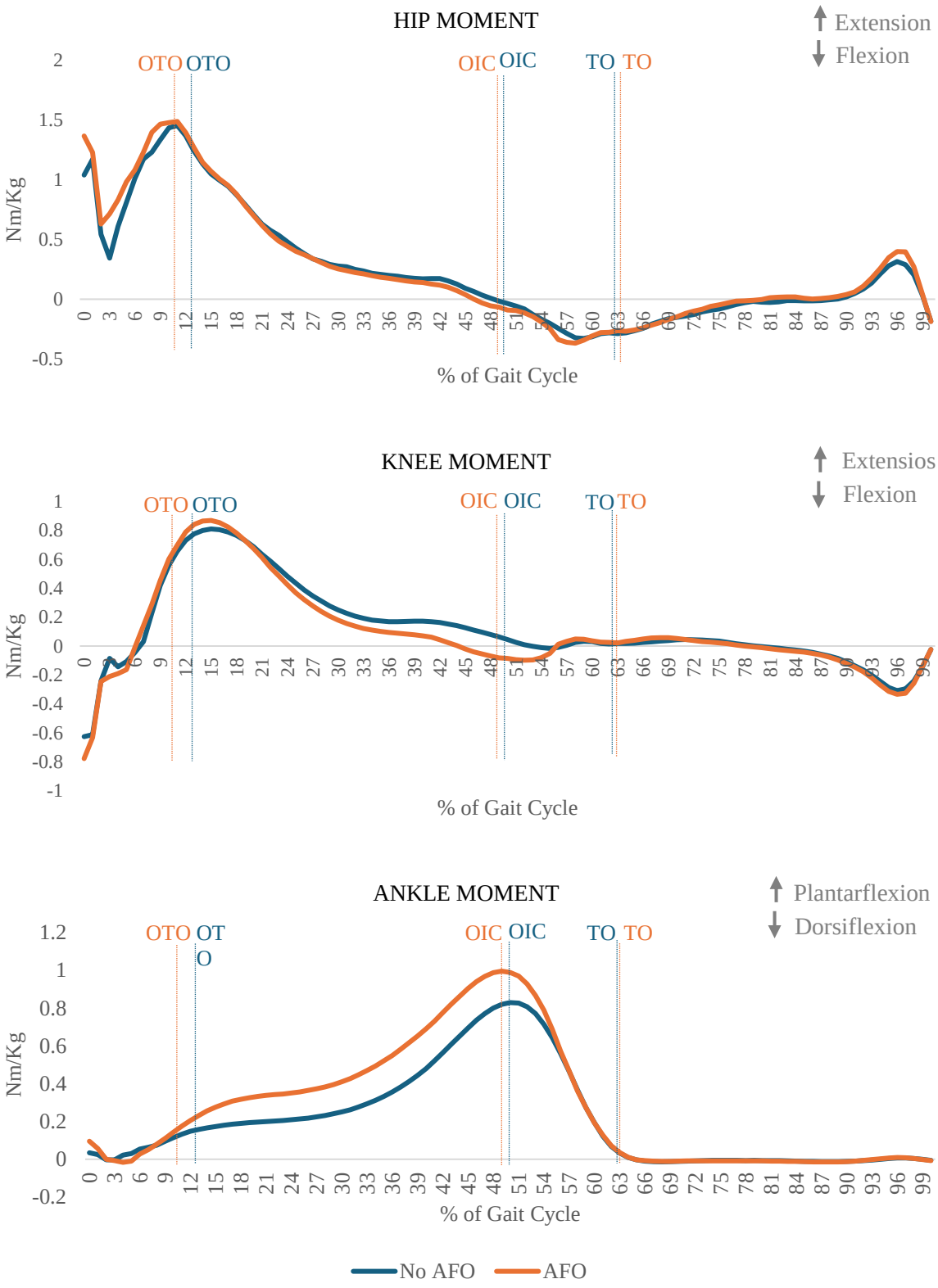


Figure 64:Grand average joint moments in the sagittal plane over one gait cycle for the ankle, knee and hip (average of left and right sides). Comparison of people with DHMN walking with and without AFO.

OTO, Opposite side tore off; OIC, Opposite side initial contact; TO, Toe off, N=10.

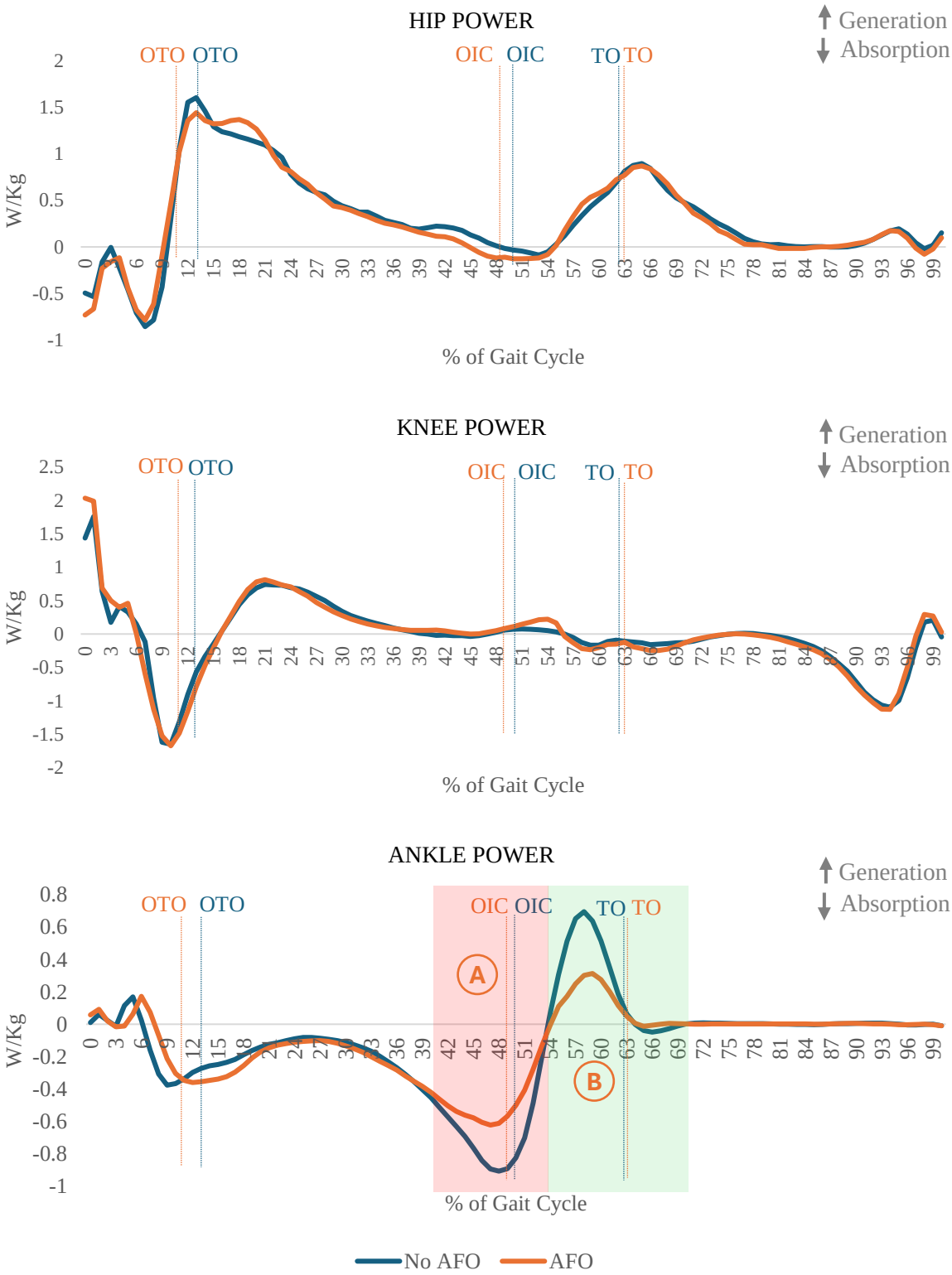


Figure 65:Grand average joint powers in the sagittal plane over one gait cycle for the ankle, knee and hip (average of left and right sides). Comparison of people with DHMN walking with and without AFO.

OTO, Opposite side tore off; OIC, Opposite side initial contact; TO, Toe off; A, absorption; B, generation, N=10.

8.4. Discussion

This current study is aimed to explore the effect of carbon fibre ankle foot orthoses on gait kinetics and kinematics in people with DHMN. This DHMN cohort presented with plantar flexor weakness that affected the stability of the ankle in stance phase (plantar flexion failure), and dorsiflexor weakness that affected foot clearance in swing phase (foot drop), resulting in slower walking gait (chapter 5).

There were promising trends towards faster gait speed, longer strides, and shorter double support, due to the biomechanical assist of the AFOs. Where there is plantar flexion weakness, the muscle group is not capable of producing a high enough isometric force to control the ankle joint angle in stance phase, therefore, the ankle falls in passive dorsiflexion as the centre of gravity progresses forward (Brockett and Chapman, 2016). The AFO compensated for the weakness by providing enough ankle support and stability, to hold the ankle in a neutral position and allow the contralateral leg to start the swing phase sooner. This finding is consistent with studies by Dufek et al. (2014), which demonstrated that custom-made carbon fibre AFOs enabled faster walking speeds and improved step length in people with CMT (Dufek et al., 2014). However, the AFO rigidity limits the range of plantar flexion in mid to late stance. This appears to inhibit plantar flexor power generation and may transfer the motion to the knee joint, so explaining the increased knee flexor moment. The difference in ankle power

generation was not significant but showed a moderate effect size so this hypothesis would need confirmation with larger sample.

Dorsiflexion weakness explains the reduction in ankle dorsiflexion angle while the leg is swinging. Therefore, knee and hip flexion is increased to clear the foot from the ground. The AFO compensated for the weakness by holding the ankle in a neutral position, which allows foot clearance and longer strides without the need to shorten the leg by excessively flexing the knee and the hip. This is in line with findings from previous research which reported that AFOs improved ankle dorsiflexion angles and reduced compensatory hip flexion especially in those with foot drop in children (Õunpuu et al., 2021), and adults (Ramdharry et al., 2012a) with CMT.

Overall, there was an improvement in walking gait with AFOs. However, there is a possibility that with restricted ankle movement, planer flexor activity might be inhibited (Figure 65). This could have long term implication for disuse weakness. Further studies are recommended with electromyography to explore changes in muscle activity. Longitudinal follow up will aid better understanding of the long term effects of wearing AFOs.

8.5. Summary

The current study explored the effect of carbon fibre AFOs on gait kinetic, kinematic, and spatiotemporal data in DHMN. The study findings are summarised in Table 57.

<table>
<tr><th colspan="9">The Normal Gait Cycle</th></tr>
<tr><th>% of cycle</th><td>0%</td><td>0-10%</td><td>10-30%</td><td>30-50%</td><td>50-60%</td><td>60-70%</td><td>70-85%</td><td>85-100%</td></tr>
<tr><th>Phase</th><td colspan="5">Stance phase</td><td colspan="3">Swing phase</td></tr>
<tr><th>Event</th><td>Initial contact</td><td colspan="2">Foot flat, Opposite toe off (OTO)</td><td colspan="2">Heel off, Opposite initial contact</td><td>Toe off</td><td>Tibia vertical, Feet adjacent</td><td>Initial contact (next cycle)</td></tr>
<tr><th>Period</th><td>Loading response</td><td colspan="2">Mid-stance</td><td>Terminal stance</td><td>Pre-swing</td><td>Initial swing</td><td>Mid-swing</td><td>Terminal swing</td></tr>
<tr><th>Support</th><td>Double support</td><td colspan="3">Single leg support</td><td>Double support</td><td colspan="3">Single leg support</td></tr>
<tr><th colspan="9">DHMN Pattern in Gait and Muscle Involvement (Chapter 5)</th></tr>
<tr><th>Muscle Involvement</th><td colspan="5">Plantar flexion (PF) weakness affected the stability of the ankle joint in stance phase</td><td colspan="3">Dorsiflexion (DF) weakness affected foot clearance in swing phase</td></tr>
<tr><th rowspan="2">Spatio Temporal Variables</th><td colspan="3">Increased double support time due to delayed OTO</td><td colspan="2">Increased stance time due to increased step and stride length</td><td colspan="3">Increased single support time</td></tr>
<tr><td colspan="8">Slow gait due to the events timing</td></tr>
<tr><th>Kinematics Variables</th><td colspan="4">Increased ankle passive dorsiflexion</td><td>Decreased ankle plantar flexion</td><td colspan="3">Decreased ankle dorsiflexion and increased hip flexion</td></tr>
<tr><th>Kinetics Variables</th><td colspan="5">Decreased plantar flexion moment and power</td><td colspan="3">Decreased ankle power generation</td></tr>
<tr><th>Gait Pattern</th><td colspan="5">Plantar flexion failure</td><td colspan="3">Foot drop</td></tr>
<tr><th colspan="9">Effect of AFO on DHMN Gait</th></tr>
<tr><th>Kinematics Variables</th><td colspan="5">Improved ankle control and decreased ankle passive dorsiflexion</td><td colspan="3">Increased ankle dorsiflexion and decreased knee and hip flexion</td></tr>
<tr><th>Kinetics Variables</th><td colspan="8">Increased knee flexion moments to pick up the foot in pre-swing</td></tr>
<tr><th>Spatio Temporal Variables</th><td colspan="5">Decreased double support time due to early OTO</td><td colspan="3">faster gait due to longer strides and steps</td></tr>
<tr><th>Comment</th><td colspan="5">Improved ankle stability</td><td colspan="3">Improved foot clearance</td></tr>
</table>

Table 57: Summary of the effect of AFO on DHMN gait.

Chapter 9: The Effect of Resistance Training on Muscle Structure, Function, and Gait Patterns in Distal Hereditary Motor Neuropathy (DHMN)

9.1. Introduction

Therapeutic exercise is one of the few available treatments for peripheral neuropathies, including distal hereditary motor neuropathy (DHMN). Studies in neurological diseases suggest that exercise can improve balance, reduce fatigue, enhance physical performance, and positively affect quality of life (Roberts-Clarke et al., 2016b, Vita et al., 2016, Ramdharry et al., 2012c, Burns et al., 2017). However, there is a lack of evidence-based exercise protocols tailored to neuropathies, leading to reliance on therapist experience for program design. Existing research on Charcot-Marie-Tooth disease (CMT) has shown the potential benefits of strengthening exercises in improving muscle strength and function. However, the long-term safety and efficacy of such interventions remain underexplored (Sman et al., 2015).

This exercise trial is aimed to evaluate the effectiveness of progressive resistance training on muscle structure, using MRI, and function, using dynamometry and 3D motion analysis. It is also aimed to evaluate the safety of progressive resistance training by evaluating changes in MRI intramuscular water as a sign of active denervation.

9.2. Methods

Ten DHMN participants recruited for the natural history study were randomly assigned to “exercise group” and “no exercise group”. Participants were eligible if they had lower limb muscle strength over grade 4 of the MRC scale. Both groups underwent an MRI scan, isokinetic and isometric dynamometry of the lower limb, and 3D motion analysis to capture kinetic and kinematic data of walking gait as described in chapter 4. The same measurements were repeated after 6 months and 12 months to explore the effect of resistance training on the condition.

The 6 months strength training program included resistance training of distal and proximal lower limb muscles, using graded ankle weights, performed 3-4 times per week. Resistance training of the muscles was initially prescribed at 25% of one repetition maximum power, measured using a handheld dynamometer (Citec CT 3001, CIT Technics BV, Groningen, The Netherland), at two sets of 8-12 repetitions. This was increased to 30% and 40% when subjects were easily completing 2 sets of 12 repetitions for a week (ACSM, 2021). Training session included 5 minutes warm up and cool down using low resistance exercises.

Adherence to the exercise programme was monitored throughout the study period by supplying subjects with exercise diaries. Subjects in the treatment group were planned to receive monthly visits from the PhD student to ensure safety of the program, re-assess strength and progress training. However, due to the COVID-19 pandemic physical contact restrictions, subjects were contacted via phone or

video call on a weekly basis to monitor participation, pain levels, and any difficulties with the training. Exercise induced pain management involved monitoring pain levels to prevent musculoskeletal injury and ensure appropriate exercise intensity. Participants use the Visual Analogue Scale (VAS) to report pain. If the reported pain level was above 4, modifications were implemented to reduce pain while maintaining training. Pain levels above 7 during or post-exercise may indicate injury and lead to stopping the exercise for assessment and potential modification of the program. Delayed onset muscle soreness (DOMS) was anticipated as a normal early response and expected to resolve within 48 hours. Persistent or new pain required a full assessment and would lead to participant withdrawal from the trial if significant injury was found (appendix IV). Exercises instructions with photos and videos were provided for participants using Physiotools platform (appendix V).

9.2.1. Data Analysis

This DHMN cohort showed predominant distal weakness more than proximal, and in plantar flexors more than dorsiflexors (chapter 5). The natural history group (chapter 6) showed longitudinal deterioration in the posterior compartment of the lower leg more than other muscle groups. Therefore, MRI parameters at the calf posterior compartment were used primarily to explore the effectiveness and safety of exercises.

The analysis plan was for paired statistical tests to compare before and after exercises, and unpaired statistical tests to compare between “exercise group” and “no exercise group”. Effect sizes were to be calculated using the Hedge’s G statistic due to the small sample size.

The level of adherence was measured according to the number of sessions completed and recorded in the exercise diary.

9.3. Results

9.3.1. Adherence to Exercises

The exercise group included 5 DHMN participants. The overall group adherence to the exercises program for 6 months was low. Two participants showed high adherence (case 1: 87.5%, case 2: 97.2%), one showed low adherence (case 3: 12.5%), and two showed no adherence (case 4 and case 5: 0%). It is important to note that case 1 followed the exercise program in the first 6 months, and case 2 followed the exercise program in the second 6 months of the study period. During the exercises period, pain was reported by 2 participants: case 2 reported 12 episodes with pain levels ranging from 2 to 7 on visual analogue scale. Case 3 reported 4 episodes with pain levels ranging from 5 to 8 on visual analogue scale. Factors that influenced adherence to exercise as reported by the “exercise group” participants are shown in Table 58. Those with high adherence were happy about the program and did not recommend any changes. Participant with low adherence recommended designing a less time consuming program. Those with no adherence were hoping for more interesting program to encourage engagement with the exercises.

High Adherence	-Career requires physical fitness. -Exercise level is minimal and able to fit into a daily exercise routine. -Medical background: aware of the importance of exercising in general. -Have a dedicated room for exercising at home, equipped properly.
Low Adherence	-knee Pain after a fall. -Long COVID-19 recovery. -Tiredness after long working days. - Travel with family.
No Adherence	-Traveling abroad for long time. -Couldn't fit exercise into daily routine. -Distracted with family situation. -Preferred to wait for study results before committing to something (exercises) that might not help.

Table 58: Factors that influenced adherence to exercise.

9.3.2. Study Participants and Clinical Assessment

Data collected at each measurement point and the difference between measurements will be presented descriptively by group, exercise (n=5), and no exercises (n=5). However, since only 2 participants in the exercise group showed high level of adherence, this was deemed too small for a group analysis so they will be presented descriptively as individual cases. Differences between measurements points for each case has been presented in comparison to the mean change in a group of 8 DHMN participants, that includes the original "No Exercise" group, plus the three cases with no or low adherence (chapter 6). A summary of the subjects' demographics and clinical assessment at baseline is presented in Table 59 and Table 60.

Demographics and clinical assessment	**Exercise**	**No Exercise**
Numbers	5	5
Gender (M/F)	(3/2)	(2/3)
Age; mean years; range	55 (44/62)	60 (44/75)
Genetic diagnosis (HSPB1/unknown)	(3/2)	(3/2)
CMTES; mean (SD)	5.2 (5.1)	7.2 (1.9)
Foot Posture Index-6 (Pronated/Normal/Supinated)	(1/3/1)	(0/4/1)
Fall Frequency (weekly/Monthly/Yearly)	(1/1/0)	(0/0/2)
Walk-12; mean (SD)	33(13)	39(14)
Range of Motion	**Exercise**	**No Exercise**
Dorsiflexion (Limited/Normal)	(5/0)	(5/0)
Plantarflexion (Limited/Normal)	(5/0)	(5/0)
Manual Muscle Testing	**Exercise**	**No Exercise**
Hip Flexion; mode; range	5(4-5)	5(3-5)
Hip Extension; mode; range	5(3-5)	5(3-5)
Knee Flexion; mode; range	5(3-5)	5(4--5)
Knee Extension; mode; range	5(4-5)	5(4-5)
Dorsiflexion; mode; range	4(0-4+)	1(1-4+)
Plantarflexion; mode; range	4+(1-4+)	4+(1-4+)
M= Male, F= Female, HSPB1= Heat-shock 27-KD Protein 1, SD= Standard Deviation, CMTES= Charcot-Marie-Tooth Disease Examination Score.		

Table 59: Summary of the subjects' demographics and clinical assessment at baseline by group, exercises (n=5) and no exercise (n=5).

Demographics and clinical assessment	**CASE 1**	**CASE 2**	**No Exercise**
Numbers	1	1	8
Gender (M/F)	M	M	(3/5)
Age; mean years; range	58	61	57 (42/75)
Genetic diagnosis (HSPB1/unknown)	unknown	unknown	(6/2)
CMTES; mean (SD)	2	2	7.25(3.23)
Foot Posture Index-6 (Pronated/Normal/Supinated)	Supinated	Normal	(1/6/1)
Fall Frequency (weekly/Monthly/Yearly)	0	0	(1/1/2)
Walk-12; mean (SD)	31	30	36(12)
Range of Motion	**CASE 1**	**CASE 2**	**No Exercise**
Dorsiflexion; count (Limited/Normal)	Limited	Limited	(8/0)
Plantarflexion; count (Limited/Normal)	Limited	Limited	(8/0)
Manual Muscle Testing	**CASE 1**	**CASE 2**	**No Exercise**
Hip Flexion; mode; (range)	5	5	5(3-5)
Hip Extension; mode; (range)	5	5	5(3-5)
Knee Flexion; mode; (range)	5	5	5(4-5)
Knee Extension; mode; (range)	5	5	5(3-5)
Dorsiflexion; mode; (range)	4	4-	4+(1-4+)
Plantarflexion; mode; (range)	4	4-	4+(0-4+)
M= Male, F= Female, HSPB1= Heat-shock 27-KD Protein 1, SD= Standard Deviation, CMTES= Charcot-Marie-Tooth Disease Examination Score.			

Table 60: Summary of the subjects' demographics and clinical assessment at baseline for exercise case 1, case 2, and no exercises group (n=8).

9.3.3. Effect of Exercises on Muscle Structure (MRI Parameters)

Comparison Between Exercises Group (N=5) and No Exercises Group (N=5):

Fat Fraction (FF): The rate of change in FF varied across different muscle groups for both exercise and no-exercise groups. In the total thigh, the exercise group showed a 7.7% increase from baseline to 6 months, followed by a 2.1% increase from 6 to 12 months, resulting in a 10% total increase over 12 months. In contrast, the no-exercise group showed a 3.8% increase at 6 months and an additional 2.9% at 12 months, resulting in a 6.1% increase. At the calf level, FF remained stable in the exercise group, with no change from baseline to 6 months and a 1.6% increase by 12 months. In contrast, the no-exercise group showed a significant 9.3% increase over 12 months, with a 3.6% increase by 6 months (Table 61).

Cross Sectional Area (CSA): At the thigh level, the exercise group generally demonstrated muscle preservation or growth, whereas the no-exercise group showed declines or minimal gains. For total thigh area, the exercise group showed a 0.7% increase at 6 months, followed by a 1.8% increase at 12 months, resulting in an increase of 2.6% over 12 months. In contrast, the no-exercise group showed a 1% decrease at 6 months and a 0.3% increase from 6 to 12 months, resulting to an overall reduction of 0.7%. At the calf level, the exercise group showed a 3.1% decrease in total calf area over 12 months, driven by declines in both plantarflexion and dorsiflexion. Plantarflexion decreased by 4.6% and dorsiflexion by 2.8%. The no-exercise group also showed declines in calf area,

with a 1.5% decrease over 12 months, but the reduction in plantarflexion was less severe (2.2%), and dorsiflexion decreased by just 0.8% (Table 62).

Remaining Muscle Area (RMA): For total thigh muscle area, the exercise group showed a 0.04% increase from baseline to 6 months, followed by a more 1.5% increase from 6 to 12 months, resulting in a gain of 1.6% over 12 months. In contrast, the no-exercise group showed a marked decline, with a 1.6% reduction at 6 months and an additional 0.3% reduction by 12 months, resulting in a total loss of 1.9%. At the calf level, the exercise group showed a 2.2% reduction in muscle area over 12 months, including a 0.7% decrease in plantarflexion and a 4.3% reduction in dorsiflexion. In comparison, the no-exercise group showed a larger 7.7% loss in total calf muscle, with a 6.3% decrease in plantarflexion and a 3.7% decline in dorsiflexion. (Table 63).

Muscle Oedema (T_{2m}): In the total calf, the exercise group showed a 2.3% reduction in oedema from baseline to 6 months, followed by a 1.6% increase from 6 to 12 months, resulting in a small overall reduction of 0.8% after 12 months. Conversely, the no-exercise group showed a 4.7% reduction in oedema from baseline to 6 months, and a significant 9.1% increase at 12 months, resulting in a 4% increase over 12 months. Similarly, in dorsiflexion, the exercise group maintained relative stability, with a minor 0.3% increase over 12 months. In contrast, the no-exercise group showed a 3.6% increase in oedema after 12 months, following an initial 2.1% reduction at 6 months (Table 64).

FAT (%) FRACTION Mean (SD)	BASELINE		6 MONTHS		12 MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Total Thigh	13 (18.2)	13.2 (6)	14.2 (20.6)	13.6 (7.0)	14.5 (20.5)	14.0 (8.0)
Knee extension	11 (17.0)	12.6 (11)	12.6 (17.9)	13.0 (12.1)	12.6 (18.0)	13.0 (12.9)
Knee flexion	15 (18.4)	13.6 (5)	17.6 (22.6)	14.1 (6.6)	17.6 (22.6)	14.2 (6.1)
Total Calf	31 (19.3)	37.8 (22)	31.9 (20.6)	41.4 (22.4)	32.4 (20.3)	42.2 (21.9)
Plantar flexion	42 (20.8)	41.5 (24)	41.8 (21.5)	46.3 (24.5)	42.5 (22.1)	47.0 (23.4)
Dorsiflexion	14 (22.4)	24.4 (16)	15.8 (22.8)	26.5 (17.4)	16.0 (22.6)	27.6 (18.0)
FOOT	45 (15.8)	54.1 (7)	43.8 (18.3)	56.0 (6.9)	45.3 (18.1)	57.3 (7.8)
FAT (%) FRACTION Difference	**BASELINE-6 MONTHS**		**6MONTHS-12MONTHS**		**BASELINE-12MONTHS**	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Total Thigh	1.0(2)	0.5 (1.1)	0.3 (1.2)	0.4 (1.1)	1.3 (2.4)	0.8 (1.3)
Knee extension	0.8(1)	0.4 (1.0)	0.0 (1.3)	0.1 (0.9)	0.7 (1.3)	0.5 (1.3)
Knee flexion	2.2(4)	0.6 (1.9)	0.1 (1.1)	0.1 (1.6)	2.2 (4.3)	0.7 (1.0)
Total Calf	0.0(2)	3.6 (1.2)	0.4 (1.5)	0.8 (1.3)	0.5 (2.2)	4.3 (1.3)
Plantar flexion	-0.6(3)	4.7 (1.8)	0.7 (3.3)	0.7 (1.9)	0.0 (3.6)	5.5 (2.1)
Dorsiflexion	1.1(1)	2.1 (2.0)	0.1 (0.9)	1.1 (1.4)	1.2 (1.0)	3.2 (2.0)
FOOT	-1.9(4)	2.0 (2.6)	1.5 (2.6)	1.3 (3.5)	-0.5 (4.9)	3.3 (1.7)
Data presented as: Mean (standers deviation).						

Table 61: Longitudinal fat fraction and differences between measurement points in exercise group (n=5) and no exercise group (n=5).

Cross Sectional Area (mm^2)	BASELINE		6 MONTHS		12 MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Total Thigh	11089 (5084)	10385 (1886)	11167 (4900)	10276 (1932)	11373 (4977)	10309 (1923)
Knee extension	5525 (2258)	4913 (859)	5419 (2182)	4882 (948)	5567 (2276)	4823 (857)
Knee flexion	3788 (2040)	4018 (1036)	4001 (2083)	4009 (969)	3954 (1942)	4038 (998)
Total Calf	4958 (1385)	5231 (1754)	4926 (1738)	5121 (1784)	4769 (1590)	5152 (1919)
Plantar flexion	3032 (938)	3566 (1346)	2990 (1199)	3440 (1355)	2894 (1138)	3504 (1435)
Dorsiflexion	1052 (431)	854 (325)	1038 (419)	843 (351)	1023 (409)	849 (361)
FOOT	476 (144)	502 (129)	460 (120)	458 (116)	458 (121)	448 (100)
Cross Sectional Area (mm^2) Difference	**BASELINE-6 MONTHS**		**6MONTHS-12MONTHS**		**BASELINE-12MONTHS**	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Total Thigh	78(369.9)	-108.9 (142.0)	206.3 (148.2)	32.9 (273.2)	284.3(360.4)	-76 (378.4)
Knee extension	-106.4 (164.6)	-30.4 (96.9)	148.4 (191.5)	-59.5 (207.2)	42.0(28.4)	-90 (188.1)
Knee flexion	213.3 (177.4)	-9.2 (121.8)	-47.7 (208.9)	29.1 (64.5)	165.7(187.1)	19.8 (115.9)
Total Calf	-31.4 (396.3)	-110.1 (219.0)	-157.7 (213.1)	30.9 (206.0)	-189.1(358.0)	-79.1 (180.3)
Plantar flexion	-42.2 (312.0)	-126.7 (149.2)	-96.4 (117.1)	64.5 (113.4)	-138.6(274.4)	-62.2 (116.3)
Dorsiflexion	-14.6 (48.2)	-10.9 (54.9)	-15.2 (31.0)	6.1 (39.7)	-29.8(78.6)	-4.8 (43.4)
FOOT	-15.7 (54.2)	-43.5 (56.9)	-2.4 (29.0)	-10.8 (24.7)	-18.1(64.2)	-54.3 (52.3)
mm^2= square millimetres, Data presented as: Mean (standers deviation).						

Table 62: Longitudinal cross sectional area and differences between measurement points in exercise group (n=5) and no exercise group (n=5).

Remaining Muscle Area (mm^2)	BASELINE		6 MONTHS		12 MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Total Thigh	20511 (11036)	17942 (3019)	20519 (11055)	17622 (2809)	20834 (11182)	17568 (2671)
Knee extension	10291 (5119)	8604 (1993)	10142 (5045)	8499 (2051)	10324 (5204)	8354 (1805)
Knee flexion	6901 (4249)	6969 (1994)	7184 (4523)	6895 (1848)	7074 (4260)	6933 (1870)
Total Calf	6938 (2659)	6740 (4034)	7048 (3121)	6243 (3805)	6784 (2961)	6218 (3991)
Plantar flexion	3599 (1700)	4382 (3059)	3696 (1977)	3934 (2896)	3573 (2013)	3962 (2975)
Dorsiflexion	1910 (1035)	1342 (644)	1862 (1021)	1302 (670)	1828 (989)	1292 (692)
FOOT	274 (150)	236 (94)	270 (132)	203 (67)	258 (121)	195 (73)
Remaining Muscle Area (mm^2) Difference	**BASELINE-6 MONTHS**		**6MONTHS-12MONTHS**		**BASELINE-12MONTHS**	
	Exercise (N=5)	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**
Total Thigh	8 (337)	-320 (365)	315 (430)	-54 (258)	323 (633)	-375 (615)
Knee extension	-150 (134)	-105 (99)	183 (177)	-145 (255)	33 (161)	-250 (223)
Knee flexion	284 (391)	-73 (228)	-111 (492)	38 (124)	173 (299)	-35 (185)
Total Calf	110 (484)	-497 (361)	-264 (287)	-25 (307)	-154 (390)	-522 (173)
Plantar flexion	97 (368)	-448 (239)	-123 (223)	28 (173)	-26 (330)	-420 (153)
Dorsiflexion	-48 (87)	-40 (107)	-34 (58)	-10 (89)	-82 (135)	-50 (69)
FOOT	-4 (40)	-33 (35)	-13 (21)	-8 (17)	-16 (36)	-41 (34)
mm^2= square millimetres, Data presented as: Mean (standers deviation).						

Table 63: Longitudinal remaining muscle area and differences between measurement points in exercise group (n=5) and no exercise group (n=5).

Muscle Oedema T_{2m} (ms)	BASELINE		6 MONTHS		12 MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Total Calf	37.9 (9.7)	40.3 (4.4)	37.0 (8.2)	38.4 (6.1)	37 (8.2)	41.9 (4.9)
Plantar flexion	38.5 (9.3)	40.9 (6.3)	37.6 (7.8)	38.6 (8.2)	37 (8.0)	41.8 (6.2)
Dorsiflexion	36.7 (10.3)	39.6 (2.3)	36.6 (9.6)	38.8 (3.7)	37 (9.4)	42.9 (4.2)
FOOT	44.9 (5.1)	46.7 (6.5)	47.4 (6.4)	46.1 (7.8)	49 (4.7)	50.4 (4.4)
Muscle Oedema T_{2m} (ms) Difference	**BASELINE-6 MONTHS**		**6MONTHS-12MONTHS**		**BASELINE-12MONTHS**	
	Exercise (N=5)	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**
Total Calf	-0.9 (1.8)	-1.9 (3.8)	0.6 (1.4)	3.5 (5.2)	-0.3 (1.6)	1.6 (2.6)
Plantar flexion	-0.9 (2.0)	-2.2 (4)	0.2 (1.9)	3.1 (5.8)	-0.7 (1.7)	0.9 (2.8)
Dorsiflexion	-0.1 (1.3)	-0.8 (2.6)	0.4 (0.5)	4.1 (3.4)	0.2 (1.5)	3.2 (2.7)
FOOT	2.6 (5.0)	-0.6 (9)	1.5 (3.3)	4.3 (4.0)	4.1 (4.4)	3.6 (7.4)
ms= millisecond, Data presented as: Mean (standers deviation).						

Table 64: Longitudinal muscle oedema and differences between measurement points in exercise group (n=5) and no exercise group (n=5).

Case 1

Fat Fraction (FF): After 6 months of training, there was a 2.0% decrease in FF for the overall calf, with a 4.7% reduction in the plantar flexors and a 0.1% reduction in the dorsiflexors. In the thigh showed an overall increase in FF post-training, although the knee flexors showed a 0.4% decrease (Table 65) (Figure 66).

Cross Sectional Area (CSA): After 6 months of training, the overall calf CSA increased by 16 mm^2 , with a 36 mm^2 increase in the plantar flexors, and a 2 mm^2 reduction in the dorsiflexors. In the thigh CSA decreased by 257 mm^2, with a 117 mm^2 reduction in the knee extensors and a 15 mm^2 decrease in the knee flexors (Table 66) (Figure 66).

Remaining Muscle Area (RMA): After 6 months of training, there was an increase in RMA for the overall calf by 243 mm^2, with a 279 mm^2 increase in the plantar flexors, and a 2 mm^2 increase in the dorsiflexors. In the thigh RMA decreased by 564 mm^2, including a 306 mm^2 reduction in the knee extensors, while the knee flexors showed an 8 mm^2 increase (Table 67) (Figure 66).

Muscle Oedema (T_{2m}): After 6 months of training, Muscle oedema in the overall calf increased by 0.6 ms, with a 0.5 ms increase in the plantar flexores, and a 1 ms increase in dorsiflexors (Table 68) (Figure 66).

Case 2

Fat Fraction (FF): After 6 months of training, there was a 1.7% decrease in FF for the overall calf, with a 3.3% reduction in the plantar flexors and a 0.6% reduction in the dorsiflexors. In the thigh there was an overall decrease in FF by 1.4%, with a 1.5% reduction in the knee extensors, and a 1.3% decrease in the knee flexors (Table 65) (Figure 66).

Cross Sectional Area (CSA): After 6 months of training, the overall calf CSA decreased by 473 mm^2 , with a 227 mm^2 decrease in the plantar flexors, and a 67 mm^2 reduction in the dorsiflexors. In the thigh CSA increased by 300 mm^2, with a 26 mm^2 increase in the knee extensors and a 66 mm^2 increase in the knee flexors (Table 66) (Figure 66).

Remaining Muscle Area (RMA): After 6 months of training, there was a decrease in RMA for the overall calf by 476 mm^2, with a 9 mm^2 decrease in the plantar flexors, and a 119 mm^2 decrease in the dorsiflexors. In the thigh RMA increased by 995 mm^2, including a 276 mm^2 increase in the knee extensors, and the knee flexors showed an 260 mm^2 increase (Table 67) (Figure 66).

Muscle Oedema (T_{2m}): After 6 months of training, Muscle oedema in the overall calf decreased by 0.2 ms, with a 0.7 ms decrease in the plantar flexores, and a 1 ms increase in dorsiflexors (Table 68) (Figure 66).

No Exercise Group (n=8)

Fat Fraction (FF): During the study period, there was a 3.2% increase in FF for the overall calf, with a 3.9% increase in the plantar flexors and a 2.7% increase in the dorsiflexors. In the thigh there was an overall increase in FF by 1.2%, with a 0.6% increase in the knee extensors, and a 1.7% increase in the knee flexors (Table 65) (Figure 66).

Cross Sectional Area (CSA): During the study period, the overall calf CSA decreased by 105.4 mm^2 , with a 96.9 mm^2 decrease in the plantar flexors, and a 2 mm^2 increase in the dorsiflexors. In the thigh CSA increased by 57.1 mm^2, with a 39.9 mm^2 decrease in the knee extensors and a 80.4 mm^2 increase in the knee flexors (Table 66) (Figure 66).

Remaining Muscle Area (RMA): During the study period, there was a decrease in RMA for the overall calf by 359 mm^2, with a 287 mm^2 decrease in the plantar flexors, and a 34 mm^2 decrease in the dorsiflexors. In the thigh RMA decreased by 137 mm^2, including a 131 mm^2 decrease in the knee extensors, and the knee flexors showed an 24 mm^2 increase (Table 67) (Figure 66).

Muscle Oedema (T_{2m}): During the study period, Muscle oedema in the overall calf increased by 0.8 ms, with a 0.3 ms increase in the plantar flexors, and a 2 ms increase in dorsiflexors (Table 68) (Figure 66).

FAT (%) FRACTION	BASELINE			6 MONTHS			12 MONTHS		
	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†
Total Thigh	2.9	1.7	15.9	3.1	3.6	16.6	3.4	2.3	17.1
Knee extension	2.2	1.2	14.8	2.8	3.1	15.3	3.1	1.6	15.4
Knee flexion	3.4	2.3	17.4	3.0	4.2	18.9	3.3	2.9	19.1
Total Calf	26.8	29.0	36.6	24.7	28.5	39.2	27.2	26.8	39.8
Plantar flexion	53.7	44.9	40.2	49.0	43.5	43.5	54.9	40.2	44.0
Dorsiflexion	2.0	2.7	23.9	1.9	3.0	25.8	2.6	2.4	26.6
FOOT	31.9	27.3	55.0	24.1	27.3	56.0	23.3	29.9	57.5
FAT (%) FRACTION Difference	**BASELINE-6 MONTHS**			**6MONTHS-12MONTHS**			**BASELINE-12MONTHS**		
	CASE 1*	CASE 2	No Exercise†	CASE 1	CASE 2*	No Exercise†	CASE 1	CASE 2	No Exercise†
Total Thigh	0.3	2.0	0.6	0.3	-1.4	0.6	0.5	0.6	1.2
Knee extension	0.6	2.0	0.4	0.3	-1.5	0.2	0.9	0.5	0.6
Knee flexion	-0.4	2.0	1.5	0.3	-1.3	0.2	-0.1	0.7	1.7
Total Calf	-2.0	-0.5	2.6	2.5	-1.7	0.7	0.4	-2.2	3.2
Plantar flexion	-4.7	-1.4	3.3	5.9	-3.3	0.5	1.2	-4.7	3.9
Dorsiflexion	-0.1	0.3	1.9	0.8	-0.6	0.7	0.6	-0.3	2.7
FOOT	-7.9	0.1	1.0	-0.8	2.5	1.5	-8.6	2.6	2.5
*= after 6 months of training, No exercise group n=8, †=Mean.									

Table 65: Longitudinal fat fraction and differences between measurement points in case 1, case 2, and no exercise group (n=8).

Cross Sectional Area (mm^2)	BASELINE			6 MONTHS			12 MONTHS		
	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†
Total Thigh	12928	14362	10010	12671	14800	9968	12774	15101	10067
Knee extension	6666	7455	4758	6550	7463	4686	6712	7489	4718
Knee flexion	4644	4856	3692	4629	5141	3785	4577	5207	3772
Total Calf	5400	6143	4925	5416	6311	4814	5208	5837	4820
Plantar flexion	2568	3805	3328	2604	3946	3200	2426	3719	3231
Dorsiflexion	1681	1223	829	1679	1123	825	1659	1056	831
FOOT	492	670	466	440	581	447	392	596	443
Cross Sectional Area (mm^2) Difference	**BASELINE-6 MONTHS**			**6MONTHS-12MONTHS**			**BASELINE-12MONTHS**		
	CASE 1*	CASE 2	No Exercise†	CASE 1	CASE 2*	No Exercise†	CASE 1	CASE 2	No Exercise†
Total Thigh	-257	438	-42	103	300	99	-154.1	738.7	57.1
Knee extension	-117	8	-72	162	26	32	45.6	33.9	-39.9
Knee flexion	-15	285	94	-52	66	-13	-66.6	350.9	80.4
Total Calf	16	168	-111	-208	-473	6	-192.2	-305.7	-105.4
Plantar flexion	36	141	-128	-178	-227	31	-142.4	-86.7	-96.9
Dorsiflexion	-2	-100	-3	-20	-67	5	-21.8	-167.5	2.0
FOOT	-52	-89	-19	-49	15	-4	-100.6	-74.3	-23.4
*= after 6 months of training, mm^2= square millimetres, No exercise group n=8, †=Mean.									

Table 66: Longitudinal cross sectional area and differences between measurement points in case 1, case 2, and no exercise group (n=8).

Remaining Muscle Area (mm^2)	BASELINE			6 MONTHS			12 MONTHS		
	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†
Total Thigh	25108	28245	17364	24544	28526	17204	24669	29521	17227
Knee extension	13038	14735	8338	12731	14458	8251	13002	14734	8207
Knee flexion	8971	9492	6360	8980	9846	6447	8852	10107	6385
Total Calf	7909	8728	6469	8152	9025	6159	7581	8549	6110
Plantar flexion	2378	4197	4166	2657	4459	3880	2189	4449	3880
Dorsiflexion	3294	2381	1323	3295	2180	1293	3231	2061	1288
FOOT	335	488	216	334	422	201	300	418	193
Remaining Muscle Area (mm^2) Difference	**BASELINE-6 MONTHS**			**6MONTHS-12MONTHS**			**BASELINE-12MONTHS**		
	CASE 1*	CASE 2	No Exercise†	CASE 1	CASE 2*	No Exercise†	CASE 1	CASE 2	No Exercise†
Total Thigh	-564	281	-160	125	995	23	-439	1276	-137
Knee extension	-306	-277	-86	270	276	-45	-36	-1	-131
Knee flexion	8	355	86	-128	260	-62	-119	615	24
Total Calf	243	297	-310	-571	-476	-49	-328	-179	-359
Plantar flexion	279	262	-287	-468	-9	0	-188	253	-287
Dorsiflexion	2	-201	-30	-65	-119	-5	-63	-320	-34
FOOT	-1	-65	-15	-34	-4	-8	-35	-70	-23
*= after 6 months of training, mm^2= square millimetres, No exercise group n=8, †=Mean.									

Table 67: Longitudinal remaining muscle area and differences between measurement points in case 1, case 2, and no exercise group (n=8).

Muscle Oedema T_{2m} (ms)	BASELINE			6 MONTHS			12 MONTHS		
	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†
Total Calf	31.3	33.6	40.8	31.9	33.5	38.9	31.6	33.3	41.5
Plantar flexion	32.2	35.5	41.1	32.7	35.8	39.1	30.9	35.1	41.5
Dorsiflexion	30.4	30.7	40.1	31.4	29.5	39.5	32.1	30.5	42.1
FOOT	40.4	44.7	46.6	51.0	42.4	46.8	49.6	42.8	50.6
Muscle Oedema T_{2m} (ms) Difference	**BASELINE-6 MONTHS**			**6MONTHS-12MONTHS**			**BASELINE-12MONTHS**		
	CASE 1*	CASE 2	No Exercise†	CASE 1	CASE 2*	No Exercise†	CASE 1	CASE 2	No Exercise†
Total Calf	0.6	-0.1	-1.8	-0.3	-0.2	2.6	0.3	-0.3	0.8
Plantar flexion	0.5	0.3	-2.1	-1.8	-0.7	2.4	-1.3	-0.4	0.3
Dorsiflexion	1.0	-1.2	-0.6	0.7	1.0	2.6	1.7	-0.2	2.0
FOOT	10.6	-2.3	0.2	-1.4	0.4	3.8	9.2	-1.9	3.9
*= after 6 months of training, ms= millisecond, No exercise group n=8, †=Mean.									

Table 68: Longitudinal muscle oedema and differences between measurement points in case 1, case 2, and no exercise group (n=8).

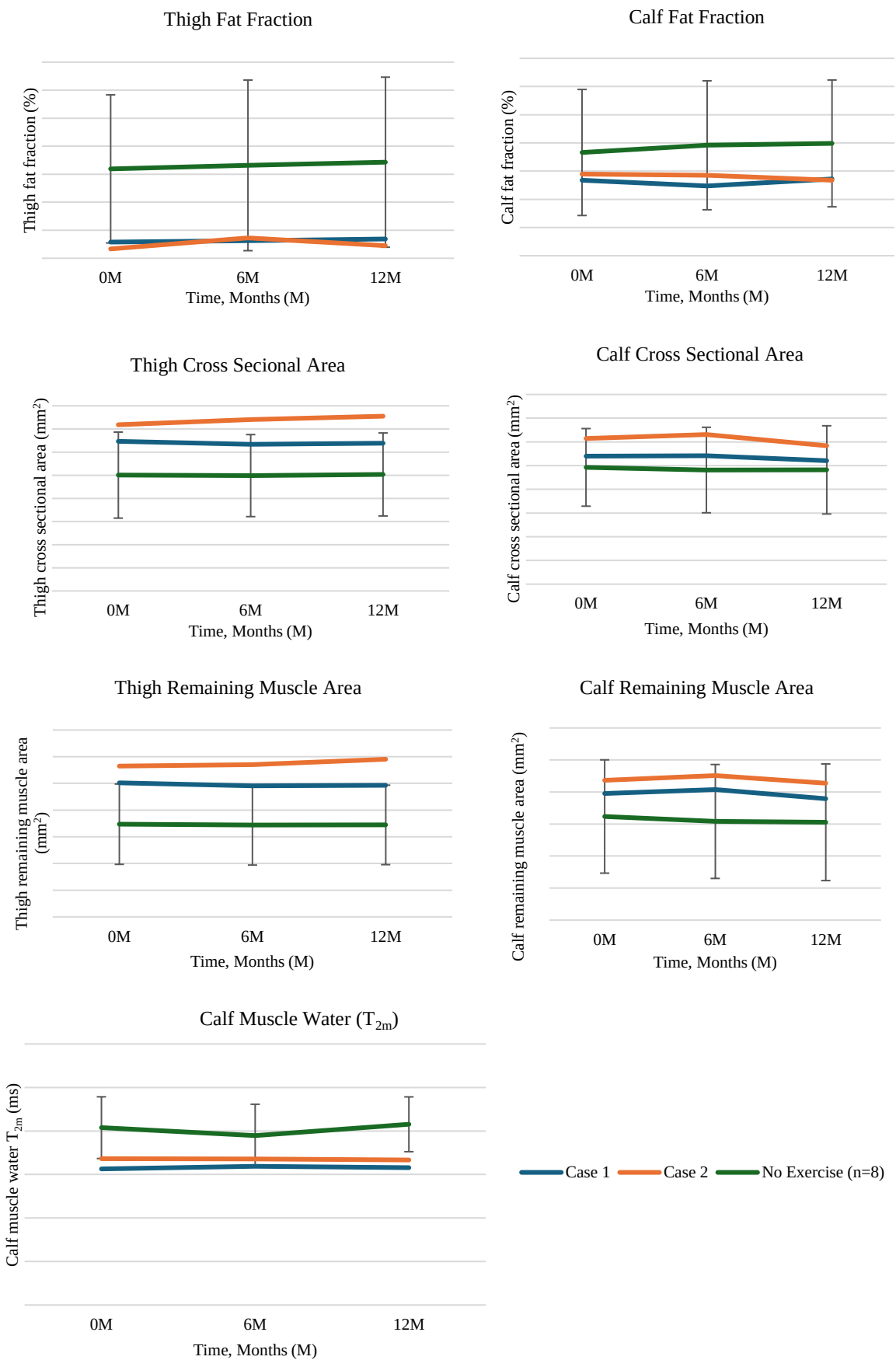


Figure 66: *Longitudinal MRI parameters in case 1, case 2, and no exercise group.*

9.3.4. Effect of Exercise on Function

9.3.4.1. Clinical Assessment

Clinical assessment at base line showed better function for the exercises group (n=5) in comparison to no exercise group (n=5) (Table 69), and for case 1 and case 2 in comparison to the no exercise group (n=8) in CMTES, FPI-6, and Walk-12 questionnaire (Table 70). Follow up assessment showed improvement in case 1 and case 2 in CMTES, Walk-12 questionnaire, and manual muscle testing.

Clinical Assessment	**BASELINE**		**6 MONTHS**		**12 MONTHS**	
	Exercise (N=5)	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**
CMTES; mean (SD)	5.2(5.1)	7.2(1.9)	5 (5.4)	6.2(1.6)	5 (5.4)	6.6 (2.3)
Foot Posture Index-6 (Pronated/Normal/Supinated)	(1/3/1)	(0/4/1)	(1/3/1)	(0/1/4)	(1/3/1)	(0/1/4)
Fall Frequency (weekly/Monthly/Yearly)	(1/1/0)	(0/0/2)	(1/1/0)	(0/0/1)	(1/1/0)	(0/0/1)
Walk-12; mean (SD)	33.4(12.9)	39(14.1)	31(13.7)	38.2(12.4)	31.4(13.2)	40.8(12.3)
Range of Motion (°)	**Exercise (N=5)**	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**
Dorsiflexion (Limited/Normal)	(5/0)	(5/0)	(5/0)	(5/0)	(5/0)	(5/0)
Plantarflexion (Limited/Normal)	(5/0)	(5/0)	(5/0)	(5/0)	(5/0)	(5/0)
Manual Muscle Testing	**Exercise (N=5)**	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**
Hip Flexion	5(4-5)	5(3-5)	5 (4-5)	5 (4-5)	5 (4-5)	5 (4-5)
Hip Extension	5(3-5)	5(3-5)	5 (3-5)	5 (3-5)	5 (3-5)	5 (3-5)
Knee Flexion	5(3-5)	5(4- -5)	5 (3-5)	5 (5-5)	5 (3-5)	5 (5-5)
Knee Extension	5(4-5)	5(4-5)	5 (4-5)	5 (5-5)	5 (4-5)	5 (5-5)
Dorsiflexion	4(0-4+)	1(1-4+)	5 (0-5)	2+ (1-4+)	5 (0-5)	2+ (1-4+)
Plantarflexion	4+(1-4+)	4+(1-4+)	4+ (1-4+)	3 (0-4+)	4+ (1-4+)	2+ (0-4+)
CMTES= Charcot-Marie-Tooth Disease Examination Score, °= degrees.						

Table 69: Longitudinal clinical assessment in exercise group (n=5) and no exercise group (n=5).

Clinical Assessment	BASELINE			6 MONTHS			12 MONTHS		
	CASE 1	**CASE 2**	**No Exercise (N=8)**	**CASE 1**	**CASE 2**	**No Exercise (N=8)**	**CASE 1**	**CASE 2**	**No Exercise (N=8)**
CMTES; mean (SD)	2	2	7.25(3.2)	1	2	6.62 (3.31)	1	2	6.87 (3.5)
Foot Posture Index-6 (Pronated/Normal/Supinated)	Supinated	Normal	(1/6/1)	Supinated	Normal	(1/3/4)	Supinated	Normal	(1/3/4)
Fall Frequency (weekly/Monthly/Yearly)	0	0	(1/1/2)	0	0	(1/1/2)	0	0	(1/1/2)
Walk-12; mean (SD)	31	30	36 (12)	29	25	35 (13)	25	26	36 (13)
Range of Motion (°)	**CASE 1**	**CASE 2**	**No Exercise** (count)	**CASE 1**	**CASE 2**	**No Exercise** (count)	**CASE 1**	**CASE 2**	**No Exercise** (count)
Dorsiflexion (Limited/Normal)	Limited	Limited	(8/0)	Limited	Limited	(8/0)	Limited	Limited	(8/0)
Plantarflexion (Limited/Normal)	Limited	Limited	(8/0)	Limited	Limited	(8/0)	Limited	Limited	(8/0)
Manual Muscle Testing	**CASE 1**	**CASE 2**	**No Exercise** Mode (range)	**CASE 1**	**CASE 2**	**No Exercise** Mode (range)	**CASE 1**	**CASE 2**	**No Exercise** Mode (range)
Hip Flexion	5	5	4+(0-4+)	5	5	4+(0-5)	5	5	4+(0-5)
Hip Extension	5	5	4+(1-4+)	5	5	4+(1-4+)	5	5	4+(1-4+)
Knee Flexion	5	5	5(4-5)	5	5	5(4-5)	5	5	5(4-5)
Knee Extension	5	5	5(3-5)	5	5	5(3-5)	5	5	5(3-5)
Dorsiflexion	4	4-	5(3-5)	5	4-	5(3-5)	5	4-	5(3-5)
Plantarflexion	4	4-	5(3-5)	4+	4	5(4-5)	4+	4	5(4-5)
CMTES= Charcot-Marie-Tooth Disease Examination Score, °= degrees, No exercise group n=8.									

Table 70: Longitudinal clinical assessment in exercise case 1, case 2, and no exercises group (n=8).

9.3.4.2. Isometric and Isokinetic Dynamometry

Comparision Between Exercises Group (N=5) and No Exercises Group (N=5):

Isometric Dynamometry: For hip extension at 45°, the exercise group showed a 24.8% decline from baseline to 6 months, followed by a 16.5% increase from 6 to 12 months, resulting in an overall 12.4% reduction over 12 months. In contrast, the no-exercise group showed a smaller 4.1% reduction at 6 months and a further 17.7% decrease by 12 months, resulting in a total 20.4% decline in strength. Knee extension at 45° showed a 13.6% decrease at 6 months in the exercise group, followed by a further 4.2% decline at 12 months, for an overall 17.3% reduction over 12 months. Conversely, the no-exercise group showed a smaller 2% increase in strength at 6 months, and a 9% decline by 12 months, resulting in a total reduction of 6.9%. The exercise group showed a 34.5% decline in ankle plantarflexion over 12 months, while the no-exercise group showed a smaller 11.1% decline. In dorsiflexion, the exercise group remained stable with a 4.3% increase, while the no-exercise group showed a larger 100% improvement in comparison to baseline (Table 71).

Isokinetic Dynamometry: For hip extension at 60°/60s, the exercise group showed a 9.2% increase from baseline to 6 months, followed by a 5.0% increase from 6 to 12 months, resulting in a 14.7% overall increase over 12 months. In contrast, the no-exercise group showed a 1.8% gain at 6 months, followed by a 5.2% decline by 12 months, resulting in a 3.5% reduction. Similarly, for hip flexion at 60°/60s, the exercise group showed a 9.3% increase at 6 months and a further 11.1% increase by 12 months, resulting in a total 21.5% gain. The no-exercise group, however, showed an initial 19.8% increase by 6 months, followed by a 6.4% reduction at 12 months, resulting in a 12.1% increase over 12 months. knee flexion strength improved by 4.7% in the exercise group compared to a 2.6% increase in the no-exercise group. Knee extension strength decreased by 8.2% in the exercise group, while the no-exercise group remained stable, with only a 1.4% decline. The exercise group showed little change in plantarflexion, with a 0% increase, while the no-exercise group experienced a 38.5% gain over 12 months. Dorsiflexion strength improved by 26.9% in the exercise group, while the no-exercise group showed a 64.3% increase (Table 72).

Isometric Dynamometry (Nm)	BASELINE		6 MONTHS		12 MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Hip EX 45°	129 (79)	147 (72)	97 (49)	141 (74)	113 (49)	117 (67)
Hip FL 45°	107 (56)	103 (48)	103 (59)	113 (56)	120 (49)	104 (49)
Knee EX 45°	110 (83)	99 (37)	95 (69)	100 (42)	91 (58)	91 (38)
Knee EX 90°	110 (77)	99 (38)	100 (71)	102 (34)	100 (73)	90 (44)
Knee FL 45°	65 (50)	53 (26)	54 (40)	56 (24)	57 (41)	53 (27)
Knee FL 90°	38 (29)	33 (15)	40 (29)	44 (18)	43 (34)	39 (17)
Ankle PF 10°	29 (20)	27 (21)	22 (13)	35 (27)	19 (12)	30 (21)
Ankle DF 10°	23 (22)	4 (6)	24 (20)	9 (9)	24 (21)	8 (6)
Ankle DF 30°	26 (22)	10 (8)	30 (21)	18 (12)	30 (23)	20 (8)
Isometric Dynamometry (Nm) Difference	**BASELINE-6 MONTHS**		**6MONTHS-12MONTHS**		**BASELINE-12MONTHS**	
	Exercise (N=5)	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**
Hip EX 45°	-32 (49)	-6 (18)	16 (30)	-25 (45)	-16 (42)	-30 (36)
Hip FL 45°	-4 (23)	10 (9)	17 (33)	-9 (10)	13 (18)	1 (8)
Knee EX 45°	-15 (27)	2 (15)	-4 (19)	-9 (16)	-19 (28)	-8 (15)
Knee EX 90°	-10 (15)	3 (10)	0 (16)	-12 (20)	-10 (6)	-9 (16)
Knee FL 45°	-11 (26)	4 (6)	3 (21)	-4 (14)	-8 (12)	0 (11)
Knee FL 90°	2 (6)	11 (10)	4 (18)	-5 (10)	5 (20)	6 (11)
Ankle PF 10°	-7 (12)	8 (9)	-4 (6)	-5 (11)	-10 (13)	2 (7)
Ankle DF 10°	1 (4)	5 (4)	0 (3)	0 (6)	1 (3)	4 (4)
Ankle DF 30°	4 (7)	8 (5)	0 (4)	2 (8)	3 (5)	10 (8)
Nm= Newton meter, °= degrees, Data presented as: Mean (standers deviation).						

Table 71: Longitudinal isomeric dynamometry and differences between measurement points in exercise group (n=5) and no exercise group (n=5).

Isokinetic Dynamometry (Nm)	BASELINE		6 MONTHS		12 MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Hip EX 60°/60s	109 (82)	113 (77)	119 (86)	115 (85)	125 (77)	109 (79)
Hip FL 60°/60s	107 (62)	91 (45)	117 (74)	109 (59)	130 (58)	101 (39)
Knee EX 60°/60s	85 (67)	73 (39)	77 (64)	78 (38)	78 (50)	74 (39)
Knee FL 60°/60s	43 (36)	39 (22)	37 (30)	44 (25)	45 (32)	41 (23)
Knee EX120°/120s	61 (57)	51 (33)	60 (52)	58 (31)	59 (39)	53 (30)
Knee FL 120°/120s	35 (33)	28 (16)	33 (29)	34 (23)	35 (28)	30 (18)
Ankle PF 60°/60°	21 (24)	13 (7)	23 (20)	21 (15)	21 (17)	17 (5)
Ankle DF 60°/60°	15 (11)	14 (6)	21 (12)	18 (10)	22 (13)	23 (9)
Isokinetic Dynamometry (Nm) Difference	**BASELINE-6 MONTHS**		**6MONTHS-12MONTHS**		**BASELINE-12MONTHS**	
	Exercise (N=5)	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**
Hip EX 60°/60s	10 (19)	2 (20)	6 (22)	-6 (20)	16 (12)	-4 (8)
Hip FL 60°/60s	10 (18)	18 (21)	13 (31)	-7 (22)	23 (16)	11 (11)
Knee EX 60°/60s	-8 (19)	5 (9)	1 (25)	-4 (9)	-7 (20)	1 (15)
Knee FL 60°/60s	-6 (8)	5 (7)	8 (12)	-3 (8)	2 (11)	2 (7)
Knee EX120°/120s	-1 (6)	7 (12)	-1 (21)	-6 (5)	-2 (25)	1 (12)
Knee FL 120°/120s	-2 (6)	6 (10)	2 (11)	-3 (10)	0 (13)	3 (9)
Ankle PF 60°/60°	2 (8)	9 (8)	-2 (5)	-4 (10)	0 (9)	5 (3)
Ankle DF 60°/60°	6 (7)	4 (10)	1 (4)	5 (14)	7 (7)	9 (7)
Nm= Newton meter, °= degrees, Data presented as: Mean (standers deviation).						

Table 72: Longitudinal isokinetic dynamometry and differences between measurement points in exercise group (n=5) and no exercise group (n=5).

Case 1

Isometric Dynamometry: After 6 months of training, there was a decrease in plantar flexion at 10° strength by 5 Nm and dorsiflexor at 10° strength by 4 Nm. There was an increase in dorsiflexor strength at 30° by 8 Nm. Proximally, knee flexion showed an increase in strength at 45° and 90°by 6 Nm and 10 Nm, respectively. Knee extension showed increase in strength at 90° by 10 Nm, and a decrease in strength at 45° by 49 Nm. Hip extension and flexion showed a decrease in strength by 62 Nm and 33 Nm, respectively (Table 73) (Figure 67).

Isokinetic Dynamometry: After 6 months of training, there was an increses in strength in dorsiflexion with 8 Nm only, no change in knee extension at 120°/120s speed, and a decrese in the rest of the parameters (Table 74) (Figure 67).

Case 2

Isometric Dynamometry: After 6 months of training, there was a decrease in plantar flexion at 10° strength by 9 Nm. There was an increase in dorsiflexor strength at 30° by 6 Nm and dorsiflexor at 10° strength by 1 Nm. Proximally, knee flexion showed an increase in strength at 45° and 90° by 33 Nm and 34 Nm, respectively. Knee extension showed increase in strength at 90° by 18 Nm, and a decrease in strength at 45° by 26 Nm. Hip flexion showed a decrease in strength by 27 Nm. Hip extension showed an increase in strength by 13 Nm. (Table 73) (Figure 67).

Isokinetic Dynamometry: After 6 months of training, there was an increse in strength in dorsiflexion with 6 Nm and knee flexion at 60°/60s speed with 13 Nm, no change in knee flexion at 120°/120s speed, and a decrese in the rest of the parameters (Table 74) (Figure 67).

No Exercise Group (n=8)

Isometric Dynamometry: During the study period, there was no change in plantar flexion at 10° strength. There was an increase in dorsiflexor strength at 30° by 8 Nm and dorsiflexor at 10° strength by 3 Nm. Proximally, knee flexion showed an increase in strength at 90° by 2 Nm and a decrease at 45° by 3 Nm. Knee extension showed a decrease in strength at 90° and 45° by 9 Nm and 6 Nm, respectively. Hip extension showed a decrease in strength by 19 Nm. Hip flexion showed an increase in strength by 9 Nm. (Table 73) (Figure 67).

Isokinetic Dynamometry: During the study period, there was an increses in strength in dorsiflexion with 9 Nm, plantar flexion with 5 Nm, Hip extension with 3 Nm, Hip flexion with 18 Nm, and a minimal increase in the rest of the parameters (Table 74) (Figure 67).

Isometric Dynamometry (Nm)	BASELINE			6 MONTHS			12 MONTHS		
	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†
Hip EX 45°	122	249	126	61	149	123	129	162	107
Hip FL 45°	121	176	94	89	199	99	150	172	100
Knee EX 45°	121	234	86	72	194	89	97	168	81
Knee EX 90°	125	223	87	135	191	85	116	209	78
Knee FL 45°	61	135	49	67	79	51	65	112	46
Knee FL 90°	54	50	31	64	54	38	59	87	33
Ankle PF 10°	29	46	26	24	34	29	14	25	25
Ankle DF 10°	36	54	6	32	55	10	35	55	9
Ankle DF 30°	36	59	11	44	54	18	42	60	18
Isometric Dynamometry (Nm) Difference	**BASELINE-6 MONTHS**			**6MONTHS-12MONTHS**			**BASELINE-12MONTHS**		
	CASE 1*	CASE 2	No Exercise†	CASE 1	CASE 2*	No Exercise†	CASE 1	CASE 2	No Exercise†
Hip EX 45°	-62	-100	-3	69	13	-16	7	-87	-19
Hip FL 45°	-33	23	5	61	-27	1	29	-5	6
Knee EX 45°	-49	-40	3	25	-26	-8	-24	-66	-6
Knee EX 90°	10	-32	-2	-19	18	-7	-10	-14	-9
Knee FL 45°	6	-56	2	-2	33	-4	4	-23	-3
Knee FL 90°	10	4	6	-5	34	-4	5	37	2
Ankle PF 10°	-5	-12	3	-10	-9	-3	-15	-21	0
Ankle DF 10°	-4	1	4	3	1	-1	-1	1	3
Ankle DF 30°	8	-5	7	-2	6	1	6	1	8
*= after 6 months of training, Nm= Newton meter, °= degrees, No exercise group n=8, †=Mean.									

Table 73: Longitudinal isomeric dynamometry and differences between measurement points in case 1, case 2, and no exercise group (n=8).

Isokinetic Dynamometry (Nm)	BASELINE			6 MONTHS			12 MONTHS		
	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†	CASE 1	CASE 2	No Exercise†
Hip EX 60°/60s	106	231	97	99	260	102	137	239	99
Hip FL 60°/60s	108	194	86	94	231	101	130	196	104
Knee EX 60°/60s	96	186	63	55	178	68	92	147	65
Knee FL 60°/60s	42	94	34	35	75	37	58	88	35
Knee EX120°/120s	34	152	47	34	141	52	65	114	47
Knee FL 120°/120s	23	86	25	23	74	29	42	74	26
Ankle PF 60°/60°	20	63	11	16	56	19	19	48	16
Ankle DF 60°/60°	20	30	12	28	29	17	27	35	20
Isokinetic Dynamometry (Nm) Difference	**BASELINE-6 MONTHS**			**6MONTHS-12MONTHS**			**BASELINE-12MONTHS**		
	CASE 1*	CASE 2	No Exercise†	CASE 1	CASE 2*	No Exercise†	CASE 1	CASE 2	No Exercise†
Hip EX 60°/60s	-8	29	5	39	-21	-2	31	8	3
Hip FL 60°/60s	-14	38	14	36	-36	3	22	2	18
Knee EX 60°/60s	-41	-8	4	37	-32	-3	-4	-40	1
Knee FL 60°/60s	-7	-20	3	23	13	-2	16	-7	1
Knee EX120°/120s	0	-11	5	31	-28	-5	31	-39	1
Knee FL 120°/120s	-1	-12	4	20	0	-3	19	-12	1
Ankle PF 60°/60°	-4	-8	8	3	-8	-3	-1	-15	5
Ankle DF 60°/60°	8	-1	6	-1	6	3	7	5	9
*= after 6 months of training, Nm= Newton meter, °= degrees, s= seconds, No exercise group n=8, †=Mean.									

Table 74: Longitudinal isokinetic dynamometry and differences between measurement points in case 1, case 2, and no exercise group (n=8).

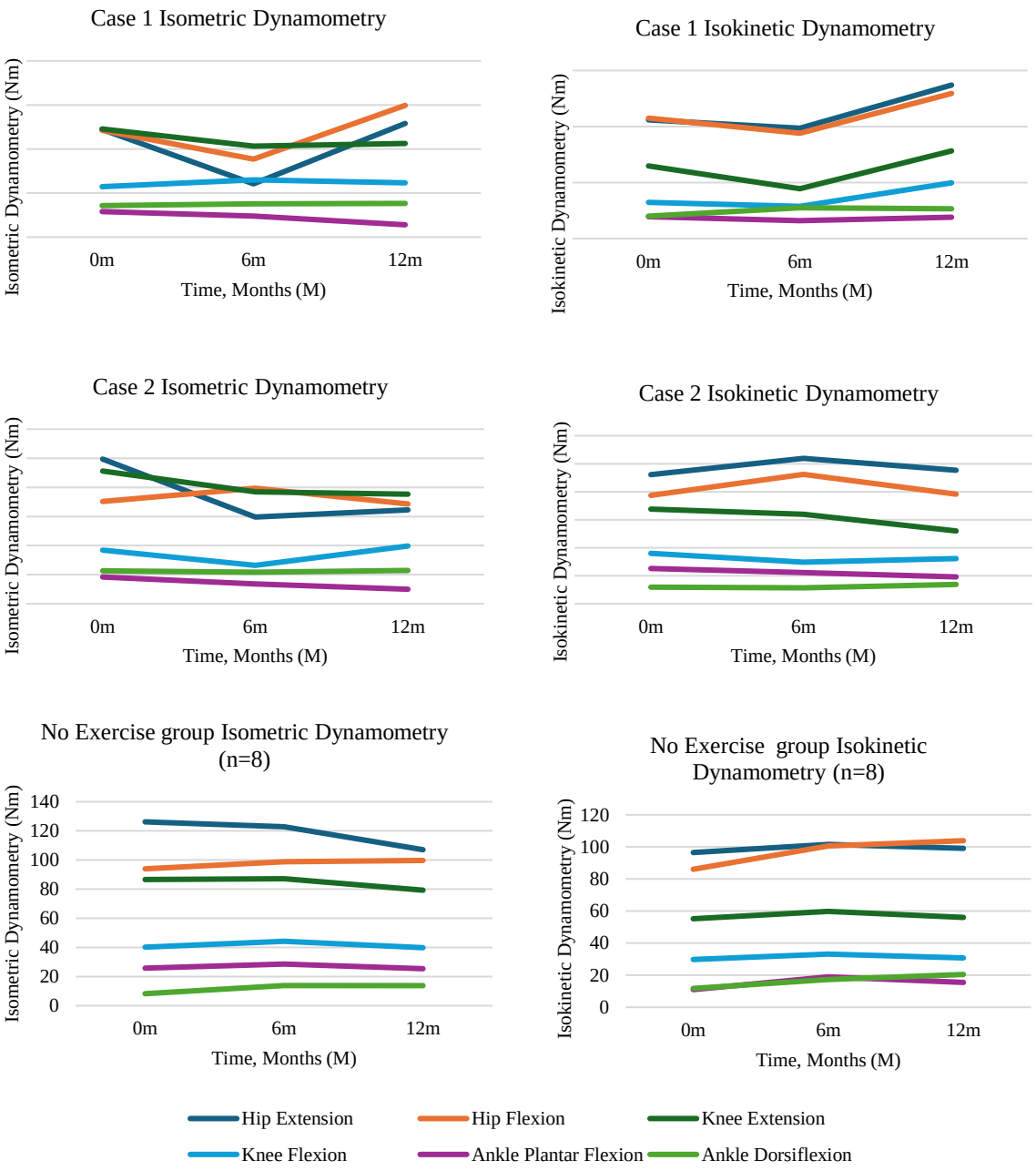


Figure 67: Longitudinal isometric and isokinetic strength in case 1, case 2, and no exercises group.

9.3.4.3. Three Dimensional Motion Analysis

Comparision Between Exercises Group (N=5) and No Exercises Group (N=5):

The exercise group showed consistent improvements in gait speed and stride length, whereas the no-exercise group showed minimal improvements or declines over time. For gait speed, the exercise group showed a 4.6% increase from baseline to 6 months, followed by a 1.7% increase from 6 to 12 months, resulting in an increase of 6.5% over 12 months. In contrast, the no-exercise group demonstrated a similar 6.7% increase at 6 months, but this was followed by a 6.3% reduction by 12 months, resulting in no net change. In stride length, the exercise group showed a 4.7% increase at 6 months, followed by a 1.5% increase at 12 months, for a total increase of 6.3%. The no-exercise group showed a smaller 2.8% increase at 6 months, followed by a 1.8% decline by 12 months, resulting in a total 0.9% increase (Table 75).

Gait Spatiotemporal Parameters	BASELINE		6 MONTHS		12 MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Speed; m/s	1.08 (0.34)	0.89 (0.21)	1.13 (0.35)	0.95 (0.19)	1.15 (0.38)	0.88 (0.25)
Stride Length; m	1.27 (0.25)	1.07 (0.21)	1.33 (0.31)	1.10 (0.18)	1.35 (0.35)	1.08 (0.21)
Stride Time; s	1.23 (0.23)	1.22 (0.10)	1.22 (0.18)	1.18 (0.08)	1.21 (0.18)	1.26 (0.16)
Strides Per Minute	50.17 (7.42)	49.56 (3.81)	49.9 (6.35)	51.26 (3.15)	50.27 (6.67)	48.42 (5.49)
Step Length; m	0.63 (0.13)	0.54 (0.11)	0.66 (0.15)	0.55 (0.09)	0.67 (0.17)	0.54 (0.11)
Step Time; s	0.62 (0.12)	0.61 (0.05)	0.61 (0.09)	0.59 (0.04)	0.61 (0.09)	0.63 (0.08)
Steps Per Minute	100.35 (14.83)	99.13 (7.62)	99.9 (12.70)	102.52 (6.29)	100.55 (13.34)	96.84 (10.99)
Percent Stance	0.63 (0.03)	0.64 (0.02)	0.63 (0.02)	0.64 (0.02)	0.63 (0.02)	0.65 (0.01)
Single Support Time; s	0.61 (0.06)	0.61 (0.05)	0.63 (0.08)	0.58 (0.05)	0.63 (0.09)	0.65 (0.11)
Double Support Time; s	0.17 (0.12)	0.17 (0.03)	0.14 (0.06)	0.17 (0.03)	0.14 (0.07)	0.17 (0.02)

Percent Opposite Toe Off	13.36 (7.05)	13.92 (1.25)	13.4 (3.43)	14.64 (1.58)	12.47 (4.38)	13.47 (1.29)
Percent Opposite Foot Contact	49.68 (0.77)	49.51 (0.68)	49.9 (0.72)	50.13 (0.78)	49.82 (0.37)	50.20 (1.00)
Gait Spatiotemporal Parameters Difference	**BASELINE-6 MONTHS**		**6MONTHS-12MONTHS**		**BASELINE-12MONTHS**	
	Exercise (N=5)	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**	**Exercise (N=5)**	**No Exercise (N=5)**
Speed; m/s	0.05 (0)	0.06 (0.0)	0.02 (0.06)	-0.06 (0.07)	0.07 (0.09)	0.00 (0.11)
Stride Length; m	0.06 (0)	0.03 (0.0)	0.02 (0.06)	-0.02 (0.04)	0.08 (0.14)	0.01 (0.05)
Stride Time; s	-0. (0.07)	-0.04 (0.0)	-0.01 (0.02)	0.08 (0.08)	-0.02 (0.06)	0.04 (0.09)
Strides Per Minute	-0.19 (2)	1.70 (1.5)	0.29 (0.84)	-2.84 (2.67)	0.10 (2.04)	-1.14 (3.44)
Step Length; m	0.03 (0)	0.02 (0.0)	0.01 (0.03)	-0.01 (0.02)	0.04 (0.07)	0.01 (0.03)
Step Time; s	-0. (0.03)	-0.02 (0.0)	0.00 (0.01)	0.04 (0.04)	-0.01 (0.03)	0.02 (0.04)
Steps Per Minute	-0.38 (4)	3.39 (3.1)	0.58 (1.68)	-5.68 (5.34)	0.20 (4.07)	-2.28 (6.89)
Percent Stance	0.00 (0)	0.01 (0.0)	0.00 (0.01)	0.01 (0.01)	0.00 (0.02)	0.01 (0.01)
Single Support Time; s	0.0 (0.04)	-0.02 (0.0)	0.00 (0.03)	0.06 (0.07)	0.03 (0.04)	0.04 (0.07)
Double Support Time; s	-0.03 (0)	0.00 (0.0)	0.00 (0.02)	-0.01 (0.03)	-0.04 (0.06)	0.00 (0.03)
Percent Opposite Toe Off	0.11 (7)	0.73 (1.4)	-1.01 (6.47)	-1.18 (2.84)	-0.90 (5.75)	-0.45 (2.35)
Percent Opposite Foot Contact	0.28 (0)	0.63 (0.7)	-0.13 (1.06)	0.07 (0.99)	0.14 (1.07)	0.70 (0.54)
m= meters, s= seconds, Data presented as: Mean (standers deviation).						

Table 75: *Longitudinal spatiotemporal gait parameters and differences between measurement points in exercise group (n=5) and no exercise group (n=5).*

Comparision Between Case 1, Case 2, and No Exercises Group (N=8):

Three dimensional motion analysis showed increase in speed, stride and step length, and a decrease in double support time, in comparison to no exercise group (n=8) (Table 76) (Figure 68). However, changes in kinetics and kinematics parameters were random and not consistence. Moreover, there was a technical issue when measuring gait at baseline for case 1 that did not allow the software to calculate kinetics and kinematics, but 6 months and 12 months follow ups were measures successfully. Detailed tables of the kinetic and kinematic data differences are presented in appendix VI.

Gait Spatiotemporal Parameters	**BASELINE**			**6 MONTHS**			**12 MONTHS**		
	CASE 1	**CASE 2**	**No Exercise†**	**CASE 1**	**CASE 2**	**No Exercise†**	**CASE 1**	**CASE 2**	**No Exercise†**
Speed; m/s	1.34	1.28	0.90	1.37	1.44	0.95	1.50	1.43	0.90
Stride Length; m	1.40	1.51	1.10	1.53	1.68	1.12	1.65	1.71	1.10
Stride Time; s	1.05	1.18	1.25	1.12	1.17	1.21	1.10	1.19	1.26
Strides Per Minute	57.60	50.77	48.79	53.72	51.40	50.14	54.47	50.35	48.58
Step Length; m	0.70	0.76	0.55	0.76	0.84	0.56	0.82	0.85	0.55
Step Time; s	0.52	0.59	0.63	0.56	0.58	0.61	0.55	0.60	0.63
Steps Per Minute	115.21	101.54	97.58	107.43	102.80	100.27	108.93	100.69	97.16
Percent Stance	0.60	0.64	0.64	0.61	0.63	0.64	0.62	0.63	0.65
Single Support Time; s	0.55	0.67	0.61	0.56	0.66	0.61	0.60	0.68	0.64
Double Support Time; s	0.09	0.09	0.19	0.12	0.08	0.17	0.09	0.07	0.17
Percent Opposite Toe Off	8.24	7.44	15.09	10.89	17.75	13.99	15.45	5.58	13.58
Percent Opposite Foot Contact	50.56	49.54	49.48	51.12	49.67	49.96	49.24	50.15	50.09
Gait Spatiotemporal Parameters Difference	**BASELINE-6 MONTHS**			**6MONTHS-12MONTHS**			**BASELINE-12MONTHS**		
	CASE 1*	**CASE 2**	**No Exercise†**	**CASE 1**	**CASE 2***	**No Exercise†**	**CASE 1**	**CASE 2**	**No Exercise†**
Speed; m/s	0.03	0.16	0.05	0.13	0.00	-0.04	0.16	0.16	0.00

Stride Length; m	0.13	0.16	0.02	0.12	0.03	-0.02	0.25	0.20	0.00
Stride Time; s	0.07	-0.01	-0.04	-0.01	0.02	0.04	0.06	0.01	0.00
Strides Per Minute	-3.89	0.63	1.35	0.75	-1.05	-1.55	-3.14	-0.42	-0.21
Step Length; m	0.07	0.08	0.01	0.06	0.02	-0.01	0.12	0.10	0.00
Step Time; s	0.04	-0.01	-0.02	-0.01	0.01	0.02	0.03	0.01	0.00
Steps Per Minute	-7.78	1.26	2.70	1.50	-2.11	-3.11	-6.28	-0.85	-0.41
Percent Stance	0.01	-0.01	0.00	0.01	-0.01	0.00	0.02	-0.02	0.01
Single Support Time; s	0.01	-0.01	0.00	0.04	0.02	0.03	0.05	0.01	0.04
Double Support Time; s	0.04	-0.01	-0.02	-0.03	-0.01	0.00	0.00	-0.02	-0.02
Percent Opposite Toe Off	2.64	10.32	-1.10	4.56	-12.17	-0.42	7.21	-1.85	-1.51
Percent Opposite Foot Contact	0.56	0.13	0.48	-1.88	0.48	0.14	-1.32	0.61	0.61
*= after 6 months of training, m= meters, s= seconds, No exercise group n=8, †=Mean.									

Table 76: Longitudinal spatiotemporal gait parameters and differences between measurement points in case 1, case 2, and no exercise group (n=8).

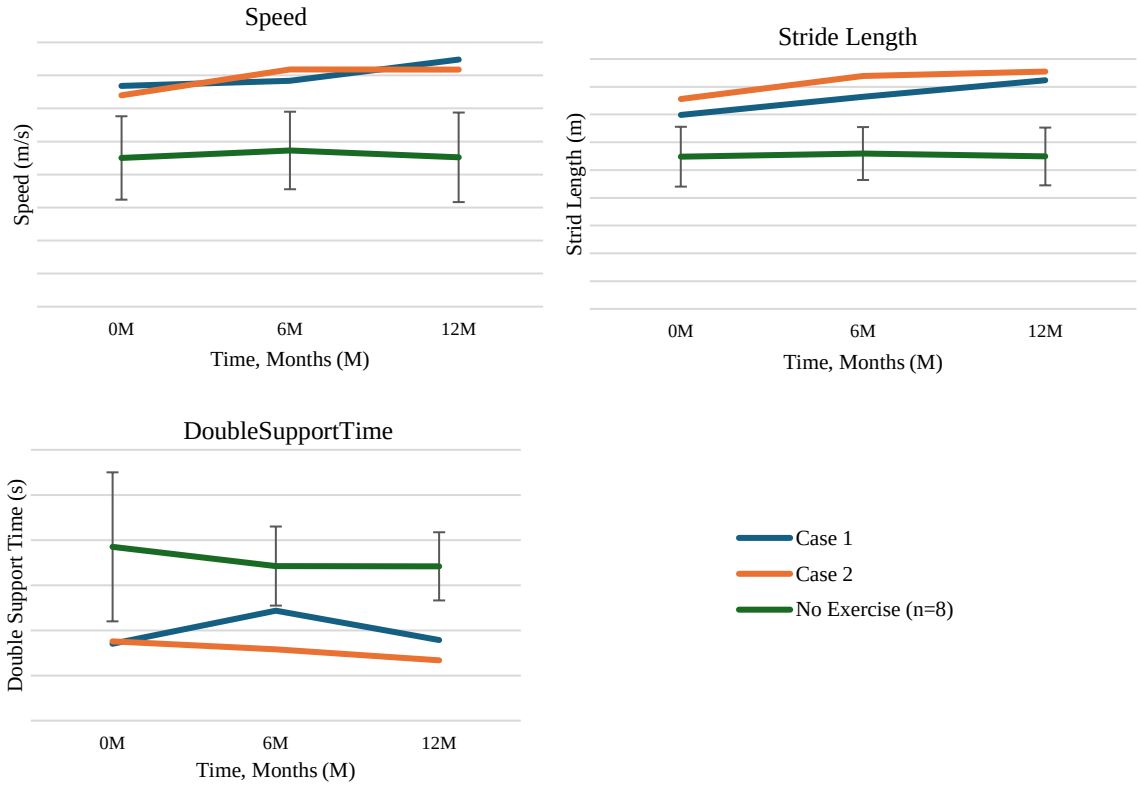


Figure 68: Longitudinal spatiotemporal parameters in case 1, case 2, and no exercises group.

9.4. Discussion

This study aimed to explore the effectiveness and safety of resistance exercise program in people with DHMN. The COVID-19 pandemic disrupted the study protocol and planned support for participants. This may have led to low adherence in the exercise group, limiting the trial's success. Nevertheless, it provided valuable preliminary data on the potential benefits of exercise and insights for future trial design.

9.4.1. Effectivness and Safety of Resistive Exercises

Changes in MRI parameters showed possible eveidance of effect and safetty of the exercise in these two cases. In the exercising cases, there were possible, positive effects on fat fraction levels at the calf and thigh, while “No Exercises” group showed an increase with the expected natural history. This might suggest that exercising could be used to slow the neuropathy progression, but would require more extensive testing.

At the thigh level case 1 and case 2 showed increase in cross sectional area (CSA) and the remaining muscle area (RMA) during the exercise period while CSA and RMA in “No Exercises” group showed no change. This finding is consistent with Chetlin et al. (2004), who found improvements in muscle fiber diameter following a home-based resistance training program in CMT patients (Chetlin et al., 2004b). At the calf level there was a trend of decrease in CSA and RMA in both exercise cases and “No Exercise” group. This suggests that when muscles

more midly affected by the neuropathy, the opportunities to gain muscle using resistive exercises could be higher, supporting the importance of early intervention.

Changes in muscle oedema was minimal in both case 1 and case 2 at the calf level, unlike the "No Exercise" group, that showed higher rates of muscle oedema. This finding may suggest that resistance exercises are safe, do not induce neurodegeneration, and are unlikely to cause overwork weakness in people with DHMN. This aligns with studies by Burns et al. (2017), which reported no adverse effects on muscle volume or signs of acute denervation following resistance exercises in children with Charcot-Marie-Tooth (CMT) disease (Burns et al., 2017).

Dynamometry showed individual improvement in strength with exercise. In both case 1 and case 2, dorsiflexor isometric and isokinetic strength slightly improved. This observation aligns with the findings of Burns et al. (2017), who reported 30% improvements in dorsiflexor strength following 6 months resistance training (Burns et al., 2017). However, plantar flexor continued to deteriorate in the exercise cases. Plantar flexors are a more affected group of muscles in DHMN, and exercising this group when they are advanced in neuropathy may not show improvement in strength. These findings are consistence with the MRI muscle area that showed a decrease in the plantar flexors muscle area even with exercising. The "No Exercise" group showed improved isometric and isokinetic

strength in dorsiflexors and plantar flexors. However, these results as discussed in chapter 6 are compromised a small sample size and high inter-group variability. Larger natural history studies are required to understand the average changes in muscles over time.

Proximally, isometric and isokinetic strength in both exercise cases showed improvements in strength, however, the pattern was not the same between cases. Proximal improvement in the case 1 and case 2 is consistence with the MRI findings. In the "No Exercise" group, isometric strength proximally showed continued deterioration, and isokinetic strength showed selective improvement in hip flexors. Improvement in hip flexors in the "No Exercise" group could be a false finding, but the compensatory function of this group is shown in chapter 5. The DHMN cohort used their hip flexors to compensate for distal weakness in pre-swing and swing phase. Inclusion of more participants and more measurement points would be recommended for future research to ascertain the effect of exercises on strength.

Exercising seemed to show a positive impact on the general function in the exercising cases. Three dimensional motion analysis showed increase in speed, stride and step length, and a decrease in double support time, in comparison to the "No Exercise" group. Moreover, clinical assessment showed improvement in exercises cases in CMTES, Walk-12 questionnaire, and manual muscle testing.

Larger studies are required to understand the impact of strength gain on general function.

9.4.2. Factors to Improve Adherence for Successful Exercise Trials

Clinical assessment at baseline showed better function in both exercise cases in comparison to the "No Exercise" group in CMTES, FPI-6, and Walk-12 questionnaire. It is possible that the more mildly affected cases were more likely to engage in exercise in comparison to those with more advanced neuropathy. This is consistent with the findings of Menotti et al. (2014), who reported that physical activity levels correlated with muscle strength in CMT patients (Menotti et al., 2014a). Motivation to exercise was influenced by occupational and environmental factors. Both cases with high adherence have physically demanding occupations and both were already engaging in other exercise. One of them had a medical background, so education in understanding the benefits could be important. Also, having an equipped room at home dedicated for exercise motivated them to keep engaged, despite pain or time limitations. This aligns with the conclusions of Anens et al. (2015), who identified personal and socio-environmental factors as key influences on physical activity levels in individuals with CMT (Anens et al., 2015).

Available evidence on exercise benefits in peripheral neuropathy, pain, and sociopsychological factors can also influence motivation to exercises. Participants with low and no adherence reported more pain levels and time

limitation because of work or family situations, reflecting the findings of Roberts-Clarke et al. (2016), who noted that barriers such as fatigue and pain negatively impacted exercise adherence (Roberts-Clarke et al., 2016b). Some cases preferred to wait for the study findings before committing to an exercises program, indicating a need for better motivational strategies and support systems, as suggested by Buscemi et al. (Buscemi et al., 2023).

While this study provided initial insights into the effects of resistance exercises, uncertainties about their long-term effectiveness and safety remain. Future research should include a two-phase trial to address these questions comprehensively. The first phase of the study is aimed to ascertain the effectiveness and safety of progressive resistive exercises in DHMN with a supervised exercises sessions at an equipped facility to ascertain exercises application and motivate participants to engage in the program. Once the safety and efficacy are established, the second phase should explore the feasibility of home-based exercises using a program co-designed with participants, incorporating home visits, weekly check-ins, and exercise diaries to enhance adherence. Wearable monitors could be used to track engagement and progress, as recommended by Wallace et al. (Wallace et al., 2019).

The DHMN cohort identified personal beliefs and socio-environmental factors that influenced adherence to physical activity and exercise. To address these issues, approaches such as the Behaviour Change Wheel (BCW) (Figure 69)

(Michie S, 2014) could be used when designing the exercise protocol. The BCW framework can help identify key barriers and facilitators of exercise adherence, enabling the development of tailored interventions that support long-term engagement in physical activity for individuals with DHMN. This approach could enhance motivation, adherence, and ultimately, the quality of life for those affected by this condition. It encompasses three core components: the COM-B model (Capability, Opportunity, Motivation, and Behaviour), intervention functions, and policy categories. This framework is particularly valuable in designing interventions for complex behaviours like adherence to exercise programs in individuals with DHMN (Table 77).

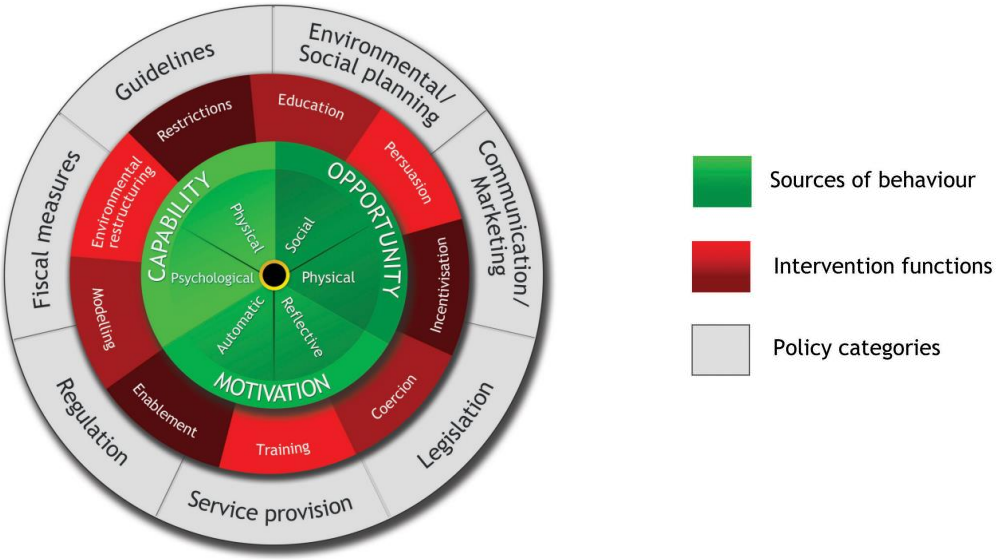


Figure 69: The Behaviour Change Wheel (BCW)

COM-B	Intervention Functions	Policy Categories
Capability (C) -Physical Capability: DHMN patients often have significant muscle weakness and fatigue. Exercise programs should be adapted to their current physical abilities, ensuring that exercises are feasible and safe. -Psychological Capability: Many patients may lack the knowledge or confidence to exercise effectively. Providing clear instructions and education about exercise benefits can enhance their understanding and skills. **Opportunity (O)** -Physical Opportunity: Access to exercise facilities and equipment is crucial. Providing exercise equipment for home use and modifying environments to make exercise more accessible can help. -Social Opportunity: Social support from family, friends, and healthcare providers is essential. Creating a supportive environment and opportunities for group exercises can encourage adherence. **Motivation (M)** -Reflective Motivation: Educating patients about the benefits of exercise and helping them set realistic goals can enhance their motivation. -Automatic Motivation: Incorporating regular feedback, rewards, and integrating exercise into daily routines can help make exercise a habitual part of their life.	**Education:** Provide comprehensive information about the benefits of exercise and its role in managing DHMN. This can include informational sessions, educational materials, and personalized advice from healthcare professionals. **Training:** Offer training sessions to teach proper exercise techniques and safe practices. This can help build confidence and ensure that participants perform exercises correctly, reducing the risk of injury and enhancing the effectiveness of the program. **Persuasion:** Use motivational interviewing techniques to encourage commitment to the exercise program. This involves exploring participants' values, goals, and potential barriers to adherence, and helping them find personal motivations to engage in regular exercise. **Incentivization:** Implement reward systems to encourage adherence. This could include tangible rewards, such as vouchers or discounts, as well as intangible rewards like praise, recognition, and the intrinsic satisfaction of achieving personal health goals. **Environmental Restructuring:** Modify physical and social environments to make exercise more accessible and enjoyable. This might involve setting up exercise spaces at home, providing access to community facilities, or organizing group exercise sessions to build a sense of community and support. **Modelling:** Use role models to demonstrate the desired behaviour. Sharing success stories of individuals who have successfully incorporated exercise into their routines can inspire and motivate participants. **Enablement:** Provide practical support to overcome barriers to exercise. This can include assistance with transportation, scheduling, and finding time to exercise, as well as addressing any physical limitations through adaptive equipment or tailored exercise plans.	**Communication and Marketing:** Develop public health campaigns to raise awareness about the benefits of exercise for DHMN and other neuropathies. This can help create a positive cultural attitude towards physical activity and encourage more people to participate. **Guidelines:** Establish evidence-based guidelines for exercise in DHMN. These guidelines can provide healthcare providers with the information they need to recommend appropriate exercise regimens and support patients in their adherence efforts. **Environmental Planning:** Advocate for the creation of more accessible exercise facilities and spaces. Ensuring that community gyms, parks, and other recreational areas are inclusive and equipped to meet the needs of individuals with mobility challenges can facilitate regular physical activity. **Service Provision:** Increase the availability of exercise programs specifically designed for individuals with DHMN. This could include offering specialized classes in community centres, integrating exercise programs into healthcare settings, and providing resources for home-based exercise. **Legislation and Regulation:** Support policies that promote physical activity and reduce barriers to exercise. This might include advocating for insurance coverage for exercise programs, supporting workplace wellness initiatives, and promoting active transportation options.

Table 77: Applying the Behaviour Change Wheel Model to DHMN Exercise Adherence.

9.5. Summary

The current study explored the effect of resistance exercises on muscle structure and function in DHMN. The study findings are summarised below:

- In two cases, MRI indicated that resistance exercises may impact neuropathy progression, especially with mildly affected muscles. Exercise cases showed decreased intramuscular fat in calf and thigh, and increased thigh muscle area.
- Exercises were safe with no evidence of overwork weakness in two DHMN cases.
- Clinical assessments showed functional gains even with minimal strength increase. Two exercise cases had increased walking speed, stride length, and reduced double support time.
- Better function at baseline was linked to higher exercise adherence for the two exercises cases.
- Motivation to engage is influenced by occupational demands, prior exercise habits, and home equipment.
- Low adherence was due to higher pain, time constraints, and limited evidence.
- Behaviour change approaches will be important to design tailored interventions to enhance long-term exercise engagement.

Chapter 10: Overview

This thesis has focused on the relationship between muscle structure and function in individuals with Distal Hereditary Motor Neuropathies (DHMN). By examining various observational methods, including MRI, dynamometry, and 3D motion analysis, this study has provided a comprehensive overview of muscle changes over a period of one year. The primary aim was to understand the pattern and progression of muscle degeneration, with secondary aims to assess the impact of orthotics and therapeutic exercises on muscle function and gait in people with DHMN.

10.1. Muscle Structure and MRI as an Outcome Measure in DHMN

Chapters 5 and 6 discussed the use of MRI to describe the pattern of muscle involvement and the longitudinal changes in DHMN patients. Quantitative MRI analysis confirmed higher rates of fatty infiltration and signs of active denervation distally in the foot and calf muscles. Over 12 months, MRI fat fraction at the calf level was the most sensitive measure, showing significant deterioration in muscle tissue with an increase in fatty infiltration. These findings are consistent with previous studies (Esteller et al., 2023, O'Donnell et al., 2022, Morrow et al., 2016), and highlight the importance of quantitative MRI as an outcome measure in DHMN research.

One significant aspect of these findings is the differential pattern of muscle involvement observed through MRI. The distal muscles, particularly the plantar

flexors, exhibited higher rates of fatty infiltration compared to proximal muscles. This pattern of distal predominance aligns with the clinical manifestations of DHMN, where distal muscle weakness and atrophy are more pronounced. The study also revealed that within the calf muscles, the posterior compartment, including the Medial Gastrocnemius and Soleus, was more affected than the anterior compartment. This specific involvement pattern underscores the importance of targeted rehabilitation strategies, focusing on these muscles to explore the possibility of slowing the progression of muscle degeneration and maintain function.

10.2. Muscle Function and Weakness Patterns in DHMN

Chapters 5 and 6 also explored muscle function using dynamometry. The results indicated predominant distal weakness, particularly in the plantar flexors. Longitudinally, muscle strength deterioration was detectable using manual muscle testing more than dynamometry. Quantitative muscle strength measures have been shown to be more sensitive in CMT (Burns et al., 2005). The DHMN cohort in the current study showed a high standard deviation (table 35, page 137) due to the small sample and high intragroup variability. Moreover, the presence of significant weakness and limited joint mobility affected the test application. This highlights the need for adapted testing protocols in future studies to accommodate the specific limitations of DHMN patients.

The decline in muscle strength over 12 months, as detected by both isometric and isokinetic dynamometry, was more pronounced in the distal muscles, consistent with the MRI findings of increased fatty infiltration. Similar relations were shown in people with peripheral neuropathies (O'Donnell et al., 2023, Morrow et al., 2016). The discrepancy between isometric and isokinetic strength in dorsiflexion suggests that muscle testing protocols must consider joint positioning and range of motion limitations in DHMN patients. For instance, the reduced isometric strength observed for ankle dorsiflexion may be partly due to the angle chosen for assessment combined with reduced range of ankle dorsiflexion. Earlier research highlighted the challenges of using dynamometry in the presence of significant weakness and limited joint mobility (Guillebastre et al., 2013, Reynaud et al., 2019). Future studies should explore alternative testing positions, such as seated rather than supine, to enhance the accuracy and reliability of strength measurements in this population. In the supine position, our cohort were able to engage their upper body to assess in pulling and pushing the foot plate by holding the side handles. This mechanism was eliminated by changing the position to 45° seated position.

10.3. Walking Gait in DHMN

Chapter 5 described the pattern of gait deviations in DHMN patients using 3D motion analysis. The findings indicated slower walking speeds, increased double support time, and compensatory hip flexion due to plantar flexion failure and foot

drop. This pattern has been described in CMT (Ramdharry et al., 2009, Don et al., 2007, Vinci and Perelli, 2002).

In foot drop pattern observed in our cohort showed a smaller ankle dorsiflexion angle in swing phase (Figure 15, A, page 116) alongside increased hip flexion angle and decreased ankle dorsiflexors power generation in swing phase. Additionally, there was an increase in plantar flexion angle at initial contact and loading response (Figure 15, B, page 116) indicating the inability to maintain the dorsiflexion angle and use the heel as a fulcrum for the first rocker. This further indicates a dropped foot pattern, although it was not statistically significant in this cohort.

A plantar flexion failure pattern was demonstrated by reduced ankle control, with increased passive dorsiflexion angle in terminal stance and decreased ankle plantar flexion in pre-swing (Figure 15, C, page 116). Kinetic data showed a decrease in plantar flexion moment and power generation in terminal stance, which confirms the presence of plantar flexion failure.

10.4. Relationships Between Intramuscular Fat Fraction, Muscle Volume, Isokinetic and Isometric Muscle Strength, and Kinetics of Gait

Chapter 7 focused on the relationships between MRI, dynamometry, and gait parameters. It was found that increased intramuscular fat fraction correlated with decreased muscle strength and altered gait kinetics. These relationships

emphasize the potential of using MRI dynamometry and gait kinetic parameters as outcome measures in clinical trials, to assess muscle changes in structure and performance.

At both distal and proximal levels, the current study showed muscle fat fraction correlating negatively with muscle area and cross sectional area, as expected with peripheral motor neuropathy. At the proximal level, increased fat fraction led to decreased muscle capacity, strength, and power during gait. On the other hand, greater muscle area correlated with higher muscle performance. Distally, higher muscle area in plantar flexors and dorsiflexors results in greater isometric ankle strength. Increased fat fraction, however, decreases dorsiflexion isometric strength. The findings align with research on hereditary peripheral neuropathies by O'Donnell et al. (2023) and Morrow et al. (2016), confirming similar relationships between muscle fat fraction, muscle area, and strength (Morrow et al., 2016, O'Donnell et al., 2023).

Increased fat fraction in dorsiflexors correlated with reduced dorsiflexor power generation during the swing phase, explaining the foot drop gait pattern. Decreased eccentric plantar flexor activity correlated positively with the reduction in plantar flexor moments in stance phase. This reduction in plantar flexor capacity likely contributed to the plantar flexor failure pattern with the inability to control ankle dorsiflexion angle in terminal stance plus delayed heel raise and toe off. Moreover, higher disease severity (CMTES) correlates with

slower gait speed and shorter step and stride lengths, indicating that neuropathy affects spatial more than temporal gait parameters.

However, due to the high variability in a small sample, some significant correlations shown in this study do not make functional sense. For example, the relation between dorsiflexors strength and plantar flexors moments during gait. Future studies with larger samples and a control group are recommended to ascertain relationships with more accuracy.

10.5. The Effect of Rehabilitation Strategies in Management of DHMN

10.5.1. Effect of Carbon Fibre AFOs on Gait Parameters in DHMN

Chapter 8 further explored the effect of carbon fiber ankle foot orthoses (AFOs) on gait kinetics and kinematics, demonstrating improvements in walking gait with AFOs, but also suggesting the need for further studies to assess the long-term effects of AFOs. The use of AFOs compensated for dorsiflexion weakness by providing ankle support and stability, which allowed for a more normal ankle position at in initial contact (Figure 63, B, page 195), improved foot clearance (Figure 63, A, page 195), and longer strides without excessive hip and knee flexion in swing phase. This is in line with findings from previous research in children (Õunpuu et al., 2021), and adults (Ramdharry et al., 2012a) with CMT. However, the rigidity of the AFOs did prevent excessive forward progression of the tibia in mid to late stance phase (Figure 63,C, page 195), but also appeared to

restrict ankle movement at pre-swing, potentially inhibiting plantar flexor activity (Figure 65, page 197). The reduction in ankle power generation at pre-swing supports this hypothesis, in that the plantar flexors reduced activation, though electromyography (EMG) data was not available to confirm this. These findings suggest that while AFOs can improve gait function, their design should balance stability with the need for natural ankle movement to avoid long-term muscle disuse and atrophy.

10.5.2. Effectiveness and Safety of Resistive Exercises

Chapter 9 explored the impact of a resistive exercise program on muscle structure and function for DHMN participants. Although the COVID-19 pandemic disrupted the study protocol and led to low adherence, the preliminary data suggested potential benefits of exercise in two cases. MRI parameters showed improvement in muscle structure in the individuals who exercised.

The exercise intervention showed improvement in muscle area and a decrease in fat fraction levels, particularly in the thigh muscles, which are less affected by neuropathy, compared to the calf. These findings may indicate that early intervention with resistance exercises could potentially slow the progression of muscle degeneration. Moreover, the safety of resistance exercises was supported by the minimal increase in muscle edema, indicating that such interventions do not aggravate neurodegeneration or induce overwork weakness. These findings

align with previous research in children with CMT that showed improvement in strength after completing resistive exercises program without signs of active denervation on MRI (Burns et al., 2017).

However, these findings were limited by the problems recruiting and low exercise adherence, highlighting the need for larger and more controlled trials to validate the effectiveness of exercise interventions in DHMN. The study revealed challenges in implementing exercise interventions in this population. The low adherence rate, partly due to the COVID-19 pandemic, highlights the need for strategies to improve patient engagement and adherence in future trials. Maintaining physically active lifestyles has been reported as particularly challenging for people with rare neurological conditions (Buscemi et al., 2024). Behavioural models and tailored exercise programs that consider the specific limitations and needs of DHMN patients could enhance adherence and maximize the benefits of exercise interventions (Busse and Ramdharry, 2020).

10.6. Study Limitations and Recommendations for Future Work

The results in a small and variable sample for the natural history study, and in two individual cases for the exercises trial, are insufficient to generalize to a broader DHMN population. The COVID-19 pandemic significantly impacted the study, leading to delays, low recruitment rates, and reduced exercise adherence. Methodological issues such as long visit times, extended MRI scanning periods,

and limited gait analysis space also posed challenges. These factors highlight the need for improved study designs and protocols in future research. Challenges and recommendations are discussed below.

10.6.1. Impact of COVID-19 Pandemic on the Study

The COVID-19 pandemic had a significant impact on the study. Due to lockdowns and social distancing measures, there were substantial delays in the research timeline, leading to shorter study periods and low recruitment rates. Planned home visits for exercise participants were cancelled, resulting in reduced engagement and adherence to the exercise protocol. These disruptions led to a reduction in the overall strength of the study findings and affected the ability to collect data as planned. Due to the pandemic and other factors, exercise adherence was low, making it difficult to accurately assess the impact of the exercise intervention. This limited the potential to calculate meaningful statistical differences between the intervention and control groups. These challenges highlight the importance of developing tools to remotely monitor adherence, progression, and functional improvement. Development of such tools can encourage the inclusion of participants who do not have access to research facilities. Efforts to adapt to remote research have been taken to develop a virtual version of the CMTES (Prada et al., 2022). The virtual version was shown to be reproducible and reliable, however, the responsiveness for longitudinal trials haven’t been explored yet (Prada et al., 2022).

10.6.2. Methodological Challenges

Several methodological issues were encountered during the study, the most important is the long visit time. Each visit is almost a full day to complete all study measurements including clinical assessments, MRI, dynamometry and motion analysis, which posed a challenge for participants. This led to two participants withdrawing from the study. Trials should consider identifying funding for accommodation to split the visit activity over two days, and vouchers to account for the participant’s time.

MRI scans required extended periods, which posed a challenge for participants and led to shortening the MRI protocol, discarding scans covering thigh muscle which would provide valuable data. Splitting the scanning time over two session might be more practical to collect needed data without compromising participants convenience.

The dynamometry assessments were constrained by participants' limited ability to move due to muscle weakness or a restricted joint range of motion. This affected the accuracy of muscle strength measurements and required modifications to the testing protocol to accommodate these limitations such as changing the testing position. Future studies targeting participants with severe distal weakness and joint movement limitations could consider using alternative muscle strength quantification options, such as hand held dynamometry, as it can be triggered with minimum strength and shown to be valid and reliable in CMT

(Burns et al., 2005). However, it is limited to measuring isometric muscle strength only.

The gait analysis was conducted in a relatively small space with a short walkway, which affected the marker visibility and limited the ability to capture a full gait cycle for both sides at the same time. This led to repeating data collection captures that extended the gait assessment may have been more tiring for some participants.

10.6.3. Research and Clinical Implications

Future research should focus on extending the study period and increasing the frequency of follow-up visits to better capture the natural history of DHMN (Fridman et al., 2023). Longitudinal studies with larger sample sizes and inclusion of control participants are needed to validate the findings and provide more robust data on the progression of muscle degeneration and the effectiveness of interventions.

Calf muscle quantitative fat fraction using the Dixon method has been proposed as the most responsive outcome measure in natural history studies in CMT, IBM (Morrow et al., 2016), and in hereditary sensory neuropathy type 1 (HSN1) (Kugathasan et al., 2019). The current study showed calf muscle MRI quantitative fat fraction to have greater responsiveness (higher SRM) than dynamometry and 3D motion analysis. This finding has important implications for future trial

design. Standardized Response Mean (SRM) is a measure of effect, in this context referring to DHMN natural history. A high SRM indicates that fewer participants are needed to determine if a new treatment effectively slows disease progression when the primary outcome measure of the trial is calf MRI fat fraction. On the contrary, the more modest SRM for muscle function and gait parameters suggests that larger sample sizes are necessary if the primary outcome of a trial is dynamometry or 3D motion analysis. For a hypothetical DHMN treatment trial using calf muscle MRI fat fraction as the primary outcome measure (SRM = 1.48), approximately 8 participants per group (active and placebo) are needed to detect a 50% reduction in disease progression over one year with 80% power at a significance level of $p < 0.05$ (Lehr, 1992).

Investigating the role of different types of exercise and rehabilitation techniques may provide insights into more effective management strategies for DHMN. If there is possibility that resistance exercise could slow progression of muscle impairment, then this will be an important recommendation in the current landscape where there are no other disease modifying therapies. Future research could include a two-phase trial to address these questions comprehensively. The first phase of the study would aim to ascertain the effectiveness and safety of progressive resistance exercises in DHMN with a supervised intensive exercises sessions, at an equipped facility, to ascertain exercises application and motivate participants to engage in the program. Once the safety and efficacy are

established, the second phase should explore the feasibility of home-based exercises using program co-designed with participants, incorporating home visits, weekly check-ins, and exercise diaries to enhance adherence. Wearable monitors could be used to track engagement and progress, as recommended by Wallace et al. (Wallace et al., 2019). Additionally, applying models for behavioural changes to improve exercise adherence in trials could enhance the outcomes of exercise interventions. Using a crossover study design is suitable in rare conditions with high variability, such as DHMN, as fewer participants are needed, variability between groups is eliminated, and participants act as their own control. This study design has been used to assess the feasibility of community-based aerobic exercise training for people with CMT (Wallace et al., 2019). Although blinding participants and incorporating washout periods in exercises trials are challenging to apply, blinding the assessor to the group can improve the muscle strength assessment accuracy.

Understanding the biomechanical effect of AFOs can inform industrial research to improve design and material. Our data suggest that carbon fibre AFOs may restrict plantar flexion muscle activity, so it will be important to ensure that when they are prescribed, duration of wearing is considered, plus a specific program of plantar flexor strengthening exercise to negate the possibility of disuse atrophy. Hedge's G showed the effect size to be the largest in ankle angles; dorsiflexion and plantar flexion. This informs future research in AFO development, if they

target ankle angles, a smaller sample size is required. However, if the focus is on proximal compensatory mechanisms, larger sample sizes will be necessary to determine the effectiveness of the AFO.

Overall, this thesis provides valuable insights into the natural history of DHMN and the effectiveness of rehabilitation interventions. The findings highlight the importance of improving orthotic designs, and early exercise interventions to manage DHMN. Future research should focus on larger trials to validate these findings and develop effective management strategies for this condition.

Chapter 11: Conclusion

In conclusion, this thesis has provided valuable insights into the presentation and progression of muscle degeneration and functional impairment in individuals with Distal Hereditary Motor Neuropathies (DHMN). The use of advanced observational techniques, such as MRI, dynamometry, and 3D motion analysis, has allowed for a detailed examination of muscle changes over time, the functional consequences and the impact of targeted exercise interventions.

The study has highlighted several key findings, including the significant increase in intramuscular fat and muscle atrophy, the decline in muscle strength, and the altered gait patterns in people with DHMN. These findings highlight the importance of early diagnosis and intervention to manage the progression of muscle degeneration, and maintain mobility and quality of life in affected individuals.

The impact of the COVID-19 pandemic on the study has been considerable, leading to methodological challenges and limitations in data collection and participant recruitment. Despite these obstacles, the study has demonstrated possible benefits of exercise interventions in improving muscle function and gait in people with DHMN.

Future research should build on these findings by extending the study period, increasing follow-up visits, and applying behavioural models to improve exercise

adherence. Longitudinal studies with larger sample sizes and diverse populations are needed to validate the findings and provide more comprehensive data on the natural history of DHMN and the effectiveness of rehabilitation interventions.

Overall, this thesis has contributed to a better understanding of DHMN and its impact on muscle function and mobility, providing a foundation for future research and clinical practice aimed at improving outcomes for individuals living with this condition.

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Appendix I: Difference Between Right and Left Side in The Primary Parameters and Application of the Modified Bonferroni Correction

Introduction

Distal hereditary motor neuropathy lays under the CMT umbrella which is characterized by its symmetrical nature. The impact of the COVID-19 pandemic significantly influenced the recruitment rate for this project, resulting in a smaller sample size that may not accurately reflect the population. Additionally, smaller sample sizes have a higher risk of deviating from the norm due to chance alone. Therefore, right and left symmetry of the primary parameters in our sample had to be ascertained before proceeding with the analysis.

Methods

To ascertain symmetry in our sample, difference between right and left side in the primary parameters was tested using unpaired T.Test for normally distributed data and Mann-Whitney test for non-normally distributed data. Consideration to correction for the multiple comparisons to decrease the chance of type I and type II error was given using modified Bonferroni procedure. Pelvic segment is not independent left to right due to the structure of the pelvic ring therefore differences at the pelvic level will not be considered.

The modified Bonferroni correction (Simes, 1986) is based on the ordered P-values of individual tests. $T_1,...,T_n$ is a set of statistics with corresponding p-values $P_1,...,P_n$ for testing hypothesis $H_1,\ldots,H_n$. The p-values were ordered as $P_{(1)},...,P_{(n)}$ to test the hypothesis $H0=[H(1),...H(n)]$. The null hypothesis H_0 was rejected if $P_{(j)} \leq j\alpha/n$ for any $j = 1,\ldots,n$. (Simes, 1986).

Results:

The test showed no significant difference in all primary parameters with modified Bonferroni correction for multiple testing except in pelvis angle at Z axis. However, the pelvic segment is not independent left to right due to the structure of the pelvic ring. Moreover, this difference could be caused by the lost visibility due to arm swing during walking. Test results and application of modified Bonferroni correction are shown in the table below.

Test rank	Fat Fraction Variables	P-value	Modified Bonferroni Threshold	Modified Bonferroni Outcome
1	Adductor Magnus	0.6994	0.0028	ACCEPT H_0
2	Biceps Femoris	0.5619	0.0056	ACCEPT H_0
3	Femur	0.7477	0.0083	ACCEPT H_0
4	Gracilis	0.5619	0.0111	ACCEPT H_0
5	Lateral Gastrocnemius	0.7969	0.0139	ACCEPT H_0
6	Medial Gastrocnemius	0.8731	0.0167	ACCEPT H_0
7	Peroneus Longus	0.7477	0.0194	ACCEPT H_0
8	Rectus Femoris	0.9487	0.0222	ACCEPT H_0
9	Soleus	0.7231	0.0250	ACCEPT H_0
10	Sartorius	0.6522	0.0278	ACCEPT H_0
11	Semimembranosus	0.3653	0.0306	ACCEPT H_0
12	Semitendinosus	0.7477	0.0333	ACCEPT H_0
13	Tibialis Anterior	0.9487	0.0361	ACCEPT H_0
14	Tibia	0.7477	0.0389	ACCEPT H_0

15	Tibialis Posterior	0.7477	0.0417	ACCEPT H_0
16	Vastus Intermedius	0.7477	0.0444	ACCEPT H_0
17	Vastus Lateralis	0.7477	0.0472	ACCEPT H_0
18	Vastus Medialis	0.519	0.0500	ACCEPT H_0
Test rank	Volume Variables	P-value	Modified Bonferroni Threshold	Modified Bonferroni Outcome
1	Adductor Magnus	0.7917	0.0028	ACCEPT H_0
2	Biceps Femoris	0.6306	0.0056	ACCEPT H_0
3	Femur	0.3851	0.0083	ACCEPT H_0
4	Gracilis	0.8442	0.0111	ACCEPT H_0
5	Lateral Gastrocnemius	0.9487	0.0139	ACCEPT H_0
6	Medial Gastrocnemius	0.9516	0.0167	ACCEPT H_0
7	Peroneus Longus	0.914	0.0194	ACCEPT H_0
8	Rectus Femoris	0.47	0.0222	ACCEPT H_0
9	Soleus	0.9859	0.0250	ACCEPT H_0
10	Sartorius	0.4672	0.0278	ACCEPT H_0
11	Semimembranosus	0.9883	0.0306	ACCEPT H_0
12	Semitendinosus	0.8641	0.0333	ACCEPT H_0
13	Tibialis Anterior	0.9294	0.0361	ACCEPT H_0
14	Tibia	0.9218	0.0389	ACCEPT H_0
15	Tibialis Posterior	0.9563	0.0417	ACCEPT H_0
16	Vastus Intermedius	0.912	0.0444	ACCEPT H_0
17	Vastus Lateralis	0.9367	0.0472	ACCEPT H_0
18	Vastus Medialis	0.7932	0.0500	ACCEPT H_0
Test rank	Kinematic Gait Variables	P-value	Modified Bonferroni Threshold	Modified Bonferroni Outcome
1	Ankle Max	0.8205	0.0042	ACCEPT H_0
2	Ankle Min	0.9487	0.0083	ACCEPT H_0
3	Hip Max	0.7756	0.0125	ACCEPT H_0
4	Hip Min	0.6225	0.0167	ACCEPT H_0
5	Knee Max	0.875	0.0208	ACCEPT H_0
6	Knee Min	0.5888	0.0250	ACCEPT H_0
7	Pelvis X Max	0.5642	0.0292	ACCEPT H_0
8	Pelvis X Min	0.3383	0.0333	ACCEPT H_0
9	Pelvis Y Max	0.7969	0.0375	ACCEPT H_0
10	Pelvis Y Min	0.9984	0.0417	ACCEPT H_0
11	Pelvis Z Max	0.0073	0.0458	REJECT H_0
12	Pelvis Z Min	0.0192	0.0500	REJECT H_0
Test rank	Kinetic Gait Variables	P-value	Modified Bonferroni Threshold	Modified Bonferroni Outcome

1	Ankle Moment Y Max	0.4385	0.0033	ACCEPT H_0
2	Ankle Moment Y Min	0.9487	0.0067	ACCEPT H_0
3	Ankle Power Max During Stance	0.4385	0.0100	ACCEPT H_0
4	Ankle Power Max During Swing	0.4779	0.0133	ACCEPT H_0
5	Ankle Power Min During Stance	0.519	0.0167	ACCEPT H_0
6	Hip Moment Y Max	0.6882	0.0200	ACCEPT H_0
7	Hip Moment Y Min	0.7969	0.0233	ACCEPT H_0
8	Hip Power Max During Stance	0.9955	0.0267	ACCEPT H_0
9	Hip Power Max During Swing	0.8133	0.0300	ACCEPT H_0
10	Hip Power Min During Stance	0.8977	0.0333	ACCEPT H_0
11	Knee Moment Y Max	0.6063	0.0367	ACCEPT H_0
12	Knee Moment Y Min	0.4457	0.0400	ACCEPT H_0
13	Knee Power Max During Stance	0.5527	0.0433	ACCEPT H_0
14	Knee Power Max During Swing	0.6935	0.0467	ACCEPT H_0
15	Knee Power Min During Stance	0.4779	0.0500	ACCEPT H_0
Test rank	Spatiotemporal Gait Variables	P-value	Modified Bonferroni Threshold	Modified Bonferroni Outcome
1	Double Support Time	0.66	0.00417	ACCEPT H_0
2	Percent Opposite Foot Contact	0.8714	0.00833	ACCEPT H_0
3	Percent Opposite Toe Off	0.3316	0.01250	ACCEPT H_0
4	Percent Stance	0.7676	0.01667	ACCEPT H_0
5	Single Support Time	0.5113	0.02083	ACCEPT H_0
6	Speed	0.9384	0.02500	ACCEPT H_0
7	Step Length	0.9177	0.02917	ACCEPT H_0
8	Steps Per Minute	0.9487	0.03333	ACCEPT H_0
9	Step Time	0.8955	0.03750	ACCEPT H_0
10	Stride Length	0.9193	0.04167	ACCEPT H_0
11	Strides Per Minute	0.9487	0.04583	ACCEPT H_0
12	Stride Time	0.9215	0.05000	ACCEPT H_0
Test rank	Isometric dynamometry Variables	P-value	Modified Bonferroni Threshold	Modified Bonferroni Outcome
1	Ankle ISOM DF 10°	0.8935	0.00556	ACCEPT H_0
2	Ankle ISOM DF 30°	0.7479	0.01111	ACCEPT H_0
3	Ankle_ISOM_PF_10°	0.6932	0.01667	ACCEPT H_0
4	Hip ISOM EX 45°	0.9783	0.02222	ACCEPT H_0
5	Hip ISOM FL 45°	0.7289	0.02778	ACCEPT H_0
6	Knee_ISOM_EX_45°	0.975	0.03333	ACCEPT H_0
7	Knee ISOM EX 90°	0.8972	0.03889	ACCEPT H_0
8	Knee ISOM FL 45°	0.9499	0.04444	ACCEPT H_0
9	Knee_ISOM_FL_90°	0.9873	0.05000	ACCEPT H_0

Test rank	Isokinetic dynamometry Variables	P-value	Modified Bonferroni Threshold	Modified Bonferroni Outcome
1	Ankle ISOK DF 60°/60°	0.3905	0.0063	ACCEPT H_0
2	Ankle ISOK PF 60°/60°	0.6442	0.0125	ACCEPT H_0
3	Hip ISOK EX 60°/60s	0.9607	0.0188	ACCEPT H_0
4	Hip ISOK FL 60°/60s	0.8906	0.0250	ACCEPT H_0
5	Knee ISOK EX 120°/120s	0.7929	0.0313	ACCEPT H_0
6	Knee ISOK EX 60°/60s	0.886	0.0375	ACCEPT H_0
7	Knee ISOK FL 120°/120s	0.7352	0.0438	ACCEPT H_0
8	Knee ISOK FL 60°/60s	0.8236	0.0500	ACCEPT H_0

Conclusion

The DHMN sample recruited for this project showed symmetry between right and left side, therefore, the decision was made to analyse the primary and secondary parameters for each objective using the average of both sides rather than analysing both sides separately to eliminate type 1 error caused by multiple comparisons.

Appendix II: Friedman Test Manual Calculation

Introduction

The Friedman test is a non-parametric statistical test used for analysing repeated measures data. It is mainly used when the assumptions of normality and homogeneity of variances are not met, making it an alternative to repeated measures ANOVA. The analysis of variance (ANOVA) tests the extent to which the measured values of the dependent sample differ. However, The Friedman test uses ranks rather than the actual measured values which make it a valid tool for analysing ordinal data (Friedman, 1940, Friedman, 1937). The null hypothesis for Friedman test is there is no significant difference between the dependent groups. And the alternative hypothesis is there is a significant difference between the dependent groups (Conover, 1999).

Analysis of the longitudinal MRI Muscle Fat qualitative score at the foot level with Modified Mercuri's Scale of DHMN using statistical software (© 2024 MedCalc Software Ltd) showed significant difference although the pairwise analysis showed no difference across all measurement points (chapter 6). This manual calculation is aimed to confirm the false positive type I error occurred in the analysis.

Manual Calculation Procedure

A. Data Set and Ranking the Values: The point in time where a participant has the highest value gets rank 1, the point in time with the second highest value gets rank 2 and the point in time with the smallest value gets rank 3. To ensure correct handling of ties (when values in two or more time points are the same), the average rank is used. This process is repeated for all rows. Afterwards the ranks of each single point of time are combined.

Since all values for each participant are the same at all measurement points, they receive the average rank. For three measurements, the average rank would be:

$$Average\ rank = \frac{1+2+3}{3} = 2$$

Baseline			6 Months			12 Months		
Mercuri Score	Rank	Average rank	Mercuri Score	Rank	Average rank	Mercuri Score	Rank	Average rank
6	1	2	6	2	2	6	3	2
6	1	2	6	2	2	6	3	2
6	1	2	6	2	2	6	3	2
6	1	2	6	2	2	6	3	2
6	1	2	6	2	2	6	3	2
6	1	2	6	2	2	6	3	2
6	1	2	6	2	2	6	3	2
6	1	2	6	2	2	6	3	2
Sum rank	8	16	Sum rank	16	16	Sum rank	24	16

Since each time point (k=3) in every participant (n =8) has the rank of 2, the sum of ranks for each time point (R_j) would be:

$$R_1=2\times8=16,\quad R_2=2\times8=16,\quad R_3=2\times8=16$$

B. Compute the Test Statistic: The test statistic for the Friedman test is calculated using the formula:

$$x^2 = \frac{12}{N \cdot k \cdot (k+1)} \cdot \sum R_j^2 - 3 \cdot N \cdot (k+1)$$

where:

n is the number of blocks (participants), k is the number of treatments (time points), R_j is the sum of ranks for the j-th treatment.

The test statistic χ^2 is then compared to the critical value from the chi-square distribution with $k-1$degrees of freedom to determine the p-value.

Plugging in the values to calculate χ^2:

$$n=8,\ k=3,\ R_1=16,\ R_2=16,\ R_3=16$$

$$\chi^2=12/8\times3\times4(16^2+16^2+16^2)-3\times8\times4$$

$$\chi^2=12/96(256+256+256)-96$$

$$\chi^2=12/96\times768-96$$

$$\chi^2=9216/96-96$$

$$\chi^2=96-96$$

$$\boldsymbol{\chi^2= 0}$$

In this specific case, because all the values are the same across all time points and participants, the calculated Friedman test statistic (χ^2) is 0. This result indicates that there are no differences between the treatments, as expected.

C. Calculate the P-Value:

1.Determine the Chi-Square Test Statistic (χ^2):

Based on the previous calculation, χ^2 =0

2.Determine the Degrees of Freedom (df):

$$df = k - 1 = 3 - 1 = 2.$$

3.Using the Cumulative Distribution Function (CDF):

The *p*-value for a chi-square statistic is the area under the chi-square distribution curve to the right of the given test statistic. For χ^2=0 with any degrees of freedom, this area is essentially the entire area under the curve since the chi-square distribution starts at 0 and is always positive.

For a chi-square statistic of 0, the cumulative probability up to 0 is 0. Therefore, the *p*-value is 1, which indicates that a test statistic as extreme as or more extreme than 0 would occur with certainty under the null hypothesis.

4. Formula and Calculation:

The cumulative distribution function (CDF) for the chi-square distribution at 0 is:

$$P(\chi^2 \leq 0)$$

Given that the chi-square distribution is non-negative, the probability that a chi-square statistic is less than or equal to 0 is 0. Thus, the p-value is:

$$p\text{-value} = 1 - P(\chi^2 \leq 0) = 1 - 0 = 1$$

For a chi-square statistic of 0 with 2 degrees of freedom, the p-value is 1. This indicates that observing a test statistic of 0 or more extreme under the null hypothesis is certain, which makes sense since a chi-square statistic of 0 indicates no deviation from the expected values.

Conclusion:

In this specific case, because all the values are the same across all time points and participants, the manually calculated Friedman test statistic is 0. This result indicates that there are no differences in Mercuri score between the time points, as expected. When all values are the same for all time points, significant *p*-value suggests an issue with the implementation or the interpretation of the test. The Friedman test statistic calculation using statistical software in this scenario might leads to an invalid result such as type 1 error. Small sample size, outliers, and inadequate handling of ties are possible explanations for type 1 error in Fridman test (Hollander, 2013, Conover, 1999).

Appendix III: Correlograms Showing the Correlation and Significance Level for All Parameters Comparisons in Chapter 7

Relationships Between Intramuscular Fat Fraction, Muscle Volume, Isokinetic and Isometric Muscle Strength, And Kinetics of Gait

Plantar Flexors

	Ankle ISOM PF	PF CSA	PF RMA	Ankle Moment Y Max	Ankle Power Max During Stance	Ankle ISOK PF	Ankle Power Max During Swing	Ankle Power Min During Stance	Ankle Moment Y Min	PF FF
Ankle ISOM PF	1	0.827 P=0.0031 n=10	0.754 P=0.0118 n=10	0.413 P=0.2359 n=10	0.391 P=0.2645 n=10	0.56 P=0.0926 n=10	0.343 P=0.3314 n=10	0.161 P=0.6564 n=10	0.05 P=0.8911 n=10	-0.4 P=0.2516 n=10
PF CSA	0.827 P=0.0031 n=10	1	0.843 P=0.0022 n=10	0.503 P=0.1382 n=10	0.275 P=0.4420 n=10	0.368 P=0.2948 n=10	0.017 P=0.9620 n=10	-0.001 P=0.9971 n=10	-0.11 P=0.7626 n=10	-0.375 P=0.2852 n=10
PF RMA	0.754 P=0.0118 n=10	0.843 P=0.0022 n=10	1	0.391 P=0.2643 n=10	0.361 P=0.3059 n=10	0.293 P=0.4112 n=10	0.059 P=0.8705 n=10	-0.31 P=0.3827 n=10	-0.108 P=0.7672 n=10	-0.791 P=0.0065 n=10
Ankle Moment Y Max	0.413 P=0.2359 n=10	0.503 P=0.1382 n=10	0.391 P=0.2643 n=10	1	0.547 P=0.1016 n=10	0.599 P=0.0671 n=10	0.487 P=0.1529 n=10	0.472 P=0.1680 n=10	0.413 P=0.2353 n=10	-0.27 P=0.4513 n=10
Ankle Power Max During Stance	0.391 P=0.2645 n=10	0.275 P=0.4420 n=10	0.361 P=0.3059 n=10	0.547 P=0.1016 n=10	1	0.383 P=0.2745 n=10	0.662 P=0.0372 n=10	0.259 P=0.4703 n=10	0.168 P=0.6429 n=10	-0.539 P=0.1077 n=10
Ankle ISOK PF	0.596 P=0.0692 n=10	0.529 P=0.1160 n=10	0.426 P=0.2202 n=10	0.778 P=0.0080 n=10	0.389 P=0.2665 n=10	1	0.401 P=0.2505 n=10	0.292 P=0.4133 n=10	0.389 P=0.2665 n=10	-0.419 P=0.2276 n=10
Ankle Power Max During Swing	0.343 P=0.3314 n=10	0.017 P=0.9620 n=10	0.059 P=0.8705 n=10	0.487 P=0.1529 n=10	0.662 P=0.0372 n=10	0.41 P=0.2388 n=10	1	0.64 P=0.0464 n=10	0.323 P=0.3621 n=10	-0.187 P=0.6051 n=10
Ankle Power Min During Stance	0.161 P=0.6564 n=10	-0.001 P=0.9971 n=10	-0.31 P=0.3827 n=10	0.472 P=0.1680 n=10	0.259 P=0.4703 n=10	0.162 P=0.6545 n=10	0.64 P=0.0464 n=10	1	0.041 P=0.9101 n=10	0.418 P=0.2297 n=10
Ankle Moment Y Min	-0.067 P=0.8548 n=10	-0.018 P=0.9602 n=10	0.03 P=0.9338 n=10	0.43 P=0.2145 n=10	0.042 P=0.9074 n=10	0.389 P=0.2665 n=10	0.236 P=0.5109 n=10	-0.067 P=0.8548 n=10	1	-0.139 P=0.7009 n=10
PF FF	-0.4 P=0.2516 n=10	-0.375 P=0.2852 n=10	-0.791 P=0.0065 n=10	-0.27 P=0.4513 n=10	-0.539 P=0.1077 n=10	-0.178 P=0.6236 n=10	-0.187 P=0.6051 n=10	0.418 P=0.2297 n=10	0.053 P=0.8845 n=10	1
	Ankle ISOM PF	**PF CSA**	**PF RMA**	**Ankle Moment Y Max**	**Ankle Power Max During Stance**	**Ankle ISOK PF**	**Ankle Power Max During Swing**	**Ankle Power Min During Stance**	**Ankle Moment Y Min**	**PF FF**

Table 78:Correlation between baseline plantar flexors measurements.

Dorsiflexors

	DF RMA	Ankle ISOM DF	DF CSA	Ankle ISOK DF	Ankle Moment Y Max	Ankle Power Max During Swing	Ankle Power Max During Stance	Ankle Power Min During Stance	Ankle Moment Y Min	DF FF
DF RMA	1	0.764 P=0.0102 n=10	0.981 P<0.0001 n=10	0.53 P=0.1153 n=10	0.381 P=0.2769 n=10	0.615 P=0.0587 n=10	0.222 P=0.5373 n=10	0.355 P=0.3147 n=10	0.326 P=0.3576 n=10	-0.82 P=0.0037 n=10
Ankle ISOM DF	0.891 P=0.0005 n=10	1	0.733 P=0.0158 n=10	0.612 P=0.0600 n=10	0.77 P=0.0092 n=10	0.673 P=0.0330 n=10	0.382 P=0.2763 n=10	0.479 P=0.1615 n=10	0.418 P=0.2291 n=10	-0.867 P=0.0012 n=10
DF CSA	0.981 P<0.0001 n=10	0.703 P=0.0235 n=10	1	0.452 P=0.1896 n=10	0.273 P=0.4450 n=10	0.554 P=0.0969 n=10	0.08 P=0.8256 n=10	0.353 P=0.3176 n=10	0.257 P=0.4734 n=10	-0.719 P=0.0190 n=10
Ankle ISOK DF	0.53 P=0.1153 n=10	0.703 P=0.0235 n=10	0.452 P=0.1896 n=10	1	0.591 P=0.0720 n=10	0.299 P=0.4013 n=10	0.386 P=0.2706 n=10	0.25 P=0.4867 n=10	0.445 P=0.1975 n=10	-0.553 P=0.0976 n=10
Ankle Moment Y Max	0.381 P=0.2769 n=10	0.666 P=0.0354 n=10	0.273 P=0.4450 n=10	0.591 P=0.0720 n=10	1	0.487 P=0.1529 n=10	0.547 P=0.1016 n=10	0.472 P=0.1680 n=10	0.413 P=0.2353 n=10	-0.403 P=0.2477 n=10
Ankle Power Max During Swing	0.615 P=0.0587 n=10	0.612 P=0.0598 n=10	0.554 P=0.0969 n=10	0.299 P=0.4013 n=10	0.487 P=0.1529 n=10	1	0.662 P=0.0372 n=10	0.64 P=0.0464 n=10	0.323 P=0.3621 n=10	-0.592 P=0.0716 n=10
Ankle Power Max During Stance	0.222 P=0.5373 n=10	0.384 P=0.2731 n=10	0.08 P=0.8256 n=10	0.386 P=0.2706 n=10	0.547 P=0.1016 n=10	0.662 P=0.0372 n=10	1	0.259 P=0.4703 n=10	0.168 P=0.6429 n=10	-0.595 P=0.0697 n=10
Ankle Power Min During Stance	0.355 P=0.3147 n=10	0.4 P=0.2516 n=10	0.353 P=0.3176 n=10	0.25 P=0.4867 n=10	0.472 P=0.1680 n=10	0.64 P=0.0464 n=10	0.259 P=0.4703 n=10	1	0.041 P=0.9101 n=10	-0.07 P=0.8466 n=10
Ankle Moment Y Min	0.43 P=0.2145 n=10	0.418 P=0.2291 n=10	0.2 P=0.5796 n=10	0.345 P=0.3282 n=10	0.43 P=0.2145 n=10	0.236 P=0.5109 n=10	0.042 P=0.9074 n=10	-0.067 P=0.8548 n=10	1	-0.455 P=0.1869 n=10
DF FF	-0.867 P=0.0012 n=10	-0.867 P=0.0012 n=10	-0.673 P=0.0330 n=10	-0.588 P=0.0739 n=10	-0.612 P=0.0600 n=10	-0.673 P=0.0330 n=10	-0.527 P=0.1173 n=10	-0.406 P=0.2443 n=10	-0.455 P=0.1869 n=10	1
	DF RMA	**Ankle ISOM DF**	**DF CSA**	**Ankle ISOK DF**	**Ankle Moment Y Max**	**Ankle Power Max During Swing**	**Ankle Power Max During Stance**	**Ankle Power Min During Stance**	**Ankle Moment Y Min**	**DF FF**

Table 79:Correlation between baseline dorsiflexors measurements.

Knee Extensors

	Knee ISOM EX	Knee Moment Y Max	Knee ISOK EX	EX CSA	EX RMA	Knee Power Max During Swing	Knee Power Max During Stance	Knee Moment Y Min	EX FF	Knee Power Min During Stance
Knee ISOM EX	1	0.902 P=0.0004 n=10	0.982 P<0.0001 n=10	0.855 P=0.0016 n=10	0.844 P=0.0021 n=10	0.566 P=0.0881 n=10	0.5 P=0.1409 n=10	0.43 P=0.2149 n=10	-0.592 P=0.0711 n=10	0.752 P=0.0121 n=10
Knee Moment Y Max	0.902 P=0.0004 n=10	1	0.867 P=0.0012 n=10	0.876 P=0.0009 n=10	0.818 P=0.0038 n=10	0.715 P=0.0200 n=10	0.715 P=0.0202 n=10	0.585 P=0.0755 n=10	-0.507 P=0.1350 n=10	0.872 P=0.0010 n=10
Knee ISOK EX	0.982 P<0.0001 n=10	0.867 P=0.0012 n=10	1	0.785 P=0.0071 n=10	0.761 P=0.0105 n=10	0.492 P=0.1490 n=10	0.422 P=0.2250 n=10	0.43 P=0.2154 n=10	-0.48 P=0.1600 n=10	0.754 P=0.0118 n=10
EX CSA	0.855 P=0.0016 n=10	0.876 P=0.0009 n=10	0.785 P=0.0071 n=10	1	0.977 P<0.0001 n=10	0.629 P=0.0512 n=10	0.659 P=0.0384 n=10	0.595 P=0.0695 n=10	-0.695 P=0.0257 n=10	0.865 P=0.0012 n=10
EX RMA	0.844 P=0.0021 n=10	0.818 P=0.0038 n=10	0.761 P=0.0105 n=10	0.977 P<0.0001 n=10	1	0.596 P=0.0692 n=10	0.657 P=0.0390 n=10	0.55 P=0.0992 n=10	-0.812 P=0.0044 n=10	0.754 P=0.0117 n=10
Knee Power Max During Swing	0.566 P=0.0881 n=10	0.715 P=0.0200 n=10	0.492 P=0.1490 n=10	0.629 P=0.0512 n=10	0.596 P=0.0692 n=10	1	0.87 P=0.0011 n=10	0.682 P=0.0300 n=10	-0.251 P=0.4848 n=10	0.584 P=0.0762 n=10
Knee Power Max During Stance	0.5 P=0.1409 n=10	0.715 P=0.0202 n=10	0.422 P=0.2250 n=10	0.659 P=0.0384 n=10	0.657 P=0.0390 n=10	0.87 P=0.0011 n=10	1	0.835 P=0.0026 n=10	-0.398 P=0.2549 n=10	0.612 P=0.0599 n=10
Knee Moment Y Min	0.43 P=0.2149 n=10	0.585 P=0.0755 n=10	0.43 P=0.2154 n=10	0.595 P=0.0695 n=10	0.55 P=0.0992 n=10	0.682 P=0.0300 n=10	0.835 P=0.0026 n=10	1	-0.116 P=0.7487 n=10	0.728 P=0.0171 n=10
EX FF	-0.592 P=0.0711 n=10	-0.507 P=0.1350 n=10	-0.48 P=0.1600 n=10	-0.695 P=0.0257 n=10	-0.812 P=0.0044 n=10	-0.251 P=0.4848 n=10	-0.398 P=0.2549 n=10	0.116 P=0.7487 n=10	1	-0.291 P=0.4154 n=10
Knee Power Min During Stance	0.752 P=0.0121 n=10	0.872 P=0.0010 n=10	0.754 P=0.0118 n=10	0.865 P=0.0012 n=10	0.754 P=0.0117 n=10	0.584 P=0.0762 n=10	0.612 P=0.0599 n=10	0.728 P=0.0171 n=10	-0.291 P=0.4154 n=10	1
	Knee ISOM EX	**Knee Moment Y Max**	**Knee ISOK EX**	**EX CSA**	**EX RMA**	**Knee Power Max During Swing**	**Knee Power Max During Stance**	**Knee Moment Y Min**	**EX FF**	**Knee Power Min During Stance**

Table 80:Correlation between baseline knee extensors measurements.

Knee Flexors

	Knee ISOM FL	Knee Moment Y Max	FL RMA	Knee ISOK FL	FL CSA	Knee Power Max During Swing	Knee Power Max During Stance	Knee Moment Y Min	FL FF	Knee Power Min During Stance
Knee ISOM FL	1	0.856 P=0.0016 n=10	0.918 P=0.0002 n=10	0.935 P=0.0001 n=10	0.897 P=0.0004 n=10	0.492 P=0.1488 n=10	0.519 P=0.1243 n=10	0.529 P=0.1160 n=10	-0.71 P=0.0213 n=10	0.81 P=0.0045 n=10
Knee Moment Y Max	0.856 P=0.0016 n=10	1	0.846 P=0.0021 n=10	0.867 P=0.0012 n=10	0.809 P=0.0046 n=10	0.715 P=0.0200 n=10	0.715 P=0.0202 n=10	0.585 P=0.0755 n=10	-0.783 P=0.0074 n=10	0.872 P=0.0010 n=10
FL RMA	0.918 P=0.0002 n=10	0.846 P=0.0021 n=10	1	0.831 P=0.0029 n=10	0.99 P<0.0001 n=10	0.4 P=0.2520 n=10	0.526 P=0.1181 n=10	0.501 P=0.1404 n=10	-0.798 P=0.0056 n=10	0.818 P=0.0039 n=10
Knee ISOK FL	0.935 P=0.0001 n=10	0.867 P=0.0012 n=10	0.831 P=0.0029 n=10	1	0.806 P=0.0049 n=10	0.432 P=0.2124 n=10	0.412 P=0.2365 n=10	0.454 P=0.1874 n=10	-0.649 P=0.0421 n=10	0.792 P=0.0064 n=10
FL CSA	0.897 P=0.0004 n=10	0.809 P=0.0046 n=10	0.99 P<0.0001 n=10	0.806 P=0.0049 n=10	1	0.354 P=0.3159 n=10	0.451 P=0.1904 n=10	0.467 P=0.1740 n=10	-0.735 P=0.0155 n=10	0.829 P=0.0031 n=10
Knee Power Max During Swing	0.492 P=0.1488 n=10	0.715 P=0.0200 n=10	0.4 P=0.2520 n=10	0.432 P=0.2124 n=10	0.354 P=0.3159 n=10	1	0.87 P=0.0011 n=10	0.682 P=0.0300 n=10	-0.356 P=0.3120 n=10	0.584 P=0.0762 n=10
Knee Power Max During Stance	0.519 P=0.1243 n=10	0.715 P=0.0202 n=10	0.526 P=0.1181 n=10	0.412 P=0.2365 n=10	0.451 P=0.1904 n=10	0.87 P=0.0011 n=10	1	0.835 P=0.0026 n=10	-0.535 P=0.1111 n=10	0.612 P=0.0599 n=10
Knee Moment Y Min	0.529 P=0.1160 n=10	0.585 P=0.0755 n=10	0.501 P=0.1404 n=10	0.454 P=0.1874 n=10	0.467 P=0.1740 n=10	0.682 P=0.0300 n=10	0.835 P=0.0026 n=10	1	-0.241 P=0.5017 n=10	0.728 P=0.0171 n=10
FL FF	-0.71 P=0.0213 n=10	-0.783 P=0.0074 n=10	-0.798 P=0.0056 n=10	-0.649 P=0.0421 n=10	-0.735 P=0.0155 n=10	-0.356 P=0.3120 n=10	-0.535 P=0.1111 n=10	-0.241 P=0.5017 n=10	1	-0.56 P=0.0925 n=10
Knee Power Min During Stance	0.81 P=0.0045 n=10	0.872 P=0.0010 n=10	0.818 P=0.0039 n=10	0.792 P=0.0064 n=10	0.829 P=0.0031 n=10	0.584 P=0.0762 n=10	0.612 P=0.0599 n=10	0.728 P=0.0171 n=10	-0.56 P=0.0925 n=10	1
	Knee ISOM FL	**Knee Moment Y Max**	**FL RMA**	**Knee ISOK FL**	**FL CSA**	**Knee Power Max During Swing**	**Knee Power Max During Stance**	**Knee Moment Y Min**	**FL FF**	**Knee Power Min During Stance**

Table 81: Correlation between baseline knee flexors measurements.

Between Clinical Measurements

	CMTES	WALK-12	FPI-6
CMTES	1	0.671 P=0.0337 n=10	-0.358 P=0.3100 n=10
WALK-12	0.671 P=0.0337 n=10	1	-0.494 P=0.1470 n=10
FPI-6	-0.358 P=0.3100 n=10	-0.494 P=0.1470 n=10	1
	CMTES	**WALK-12**	**FPI-6**

Table 82:Correlation between baseline clinical measurements.

Gait and Strength Measurements

	Step Length	Stride Length	Speed	Steps Per Minute	Strides Per Minute	Stride Time	Step Time
Hip ISOK FL	0.753 P=0.0119 n=10	0.753 P=0.0119 n=10	0.748 P=0.0128 n=10	0.575 P=0.0821 n=10	0.527 P=0.1173 n=10	-0.576 P=0.0816 n=10	-0.576 P=0.0816 n=10
Hip ISOK EX	0.724 P=0.0179 n=10	0.724 P=0.0179 n=10	0.715 P=0.0202 n=10	0.555 P=0.0957 n=10	0.442 P=0.2004 n=10	-0.491 P=0.1497 n=10	-0.491 P=0.1497 n=10
Hip ISOM FL	0.683 P=0.0295 n=10	0.683 P=0.0295 n=10	0.695 P=0.0258 n=10	0.573 P=0.0831 n=10	0.515 P=0.1276 n=10	-0.552 P=0.0984 n=10	-0.552 P=0.0984 n=10
Hip ISOM EX	0.651 P=0.0416 n=10	0.65 P=0.0417 n=10	0.647 P=0.0433 n=10	0.506 P=0.1354 n=10	0.442 P=0.2004 n=10	-0.491 P=0.1497 n=10	-0.491 P=0.1497 n=10
Knee ISOK FL	0.728 P=0.0170 n=10	0.728 P=0.0171 n=10	0.718 P=0.0193 n=10	0.543 P=0.1049 n=10	0.479 P=0.1615 n=10	-0.527 P=0.1173 n=10	-0.527 P=0.1173 n=10
Knee ISOK EX	0.724 P=0.0180 n=10	0.723 P=0.0181 n=10	0.697 P=0.0251 n=10	0.489 P=0.1519 n=10	0.43 P=0.2145 n=10	-0.467 P=0.1739 n=10	-0.467 P=0.1739 n=10
Knee ISOM FL	0.742 P=0.0139 n=10	0.742 P=0.0139 n=10	0.765 P=0.0100 n=10	0.628 P=0.0518 n=10	0.515 P=0.1276 n=10	-0.564 P=0.0897 n=10	-0.564 P=0.0897 n=10
Knee ISOM EX	0.74 P=0.0145 n=10	0.74 P=0.0145 n=10	0.731 P=0.0164 n=10	0.549 P=0.0999 n=10	0.515 P=0.1276 n=10	-0.564 P=0.0897 n=10	-0.564 P=0.0897 n=10
Ankle ISOK PF	0.559 P=0.0928 n=10	0.559 P=0.0928 n=10	0.65 P=0.0417 n=10	0.176 P=0.6261 n=10	0.176 P=0.6261 n=10	-0.274 P=0.4444 n=10	-0.274 P=0.4444 n=10
Ankle ISOK DF	0.486 P=0.1540 n=10	0.486 P=0.1545 n=10	0.521 P=0.1223 n=10	0.535 P=0.1112 n=10	0.285 P=0.4250 n=10	-0.382 P=0.2763 n=10	-0.382 P=0.2763 n=10
Ankle ISOM PF	0.62 P=0.0560 n=10	0.62 P=0.0559 n=10	0.645 P=0.0439 n=10	0.568 P=0.0865 n=10	0.479 P=0.1615 n=10	-0.503 P=0.1383 n=10	-0.503 P=0.1383 n=10
Ankle ISOM DF	0.818 P=0.0038 n=10	0.818 P=0.0038 n=10	0.794 P=0.0061 n=10	0.176 P=0.6272 n=10	0.176 P=0.6272 n=10	-0.261 P=0.4671 n=10	-0.261 P=0.4671 n=10

Table 83: Correlation between baseline strength measurements and spatiotemporal parameters.

Appendix IV: Exercise Induced Pain Management

Pain levels can be an indication of musculoskeletal injury or working at an intensity that is too high for the individual's ability and level of fitness. The perception of pain is a very personal thing and that the visual analogue scale (VAS) rating is very subjective. Participants were given the relevant information and support to help them take the decision whether or not to stop rather than the researcher taking the unsolicited decision for them based on a formal criterion. It is, however, important to distinguish between a temporary increase in background pain levels during training versus new musculoskeletal pain signifying injury.

The following steps were taken to ensure these points are clear to participants from the outset:

1. VAS level of pain was explored at the consent meeting and some discussion had about how it will be managed when doing exercise.
2. VAS levels of 4 and above when doing exercise triggered a discussion with the trial physiotherapist around the participant's pain levels and if they feel it is appropriate to continue.
3. All means of support or modification (e.g. alternative position for the equipment to see if it reduces strain and makes them more comfortable) will be explored and implemented if it helps to reduce pain levels whilst maintaining the ability to train.

4. Anyone reported VAS level 7 or above during training were informed that this was set as a cut-off point because we were concerned that pain levels as high as this might indicate that damage is occurring. However, if there is no evidence that this is the case and so, after discussion with their therapist, we let the participants decide if they wish to continue.
5. The one exception to point 4 would be where pain greater than 7/10 continues for more than a couple of hours afterwards (most people with higher levels report it decreases as soon as they stop exercising) or if it is a new, acute onset of a different pain than what they normally experience. Either of these events might indicate injury to musculoskeletal structures and would prompt a full assessment by an experienced trial physiotherapist. If no significant injury is found, the training will be stopped for two weeks and then gradually restarted starting at two-thirds of the intensity first started. Exercises were definitely stopped if musculoskeletal pain occurs again after restarting the regime (challenge/re-challenge principle).
6. Finally, our management of delayed onset muscle soreness (DOMS) will remain the same i.e. we expect some participants to report mild to moderate DOMS, this is a normal response to new exercise, but this should not last longer than 48 hours and should not be severe or debilitating (they should be able to carry out their normal activities).

If pain cannot be explained by DOMS, the research physiotherapist will perform a full neuro-musculoskeletal assessment. If significant musculoskeletal injury was found on assessment the participant is removed from the trial and referred to their GP or local accident and emergency department for appropriate treatment. The methods and timing for assessing, recording, and analysing safety parameters were then followed by the research physiotherapist.

Appendix V: Exercise Program

PHYSIOTOOLS

Personal exercise program

MRI and Gait in dHMN

The National Hospital for Neurology & Neurosurgery

The National Hospital for Neurology & Neurosurgery
Queen Square, WC1N 3BG, London, United Kingdom

Provided by Aljwhara Alangary

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Warm Up

Use cardiovascular and body exercises in the following order for warm up:

1) Cardiovascular exercises: 5 to 10 minutes with moderate intensity!

2) Flexibility exercises: Start stretching exercises by carefully entering the stretch position and pause at a moderate stretch intensity for about 4 breaths. Relax all muscles as much as you can and concentrate on the muscle being stretched. Carefully increase the stretch while relaxing all non-involved muscles.

3) Resistance exercises: Always perform last! Try these exercises at least every other day.
Start light with the resistance and you can gradually increase the resistance as it gets easier.
Start with 2 sets of 8 repetitions (with a 1-2 minute rest between sets) and gradually increase the number of repetitions by 1-2 per week until you can do 3 sets of 15. If you are increasing the resistance (Weight), drop back to 2 sets of 8 and gradually increase again.

1

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Jogging in Place

Start by standing on a flat surface.

Start jogging/running in place.

Continue for 5-10 minute.

2

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Stand holding on to a support with one hand and to the ankle with the other hand.

Pull the ankle towards your bottom, then try to straighten the knee approx. 10 secs. while resisting with your hand. Relax your leg and repeat the exercise pulling the ankle up a bit further. Return to starting position.

Repeat for the other side.

3

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Stand in a walking position with the leg to be stretched straight behind you and the other leg bent in front of you. Take support from a wall or chair.

Lean your body forwards and down until you feel the stretching in the calf of the straight leg. Hold approx. 10 secs. - relax. Stretch the other leg.

4

©Physiotools

Sit on side of bench with on leg bent and one straight.

Lean forwards from pelvis to feel stretch at back of thigh. Hold for 10 sec.

Repeat for the other side

5

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Sit. Put a weight on top of your foot.

Bend the ankle up and down.

2 sets of 8 repetitions (with a 1-2 minute rest between sets)

6

©PhysioTools

Put one knee on a chair. Ankle weight is wrapped around the foot.

Bend the foot back.

2 sets of 8 repetitions (with a 1-2 minute rest between sets)

7

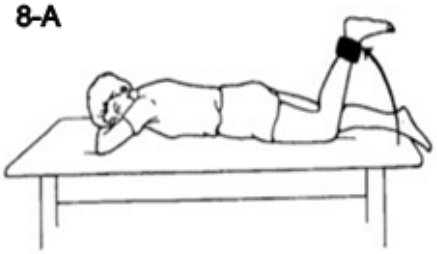
©Physiotools

Sit up straight on a chair, with a weight attached to your ankle.

Fully straighten your knee. Return back to the starting position in a controlled manner.

2 sets of 8 repetitions (with a 1-2 minute rest between sets)

8-A



Start by lying on your front with a weight around your ankle.

Tighten your buttocks, keep your hips straight. Try to bend the knee and bring your foot towards the buttocks. Return back to the starting position in a controlled manner.

2 sets of 8 repetitions (with a 1-2 minute rest between sets)

8-B

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Stand up with a weight around your ankle. Support yourself on a chair.

Tighten your buttocks, keep your hips straight. Try to bend the knee and bring your foot towards the buttocks. Return back to the starting position in a controlled manner.

2 sets of 8 repetitions (with a 1-2 minute rest between sets)

9

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Start by lying on your back.

Bend your knee and bring it towards your chest. Lower the leg to the starting position in a controlled manner.

Note: Keep your pelvis level and maintain neutral spine.

2 sets of 8 repetitions (with a 1-2 minute rest between sets)

10

©Physiotools

Lie face down on a table, with hips over the edge and toes on the floor. Ankle weight attached to one ankle.

Straighten your hip. Focus on using your buttocks and control the neutral position of your lower back. Return to the starting position.

2 sets of 8 repetitions (with a 1-2 minute rest between sets)

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Cool Down

Use cardiovascular and flexibility exercises for Cool down!

The main purpose of the cool down is to relax the increased muscle tension caused by resistance training, lower the heart rate, and reduce metabolic fatigue

Cardiovascular exercises: 5 minutes at a very low intensity level!

Flexibility exercises: Use a very comfortable position with a soft stretch. Concentrate on long exhalations and completely relax while stretching your muscles!

11

Dynamic Anterior and Posterior Chain Stretch

Start in half-kneeling. Bend yourself over the front leg and take support from the floor.

Shift your weight backwards and let the leg in front straighten. Feel the stretch at the back of the thigh and knee. Then shift your weight forwards, so that the hip of the rear leg straightens. Push your pelvis towards the floor and feel the stretch at the front of your hip. Continue shifting your weight back and forth.

Repeat 3 times for each side.

12

Stand holding on to a support with one hand and to the ankle with the other hand.

Pull the ankle towards your bottom, then try to straighten the knee approx. 15 secs. while resisting with your hand. Relax your leg and repeat the exercise pulling the ankle up a bit further. Return to starting position.

Repeat 3 times for each side.

13

Stand in a walking position with the leg to be stretched straight behind you and the other leg bent in front of you. Take support from a wall or chair.

Lean your body forwards and down until you feel the stretching in the calf of the straight leg. Hold approx. 15 secs. - relax. Stretch the other leg.

Repeat 3 times for each side.

Appendix VI : Kinematic and Kinetic Gait Measurements of the Exercising Participants

KINEMATICS	BASELINE		6 MONTHS		12 MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Pelvis X Max	4.17 (1.06)	4.14 (0.72)	4.84 (1.83)	4.01 (1.32)	5.16 (2.49)	4.19 (0.92)
Pelvis X Min	-4.41 (1.39)	-4.32 (1.05)	-5.3 (2.44)	-4.01 (1.45)	-5.75 (2.24)	-4.39 (0.96)
Pelvis Y Max	16.23 (6.26)	14.36 (6.05)	15.6 (6.28)	16.64 (3.51)	19.61 (4.61)	15.83 (4.52)
Pelvis Y Min	11.71 (4.46)	9.18 (4.99)	10.0 (4.94)	11.41 (3.04)	13.23 (2.82)	11.14 (3.67)
Pelvis Z Max	7.19 (3.33)	6.51 (2.44)	7.90 (4.61)	6.46 (1.76)	9.20 (4.71)	6.23 (3.55)
Pelvis Z Min	-7.66 (3.99)	-6.37 (2.67)	-8.2 (4.52)	-5.64 (1.40)	-8.04 (4.56)	-7.21 (2.11)
Hip Max	49.27 (4.79)	44.59 (5.85)	46.1 (9.31)	46.72 (2.02)	51.39 (4.76)	47.51 (3.98)
Hip Min	2.94 (4.27)	4.26 (4.85)	-2.0 (4.61)	3.70 (4.35)	0.36 (3.08)	4.86 (2.58)
Knee Max	75.08 (4.09)	72.20 (5.22)	73.6 (3.47)	75.13 (3.34)	73.37 (3.86)	74.62 (3.84)
Knee Min	5.45 (5.58)	8.10 (5.15)	2.55 (3.92)	8.43 (5.03)	0.51 (5.61)	9.77 (5.76)
Ankle Max	26.86 (4.78)	22.76 (6.64)	24.9 (6.08)	25.51 (3.29)	27.24 (7.24)	23.64 (3.26)
Ankle Min	-10.26 (2.95)	-11.83 (4.87)	-9.6 (4.82)	-12.33 (7.47)	-9.46 (3.45)	-14.08 (6.81)
KINEMATICS DIFFERENCE	BASELINE-6 MONTHS		6MONTHS-12MONTHS		BASELINE-12MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Pelvis X Max	1.5 (3.42)	-0.13 (0.7)	0.33 (0.94)	0.17 (1.03)	1.83 (4.24)	0.05 (0.84)
Pelvis X Min	-1.78 (4)	0.31 (1.3)	-0.45 (0.93)	-0.39 (1.39)	-2.22 (3.57)	-0.07 (0.34)
Pelvis Y Max	2.63 (12)	2.28 (3.6)	4.00 (5.83)	-0.82 (3.8)	6.63 (12.92)	1.46 (5.07)
Pelvis Y Min	0.66 (9)	2.23 (3.6)	3.20 (5.40)	-0.27 (4.17)	3.86 (8.99)	1.96 (4.61)
Pelvis Z Max	2.1 (2.95)	-0.05 (1.7)	1.31 (2.89)	-0.23 (2.69)	3.45 (5.26)	-0.28 (1.80)
Pelvis Z Min	-2.08 (3)	0.72 (1.6)	0.17 (0.87)	-1.57 (1.37)	-1.91 (3.81)	-0.84 (1.55)
Hip Max	6.73 (27)	2.12 (5.7)	5.24 (6.99)	0.8 (5.31)	11.98 (26.81)	2.92 (5.54)
Hip Min	-4. (5.78)	-0.56 (5.9)	2.46 (6.95)	1.17 (4.67)	-1.99 (6.16)	0.60 (6.74)
Knee Max	13.53 (36)	2.93 (4.7)	-0.22 (2.05)	-0.51(3.51)	13.31 (37.12)	2.41 (4.49)
Knee Min	-1.82 (3)	0.33 (2.6)	-2.03 (5.63)	1.34 (1.31)	-3.85 (7.26)	1.67 (3.52)
Ankle Max	3.45 (14)	2.75 (4.8)	2.30 (5.26)	-1.87 (1.5)	5.75 (11.85)	0.88 (3.96)
Ankle Min	-1. (2.03)	-0.50 (5.2)	0.21 (2.82)	-1.75 (5.99)	-1.25 (2.89)	-2.25 (2.54)

Pelvic z Maximum= Rotation forwards, Pelvic z Minimum= Rotation backwords, Pelvic x Maximum= lateral raise, Pelvic x Minimum= lateral drop, Pelvic y Maximum= Anterior tilt, Pelvic y Minimum = Posterior tilt, Hip y Maximum= Flexion, Hip y Minimum= Extension, Knee y Maximum= Flexion, Knee y Minimum= Extension, Ankle y Maximum= Dorsiflexion, Ankle y Minimum= Plantarflexion, Data presented as: Mean (standers deviation).

Table 84: Longitudinal kinematic gait parameters and differences between measurement points in exercise group (n=5) and no exercise group (n=5).

KINEMATICS	BASELINE				6 MONTHS				12 MONTHS			
	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group
Pelvis X Max		4.72	4.08	0.85	7.52	5.64	3.89	1.11	9.12	6.13	3.94	0.79
Pelvis X Min		-5.18	-4.26	1.16	-8.71	-6.80	-3.88	1.22	-7.96	-8.34	-4.30	0.82
Pelvis Y Max		10.73	15.75	5.97	21.47	19.94	14.99	4.72	27.81	16.72	16.58	3.58
Pelvis Y Min		6.98	10.72	4.80	13.60	15.16	9.80	3.87	17.25	11.97	11.58	3.11
Pelvis Z Max		4.97	7.04	2.78	6.19	4.80	7.60	3.67	12.00	7.34	7.23	4.49
Pelvis Z Min		-3.87	-7.33	3.15	-7.08	-5.33	-7.11	3.84	-7.82	-5.79	-7.83	3.76
Hip Max		45.58	46.81	6.01	53.21	55.22	44.49	5.48	58.91	52.93	47.84	3.15
Hip Min		-0.38	4.18	4.38	0.19	2.94	0.61	5.84	3.08	-2.37	3.18	3.54
Knee Max		75.44	73.24	4.97	78.68	73.42	73.94	3.33	79.51	69.82	73.83	3.26
Knee Min		10.58	6.47	5.35	2.81	8.29	5.47	5.78	1.59	6.51	5.41	8.09
Ankle Max		28.56	24.09	6.15	29.47	26.55	24.52	4.90	25.23	36.60	24.07	4.55
Ankle Min		-6.67	-11.69	3.86	-3.73	-7.54	-12.35	6.03	-5.62	-5.82	-13.29	5.28
KINEMATICS DIFFERENCE	BASELINE-6 MONTHS				6MONTHS-12MONTHS				BASELINE-12MONTHS			
	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group
Pelvis X Max	7.52	0.92	-0.20	0.49	1.60	0.48	0.05	0.78	9.12	1.40	-0.14	0.99
Pelvis X Min	-8.71	-1.62	0.38	0.65	0.75	-1.53	-0.42	0.61	-7.96	-3.16	-0.05	0.71
Pelvis Y Max	21.47	9.21	-0.77	5.46	6.34	-3.22	1.60	5.82	27.81	5.99	0.83	5.32
Pelvis Y Min	13.60	8.18	-0.92	5.51	3.65	-3.19	1.78	5.52	17.25	4.99	0.86	4.83
Pelvis Z Max	6.19	-0.18	0.56	1.97	5.81	2.55	-0.37	2.16	12.00	2.37	0.19	2.33
Pelvis Z Min	-7.08	-1.45	0.22	1.88	-0.73	-0.46	-0.72	1.62	-7.82	-1.92	-0.50	1.93
Hip Max	53.21	9.64	-2.32	8.21	5.70	-2.29	3.35	7.32	58.91	7.35	1.03	5.89
Hip Min	0.19	3.32	-3.57	6.57	2.89	-5.32	2.57	6.07	3.08	-2.00	-1.00	7.29
Knee Max	78.68	-2.02	0.70	5.17	0.82	-3.60	-0.11	1.61	79.51	-5.61	0.59	4.97
Knee Min	2.81	-2.29	-0.99	3.34	-1.22	-1.78	-0.06	5.13	1.59	-4.07	-1.05	7.31
Ankle Max	29.47	-2.01	0.44	5.91	-4.23	10.06	-0.46	2.67	25.23	8.05	-0.02	3.88
Ankle Min	-3.73	-0.87	-0.66	4.01	-1.89	1.72	-0.94	4.64	-5.62	0.85	-1.60	2.41

Pelvic z Maximum= Rotation forwards, Pelvic z Minimum= Rotation backwords, Pelvic x Maximum= lateral raise, Pelvic x Minimum= lateral drop, Pelvic y Maximum= Anterior tilt, Pelvic y Minimum = Posterior tilt, Hip y Maximum= Flexion, Hip y Minimum= Extension, Knee y Maximum= Flexion, Knee y Minimum= Extension, Ankle y Maximum= Dorsiflexion, Ankle y Minimum= Plantarflexion, No Exercise Group (n=8).

Table 85: Longitudinal kinematic gait parameters and differences between measurement points in case 1, case 2, and no exercise group.

KINETICS	BASELINE		6 MONTHS		12 MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Hip Moment Y Max	1.57 (0.63)	1.25 (0.36)	1.74 (0.72)	1.23 (0.37)	1.85 (0.88)	1.24 (0.62)
Hip Moment Y Min	-0.51 (0.24)	-0.47 (0.14)	-0.5 (0.19)	-0.99 (1.06)	-0.69 (0.34)	-0.53 (0.18)
Knee Moment Y Max	0.67 (0.45)	0.64 (0.23)	0.78 (0.42)	1.35 (1.67)	0.80 (0.42)	0.79 (0.24)
Knee Moment Y Min	-0.84 (0.21)	-0.47 (0.19)	-0.8 (0.29)	-0.52 (0.22)	-0.89 (0.35)	-0.54 (0.27)
Ankle Moment Y Max	1.09 (0.28)	0.95 (0.28)	1.13 (0.27)	0.76 (0.48)	1.13 (0.27)	0.99 (0.29)
Ankle Moment Y Min	-0.12 (0.10)	-0.03 (0.02)	-0.1 (0.08)	-0.72 (1.55)	-0.13 (0.10)	-0.02 (0.01)
Hip Power Max Swing	0.95 (0.32)	0.95 (0.21)	1.08 (0.46)	1.09 (0.23)	1.00 (0.30)	1.00 (0.36)
Hip Power Min Stance	-1.21 (0.89)	-0.53 (0.32)	-1.3 (0.78)	-1.53 (1.93)	-1.99 (1.21)	-0.63 (0.43)
Hip Power Max Stance	1.84 (0.83)	1.59 (0.53)	2.06 (1.03)	2.04 (0.83)	2.62 (1.29)	1.63 (0.76)
Knee Power Max Swing	0.37 (0.11)	0.30 (0.08)	0.48 (0.27)	0.48 (0.41)	0.56 (0.38)	0.35 (0.13)
Knee Power Min Stance	-1.68 (1.19)	-1.32 (0.82)	-1.7 (0.93)	-3.57 (5.32)	-2.04 (1.14)	-1.81 (1.10)
Knee Power Max Stance	1.90 (0.93)	0.88 (0.44)	2.55 (1.56)	1.65 (1.47)	2.65 (1.70)	1.08 (0.78)
Ankle Power Max Swing	0.05 (0.03)	0.03 (0.01)	0.08 (0.04)	0.04 (0.02)	0.13 (0.10)	0.03 (0.02)
Ankle Power Min Stance	-1.18 (0.25)	-1.03 (0.42)	-1.2 (0.32)	-1.85 (1.84)	-1.35 (0.30)	-1.04 (0.38)
Ankle Power Max Stance	1.29 (0.63)	0.88 (0.11)	1.17 (0.53)	2.69 (3.41)	1.22 (0.54)	1.11 (0.13)
KINETICS DIFFERENCE	BASELINE-6 MONTHS		6MONTHS-12MONTHS		BASELINE-12MONTHS	
	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)	Exercise (N=5)	No Exercise (N=5)
Hip Moment Y Max	0.4 (1.06)	-0.03 (0.2)	0.10 (0.23)	0.01 (0.61)	0.59 (1.29)	-0.01 (0.34)
Hip Moment Y Min	-0. (0.23)	-0.52 (1.0)	-0.10 (0.24)	0.46 (0.96)	-0.28 (0.24)	-0.06 (0.08)
Knee Moment Y Max	0.24 (0)	0.71 (1.5)	0.02 (0.14)	-0.56 (1.58)	0.26 (0.39)	0.15 (0.24)
Knee Moment Y Min	-0. (0.50)	-0.04 (0.1)	-0.01 (0.08)	-0.02 (0.22)	-0.22 (0.54)	-0.06 (0.19)
Ankle Moment Y Max	0.2 (0.51)	-0.19 (0.5)	0.00 (0.09)	0.24 (0.59)	0.26 (0.55)	0.04 (0.12)
Ankle Moment Y Min	-0. (0.06)	-0.68 (1.5)	-0.02 (0.02)	0.70 (1.54)	-0.03 (0.06)	0.01 (0.02)
Hip Power Max Swing	0.32 (0)	0.14 (0.2)	-0.08 (0.28)	-0.09 (0.27)	0.24 (0.55)	0.05 (0.17)
Hip Power Min Stance	-0. (0.35)	-1.00 (2.0)	-0.67 (0.88)	0.90 (1.91)	-1.02 (1.17)	-0.09 (0.31)
Hip Power Max Stance	0.59 (1)	0.46 (0.3)	0.56 (0.83)	-0.41 (0.33)	1.15 (1.55)	0.05 (0.26)
Knee Power Max Swing	0.18 (0)	0.18 (0.4)	0.08 (0.30)	-0.14 (0.34)	0.26 (0.53)	0.04 (0.12)
Knee Power Min Stance	-0. (0.91)	-2.26 (5.2)	-0.32 (0.43)	1.77 (5.04)	-0.70 (0.62)	-0.49 (0.80)
Knee Power Max Stance	1.03 (2)	0.78 (1.2)	0.10 (0.58)	-0.57 (0.79)	1.13 (2.26)	0.20 (0.54)
Ankle Power Max Swing	0.04 (0)	0.02 (0.0)	0.05 (0.12)	-0.01 (0.02)	0.09 (0.11)	0.00 (0.01)
Ankle Power Min Stance	-0. (0.80)	-0.82 (1.9)	-0.07 (0.18)	0.80 (1.79)	-0.41 (0.67)	-0.02 (0.20)
Ankle Power Max Stance	0.14 (0)	1.80 (3.4)	0.05 (0.16)	-1.58 (3.43)	0.19 (0.56)	0.22 (0.17)

Hip Moments Maximum= Extension, Hip Moments Minimum= Flexion, Knee Moments Maximum= Extension, Knee Moments Minimum= Flexion, Ankle Moments Maximum= Plantarflexion, Ankle Moments Minimum= Dorsiflexion, Hip Power Maximum = generation in swing phase, Hip Power Minimum= absorption in stance phase, Hip Power Maximum= generation in stance phase, Knee Power Maximum= generation in stance phase, Knee Power Maximum= generation in swing phase, Knee Power Minimum = absorption in stance phase, Ankle Power Maximum= generation in stance phase, Ankle Power Minimum= absorption in stance phase, Ankle Power Maximum= generation in swing phase, Data presented as: Mean (standers deviation).

Table 86: Longitudinal kinetic gait parameters and differences between measurement points in exercise group (n=5) and no exercise group (n=5).

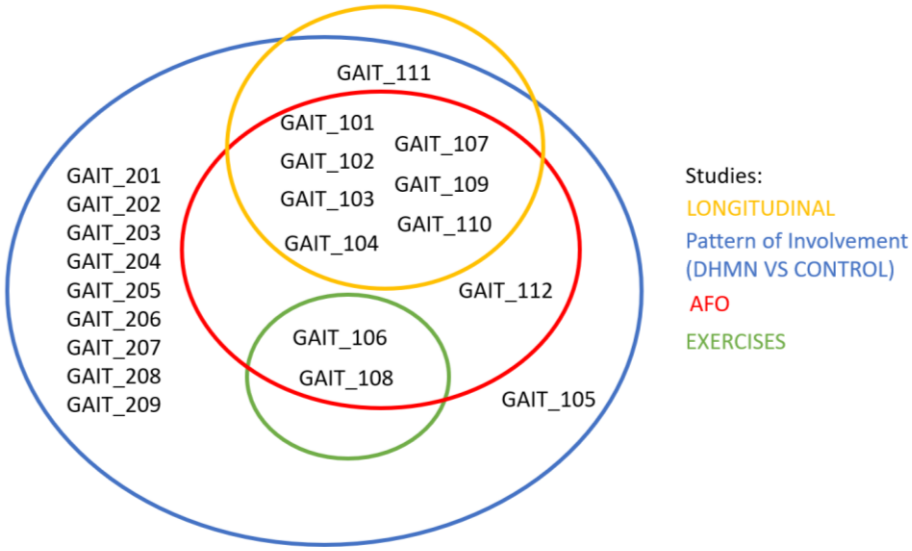
KINETICS	BASELINE				6 MONTHS				12 MONTHS			
	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group
Hip Moment Y Max		2.23	1.29	0.41	2.37	2.23	1.28	0.48	2.88	2.28	1.28	0.61
Hip Moment Y Min		-0.76	-0.45	0.16	-0.54	-0.91	-0.81	0.84	-0.62	-1.01	-0.56	0.26
Knee Moment Y Max		1.21	0.58	0.26	1.00	1.18	1.06	1.34	0.89	1.22	0.73	0.32
Knee Moment Y Min		-0.94	-0.60	0.26	-1.09	-0.91	-0.62	0.30	-1.14	-0.84	-0.65	0.35
Ankle Moment Y Max		1.33	0.97	0.26	1.17	1.40	0.86	0.42	1.22	1.45	1.00	0.26
Ankle Moment Y Min		-0.26	-0.05	0.04	-0.10	-0.25	-0.47	1.22	-0.10	-0.29	-0.04	0.05
Hip Power Max Swing		1.37	0.90	0.20	1.66	1.41	0.97	0.27	1.17	1.19	0.95	0.33
Hip Power Min Stance		-1.58	-0.74	0.67	-0.91	-1.77	-1.44	1.56	-2.93	-2.82	-0.91	0.85
Hip Power Max Stance		2.39	1.61	0.63	3.10	3.02	1.80	0.80	3.72	2.84	1.84	1.05
Knee Power Max Swing		0.53	0.31	0.06	0.64	0.86	0.41	0.33	1.20	0.60	0.34	0.11
Knee Power Min Stance		-2.64	-1.33	0.91	-1.97	-2.37	-2.77	4.22	-1.71	-3.10	-1.80	1.11
Knee Power Max Stance		2.87	1.14	0.66	4.71	3.16	1.64	1.24	4.97	2.44	1.41	1.10
Ankle Power Max Swing		0.06	0.04	0.02	0.06	0.07	0.06	0.04	0.06	0.06	0.09	0.10
Ankle Power Min Stance		-1.24	-1.07	0.36	-1.75	-1.27	-1.58	1.45	-1.57	-1.57	-1.11	0.35
Ankle Power Max Stance		1.43	1.02	0.46	0.76	1.65	2.11	2.72	0.99	1.74	1.11	0.35
KINETICS DIFFERENCE	BASELINE-6 MONTHS				6MONTHS-12MONTHS				BASELINE-12MONTHS			
	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group	Case 1	Case 2	Mean No Exercise Group	SD No Exercise Group
Hip Moment Y Max	2.37	0.01	-0.01	0.14	0.51	0.04	0.00	0.24	2.88	0.05	-0.01	0.19
Hip Moment Y Min	-0.54	-0.15	-0.36	0.08	-0.08	-0.10	0.25	0.21	-0.62	-0.25	-0.11	0.15
Knee Moment Y Max	1.00	-0.03	0.47	0.11	-0.11	0.04	-0.33	0.15	0.89	0.01	0.14	0.23
Knee Moment Y Min	-1.09	0.02	-0.03	0.13	-0.04	0.08	-0.02	0.11	-1.14	0.10	-0.05	0.13
Ankle Moment Y Max	1.17	0.07	-0.11	0.09	0.05	0.05	0.13	0.09	1.22	0.12	0.03	0.14
Ankle Moment Y Min	-0.10	0.01	-0.42	0.03	0.00	-0.04	0.43	0.02	-0.10	-0.03	0.01	0.04
Hip Power Max Swing	1.66	0.04	0.08	0.21	-0.49	-0.21	-0.02	0.23	1.17	-0.18	0.06	0.13
Hip Power Min Stance	-0.91	-0.19	-0.70	0.27	-2.02	-1.05	0.53	0.30	-2.93	-1.24	-0.17	0.32
Hip Power Max Stance	3.10	0.64	0.19	0.65	0.63	-0.19	0.03	0.92	3.72	0.45	0.23	0.58
Knee Power Max Swing	0.64	0.33	0.10	0.09	0.56	-0.26	-0.07	0.06	1.20	0.07	0.03	0.10
Knee Power Min Stance	-1.97	0.28	-1.43	0.38	0.26	-0.74	0.96	0.53	-1.71	-0.46	-0.47	0.66
Knee Power Max Stance	4.71	0.30	0.50	0.30	0.26	-0.73	-0.24	0.47	4.97	-0.43	0.27	0.54
Ankle Power Max Swing	0.06	0.01	0.03	0.05	0.00	-0.01	0.03	0.10	0.06	0.00	0.05	0.10
Ankle Power Min Stance	-1.75	-0.03	-0.50	0.31	0.18	-0.29	0.47	0.24	-1.57	-0.32	-0.03	0.17
Ankle Power Max Stance	0.76	0.22	1.09	0.28	0.23	0.09	-1.00	0.10	0.99	0.31	0.09	0.32

Hip Moments Maximum= Extension, Hip Moments Minimum= Flexion, Knee Moments Maximum= Extension, Knee Moments Minimum= Flexion, Ankle Moments Maximum= Plantarflexion, Ankle Moments Minimum= Dorsiflexion, Hip Power Maximum = generation in swing phase, Hip Power Minimum= absorption in stance phase, Hip Power Maximum= generation in stance phase, Knee Power Maximum= generation in stance phase, Knee Power Maximum= generation in swing phase, Knee Power Minimum = absorption in stance phase, Ankle Power Maximum= generation in stance phase, Ankle Power Minimum= absorption in stance phase, Ankle Power Maximum= generation in swing phase. , No Exercise Group (n=8).

Table 87: Longitudinal kinetic gait parameters and differences between measurement points in case 1, case 2, and no exercise group.

Appendix VII: Participants Study Allocation and Visits Activities

	Visit1: Baseline			Visit 2: 6 Months			Visit 3: 12 Months		
	MRI	3D Motion Analysis	Dynamom etry	MRI	3D Motion Analysis	Dynamo metry	MRI	3D Motion Analysis	Dynamo metry
GAIT_101	✓	✓	✓	✓	✓	✓	✓	✓	✓
GAIT_102	✓	✓	✓	✓	✓	✓	✓	✓	✓
GAIT_103	✓	✓	✓	✓	✓	✓	✓	✓	✓
GAIT_104	✓	✓	✓	✓	✓	✓	✓	✓	✓
GAIT_105	✓	✗	✗	✗	✗	✗	✗	✗	✗
GAIT_106	✓	✓	✓	✓	✓	✓	✓	✓	✓
GAIT_107	✓	✓	✓	✓	✓	✓	✓	✓	✓
GAIT_108	✓	✓	✓	✓	✓	✓	✓	✓	✓
GAIT_109	✓	✓	✓	✓	✓	✓	✓	✓	✓
GAIT_110	✓	✓	✓	✓	✓	✓	✓	✓	✓
GAIT_111	✓	✓	✓	✓	✓	✓	✗	✗	✗
GAIT_112	✗	✓	✓	✗	✗	✗	✗	✗	✗
GAIT_201	✓	✓	✓	✗	✗	✗	✗	✗	✗
GAIT_202	✓	✓	✓	✗	✗	✗	✗	✗	✗
GAIT_203	✓	✓	✓	✗	✗	✗	✗	✗	✗
GAIT_204	✓	✓	✓	✗	✗	✗	✗	✗	✗
GAIT_205	✓	✓	✓	✗	✗	✗	✗	✗	✗
GAIT_206	✓	✓	✓	✗	✗	✗	✗	✗	✗
GAIT_207	✓	✓	✓	✗	✗	✗	✗	✗	✗
GAIT_208	✓	✓	✓	✗	✗	✗	✗	✗	✗
GAIT_209	✓	✓	✓	✗	✗	✗	✗	✗	✗
GAIT_100= DHMN, GAIT_200= Control, ✓= Completed, ✗= Not completed.									



Appendix VIII: Published Abstracts Related to This PhD

Gait Kinetics and Kinematics in Distal Hereditary Motor Neuropathy, Longitudinal study, (2023), PNS Abstracts 2023. J Peripher Nerv Syst, 28: S3-S254. https://doi.org/10.1111/jns.12585

Muscle Structure, Function, and Gait Patterns in Distal Hereditary Motor Neuropathy and The Effect of Carbon Fiber Ankle Foot Orthosis on Gait, (2022), PNS 2022 Abstract Supplement. J Peripher Nerv Syst, 27: S4-S183. https://doi.org/10.1111/jns.12506

Muscle Structure, Function, and Gait Patterns in Distal Hereditary Motor Neuropathy and the Effect of Carbon fiber Ankle Foot Orthosis on Gait, Authors, C. (2022) "NMSG 2022 Abstracts and Meeting Information", RRNMF Neuromuscular Journal, 3(3). doi: https://doi.org/10.17161/rrnmf.v3i3.19210

Effect of Distal Hereditary Motor Neuropathy on Muscle Structure, Function, and Gait Patterns: Two Case Reports, (2021), 2021 Peripheral Nerve Society virtual event. J Peripher Nerv Syst, 26: 307-440. https://doi.org/10.1111/jns.12460

Effect of Distal Hereditary Motor Neuropathy on Muscle Structure, Function, and Gait Patterns: Two Case Reports, Russo Paulk, L. (2021) "MSG 2021 Meeting Information and Abstracts ", RRNMF Neuromuscular Journal, 2(4), pp. 45–98. doi: https://doi.org/10.17161/rrnmf.v2i4.15849

Natural History and the Effect of Rehabilitation Interventions in Motor Neuropathy (Protocol), (2020), 2020 Peripheral Nerve Society Virtual Event. J Peripher Nerv Syst, 25: 438-576. https://doi.org/10.1111/jns.12416

Exploring Muscle Structure, Function, and Gait Patterns in People With Distal Hereditary Motor Neuropathy: Natural History and the Effect of Rehabilitation Interventions, Study Protocol. Barohn, R. J. (2020) "Abstracts from the 2020 Muscle Study Group Annual Scientific Meeting", RRNMF Neuromuscular Journal, 1(4), pp. 35–77. doi: https://doi.org/10.17161/rrnmf.v1i4.14646