UNIVERSITY of NICOSIA

Biomechanical and functional assessment of Multiple Sclerosis patients before and after supplementation with omega 3 and omega 6 fatty acids and antioxidant vitamins

Panayiotis Aristotelous

A thesis submitted to the University of Nicosia

in accordance with the requirements of the degree of

PhD in Physiotherapy

Department of Life and Health Sciences

School of Sciences and Engineering

January/ 2021

Abstract

Background: Multiple Sclerosis (MS) is a chronic, progressive inflammatory and neurodegenerative disease of the Central Nervous System (CNS) characterized by demyelination and axon loss resulting in a variety of motor symptoms and disabilities. The primary aim of the current study was to examine the effects of a 24 months supplementation (Neuroaspis PLP10) with a formula containing high dose of omega 3 and omega 6 polyunsaturated fatty acids and antioxidant vitamins on biomechanical and functional capacity parameters in PwMS. Secondary aims were to examine the effects of the above intervention in various quality of life and health-related parameters using the appropriate methodology.

Methodology: Fifty-one relapsing-remitting MS patients (age: 38.4 ± 7.1 yrs; 30 Female; EDSS: 2.38 ± 1.04) recruited and agreed to participate in this study. The assessment of muscle function in the upper extremities assessed using a handgrip dynamometer (Takei, Tokyo, Japan), while muscle function in the lower extremities assessed using an isokinetic dynamometer (model 770, CSMI Humac Norm, MA, USA). The evaluation of muscle fatigue assessed using an EMG (Trigno, Delsys, Natick, MA, USA). Spatiotemporal and kinematic parameters, such as the Gait deviation index (GDI) assessed using Vicon Nexus motion capture system (Vicon Motion Systems Ltd, Oxford, UK). Functional capacity examined using various functional tests commonly used in MS patients such as the six minutes' walk test, two Sit to Stand tests and the Timed up and Go test. Finally, the patients' health-related quality of life, sleep quality, depression, daily time sleepiness, cognition and fatigue levels evaluated using specific questionnaires. The treating neurologist clinically examined the expanded disability status scale (EDSS).

Results: The supplement had a positive effect in some spatiotemporal parameters of gait, such as the single support time ($p<0.05$), step and stride time ($p<0.05$) in the PLP10 group. Although due to the limited deviation from normal gait, there is small room for improvement, there were some promising positive changes in the spatiotemporal parameters. The supplement did not affect any of the kinematic parameters ($p>0.05$), in the sagittal, frontal and transverse plane. GDI showed some impressive group by time interaction. Precisely, while GDI of the placebo group decreases between the baseline and the 2 year follow up by about 10% on average across legs, the opposite happens in the PLP10 group. The GDI in the PLP10 group increased by about 4% on average across both legs in the same period ($p<0.05$). This outcome shows a promising significant positive effect of the medication in the gait of the PLP10 group. Moreover, the

supplement had a positive effect on some tests of functional capacity such as the STS test ($p<0.05$). A tendency of more significant improvement in the PLP10 group in contrast with the placebo group was found on the 6MWT and the TUG test. In contrast, the supplement did not have any effect in handgrip strength ($p>0.05$), knee extension and flexion strength ($p>0.05$), or fatigue ($p>0.05$). In addition, the supplement did not affect any of the quality of life parameters such as fatigue, sleep quality, depression, daytime sleepiness and cognition in the PLP10 group ($p>0.05$). Finally, the disability scores remained stable in the supplement group (PLP10) over the two years intervention period.

Conclusion: The effects of a 24 months supplementation with a formula containing high dose of omega 3 and omega 6 polyunsaturated fatty acids and antioxidant vitamins improved some spatiotemporal parameters of gait, GDI and some aspects of functional capacity whilst its maintained disability status. It seems that supplementation with a high dose of omega 3 and omega 6 free fatty acids could be a promising approach in terms of improving functional capacity, some gait parameters and maintain disability status in MS patients with low disability scores.

Key words: Multiple Sclerosis, strength, biomechanics, gait, functional capacity, quality of life

Περίληψη

Υπόβαθρο: Η Πολλαπλή Σκλήρυνση (ΠΣ) είναι μια χρόνια, προοδευτικά φλεγμονώδης και νευροεκφυλιστική ασθένεια του Κεντρικού Νευρικού Συστήματος (ΚΝΣ). Χαρακτηρίζεται από απομυελίνωση και απώλεια των αξόνων των νευρικών κυττάρων με αποτέλεσμα σωρεία κινητικών συμπτωμάτων και αναπηριών. Ο πρωταρχικός στόχος της τρέχουσας διατριβής ήταν να εξετάσει την επίδραση ενός διατροφο – φαρμακευτικού συμπληρώματος (Neuroaspis PLP10) για διάρκεια 24 μηνών, το οποίο περιέχει υψηλή ποσότητα ωμέγα 3 και ωμέγα 6 πολυακόρεστων λιπαρών οξέων και αντιοξειδωτικών βιταμινών σε εμβιομηχανικές παραμέτρους και στην λειτουργική ικανότητα σε άτομα με ΠΣ. Δευτερεύοντες στόχοι ήταν να εξεταστούν οι επιπτώσεις της παραπάνω παρέμβασης σε διάφορες παραμέτρους ποιότητας ζωής και υγείας, χρησιμοποιώντας την κατάλληλη μεθοδολογία.

Μεθοδολογία: Πενήντα ένας ασθενείς με υποτροπιάζουσα-διαλειπούσα ΠΣ (ηλικία: 38,4 ± 7,1 χρονών, 30 γυναίκες, Κατάσταση Ασθενείας: 2,38 ± 1,04) συμφώνησαν να συμμετέχουν στη μελέτη αυτή. Η αξιολόγηση της μυϊκής λειτουργίας στα άνω άκρα εκτιμήθηκε με τη χρήση δυναμόμετρου χειρός (Takei, Tokyo, Ιαπωνία) και η αξιολόγηση της μυϊκής δύναμης στα κάτω άκρα εκτιμήθηκε χρησιμοποιώντας ένα ισοκινητικό δυναμόμετρο (μοντέλο 770, CSMI Humac Norm, MA, ΗΠΑ). Η αξιολόγηση της κόπωσης των μυών εκτιμήθηκε με τη χρήση Ηλεκτρομυογραφήματος (Trigno, Delsys, Natick, MA, USA). Οι χωροχρονικές και κινηματικές παράμετροι, καθώς και ο δείκτης μεταβλητότητας της βάδισης αξιολογήθηκαν χρησιμοποιώντας το σύστημα ανάλυσης κίνησης Vicon Nexus (Vicon Motion Systems Ltd, Oxford, UK). Η λειτουργική ικανότητα εξετάστηκε με τη χρήση διαφόρων λειτουργικών δοκιμασιών που χρησιμοποιούνται συνήθως σε ασθενείς με ΠΣ (εξάλεπτη δοκιμασία βάδισης 6MWT, κάθισμα-όρθια θέση-κάθισμα STS, TUG). Τέλος, χρησιμοποιώντας συγκεκριμένα ερωτηματολόγια αξιολογήθηκε η ποιότητα ζωής των ασθενών, η ποιότητα του ύπνου, η κατάθλιψη, η ημερήσια υπνηλία, η γνωστική ικανότητα καθώς και η κόπωση. Η κλίμακα ανικανότητας εξετάστηκε από νευρολόγο. **Αποτελέσματα:** Το συμπλήρωμα PLP10 είχε θετική επίδραση σε μερικές χωροχρονικές παραμέτρους της βάδισης, όπως ο χρόνος μονής στήριξης ($p < 0,05$), ο χρόνος ενός βήματος καθώς και ο χρόνος βηματισμού ($p < 0,05$). Παρουσιάστηκαν κάποιες πολλά υποσχόμενες θετικές αλλαγές στις χωροχρονικές παραμέτρους, παρότι το περιθώριο για βελτίωση ήταν μικρό λόγω της αμελητέας απόκλισης από την κανονική βάδιση. Αντιθέτως, το συμπλήρωμα PLP10 δεν είχε καμία επίδραση σε καμία από τις κινηματικές παραμέτρους ($p>0,05$), στο οβελιαίο, μετωπιαίο και εγκάρσιο επίπεδο. Ο δείκτης μεταβλητότητας της βάδισης παρουσίασε ενδιαφέρουσα αλληλεπίδραση

με βάση το χρόνο. Συγκεκριμένα, ενώ ο δείκτης μεταβλητότητας της βάδισης, της ομάδας του εικονικού φαρμάκου μειώνεται μεταξύ των 2 ετών κατά περίπου 10% κατά μέσο όρο στα πόδια, το αντίθετο συμβαίνει στην ομάδα PLP10. Ο δείκτης μεταβλητότητας της βάδισης στην ομάδα PLP10 αυξήθηκε περίπου 4% κατά μέσο όρο και στα δύο πόδια ($p < 0,05$). Αυτό πιθανόν εμφανίζει μια σημαντική θετική επίδραση του συμπληρώματος στη βάδιση της ομάδας PLP10. Επιπλέον, το συμπλήρωμα είχε θετική επίδραση στη λειτουργική ικανότητα. Συγκεκριμένα, τα αποτελέσματα αυτής της μελέτης δείχνουν ότι το συμπλήρωμα βελτίωσε την απόδοση στο STS ($p < 0,05$). Σε ορισμένες παραμέτρους (6MWT και TUG) ενώ δεν παρατηρήθηκε στατιστικά σημαντική διαφορά μεταξύ των δύο ετών, παρατηρήθηκε τάση για βελτίωση στην ομάδα PLP10 σε αντίθεση με την ομάδα του εικονικού φαρμάκου. Η δύναμη των άνω άκρων ($p > 0,05$), η δύναμη των κάτω άκρων ($p > 0,05$) αλλά και η κόπωση ($p > 0,05$) δεν επηρεάστηκαν από τη χορήγηση του συμπληρώματος PLP10. Το συμπλήρωμα PLP10 δεν είχε καμιά επίδραση σε παραμέτρους που σχετίζονται με την ποιότητα ζωής, όπως η κόπωση, η ποιότητα του ύπνου, η κατάθλιψη, η υπνηλία κατά τη διάρκεια της ημέρας και η γνωστική λειτουργία ($p > 0,05$). Τέλος, η κλίμακα ανικανότητας EDSS διατηρήθηκε σταθερή στην ομάδα του συμπληρώματος PLP10 στη διάρκεια των 2 χρόνων της παρέμβασης.

Συμπέρασμα: Η χορήγηση για διάρκεια 24 μηνών ενός συμπληρώματος που περιέχει υψηλή δόση ωμέγα 3 και ωμέγα 6 πολυακόρεστων λιπαρών οξέων και αντιοξειδωτικών βιταμινών, βελτίωσε κάποιες χωροχρονικές παραμέτρους της βάδισης, το δείκτη μεταβλητότητας της βάδισης, την απόδοση σε ορισμένα τεστ λειτουργικής ικανότητας, ενώ διατήρησε σταθερή την κατάσταση ανικανότητας των ασθενών. Συμπερασματικά το συμπλήρωμα πιθανόν να αποτελεί υποσχόμενη προσέγγιση όσον αφορά τη βελτίωση κάποιων παραμέτρων της λειτουργικής ικανότητας και της βάδισης, αλλά και όσον αφορά τη διατήρηση σταθερής κατάστασης αναπηρίας σε ασθενείς με ΠΣ χαμηλής ανικανότητας ($p<0,05$).

Λέξεις κλειδιά: Πολλαπλή Σκλήρυνση, μυϊκή δύναμη, εμβιομηχανική, βάδιση, λειτουργική ικανότητα, ποιότητα ζωής

Acknowledges

First of all, I would like to give special thanks to my thesis supervisor, Assist Prof. Dr. Christoforos Giannaki for always making time to listen to my ideas, for his enthusiasm, professional support, guidance and encouragement throughout the completion of this thesis. I deeply appreciate his many efforts to proofread my thesis over and over again.

I am extremely grateful to my co-supervisor Asst. Prof. Dr. Manos Stefanakis for introducing me to the field of gait analysis. His extensive knowledge and range of ideas have been invaluable to me during my research. I deeply appreciate his many efforts to proofread my thesis over and over again.

I would also like to express my sincere appreciation to all academicians in the fields of sport science and biomechanics who contributed to this thesis with valuable suggestions and comments.

Love and thanks to my family and my friends for their never-ending patience, support and encouragement.

The work presented in this thesis could not have been accomplished without the support from many individuals. Finally, my thanks go to all those who are not specifically mentioned here.

Aristotelous Panayiotis

Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Nicosia. This thesis has been composed solely by myself except where stated otherwise by reference or acknowledgment. It has not been previously submitted, in whole or in part, to this or any other institution for a degree, diploma or other qualifications.

Signed:  Date: 08/01/2021

Table of Contents

Page

List of Figures

Page

List of Tables

Page

Abbreviations Index

MS:	Multiple Sclerosis
CNS:	Central Nervous System
PwMS:	People with Multiple Sclerosis
RRMS:	Relapsing Remitting Multiple Sclerosis
PPMS:	Primary progressive Multiple Sclerosis
CT:	Computerized Tomography
EDSS:	Expanded Disability Status Scale
IFN:	Interferon
HC:	Healthy Controls
PUFAs:	Polyunsaturated Fatty Acids
EFA:	Essential Fatty Acids
ALA:	Alpha-Linolenic Acid
LA:	Linolenic Acid
AA:	Arachidonic Acid
DHA:	Docosahexaenoic Acid
EPA:	Eicosapentaenoic Acid
VitA:	Vitamin A
SF36:	Short Form Health Survey Questionnaire
MFIS:	Modified Fatigue Impact Scale
PDDS:	Patients Determined Disease Steps
RCTs:	Randomized Controlled Trials
RR:	Relapsing Remitting
MDD:	Major Depressive Disorder
DMT:	Disease-Modifying Treatment
QOF:	Quality of Life
sEMG:	Surface electromyography
imEMG:	intramuscular electromyography
MU:	Motor Unit
MNF:	Mean frequency
MDF:	Median Frequency
IPT-KE:	Isokinetic peak torque knee extensors

IPT-KF:	Isokinetic peak torque knee flexors
VM:	Vastus medialis
RF:	Rectus Femoris
MVIC:	Maximal Voluntary isometric contraction
Lbm:	Lean body mass
Cw:	Energetic Cost of walking
PIG:	Plug in Gait
GDI:	Gait deviation Index
CP:	Cerebral Palsy
CoM:	Center of Mass
STS:	Sit to Stand
TUG:	Time Up and Go
Reps:	Repetitions
Secs:	Seconds
6MWT:	Six Minutes' Walk Test
FSS:	Fatigue Severity Scale
QOL:	Quality of Life
HRQOL:	Health-Related Quality of Life
ESS:	Epworth Sleepiness Scale
PSQI:	Pittsburgh Sleep Quality Index
ESS:	Epworth Sleepiness Scale
BDI:	Beck Depression Inventory
PASAT:	Paced Auditory Serial Addition Test
HC:	Healthy Control
BC:	Body Composition

CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

1.0 Introduction - Multiple Sclerosis

Multiple Sclerosis (MS) is a developing inflammatory and neurodegenerative disease of the Central Nervous System. (CNS) [1, 2]. Hallmarks of the disease include demyelination and axon lysis resulting in a variety of motor symptoms and disabilities [1]. It manifests an acute or more often a recurring character. Alterations on the cellular equilibrium, vascular growth factors, changes on the metabolic demands, viral infections of the CNS and compromised immune responses are accountable for the cause and progression of MS [3]. The inflammatory process induces degeneration of the myelin sheath, which gives an inconsistent damage along the axons. Those patchy lesions described as plaques, and they are located predominantly in the white matter of the CNS. Demyelination is the process in which the myelin sheath dissolves due to inflammatory reasons [4]. The pathophysiology involves an autoimmune manner requiring activation of helper T cells against CNS protein; once triggered the activate T-cells attack and destroy myelin and the myelin-forming cell. The degenerative process of the myelin sheath is complicated as it includes a complex immune response cascade [5]. When the myelin sheath loss its continuity, the generated action potential is slowed down or even terminated; the lost myelin is then replaced with gliosis (neural scar tissue). This process gave MS its name: 'multiple' many and 'sclerosis' scar forming. Once gliosis is established, cells and axons do not fully retrieve their former function. Furthermore, as the disease progresses, oligodendrocytes and sequentially, the axons themselves are destroyed, which leads to worsening of symptoms [4]. Mainly, patients with MS (PwMS) can experience a partial or complete loss of any function that is controlled by the CNS. The nature and severity of symptoms can vary considerably depending on which regions of the CNS are affected, and how severely they are injured.

Multiple Sclerosis is one of the most common neurological diseases and a growing issue worldwide. At the same time, it considered to be the leading neurological cause of disability in young adults, associated with impairments physically and psychologically [2, 6]. MS multifactorial nature has not been clarified. Nevertheless, the interaction between genetic susceptibility and environmental factors such as geographical, nutritional and infectious is enough to dysregulate the immune response and establish its etiopathogenesis [7].

1.1 MS Classification - Different types of Multiple Sclerosis (MS)

MS classified into four major types based on the development of the disease [8].

a) Relapsing-remitting MS (RRMS)

b) Secondary Progressive MS (SPMS)

c) Primary progressive MS (PPMS)

d) Progressive-Relapsing MS (PRMS)

Relapsing-remitting MS (RRMS) is the most common form, affecting about 85% of MS patients. About 50% of RRMS population within ten years will progress to SPMS and about 85% between ages 25-35 years of RRMS patients will develop SPMS. PPMS affects about 10% of MS patients still in this form; symptoms gradually worsen from the beginning. PRMS is a different form affecting 5% of MS patients and is progressive from the beginning.

(RRMS): The majority of patients presenting with MS are initially diagnosed with relapsing/remitting MS (RRMS). The patients are experiencing a series of relapses followed by periods of remission, which also include the characteristic pattern for RRMS. The clinical signs and symptoms improve or cease until the next relapse occurs. Time intervals between relapses vary, and they can be between a few weeks to decades. The recovery from relapses can last for days, weeks or months and can be slow and gradual or almost instantaneous. During remission, the patient can recover fully or partially from the potential deficits of a previous relapse.

(SPMS): Described as a regular progression of clinical neurological injury with or without superimposed relapses, minor remissions and plateaus. Any superimposed relapses and remissions tend to tail off over time. The SPMS form tends to be correlated with less gliosis than RRMS at a time, although the total burden of disease continues to progress. The reason is the higher levels axon lysis

(PPMS): Gradual deterioration of Symptoms from the beginning. No relapses or remissions are reported, though there might be occasional plateaus. PPMS is more resistant to the drugs typically used to treat the disease and distinguished by a gradual progression of the disease involving a decline in the patients [9].

(PRMS): Characterized from early progression with recurrent flare-ups of worsening symptoms along the way. No periods of remission noticed [10].

In RRMS and SPMS, the female to male ratio is about 2:1 [11]. Although the onset of MS in woman tends to be early (ages 18 to 30), the onset of MS in men tends to be later in life (ages 30 to 40). Testosterone seems to play a statistically significant role in protecting young men from the disease [9, 11].

The onset of MS in men agrees with the initial declining in bioavailable testosterone in healthy men [11]. In PPMS sex ratio is different from other forms of the disease, and both genders involve the same risk manifesting the disease [10].

1.1.1 Diagnostic Criteria

A review set of diagnostic criteria issued by the International Panel on The Diagnosis of Multiple Sclerosis in 2001 [12].

In addition to the standard specifications, particular guidelines are provided by the review for using findings on MRI as the preferred imaging modality, cerebrospinal fluid analysis, and visual evoked potentials. Findings on those investigations provide the data for the second relapse to establish the diagnosis more quickly.

These guidelines also expedite the diagnostic method in those patients who have a steady progression of disability without discrete relapses. The MRI test is the preferred imaging modality to illustrate CNS (the brain and spinal cord) to identify the appearance of plaques or gliosis caused by MS. MRI technology is also capable of recognizing lesions in different areas of the CNS and differentiate between new or active versus old or already established lesions. MRI scan does not use radiation in comparison to computerized tomography (CT) or conventional X-ray. Alternatively, it uses magnetism and radio waves. Powerful magnetic fields interact with the hydrogen atoms detected in the water carried in all body tissues and fluids. Radiofrequency signals cause these hydrogen atoms to release energy. Computer software transpose the changes into cross-sectional images. The scanning process is sensitive enough, and can generate pictures of lesions, or areas of loss; a CT scan would miss that.

Kurtzke quantifies the degree of disability of patients with MS using an Expanded Disability Status Scale (EDSS) [13]. The EDSS quantifies disability in eight functional systems (FS) and provides to neurologists and physicians to indicate a Functional System Score in each of these. Some of the functional systems included are: pyramidal, cerebellar, brainstem, sensory bowel and bladder, visual, cerebral and other. The EDSS steps 1.0 to 4.5 refer to people with MS who are fully mobile. EDSS steps the impairment defines 5.0 to 9.5 to ambulation.

1.1.2 Treatment of Multiple Sclerosis – Therapies

Currently there is no cure for MS, though, there are treatments to slow down the progression of the disease and to manage the symptoms of MS. Medications which can modify the course

of the disease referred to disease-modifiers. There are three types of disease- modifiers which have been approved for the treatment of MS:

a) Interferon beta

b) Glatiramer acetate

c) Mitoxantrone, a chemotherapeutic substitute

Interferon (IFN) beta

Three interferon beta medications have approved for treating relapsing forms of MS: i.m. IFN beta-1a (Avonex from Biogen); s.c. IFN beta-1a (Rebif from Ares-Serono) and s.c. IFN beta-1b (Betaseron from Berlex, Betaferon from Schering); Avonex, manufactured by Biogen, is a form of beta interferon known as interferon beta-1a for intramuscular application. It is identical to the Naturally occurring protein found in the human body. Avonex is used to modify the course of MS. Clinical trials showed that Avonex reduce the average relapse rate in people with the RRMS and SPMS forms of the disease [14-16]. Rebif, manufactured by Ares-Serono, is the same substance as Avonex but injected subcutaneously. Similar to Avonex, clinical trials showed that Rebif reduce the average relapse rate in people with the RRMS and SPMS forms of the disease [17-20] . Schering and its US associate Berlex manufacture Betaseron (known as Betaferon in Europe). It is injected subcutaneously and has shown to reduce the average relapse rate in people with the RRMS and SPMS forms of the disease [17, 18, 21, 22].

Glatiramer acetate

Glatiramer acetate is a random chain polypeptide including the amino acids glutamic acid, lysine, alanine and tyrosine (GLATiramer). Originally was manufactured to simulate a myelin protein (myelin basic protein), with interferon to induce experimental autoimmune encephalomyelitis (an animal model of MS). Despite, it was observed to suppress the disease and trialled in human MS. Copaxone, glatiramer acetate brand name manufactured by Teva Marion Partners. In early analyses of the drug, was known as Copolymer-1 and Cop-1. Copaxone has been shown in clinical trials to minimise the average relapse rate in people with the RRMS and to lessen the formation of new lesions in the CNS [23-25].

Mitoxantrone

Mitoxantrone (Novantrone, from Ares-Serono), a synthetic anthracenedione derivative, is an immunomodulatory agent with antineoplastic effects. The immunomodulatory mechanisms is the presumed mechanism of action in patients with MS, yet these remain to be fully defined.

Mitoxantrone treatment involves intravenous administration of the agent, which demonstrated improved neurological impairment and delayed progression of MS in patients with the PRMS and SPMS forms of the disease [26-28]. The standard treatment for significant acute exacerbations is the administration of steroids, which exert potent anti-inflammatory effects. Steroids are allowing return to normal function to occur more rapidly and reducing the duration of the exacerbation and reduce inflammation at the site of new demyelination. Methyl-prednisolone is the preferred steroid regime and is administered intravenously in high doses for 3–5 days. Often subsequent tapering lower oral doses of prednisone for 1-2 weeks might be required. Unfortunately, the use of steroids is not recognized to have any effect on the long term course of the disease.

1.1.3 Necessity of different approach

Even though current treatments can modify the course of the disease and to manage the symptoms of MS, because there is no cure, there is a need of a separate method/treatment option which can diminish disease symptoms besides the existing treatments.

In the 1950s, MS prevalence proved by ecological studies to be independent of latitude [29, 30]. Initially, differences in saturated fat intake from animal sources proved to increase the causality of the disease. Later research detected an association between the increased intake of polyunsaturated fatty acids (PUFAs) and reduction of MS prevalence [31]. The PUFAs, such as the omega-3 and omega-6 fatty acids, have essential roles in membranes structure and function; in the regulation of gene expression and cell signaling. Moreover, they play essential roles in inflammation, immunity, and many other physiological responses as they are substrates for the synthesis of lipid mediators involved [32]. Western diets are recognized for high omega-6 (ω6) and low omega-3 (ω3) fatty acid intake. In contrast, during the Paleolithic period, when human DNA profile established, researchers believe that there was a balance between omega-6 and omega-3 fatty acids [33]. The present Western diet is characterized by a lack of intake in omega-3 fatty acids with a ratio of ω6 to ω3 of 15:1 or 20:1. A diet balance of 1:1 is necessary for wild animals and presumably human beings [34-40]. Other studies recommend optimal ratio of ω6/ω3 fatty acids with a rate of 2.3:1 [41] or 1.5:1 [42] in order to provide the ideal homeostatic level. For millions of years, a balance existed between omega-6 and omega-3 during the long evolutionary history of the genus Homo. Genetic modifications happened partly in response to these dietary influences [33]. People with Multiple Sclerosis (PwMS) ever since prospective longitudinal studies showed that PUFAs are associated with a decrease in

MS mortality have focused their interest on the function of omega 3 and omega 6 fatty acids [43].

However, because it is challenging to isolate this ratio by diet, it is essential to monitoring the additional dietary supplementation of ω3 to obtain a balanced ratio of ω3/ ω6 fatty acids [44].

1.2 Essential fatty acids: Ω3 - Ω6 and antioxidants

Nowadays, individuals live under nutritional conditions that deviate from that for which our genetic character was determined. The observational study of the Swank diet - a low-fat diet which introduced in 1950 - was the ground to identify that alterations of diet have been a standard way for PwMS to customize their treatment [45, 46]. The composition of the phospholipid membrane of polyunsaturated fatty acid (PUFA), demonstrated that plays a vital role in immune-related and non-immune-related inflammation [47]. It is confirmed that lipid and fatty acid synthesis is remodeled in white matter brain tissue with active MS compared with whole-brain white matter [48, 49]. Additionally, (PUFA) and antioxidant deficiencies, respectively, with decreased cellular antioxidant defense mechanisms have been observed in MS patients [47, 50]. Both PUFAs and dietary antioxidants are eligible to depreciate MS symptoms. The mechanism of action is complicated and involves inhibition and regulation of particular ion pumps responsible for triggering action potentials and regenerating the myelin sheath [51]. Even though dietary supplementation of PUFAs and antioxidants proven to be beneficial, the path mechanism has shown to be age-dependent. Thus, already developed adults brains take longer to recover in comparison to evolving brains and possibly recovery is dependent on the quantity of the dietary supplementation of PUFAs [51]. There are two classes of essential fatty acids (EFA), omega-6 (ω6) and omega-3 (ω3). The difference between omega-6 and omega-3 fatty acids is based on the location of the first double bond, counting from the methyl end of the fatty acid molecule [33]. The first double bond for omega-6 fatty acids, is between the 6th and 7th carbon atoms and for omega-3 fatty acids between the 3rd and 4th carbon atoms. Omega-6 and omega-3 fatty acids are necessary because humans, like all mammals, cannot produce them in situ and must receive them from their diet [33]. The ω3 class of PUFA includes alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) whereas the ω6 family includes linolenic acid (LA) and arachidonic acid (AA) [42]. The outcomes of ω3 family of PUFA on health derive from its impact on immune function and the immunomodulatory and anti-inflammatory effects [52]. ALA is an essential fatty acid that the body cannot compose itself, and it can be metabolized

to longer-chain forms (EPA) and (DHA) by saturation and elongation [53]. EPA and DHA are precursors for the anti-inflammatory lipid mediators, while AA is a precursor for pro-inflammatory lipid mediators [42]. Many chronic diseases are based on inflammatory response such as coronary heart disease, diabetes, arthritis, cancer, osteoporosis, mental health, dry eye disease and age-related macular degeneration [33]. For instance, if EPA deficiency appears during the stages of life, the outcome could be a significant delay in myelin synthesis accompanied by impairment in learning, compromised vision, impaired motor, and more additive abnormalities [54]. PUFA can be found in the seeds of most plants. In the modern Western diet, ω6 are rich in LA [33], while ω3 PUFA can be found in botanical sources, which are rich in ALA. Besides, marine sources, e.g. oily fish (e.g. salmon), crustacean (e.g. krill) and the liver of lean fish, are rich in EPA, DHA and DPA [55]. It is essential to consume ω3 PUFA to replenish our body with the amount of EPA and DHA necessary for optimal cellular equilibrium and homeostasis [56]. Moreover, evidence showed that PwMS tend to adapt very low-quality diets; one study showed that 95.7 % of PwMS did not meet the recommended USDA fruit/vegetable guidelines, while 87 % did not meet the whole grain guidelines [57].

Additionally, since oxidative stress implemented in MS activity, the role of dietary antioxidants in the treatment of MS has been of considerable interest, including in disease progress [58]. Tocopherol (Vitamin E) acts mainly as an antioxidant preventing the PUFAs from being damaged by lipid peroxidation [59-62]. Dietary tocopherol intake and lower circulating concentrations associated with cardiovascular disease and cancer in observational studies [63-68]. A cross-sectional, biorepository study pointed out that PwMS, on average, had a reduced serum vitamin E/cholesterol ratio and total serum vitamin E was in borderline levels [69]. Two additional studies showed a potential effect of vitamin E on MS disease activity.

In a small RCT of 88 RRMS patients treated with interferon-beta and ω3 fatty acids or placebo, both supplemented with vitamin E, serum levels of tocopherol drawn. For every ten micromolar increase in serum alpha-tocopherol, there was 36.8% reduced odds of having a new T2 lesion on MRI. Nevertheless, alpha-tocopherol levels were not associated with clinical disease activity [70]. The outcome of those results restricted by the small sample size and the evidence that the association investigated in the setting of beta interferon treatment. Another ω3 fatty acid RCT study also showed a possible role for vitamin E. In this RCT; four treatment groups were studied. Two of the groups administrated vitamin E, one in the form of gamma-tocopherol and one as alpha-tocopherol. Both groups were assigned PUFA supplements. The gamma isoform chosen for its more potent antioxidant activity, and admittedly, only the group given ω3 fatty acids with the gamma-tocopherol had improved outcomes. The study

demonstrated a longer time to disability onset and a decreased likelihood ratio of disability progression [71]. Limitations and bias for the study were the small sample size, a high drop-out rate, and the multiple synchronous interventions.

Vitamin A (VitA) is a group of unsaturated monohydric alcohols that contain an alicyclic ring. VitA is insoluble in water but is fat-soluble as vitamin E [72]. The primary biological functions of VitA include preservation of vision, stimulating growth and development, and enhances epithelium or mucosa barrier [73]. VitA is known as an anti-inflammatory vitamin because of its critical role in improving immune function. VitA is required in the maturation of the immune system and plays regulatory roles in cellular immune responses and humoral immune processes. VitA correlates with a therapeutic response to various infectious diseases [74].

1.1.4 Nutrition and MS

Notwithstanding several scientific studies investigating the relationship between nutrition and MS, there is no consensus on the dietary habits to follow to improve the course and the symptoms of the disease [75]. Inconsistent results also exist from recent studies on PUFA intake and MS risk

Nevertheless, a moderate positive effect on subjective quality-of-life assessed by the Short Form Health Survey Questionnaire (SF-36), the Modified Fatigue Impact Scale (MFIS), and the Mental Health Inventory] showed on a double-blind randomized 1-year trial [76]. The trial isolates RRMS patients who underwent a low-fat diet (15% fat) supplemented with ω3, from patients who followed a 30% fat diet supplemented with olive oil. Furthermore, in a randomized double-blind placebo-controlled phase II proof of concept clinical trial by Pantzaris et al., (2013) [71], it was shown that a combination of PUFA (Ω -3 and Ω -6 at 1:1) and gamma-tocopherol, versus placebo, significantly reduced the annualized relapse rate and the probability of disability progression at two years intended as an increase of at least 1.0 point on the Expanded Disability Status Scale (EDSS), confirmed after six months in relapsing-remitting MS (RRMS) patients, without any serious adverse events.

However, the study had two considerable limitations:

a) The small sample size (20 participants randomly assigned to each experimental group)

b) There was low adherence of the participants (51% of patients completed the 30- month trial). A cross-sectional study showed that participants with ω3 fatty acid supplementation had lower disability on average and had a 44% reduced odds of a relapse based on self-report of relapse and patients determined disease steps (PDDS) as a measure of disability [77].

Moreover, a large case/control study conducted in Australia confirmed that higher intake of ω3 fatty acids resulted in a decreased probability of a clinically demyelinating event [78], in a dose-response correlation. Two interventional trials showed potentially encouraging results with PUFA supplementation. Still, both had significant design limitations that impede the development of subsequent treatment guidelines.

In the review of Mische and Mowry (2018), the role of PUFA supplementation is somewhat complicated. Many studies have examined PUFAs, from the observational setting to interventional trials. While observational studies and weakly designed randomized controlled trials (RCTs) seem to suggest that patients who consume more PUFAs tend to have less severe disease activity, the more scientifically accurate RCTs have not conferred any benefit of PUFA administration. It is unclear why this is the case. However, some suggested that a diet already rich in PUFAs is also likely a healthier diet overall or concerning other micronutrients, which themselves may decrease risk for co-morbidities that are relevant to prognosis in PwMS.

To conclude the evidence is not strong enough to recommend their supplementation at this time [79] actively. While changes in diet to "healthier eating" seem to improve quality of life and reduce perceived physical and mental disability [80] the determination of what establishes a healthy diet for PwMS is still not precise. Although many diets have been endeavored since the Swank diet publicized, there is inadequate evidence to support that dietary changes directly impact MS. A 2 year double-blind controlled trial [81] of long-chain ω3 and ω6 PUFA demonstrated beneficial trends in 145 RRMS patients receiving ω3 in terms of reduction of MS relapses duration, frequency, and severity, as compared to 147 RRMS patients of the control arm. Nonetheless, the measured parameters strived to reach the 95% statistical significance. An open-label trial done in 2000 showed a reduction in annual relapse rate and EDSS score. However, the interpretability of the results is limited by a lack of blinding and multiple simultaneous interventions (including vitamin A, E, and D supplementation, along with dietary counselling) [82].

Additionally, although the association between Omega-3 fatty acids (ω3FA) improvement of depression with low adverse effects when used as an augmentation therapy in unipolar depression [83-86], opinions vary. Another multicenter placebo-controlled clinical trial in 2012 [87], examined the effect of daily treatment with concentrated ω3 fatty acids versus placebo on radiological disease activity assessed after 6, 9, and 24 months from baseline. ω3 fatty acids were supplemented alone during the first six months of trial and in association with subcutaneously 44 mg of interferon beta-1a to all patients over the following 18 months. The study provided class 1 evidence that ω3 fatty acid supplementation did not exert beneficial

effects on disease activity in RRMS as monotherapy or in combination with a first-line immunomodulatory treatment. More recently, a double-blind, placebo-controlled trial showed that fish oil supplementation did not influence annual relapse rate or disability. However, it did affect serum inflammatory markers, including the downregulation of TNF-α, IL1, IL6, and nitric oxide catabolites after six months [88]. Another double-blind placebo-controlled RCT did not show clinically meaningful change concerning MRI lesions, disability, or relapse rate in those treated with ω3 fatty acid supplements compared to placebo [87]. While several case-control studies have reported an inverse association between food sources (or supplements) rich in PUFA [89-91], or fish and cod liver oil [92] and MS risk, these associations could be interfered by vitamin D, an established risk factor for MS [93]. A randomized double-blind, placebo-controlled pilot study investigated whether omega-3 fatty acid supplementation, as an augmentation therapy, improves treatment-resistant major depressive disorder (MDD) in people with MS [94]. Omega-3 fatty acids as an augmentation therapy for treatment-resistant depression in MS was not significantly different than placebo.

Bjornevik et al., (2017) [95], prospectively examined the association between dietary intake of PUFA and MS risk and concluded that low dietary PUFA intake might be another modifiable risk factor for MS. In contrast, Marine n-3 fatty acids have also inversely associated with MS risk independent of vitamin D [80], which is not consistent with the findings of the above study. The effect estimates were only significant for the plant-derived ALA and not for marine n-3 fatty acids. A recent case-control study that estimated PUFA intake from the overall diet described an inverse association between marine long-chain n -3 PUFA, but not for plant-derived PUFA [78]. Nevertheless, the only prospective study on PUFA and MS risk reported an inverse non-significant trend for the plant-derived ALA [96]. In conclusion, studies of varied designs have contradictory results, leading to a lack of accuracy on the efficacy of PUFA supplementation on measures of disease activity. The inconsistencies observed could, to some amount, be attributed to methodological limitations in previous research.

1.2 Purpose of the study

This study is a part of a bigger project conducted by the Cyprus Institute of Neurology and Genetics, and is follow up assessment of the same cohort the study by Pantzaris et al., 2013 [71]. The project examined the effects of a 24-months supplementation (PLP10) with a formula containing a high dose of omega 3 and omega 6 free fatty acids and antioxidant vitamins on inflammatory indices, the annualized relapse rate (ARR), the disability progression, as well as blood parameters and MRI lesions in PwMS. Patients enlisted from the neurology clinic of the Cyprus Institute of Neurology and Genetics.

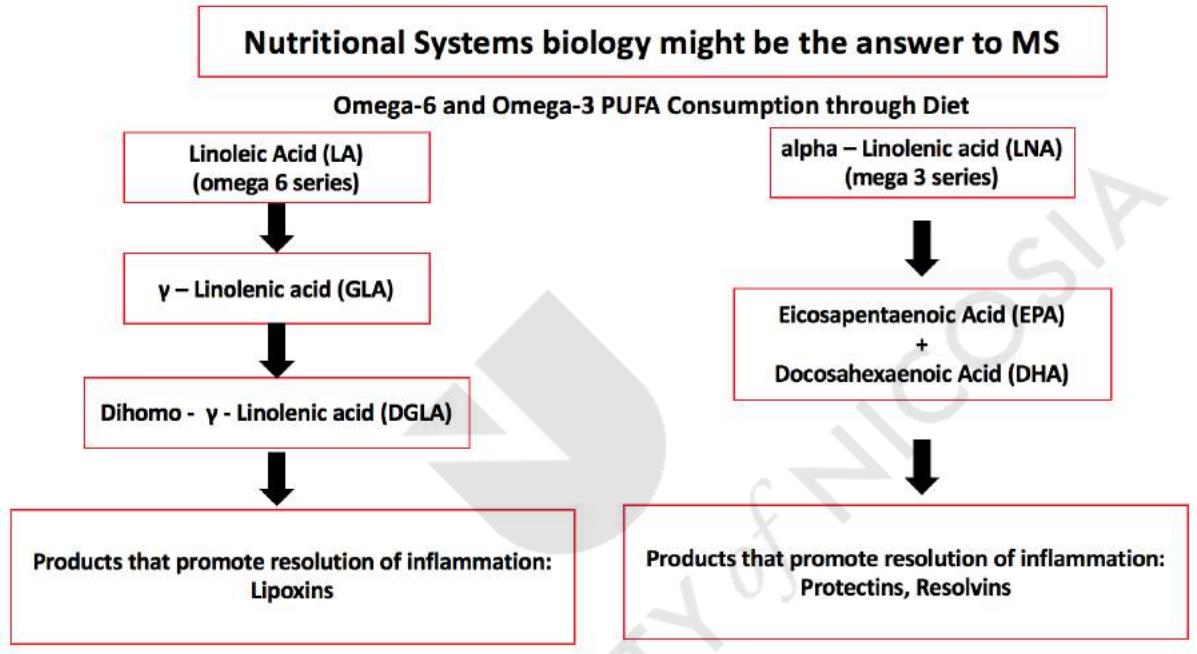

Figure 1. Ω-6 and Ω-3 and their metabolic derivatives. Also shown the relevant pathways by which the compounds can mediate inflammation and/or promote the resolution of inflammation (Pantzaris et al., (2013)) [71].

The daily dose of the compound was believed to be high enough to restore/amplify antioxidant activity of the body and ensure normalized membranes lipid profile of the cells. In addition, the active ingredients potentiate the anti-inflammatory and recovery mechanisms. Dietary fatty acid molecules need an approximately 6-month period to exert their beneficial effect, and this essential parameter was under consideration (normalization period) [97].

The primary aim of the current study was to examine the effects of a 24-months supplementation with a formula containing high dose of omega 3 and omega 6 free fatty acids and antioxidant vitamins on biomechanical and functional capacity parameters in PwMS. Secondary aims were to examine the effects of the above intervention in various quality of life and health parameters using the appropriate methodology.

We hypothesize that the functional capacity and some biomechanical (gait) related parameters would be improved after the supplementation with the above nutraceutical formula.

The assessment of muscle function in both the upper and lower extremities was evaluated during the period of 24 months and described in chapter II. Handgrip strength was evaluated by a handgrip dynamometer (Takei, Tokyo, Japan) and knee extensors and flexors strength was evaluated by an isokinetic dynamometer (Humac Norm). Additionally, muscle fatigue induced by one minute of maximum voluntary isometric contraction from an isokinetic dynamometer was evaluated via EMG.

The third chapter refers to an evaluation of gait spatiotemporal and kinematic parameters in PwMS since gait problems is one of the most commonly reported problems in MS [98]. Additionally, six minutes walk test evaluated the distance that PwMS could cover during the 3 visits (baseline, 12 and 24 months). Vicon motion capture system evaluated the spatiotemporal parameters (cadence, walking speed, step and stride length etc.) such as the joint motion angles across the averaged gait cycle.

The fourth chapter refers to an evaluation of functional capacity by a variety of functional capacity tests such as the six minutes walk test, sit to stand tests and time up and go test. PwMS in order to be able to maintain independence, must present adequate levels of functional capacity [99]. Functional capacity considered to be a highly important factor for quality of life and overall wellbeing in MS patients. Six minutes walk test (6MWT), provides an assessment of functional capacity [100] and predicts the potential decline in the ability to perform everyday activities such as habitual walking [101]. Sit to stand tests are considered an essential feature for determining the degree of independence and the quality of life of a person used to assess mobility and balance in PwMS. Time Up and go test monitor disease progression and identify potential MS fallers [102, 103].

In the fifth chapter, there is an evaluation of quality of life, daytime sleepiness, depression and cognition via a variety of specific Multiple sclerosis questionnaires.

In the sixth and final chapter of this thesis, there is a general discussion assessment where this thesis discusses the essential outcomes from the study, possible limitation factors and recommendations for future studies.

1.3 General Methods

1.3.1 Eligibility Criteria

The eligibility criteria were an age of 18–65; a diagnosis of RRMS according to the McDonald criteria; a score of 0.0–5.5 on the Expanded Disability Status Scale (EDSS) [13], a rating that ranges from 0 to 10, with higher scores indicating more severe disability; MRI showing lesions consistent with MS; at least one documented clinical relapse; and either receiving or not a disease-modifying treatment (DMT) within the 24-month period before enrolment in the study. Patients were excluded because of a recent (<30 days) relapse, prior immunosuppressant or monoclonal antibody therapy, pregnancy or nursing, other severe disease compromising organ function, progressive MS, history of recent drug or alcohol abuse, use of any additional food supplements, vitamins, or any form of PUFA and a history of severe allergic or anaphylactic reactions or known specific nutritional hypersensitivity. No monitoring or limitations on the patients daily dietary habits considered because the high quantities of the ingredients within the formula could not be significantly affected by any particular dietary pattern. The protocol approved by the Cyprus National Bioethics Committee, and overseen by an independent safety-monitoring committee evaluating the safety and over-all benefit-risk profiles. All patients gave written informed consent at the time of enrolment.

1.3.2 Randomization and masking

Patients randomly assigned to two experimental groups in a 1:1 ratio. Study data were collected by the investigators and saved as blinded codes for the study. All study personnel involved in the conduct of the study as well as the patients were blinded to the treatment throughout the study.

1.3.3 Procedures and endpoints

The specific Ω -3 and Ω -6 raw materials purchased according to the required interventions' PUFA- fraction specification (molecular structure, quantity/ratio and quality) with vitamin E (α -tocopherol) used as antioxidant stabilizer by the supplier. The vitamins and masking aroma were purchased separately [71]. The mixing of the fractions to the final required intervention composition specification was always performed by the same team of scientists under the supervision of the involved medical biochemist and lipid ology specialist and under appropriate conditions every 6 months. The interventions refrigerated in the dark until use.

The participants were randomly assigned to receive the following: group A, (Neuroaspis PLP10), a daily dose of a 20 ml cocktail formula consisting of omega-3 ((EPA (1650 mg)/DHA (4650 mg)), omega-6 ((GLA (2000 mg)/LA (3850 mg)) (1:1 w/w), vitamin A (0.6 mg), vitamin E (22 mg) plus pure γ-tocopherol (760 mg) and citrus-aroma; and group B (placebo), a daily dose of a 20 ml mixture of pure virgin olive oil plus citrus-aroma [71] (fig. 2).

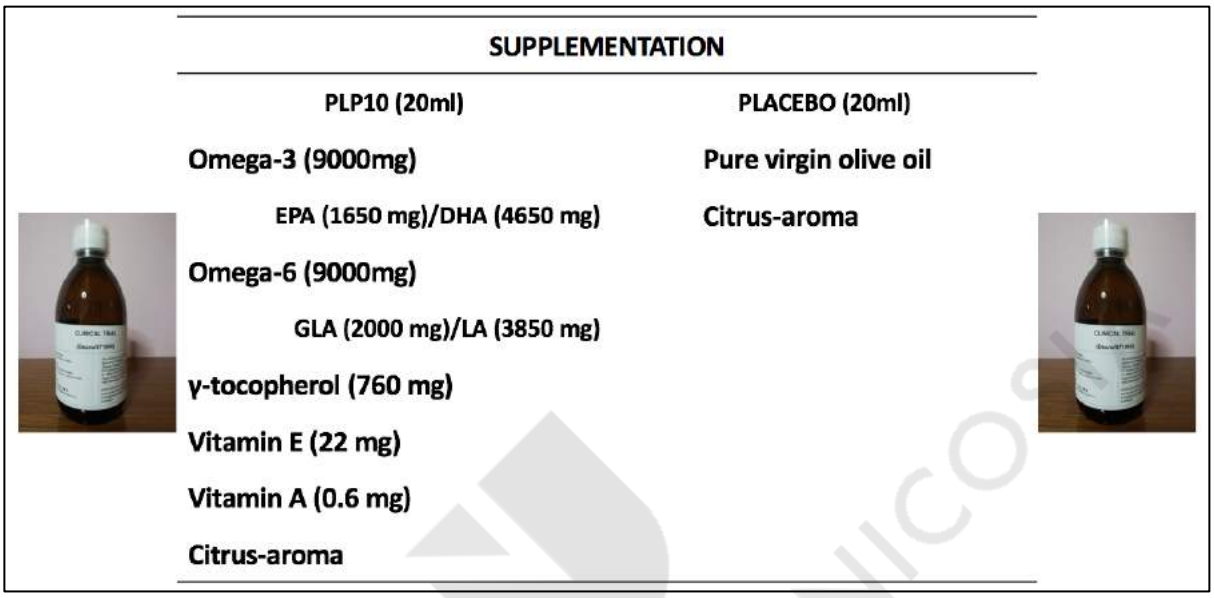

Figure 2. Contents and pictures of the PLP10 and the placebo supplementation

The pharmacist of the institution was responsible for the appropriate storage and handling of the interventions for the individual participants. The interventions were taken orally once daily 30 min before dinner using a dosage-calibrated cup for 24 months. The ingredients, ratio and dose were selected based on their biophysical interrelationship with the total known multiple MS causative factors, their biochemical importance and the role they were expected to play in the normalization and treatment of the involved complex network of events in the disease pathophysiology. Moreover, the high intervention dosage was selected with the aim of optimizing the body composition of Ω -3 to Ω -6 PUFAs to a 1:1 wt/wt ratio, irrespective of the dietary habits and geographical origin [71].

Depending on their clinical status and in accordance with common practice, the participants continued to receive their indicated regular treatment, with persistent evaluation for any side effects and adverse events. Clinical assessment visits were scheduled at baseline and at 12 and 24 months on treatment. The patients were also clinically examined by the treating neurologist within 48 h after the onset of new or recurrent neurological symptoms [71].

The involved neurologist was experienced with more than 20 years in practice. He was trained to standardize the EDSS scoring procedures, examined the patients, made all medical decisions, determined the EDSS score and reviewed the adverse effects or side effects. The patients were able to contact the involved neurologist at any time if there was any adverse event, side effect or allergic reaction. The study drug was not expected to have any clinical or laboratory adverse effects different from those of the placebo that could disturb the double-blind nature of the trial. Therefore, the study neurologist functioned as both the treating and evaluating physician [71].

The whole procedure followed the clinical trial guidelines as required by the USA Food and Drug Administration, European Medicines Agency and the Committee for Medicinal Products for Human Use [71, 104].

1.4 Participants

Fifty-one relapsing-remitting MS patients (age: 38.4 ± 7.1 yrs; 30 Female; EDSS: 2.38 ± 1.04) were enlist from the neurology clinics of the Cyprus institute of Neurology and Genetics and agreed to participate to this study [2]. The patients were randomized into the PLP10 group (n= 27, 17 female) and the placebo group (n=24, 13 female). MS diagnosed according to the revised McDonald criteria 2011 [12], while the disability level assessed by the expanded disability status scale (EDSS) [13, 105]. Inclusion criteria for this study were: patients with relapsing-remitting MS, MRI showing lesions consistent with MS and at least one documented clinical relapse with an EDSS score below 5. Exclusion criteria included experience of a relapse within the past 6 months, severely impaired visual function, severe psychiatric disorder, severe arthritis of the knee or hips, pregnancy and other neurological or vestibular disorders. All participants were given a full explanation of the purpose and procedures of the study and gave their written consent. Ethical approval was obtained by the national ethical committee [2].

At the follow-up year from the placebo group, 2 patients (1 Female) drop out from the study due to taste discomfort and relapse, whereas from the experimental group only 1 male drop out due to taste discomfort. At the 24 months assessment, from the placebo group 4 patients (3 Female) drop out from the study due to relapses and taste discomfort, whereas from the experimental group 8 patients (5 Female) drop out from the study due to pregnancies, taste discomfort and relapse. At the end of the study 36 patients completed the assessments: 18 patients (9 Female) from the placebo group and 18 patients (12 Female) from the experimental group.

In fig. 3 (flowchart) the number of PwMS at every step of the study is depicted. A total of 51 PwMS agreed to participate and met the inclusion criteria. The proportion of female/ male in the participants in the study was 21/30.

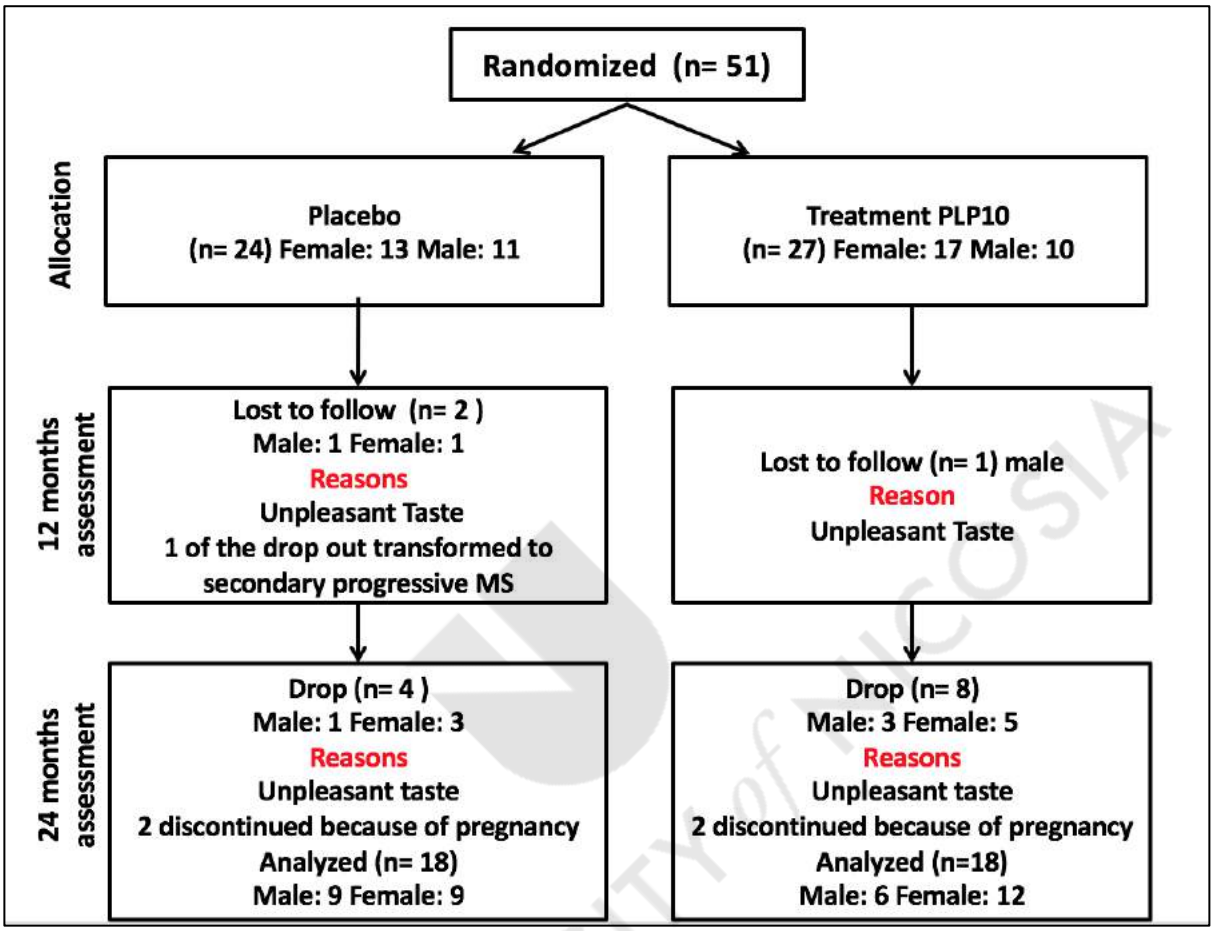

Figure 3. Flow Chart of the Participants

The time interval of the measurements is depicted in Figure 4. Patients tested at baseline (before treatment), and retested at 12 months and 24 months while continuously taking the supplements. The parameters tested in each time point include the quality of life parameters, gait analysis, functional capacity and muscle function assessment.

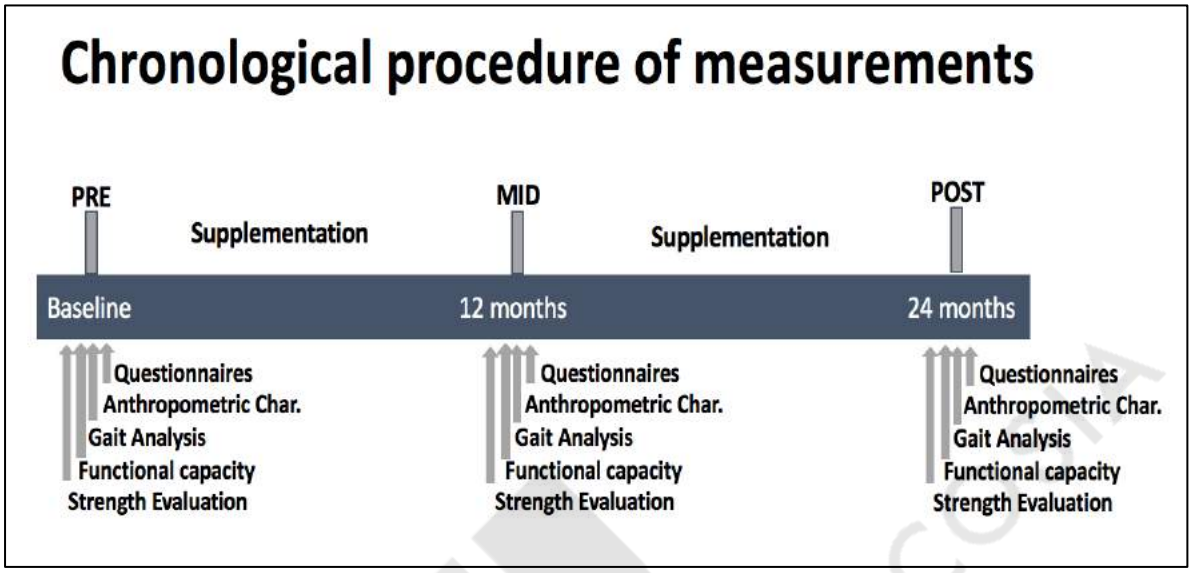

Figure 4. Chronological procedure of the measurements at three different time points

The sequence and timing of each experimental measurement is depicted in Figure 5. Initially, the evaluation of the quality of life parameters obtained by questionnaires, followed by 10 minutes of rest and then assessment of anthropometric characteristics and body composition was performed. After another 10 minutes of rest 3D gait analysis evaluation was done and completed in no more than 20 minutes (average 15 min). After another 10 minutes of rest the functional capacity evaluation (STS, TUG, 6MWT) was performed and completed within 15 min. Following another 10 minutes of rest the muscle function assessments was done (handgrip dynamometry and isokinetic evaluation). The muscle assessment was left as the last measurement as it was the most tiring for the participants and could affect the other measurements if it was done with a different sequence. The whole process was around 2 to 2.5 hrs. Detailed description of the experimental measurements is included in the relevant chapters.

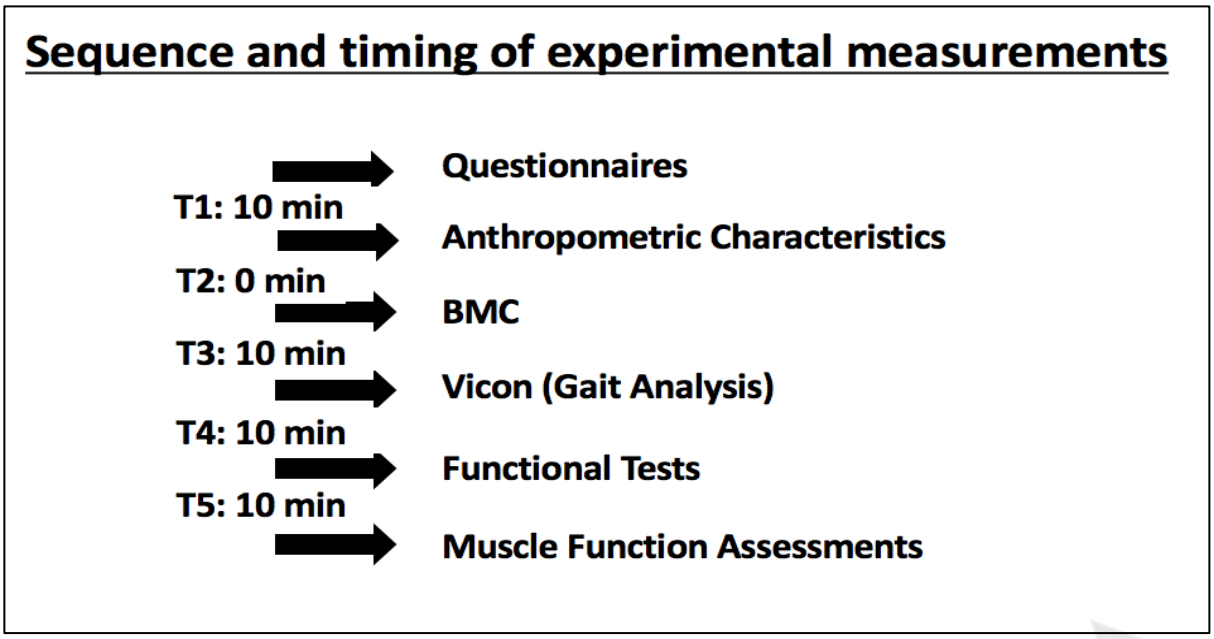

Figure 5. Timeline and rest period of Measurements

1.4.1 Anthropometric Characteristics

Anthropometric characteristics including height and body weight were measured using a standing stadiometer (Seca model 220, Germany) and an analogue scale (Seca model 755, Germany) respectively. Body mass index (BMI) was calculated as body weight divided by height squared (fig. 6).

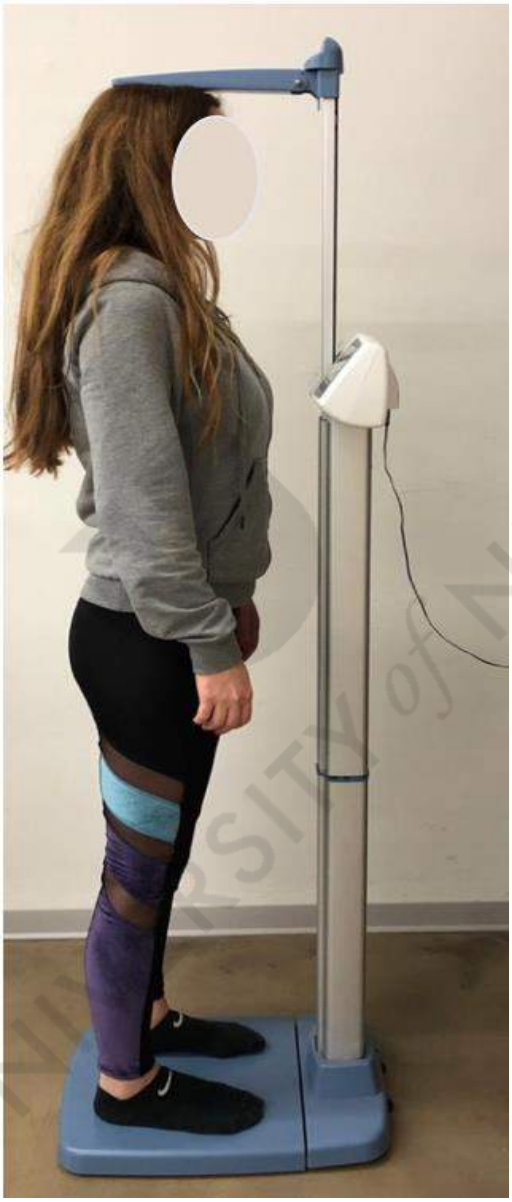

Figure 6. Height and body weight measurement. Standing stadiometer (Seca model 220, Germany) and analogue scale (Seca model 755, Germany)

1.4.2 Body Composition Assessment

Total body fat (fig. 7) assessed by the bioelectrical multi-frequency impedance analyzer (Bodystat, Quadscan 4000) with subjects in the supine position. Current-injector electrodes were placed just below the phalangeal – metacarpal joint of the subjects in the middle of the dorsal side of the right hand and below the metatarsal arch on the superior side of the right foot. Detector electrodes were placed on the posterior side of the right wrist, midline to the pisiform bone of the medial (fifth phalangeal) side with the wrist semi flexed.

Trunk fat percentage and visceral adiposity (ranging from 1 to 59 in arbitrary units) were quantified by the use of the abdominal BIA device ViScan (Tanita AB-140, Tanita Corp., Tokyo, Japan). A wireless 'electrode belt' was placed on the bare waist (fig. 8) of the subject in a supine position.

Lean Body Mass was calculated by: $\mathbf{weight} - \frac{(\mathbf{weight} * \mathbf{body\ composition})}{\mathbf{100}}$.

Figure 7. Total Body Fat assessment by Bodystat Quadscan 4000 Touch

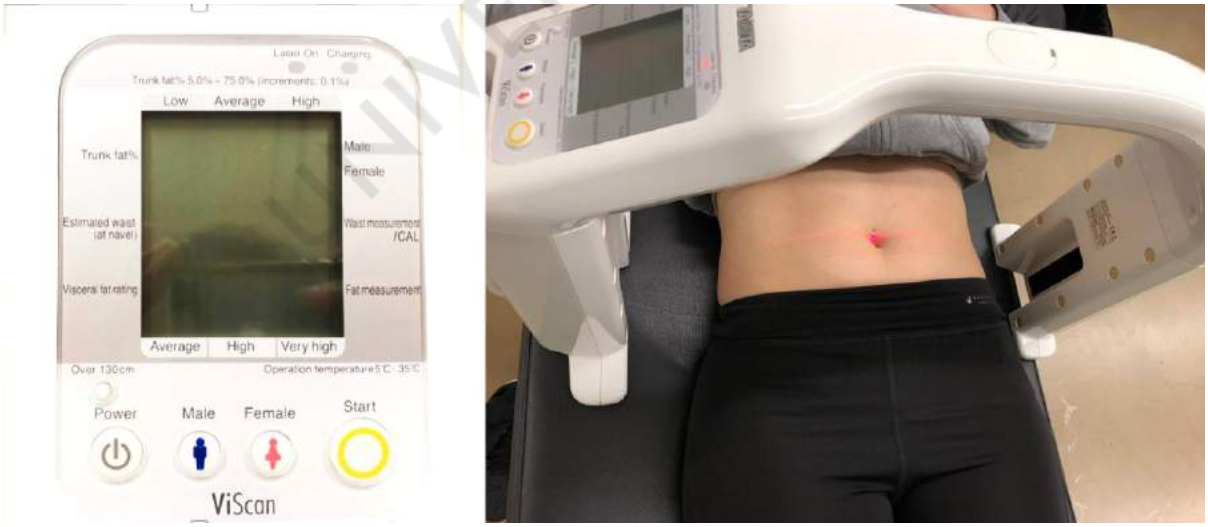

Figure 8. Trunk fat assessment by Tanita AB 140 Viscan

CHAPTER 2 ASSESSMENT OF MUSCLE FUNCTION

2.0 Introduction

Relapsing-remitting Multiple Sclerosis (RRMS) is the most common initial form of MS and a growing issue worldwide [2, 106]. At the same time, it is considered to be the leading neurological cause of disability in young adults, associated with impairments physically and psychologically [6, 107]. According to the literature, the disease affects the quality of life, resulting in a significant financial problem on the patient, family, health system and society [108]. PwMS experience depression, fatigue, poor sleep, daytime sleepiness, cognitive impairment, visual disturbances and a variety of physical symptoms including impairments of muscle function, coordination and sensory impairments [109] all significant contributing factors to reduced quality of life.

2.1 Strength

Although the precise etiology of Multiple Sclerosis (MS) remains unclear, the adverse effects from the disease are well-acknowledged, leading to a variety of impairments. One of these impairments concerns reduction in muscle function and power [110-113]. According to the International Classification of Functioning model negative alterations and reduction in muscle function have critical implications in PwMS [114]. Well documented studies in PwMS reveal that reduced lower extremity muscle function negatively affects walking performance [111, 115-117], balance [116], stair climbing and sit-to-stand ability reported both objectively [117, 118] and subjectively [119]. Reduced lower extremity muscle function could explain the lower physical activity levels observed in PwMS compared to healthy controls (HC) [120, 121].

Additionally, an early symptom of MS is weakness on one side of the body, particularly in the lower limbs [122]. Due to this unilateral weakness, PwMS often have one side more affected than the other which results in a variety of asymmetries, including measures of power, strength, muscle activity and limb loading [111, 123].

2.1.1 Upper extremity Muscle Function

Handgrip strength is a prognostic measure of more inferior health status including loss of independence and increased morbidity and mortality in studies with older adults and in clinical population (such as people with stroke incidences) [124-126]. Upper extremity motor function, coordination and sensation are largely affected in PwMS, leading to negative alterations, reduction in muscle function and dependence on others for daily life activities [127, 128] such as driving, drinking, eating and writing [129]. Yospatiran et al. (2006), compared the upper extremity index with QOF, EDSS, cognition and fatigue and the results indicated that disability levels and cognitive function in PwMS related with the upper extremity motor function [128]. As a result, they concluded that this impairment of physical domain contributes to an impairment of the quality of life.

Hand grip strength is often used to evaluate upper limb impairment in both the dominant and non-dominant hands [130]. Although the dysfunction of the upper limbs is common, its importance is under-recognized relative to the lower extremity muscle impairment, probably because the most frequently used measure of disability in MS, the Kurtzke Expanded Disability Scale [13], is heavily weighted toward lower limb function.

2.1.2 Lower extremity Muscle Function

Studies extensively focus on techniques that will potentially improve lower extremity function in PwMS in both research and clinical practice [131], since lower extremity muscle function is more affected than the upper extremity muscle function. Both quality of life and independence strongly related to adequate levels of lower extremity muscle function and power [119, 132]. In contrast, inadequate levels of lower extremity muscle function negatively influence daily activities such as walking, stair climbing and balance [111, 115]. Moreover, reduced activity levels could eventually increase the risk of all-cause mortality, metabolic syndrome, cardiovascular diseases (CVD), osteoporosis and some types of cancer [133, 134].

To date, although several studies in PwMS have consistently show the benefits of resistance training in maximal muscle strength [135, 136], not all of them reported an optimistic impact on walking or other functional tests [137, 138], in contrast with elderly and stroke patients [139, 140] were muscular strength and fitness are important factors of walking capacity [141]. Additionally, while many studies identified asymmetries, not only it is unclear whether muscle asymmetry impairs gait and balance, but also at which magnitude asymmetries become

functionally relevant and whether impairments worsen proportionally to an increase in asymmetries [142].

Muscle strength is defined as an essential predictor of ambulatory function [139]. Individuals with MS have significantly reduced muscle strength compared to their matched healthy controls [143], and their ability to generate maximal force in the lower extremities (knee flexors and extensors) is also reduced [144].

Isokinetic testing is a safe and reliable process of objective neuromuscular testing for ambulatory patients with MS [145] and is viewed as the 'gold standard' when assessing muscle strength. Researchers among the years tried to compare isometric and isokinetic upper leg muscle strength with walking capacity between a healthy population and MS patients and between mild and moderate MS patients [146, 147]. To date, several studies show that resistance training can improve muscle strength and functional capacity in MS patients [135, 148].

Muscle strength of PwMS is considered an essential determinant of walking ability based on research outcomes. For example, in the study of Thoumie et al. (2005), they observed that the walking ability in MS patients reduced and that the more reliable indicator of gait velocity was the isokinetic strength of the knee flexor muscles at 60^{o}/sec [115]. In the study of Cantalloube et al. (2006), where 21 PwMS evaluated, they reported significant correlations between gait speed and peak torque values of the quadriceps and hamstring muscles [149] while in the study of Yahia et al. (2011), they reported significant correlations between balance and gait disorders and lower extremity muscle weakness [116]. Broekmans et al. (2011), indicated that long term light to moderate resistance training improves muscle strength [137]. Furthermore, knee extensor endurance strength, as well as isometric knee flexor strength, indicated as significant predictors for walking capacity in PwMS with moderate ambulatory dysfunction [150].

2.2 Evaluation of muscle fatigue via EMG

The summation of electrical activity that active motor units produce over a muscle of interest represented by the electromyographic signal (EMG). The electromyographic signal can be surface (sEMG) or intramuscular (imEMG) [151]. Surface electromyography (sEMG) is more frequently used than intramuscular electromyography (imEMG) when muscle function obtained. Intramuscular electromyography is invasive, requires medical personnel, can cause tissue damage, and can cause pain in muscles during muscular contraction, which limits the number of muscles that can be studied simultaneously [152].

The amplitude and the frequency domain of the EMG signal is influenced by muscles fatigue because during fatigue, more muscle fibers need to be recruited in order to sustain the desired performance [153]. Limitations in skeletal muscles or reduced neural drive are the main responsible factors for exercise-induced fatigue [154-156]. Muscle behavior depends not only on the peripheral mechanisms (contractility of muscle fibers, lactate production etc.) but also on the regulation by the central nervous system (involves events incurring in the brain and spinal cord) [157]. The terms 'peripheral fatigue' and 'central fatigue' used to discriminate the two possible causes of muscle fatigue [154, 157, 158]. Two accessible and useful frequency domain features for electromyography analysis in clinical applications are the mean frequency (MNF) and median frequency (MDF). MNF and MDF frequently used as the gold standard tool in the case of analyzing MU recruitment and assessing muscle fatigue in the muscles of interest using EMG signals [159-161]. During a procedure that induces fatigue it has been shown that the mean and median frequencies of the EMG signal decrease with time. Additionally, during a rapid voluntary contraction, the ability to increase muscle force as quickly as possible, rely on both neural and muscular factors [162]. Neural factors include motor unit firing frequency and recruitment [163, 164], while muscular factors include cross-sectional muscle area, muscle fiber type composition [165, 166] and muscle fiber contractility [167]. These elements have also been observed in PwMS [168-172] and data support that the neural factors are more affected than the muscular factors [168, 171] at least in the short term, thus corresponding well with the etiology of MS.

Fatigability of MUs and force capacity has an inverse relationship. The stronger MUs are more fatigable (fatigue more rapidly) than the weaker ones [173-175]. Additionally, there is a tendency for weaker motor units to have slower twitches (i.e. longer contraction time) than stronger MUs [173]. The neural mechanisms underlying the orderly recruitment of MUs from weakest, slowest and least fatigable to strongest, fastest and most fatigable were largely revealed by Henneman and colleagues and is referred to as the size principle [176, 177]

Once recruited, individual Motor Units increase their firing rate with increased synaptic excitation over a relatively narrow range of values before saturating at levels that appear to be inversely related to the MU's recruitment threshold [178-180]. During a given contraction, MUs within a muscle can possess a wide range of activities, from those not yet recruited to those that have reached their maximal firing rates. Motor Units will fatigue at different paces dictated both by their individual firing rates (which can vary over time) and the intrinsic fatigabilities of their innervated muscle fibers. Because of this complexity, it has been difficult to predict the time-course of muscle fatigue, even for relatively simple tasks involving sustained target forces, let alone for tasks in which force levels vary over time and include varying periods of recovery between contractions. Furthermore, when challenged with different tasks, a muscle might eventually accumulate the same level of fatigue (loss in overall muscle force capacity) but do so with very different combinations of fatigue within the individual Motor Units.

2.2.1 Median Frequency

Frequency is a crucial component in EMG testing because it shows the firing rate of action potentials over time [181]. Commonly in EMG studies, the time domain is used as a variable when doing data analysis where it shows how a signal changes over time. Total power spectrum are usually applied as indices to characterize EMG signals, especially for muscle contractions [182]. When analyzing EMG readings, fatigue is present when there is a shift to lower frequency outputs in the spectrum [183]. When considering these facts, it is vital to understand the difference between muscle fiber types during evaluation. Fibers that are categorized as fast-twitch and rely on anaerobic respiration will decrease in their activity, or could completely stop firing before fibers that categorized as slow-twitch [181]. Muscles with higher percentage of fast glycolytic and fast oxidative glycolytic fibers, exhibit greater initial values of Median frequency as well as a greater reduction over the course of the contraction[181]. Range frequencies are different depending on the fiber type that being fired. Type I or slow-twitch fibers frequency range between 70-125Hz, while Type II or fast-twitch fibers range between 126-250Hz [184].

Past studies use median frequency (MDF) as a standard measurement of fatigue in data analysis. MDF described as a frequency value obtained from the EMG total power spectrum, where the power spectrum divided in half into two regions showing equal total power [182]. As mentioned above, fatigue thought to be present when there is a shift to lower frequency outputs in the power spectrum. MDF analysis is used to estimate the magnitude of that shift

[183]. Using median frequency to determine fatigue rates can be more accurate because this value is not easily affected by extremes in range of the power spectrum [185]. The power spectrum of an EMG signal shows the frequency power dispersal on the y-axis and the frequency band on the x-axis [186]. The decrease in the conduction velocity of action potentials is directly proportional to the decrease in duration in which they contract and ultimately causes a decrease in the median frequency (MDF). This decrease can serve as an index of fatigue in EMG signals [185].

Researchers may further compute the slope values of the MDF that are analyzed. Modern biomechanical technology can compute these slope values by looking at the frequency values over a time domain, as mentioned above. When there is a negative value in the slope of the MDF computed, that is representing of fatigue in the muscle over a certain period of time. The steeper the slope, the faster the muscle is experiencing fatigue.

Multiple Sclerosis, is a demyelinating disease of the central nervous system, resulting in negative alteration in neuromuscular recruitment, which affects upper motor neurons [171, 187, 188]. Impairment in the central nervous system has been shown to decrease maximal voluntary motor unit firing rates [171, 187, 188], and negatively affects force production. This force impairment should reflected in reduced surface electromyography (sEMG), which is representative of global motor units recruitment [189]. Strong evidence suggests that fatigue in MS results from reduced voluntary activation (VA) of muscles via central mechanisms [190-193]. A study of Hameau et al. (2017), compared quadriceps fatigability in PwMS and healthy subjects during maximal concentric contractions [194]. In that study, the torque fatigue index was lower in PwMS than matched controls, but when torque normalized to maximal isometric peak torque, PwMS and matched controls finished the fatigue protocol at precisely the same level of relative strength (50% of the maximal isometric peak torque). The level of neurological disability identified as an essential factor of functional exercise capacity [195] and fatigue in MS results from reduced voluntary activation [190-193].

The existing knowledge in MS, reveals that muscular strength is an essential determinant of quality of life, and muscle fatigue is a frequent complaint of the patients with MS.

This study aims to assess if PLP10 supplement improves muscular strength in the upper and lower extremities in a period of 24 months with subsequently positive effects in the quality of life of PwMS. Additionally, this study aims to investigate if PLP10 supplementation improves muscle fatigue during a 60s of maximum voluntary isometric contraction in a period of 24 months.

2.3 Methodology

2.3.1 Anthropometry and body composition assessment

Anthropometric characteristics, including height and body weight, are shown in Chapter I, General Methods, Anthropometric Characteristics.

Sequence and timings of muscle function assessment is depicted in figure 9. Handgrip dynamometry was followed by 5 minutes of rest and then knee extensors and flexors isometric and isokinetic evaluation was obtained in both sides. Three minutes of rest before every isometric evaluation and 5 minutes before every isokinetic evaluation was considered sufficient for muscle recovery.

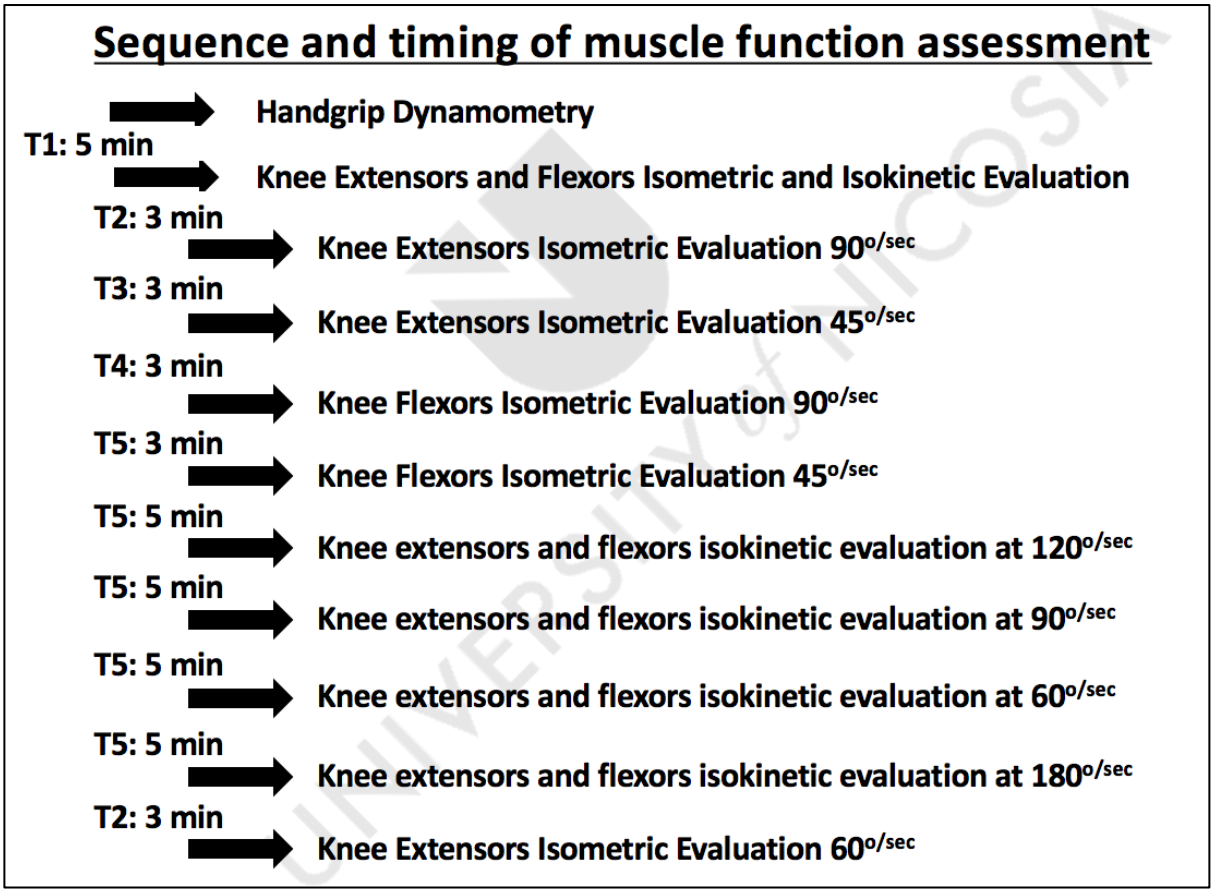

Figure 9. Sequence and timing of muscle function assessment

2.3.2 Handgrip strength assessment

A portable handgrip dynamometer (Takei, Tokyo, Japan) measured the maximal handgrip strength (kg) of both hands (fig. 10). Participants were seated in a standard chair without armrests (seat height 0.43m, seat width 0.45m). They instructed to sit in the middle of the chair, with their back straight, with the shoulder in neutral position and elbow in 90^{o} flexion position holding the dynamometer, not touching the trunk. The handle, adjusted to the participant's hand size, between the first metacarpal (heel of palm) and the middle of the four fingers (proximal interphalangeal joints). Three maximal effort trials lasting 4 to 5 s interspersed with 60-second rests were performed, and the highest value retained for the analysis.

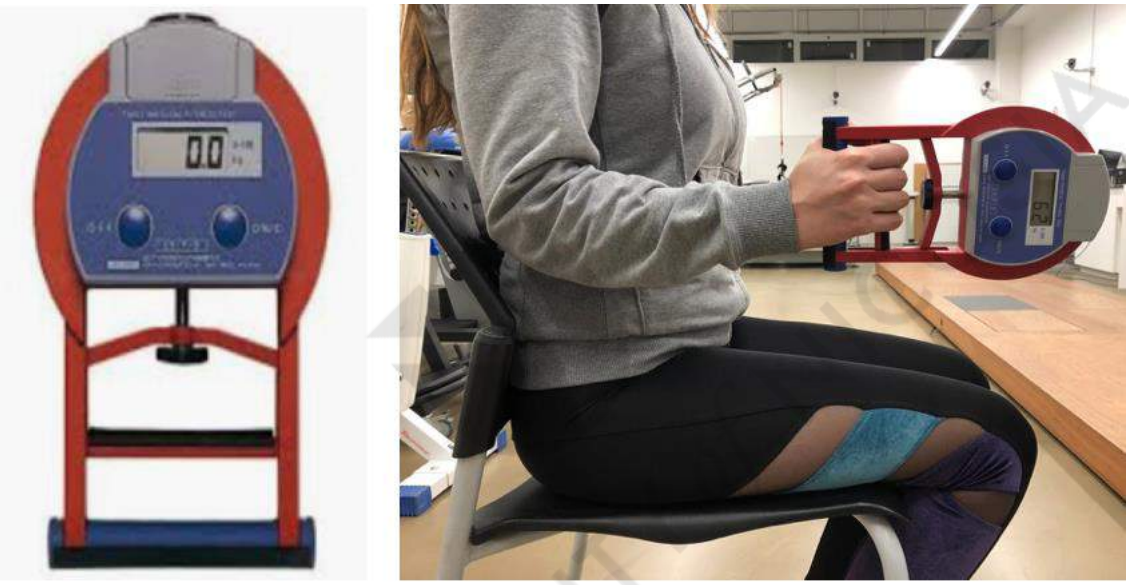

Figure 10. Handgrip dynamometer (left) and position of the subject during testing of grip strength (right)

2.3.3 Isokinetic Dynamometry

Maximal voluntary strength of both legs assessed using an isokinetic dynamometer (model 770, CSMI Humac Norm, MA, USA) which allowed recording of instantaneous isokinetic torque (Fig. 11). After a five-minute standardized warm-up on a bicycle, strength tests performed on an adjustable chair (backwards inclined 5^{o}) in a seated position. The alignment between the knee joint and the dynamometer rotational axis was adjusted to correspond to the femoral condyle axis, and the lever arm was secured 10 cm above the ankle. All the positional adjustment measures recorded for future use and standardization of the testing conditions. The upper leg, hip and shoulders were secured to the equipment with safety straps [196]. The range of motion set at 0–90° (0^{o} corresponding to full knee extension). Before each test, individual calibration completed for gravity correction [197]. This correction determined at the position of 30˚of knee flexion. During the test, participants instructed to keep the arms crossed with the hands-on the opposite shoulder to isolate the quadriceps during the torque production [198]. Following 3 submaximal knee isokinetic trial contractions and 1-minute rest period, bilateral isokinetic (concentric/concentric) flexion and extension of the right and left knee at 60°/s performed five times. Patients had periods of rest between the sessions, and verbal encouragement was standardized. The highest of five isokinetic extension and flexion torques (Nm) selected as the peak dynamic torque.

Absolute values of muscle torque (Nm) subsequently normalized to the lean body mass. Normalization to lean body mass is not only useful for comparing groups with different body sizes; it also reflects how well an individual cope with performing whole-body movement tasks [113].

Lean Body Mass was calculated by: $\mathbf{weight} - \frac{(\mathbf{weight} * \mathbf{body\ composition})}{\mathbf{100}}$

Body composition calculations shown in Chapter I, General methods subchapter, Body Composition Assessment.

Figure 11. Positioning of the subject during Isokinetic Dynamometry

2.3.4 Electromyographic activity

EMG activity of the Rectus femoris and Vastus medialis muscles, recorded simultaneously during the 60 seconds of maximal voluntary isometric contraction using an isokinetic dynamometer (model 770, CSMI Humac Norm, MA, USA) at 60º. EMG data collected using 2 wireless EMG sensors (Trigno, Delsys, Natick, MA, USA) in each limb at a sample rate of 1000 Hz. Bipolar surface EMG electrodes positioned over the vastus medialis (VM) and rectus femoris (RF) according to the SENIAM (surface electromyography for non-invasive assessment of muscles) guidelines [199, 200]. Trigno Wireless Sensors contain two differential EMG inputs with patented stabilizing references. This proprietary design allows the sensor to react instantaneously to disturbances detected on the surface of the skin, dramatically reducing the impact of these noise sources on the detected EMG signal quality. This extremely low noise baseline allows individual motor unit action potentials to be identified from minimal contractions when sensors adequately placed on the skin.

Before electrode placement, the skin area was shaved, abraded and cleaned with alcohol in order to reduce skin impedance and ensure good adhesion of the electrodes. In a sitting position, on a table, with the knees in slight flexion and the upper body slightly bend backward;

For the rectus femoris placement, sensors were placed at the 50% of the line from the anterior superior iliac spine to the superior part of the patella (fig. 12a)

and

For the vastus medialis placement, sensors were placed at the 80% of the line between the anterior superior iliac spine and the joint space in front of the anterior border of the medial ligament (fig. 12b).
All sensors were secured to the skin by a double sided adhesive interface.

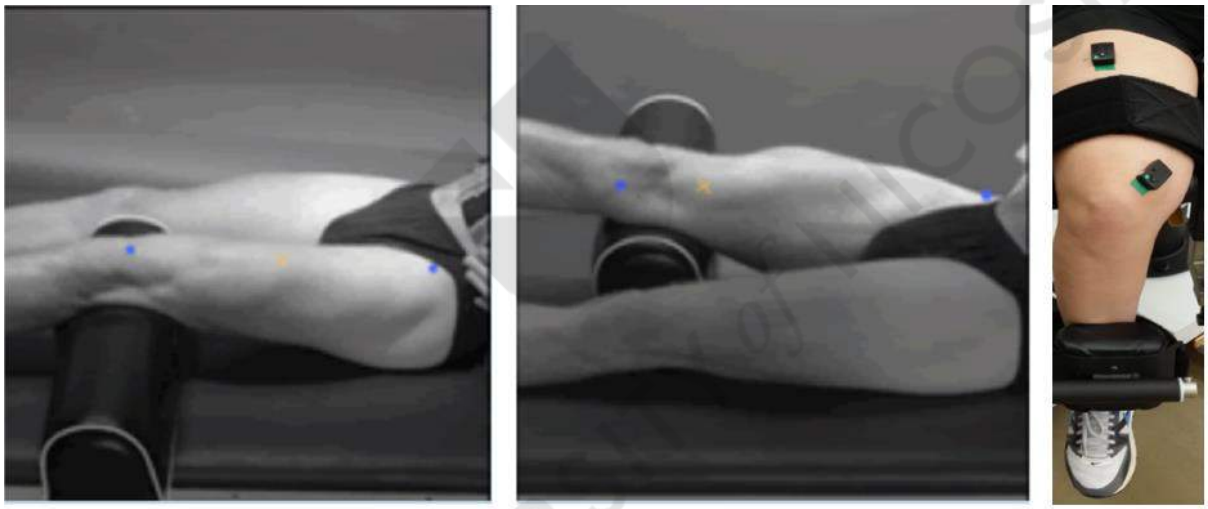

Figure 12. EMG sensors placement. a. Rectus Femoris b. Vastus Medialis c. EMG sensors' placement of Rectus Femoris and Vastus Medialis before the 60s maximum voluntary isometric contraction test in the isokinetic dynamometer

a and b Adapted from (www.seniam.org)

2.3.5 Torque production

The strength of the participants measured by an isokinetic dynamometer (model 770, CSMI Humac Norm, MA, USA). Participants instructed to sit on the dynamometer with their hips, thighs and upper bodies firmly strapped to the seat. In this position, their hip angle was 90^{o} of flexion. The right leg, attached to the arm of the dynamometer, at a level slightly above the lateral malleolus of the ankle joint, and the axis of rotation of the dynamometer arm, aligned

with the lateral femoral condyle. The dynamometer arm set at an angle of 60o from full leg extension as this is in the range to produce maximal torque. The dynamometer arm was set at an angle of 60^{o} from full leg extension as this is in the range to produce maximal torque. Then, the participants performed two sets of 30 seconds sub-maximal isometric contractions to warm up the muscle and to familiarize them with the protocol before carrying out the MVC. Following 5 mins of recovery, the participants then asked to perform one set of 60 s MVC, in each leg. All participants received standardized verbal encouragement to exert maximal effort during the maximal contraction of both legs. They also used the screen of the dynamometer and the torque bar for real-time feedback and asked to keep the bar as high as possible throughout the 60 seconds.

Torque fatigue Index during the maximal isometric contraction of 60 seconds, calculated by deducting the producing average torques of the first 10 seconds (0-10 sec) from the producing average torques of the last 10 seconds (50-60 sec) and dividing the result by the average producing torques of the first 10 secs, and multiply the result by 100.

$$Fatigue\ index = \left(\frac{average\ torque\ (50 - 60\mathrm{s}) - average\ torque(0 - 10s)}{average\ torque(0 - 10s)} \right) * 100$$

Muscular fatigue analysis, obtained in Delsys EMGworks Analysis software, using the Median Frequency function. The Median Frequency is the frequency at which the EMG power spectrum divided into two regions with equal amounts of power. The analysis of the Median Frequency is one of the most effective methods for processing EMG signals during fatigue analysis [201]. During this analysis, muscle fatigue results in an aesthetically pleasing downward shift of the EMG frequency spectrum as time increases [201]. Fibers that categorized as fast-twitch and rely on anaerobic respiration will decrease in their activity, or could completely stop firing before fibers that categorized as slow-twitch [181].

Figure 13. EMG Works Software

In order to analyze muscular fatigue, the participants' data imported to the EMG Works analysis software. EMG total power spectrum of the muscle of interest was selected (rectus femoris or vastus medialis). Subsequently, the median frequency was calculated as a function of time and then data exported to excel. The script converted the data from time series to frequency series - the median frequency calculated using a moving window. The window length chosen for 30 ms (2000 samples/sec) and the calculation run for that window length. The window then moved to the chosen window overlap length, and the calculation run for that window. The window repeated until the window has moved through the entire data set of 60 seconds.

The same procedure attended for all the examined muscles (left and right rectus femoris, left and right vastus medialis) for all the participants.

The exported excel data were further analyzed for all the muscles from all the participants during baseline, 12 months and 24 months assessments. For each patient, MDF data exported to excel and a linear function fitted ($\mathbf{y = ax + b}$).

Median Frequency (MDF) reduction was estimated, using the coefficients of the linear model fitted, between two different time points of the 60 seconds maximum voluntary isometric contraction (10 secs and 50 secs). The reduction in MDF calculated by deducting the value of MDF (50) at the time point of 50 seconds from the value of MDF (10) from the time point of 10 seconds.

As mentioned above, at the initial time points of an isometric contraction that induces fatigue, MDF exhibits higher value because more fast twitch fibers are active. Throughout the contraction, a reduction in the value of MDF will occur because more and more fast-twitch

fibers which rely on anaerobic respiration will decrease in their activity or stop firing entirely. Therefore, the muscle output will be relied on less fast-twitch fibers. As mentioned above, surface EMG exhibits the summation of the electrical activity of both the fast and slow-twitch fibers when the muscle is active. Switching off, of some fast-twitch fibers during a sustained contraction results in reduction of their contribution in the MDF of the contracting muscle. Since slow-twitch fibers exhibit lower firing frequencies than fast-twitch fibers, the MDF of the muscle will decrease during a sustain contraction as fast-twitch fibers switch off while slow-twitch fibers maintain their activity. This decrease can serve as an index of fatigue in EMG signals [185]. The steeper the slope, the faster the muscle is fatiguing.

2.4 Statistical Analysis

Descriptive statistics used to calculate the mean and standard deviation of the examined variables. Baseline differences in the anthropometric characteristics of the patients in the two groups examined using Independent Sample T-test for quantitative variables or X^2 test for categorical variables. Differences between the groups tested using a repeated measure (mixed model) ANOVA design with group (control vs experimental) as between-subject factor and time (baseline, 12 months and 24 months) as the within-subject factor. Pairwise comparisons with Bonferroni correction used when ANOVA demonstrated statistically significant differences. All analyses carried out using the Statistical Package for the Social Sciences software (SPSS for Windows, version 19.0, Chicago, Illinois). Data presented as mean ± SD and the level for statistical significance set at $p \leq 0.05$.

Power calculations for the sufficiency of sample size were performed for the original study by Pantziaris et al. The current study utilized the sample of the original study only. Therefore, the power of the study based on the recruited sample of the 51 participants was performed using Power G* software. The analysis was based on the effect size on the STS60 test (estimated by $\eta^2 = 0.099$). A repeated measures model with between and within subject effects was used with a small to moderate correlation of 0.3 between repeated measures. Based on these assumptions the calculated power of the study was > 0.90.

2.5 Results

All participants were able to perform the assessments of muscle function without injuries and adverse effects. At baseline, there were no significant differences in age, height and weight between the two groups ($p>0.05$) (Table 1).

Table 1. Patients anthropometric characteristics (All data are mean ± SD)

Variables	Placebo Group	PLP10 Group	P Value
Age (yrs)	37.87 ± 5.38	39.00 ± 8.34	0.575
Weight (kg)	72.78 ± 17.10	70.95 ± 14.63	0.683
Height (cm)	165.79 ± 7.80	166.66 ± 8.22	0.700
Gender	11 males; 13 females	10 males; 17 females	0.524
EDSS	2.56 ± 1.04	2.22 ± 1.04	0.251

2.5.1 Muscle strength

Peak handgrip strength of the right arm showed an increase in the placebo group by 6.60 % during the first 12 months of the study while in the experimental group handgrip strength increased by 3.45 %, From the 12 months assessment to the 24 months follow up assessment both groups improved their strength by 6.49% and 10.51% respectively. In total, during the two years mark, there was an increase in handgrip strength of the right arm by 12.73% in the placebo and by 14.09% in the experimental group (table 2). Although there was an increase in muscle strength results did not reveal any statistically significant group effect (p = 0.796) or interaction between group and time (p=0.739). A significant time effect found between the baseline and 12 months follow up assessment and between 12 months and 24 months follow up assessment (p=0.001). The same results appeared on the peak handgrip strength of the left arm (table 2). During the first 12 months of the study there was an improvement of the peak handgrip strength by 3.51% in the placebo and by 8.06% in the experimental group. In the two years mark, both groups improved their muscle strength by 1.81% and 5.80% respectively. In total, during the 24 months intervention, there was a strength improvement by 5.38% at the placebo group and by 14.33% at the PLP10 group without any statistically significant group effect (p= 0.560) or interaction between group and time (p=0.415). A significant time effect was found between the baseline and the 24 months follow up assessment (p=0.012).

Table 2. The results of the handgrip dynamometry test in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Handgrip Strength of Right Arm (kg)					
Baseline	28.90 ± 9.60	28.24 ± 7.47			
12 months	30.81 ±11.44	29.25 ± 7.87	**0.001**	0.739	0.796
24 months	32.58 ± 9.83	32.22 ± 8.87			
Handgrip Strength of Left Arm (kg)					
Baseline	29.33 ± 9.55	26.30 ± 6.69			
12 months	30.36 ± 11.16	28.42 ± 7.02	**0.012**	0.415	0.560
24 months	30.91 ± 11.89	30.07 ± 7.60			

All isometric and isokinetic measurements revealed the same pattern of variation and change on knee maximal muscle torque and fatigue irrespective of testing angle and velocity. Only the evaluation at $60^{o/sec}$ is presented in this chapter as this velocity is considered the gold standard velocity in PwMS.

Indicative data from the right knee extensors and flexors muscles during 5 repetitions of isokinetic concentric contraction test at $60^{o/sec}$ from two individuals with MS in this study (figure 14 and 15). The participant in the placebo group is a female (age: 38 years, EDSS: 2.5, MS duration: 12 years). In comparison, the participant in the experimental (PLP10) group is a female (age: 40 years, EDSS: 2.5, MS duration 12 years).

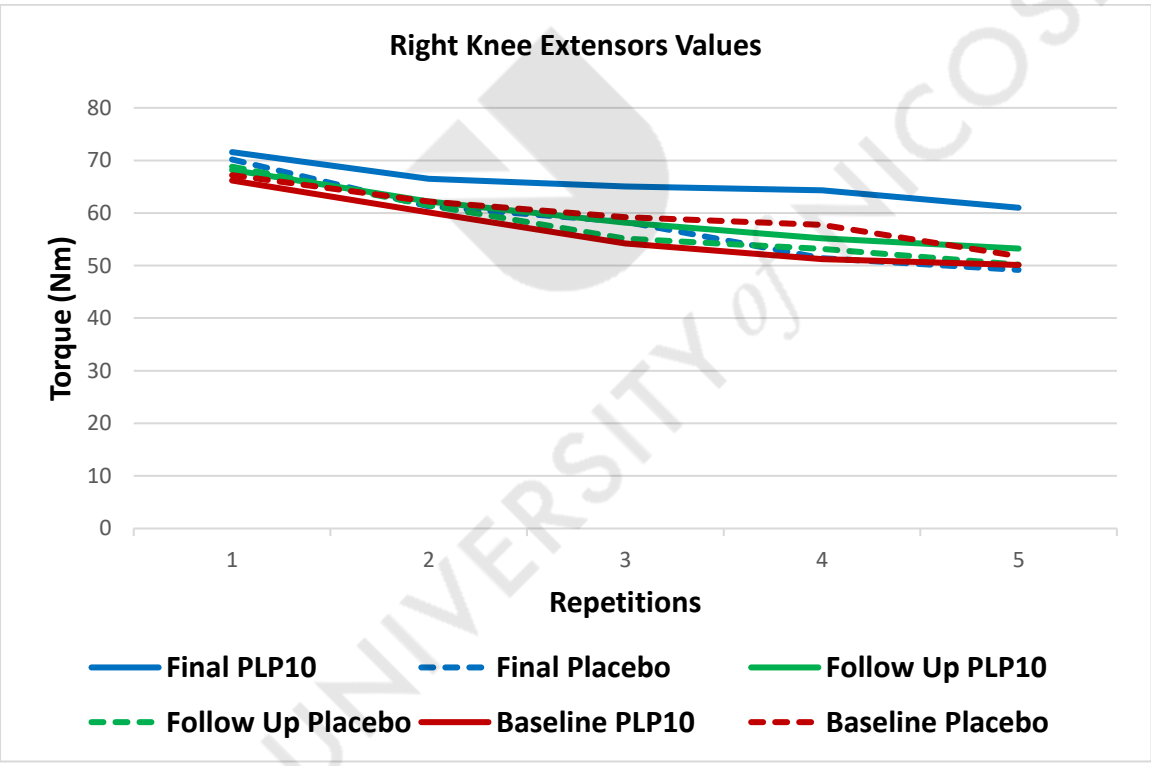

Figure 14. Right knee extensors peak values during 5 repetitions of isokinetic concentric contraction test at 60^{o}/sec from two individuals with MS in this study, for all three time-points, baseline, 12 months and 24 months follow up assessment

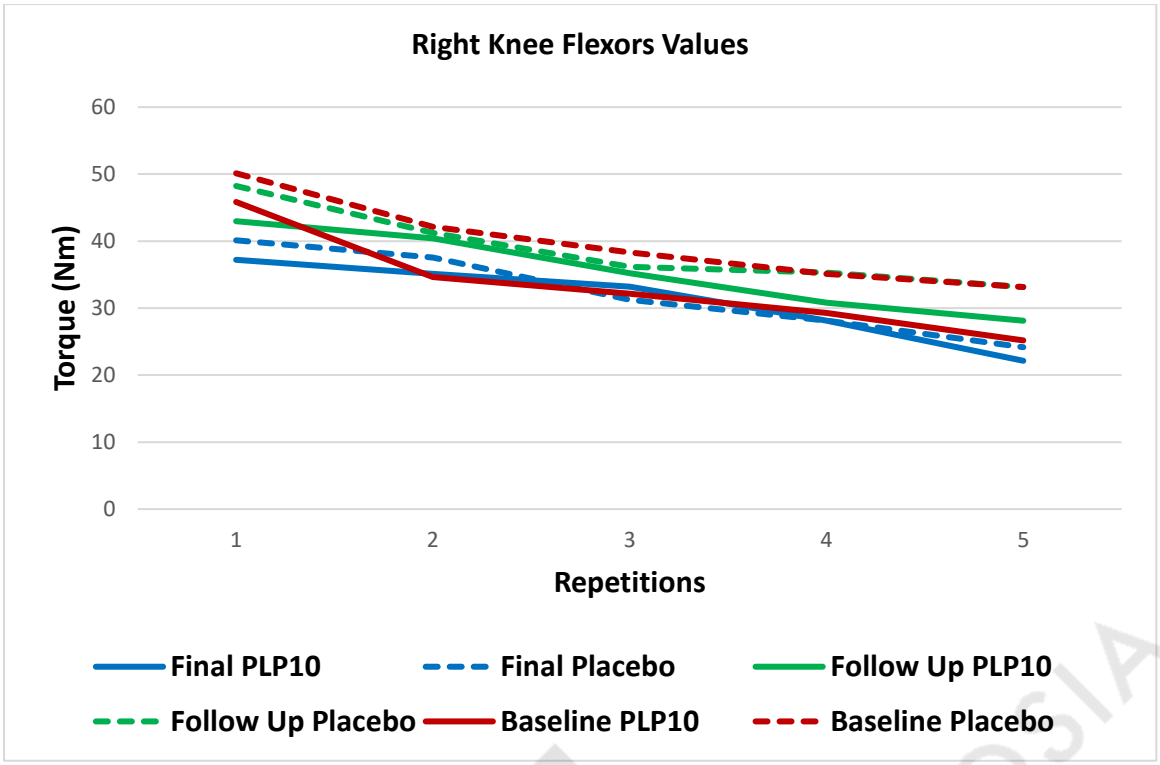

Figure 15. Right knee flexors peak values during 5 repetitions of isokinetic concentric contraction test at 60°/sec from two individuals with MS in this study, for all three time-points, baseline, 12 months and 24 months follow up assessment

Maximum right knee extension strength decreased in the placebo group by 4.56% during the first 12 months of the study, while in the experimental group improved by 4.56%. From 12 months to the 24 months follow up assessment, right knee extension strength improved in both groups, in the placebo group by 3.06% and by 0.87% in the experimental group. In total during the 24 months assessment, PwMS in the placebo group decreased their knee extension strength by 1.46% while PwMS in the experimental group improved their strength by 5.47%. Despite these changes, there was no statistically significant time effect (p= 0.774) or group effect (p=0.255), neither a statistically significant interaction between group and time (p=0.421) (table 3).

Maximum left knee extension strength decreased in the placebo group by 0.51% during the first 12 months of the study while in the experimental group improved by 3.19%. From 12 months to 24 months follow up assessment, knee extension strength increased in both groups, at the placebo group by 11.22% and by 3.60% at the PLP10 group. During the 24 months assessment, both groups improved their left knee extension strength, by 10.76% in the placebo group and by 6.97% in the experimental group (table 3). Despite these changes, results revealed only a time effect (p=0.037) between the baseline and the 24 months follow up assessment.

Table 3. The results of the maximum isokinetic knee extensors strength in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Max Isokinetic Strength of Right Knee Extensors at $60^{o/sec}$ Normalized by Lbm (N.m.kg−1)					
Baseline	2.05 ± 0.79	2.19 ± 0.62			
12 months	1.96 ± 0.89	2.29 ± 0.68	0.774	0.421	0.255
24 months	2.02 ± 0.74	2.31 ± 0.42			
Max Isokinetic Strength of Left Knee Extensors at $60^{o/sec}$ Normalized by Lbm (N.m.kg−1)					
Baseline	1.95 ± 0.62	2.15 ± 0.45			
12 months	1.94 ± 0.82	2.22 ± 0.59	**0.037**	0.618	0.284
24 months	2.16 ± 0.74	2.30 ± 0.40			

Abbreviations: Lbm, Lean body mass

Maximum right knee flexion strength progressively decreased in the placebo group from 1.31 (N.m.kg−1) at baseline to 1.30 (N.m.kg−1) in the 12 months assessment and finally to 0.97 (N.m.kg−1) in the 24 months assessment. In the experimental group maximum right knee flexion strength increased from 1.42 (N.m.kg−1) at baseline to 1.50 (N.m.kg−1) in the 12 months assessment and finally decreased to 1.12 (N.m.kg−1) in the 24 months assessment. Maximum left knee flexion strength progressively decreased in the placebo group from 1.31 (N.m.kg−1) at baseline to 1.24 (N.m.kg−1) in the 12 months' assessment and finally to 1.00 (N.m.kg−1) in the 24 months assessment. In the experimental group maximum left knee flexion strength increased from 1.36 (N.m.kg−1) at baseline to 1.39 (N.m.kg−1) in the 12 months assessment and finally decreased to 1.05 (N.m.kg−1) in the 24 months assessment. For both legs results reveal a statistically significant time effect (p=0.025) between the baseline and the 12 months assessment, between the baseline and the 24 months assessment and between the first and the 24 months assessment follow up. No statistically significant group effect (p=0.484) or interaction between group and time (p=0.373) was detected (table 4). In the two years mark, right knee flexion strength reduced by 25.95% in the placebo group and by 21.12% in the experimental group, while the left knee flexion strength reduced by 23.66% and by 22.79% respectively.

Table 4. The results of the maximum isokinetic knee flexors strength in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Max Isokinetic Strength of Right Knee Flexors at 60$^{o/sec}$ Normalized by Lbm (N.m.kg−1)					
Baseline	1.31 ± 0.45	1.42 ± 0.35			
12 months	1.30 ± 0.55	1.50 ± 0.31	**0.001**	0.718	0.217
24 months	0.97 ± 0.39	1.12 ± 0.28			
Max Isokinetic Strength of Left Knee Flexors at 60$^{o/sec}$ Normalized by Lbm (N.m.kg−1)					
Baseline	1.31 ± 0.36	1.36 ± 0.31			
12 months	1.24 ± 0.50	1.39 ± 0.36	**0.025**	0.373	0.484
24 months	1.0 ± 0.46	1.05 ± 0.26			

Abbreviations: Lbm, Lean body mass

2.5.2 Assessment of fatigue

The protocol of the study was capable of producing fatigue. Torque reduction over 60 seconds was evident by the extracted data (figures 16 - 21).

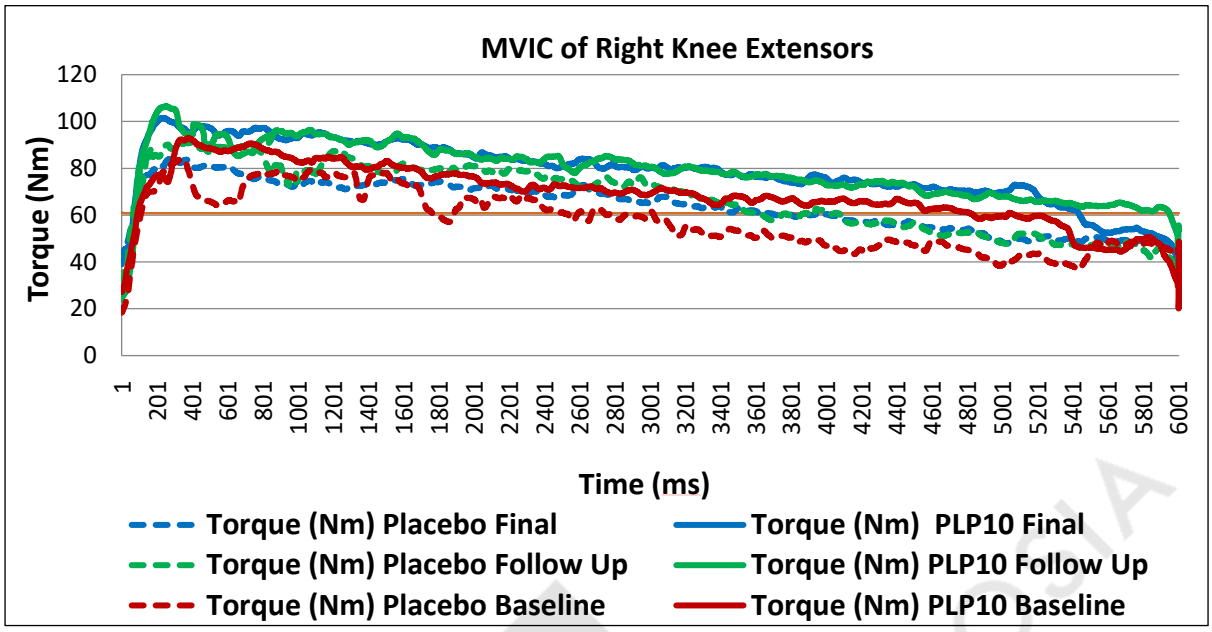

Figure 16. Average traces of the right knee extensor muscles from the placebo and PLP10 group during 60 seconds of Maximum Voluntary Isometric Contraction at baseline, 12 months (follow up) and 24 months (final). Average traces showed a steep linear decrease of torque as a consequence of the fatigue effect

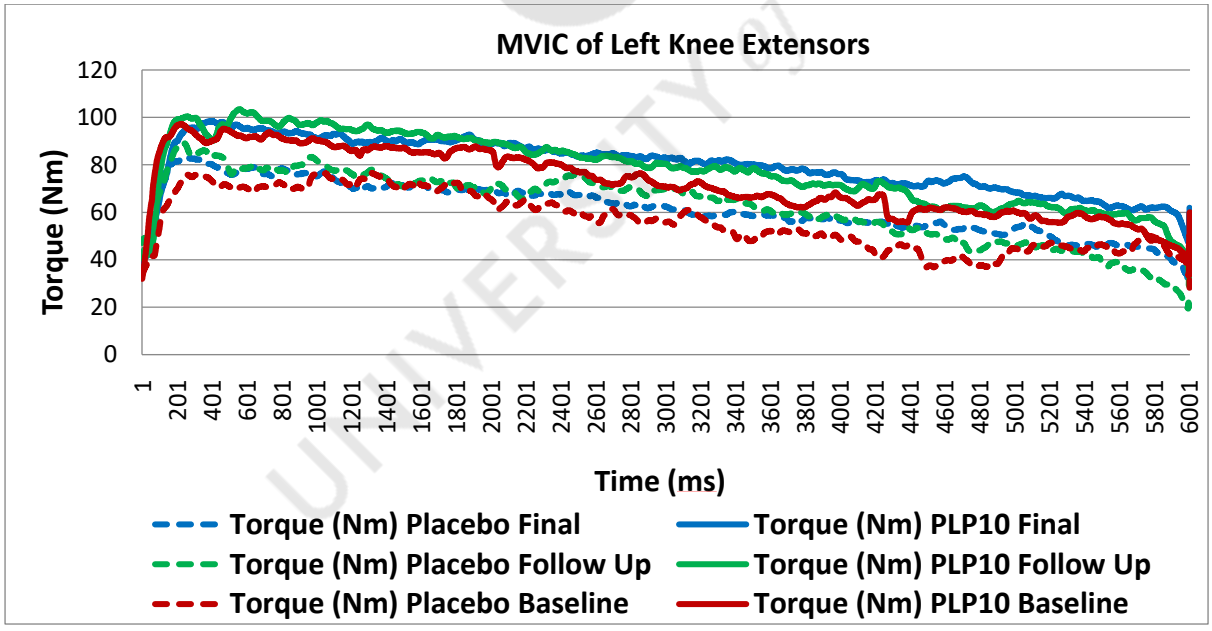

Figure 17. Average traces of the left knee extensor muscles from the placebo and PLP10 group during 60 seconds of Maximum Voluntary Isometric Contraction at baseline, 12 months (follow up) and 24 months (final). Average traces showed a steep linear decrease of torque as a consequence of the fatigue effect

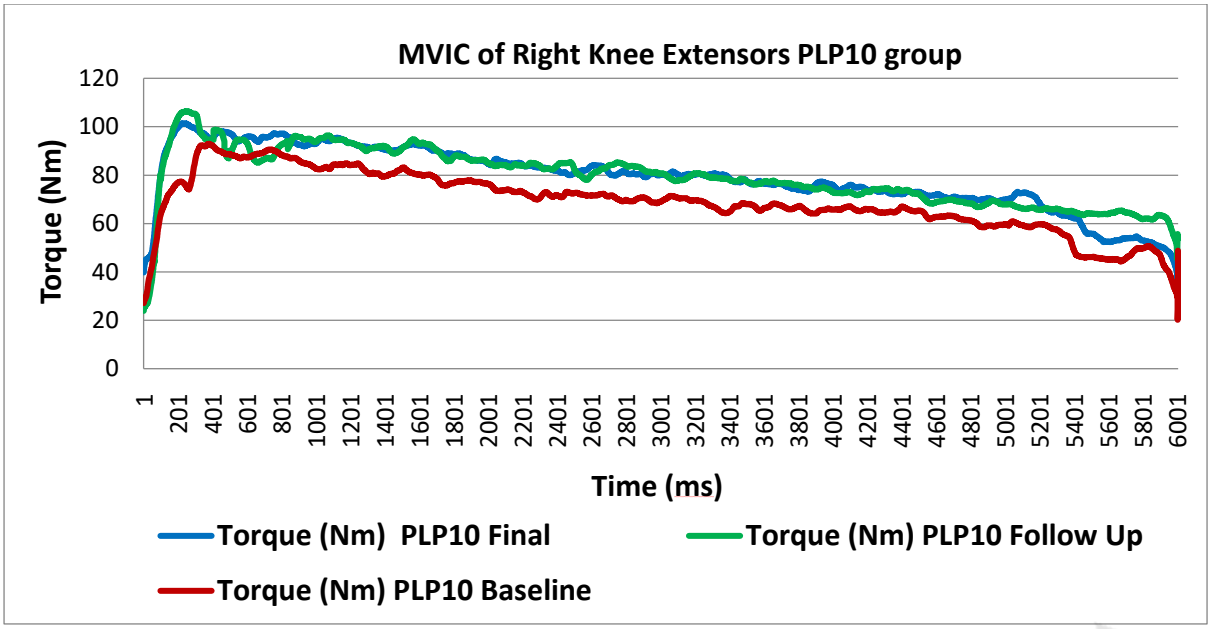

Figure 18. Average traces of the right knee extensor muscles from the PLP10 group during 60 seconds of Maximum Voluntary Isometric contraction at baseline, 12 months (follow up) and 24 months (final). Average traces showed a steep linear decrease of torque as a consequence of the fatigue effect

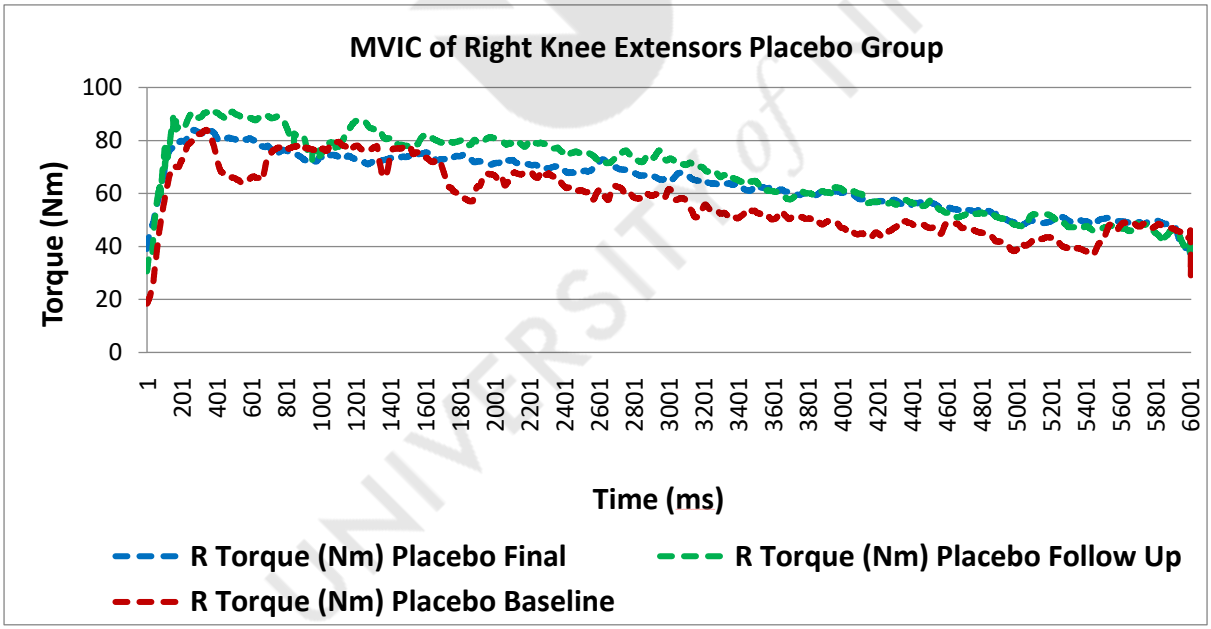

Figure 19. Average traces of the right knee extensor muscles from the placebo group during 60 seconds of Maximum Voluntary Isometric contraction at baseline, 12 months (follow up) and 24 months (final). Average traces showed a steep linear decrease of torque as a consequence of the fatigue effect

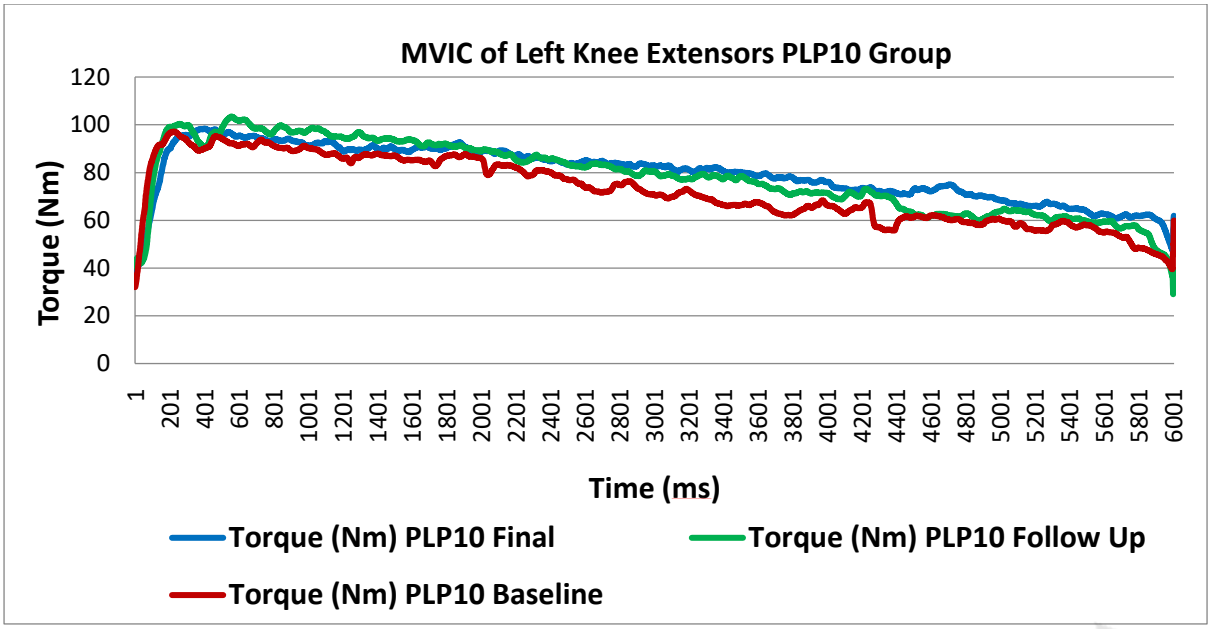

Figure 20. Average traces of the left knee extensor muscles from the PLP10 group during 60 seconds of Maximum Voluntary Isometric contraction at baseline, 12 months (follow up) and 24 months (final). Average traces showed a steep linear decrease of torque as a consequence of the fatigue effect

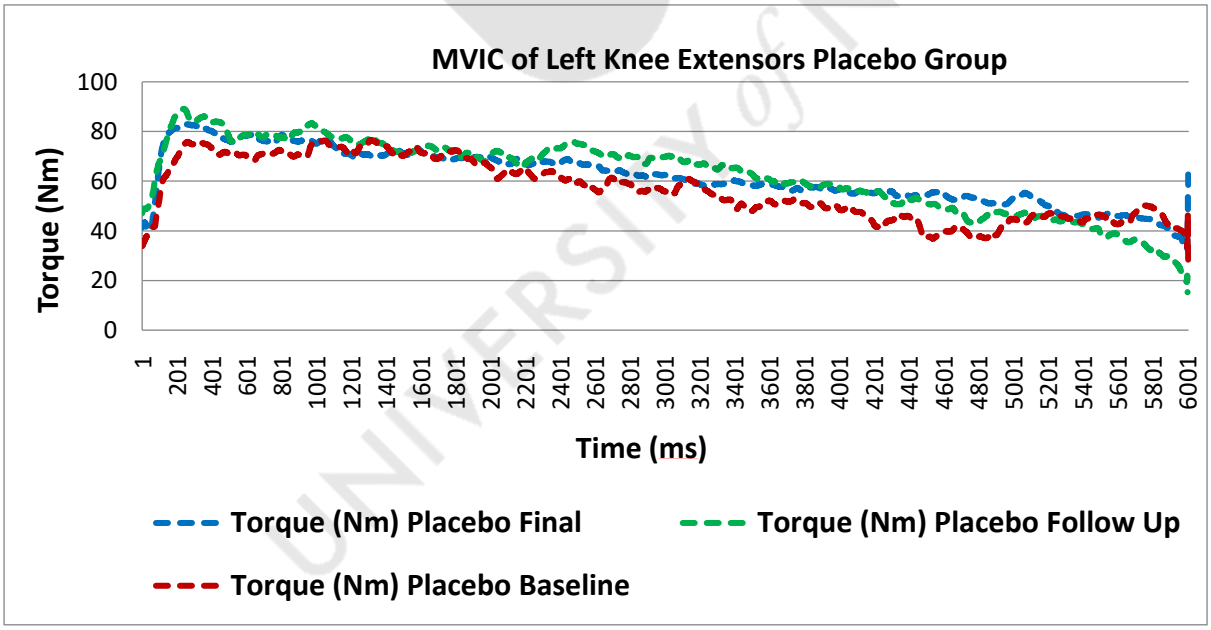

Figure 21. Average traces of the left knee extensor muscles from the placebo group during 60 seconds of Maximum Voluntary Isometric contraction at baseline, 12 months (follow up) and 24 months (final). Average traces showed a steep linear decrease of torque as a consequence of the fatigue effect

The percentage reduction of the producing average torque between the first 10 and last 10 seconds was extracted by the equation below:

$$Fatigue\ index = \left(\frac{((\ average\ torque\ (50-60\text{s}) - average\ torque(0-10s)}{average\ torque(0-10s)}\right) * 100$$

The evaluation of knee extension fatigue index during the 60 seconds maximum voluntary isometric contraction revealed an improvement in the 24 month follow up in both groups (table 5). In this study improvement in fatigue index (in absolute value) is considered a smaller decline of torque value between the last 10 and first 10 seconds of the 60 seconds maximum voluntary isometric contraction. During the 24 months, the right knee extension fatigue index improved by 15.55% in the placebo and by 17.68% in the PLP10 group, while left knee extension fatigue index improved by 19.51% and 2.98% respectively. Fatigue index of the right quadriceps progressively improved in the placebo from 40.5 % at baseline to 40.09 % in the 12 months assessment and finally to 34.22% in the 24 months assessment. In the experimental group fatigue index of the right quadriceps progressively improved from 38.16 % at baseline to 34.51% in the 12 months assessment and finally to 31.41% in the 24 months assessment. Fatigue index of the left quadriceps progressively improved in the placebo from 43.87 % at baseline to 41.86 % in the 12 months assessment and finally to 35.81% in the 24 months assessment. In the experimental group fatigue index of the left quadriceps worsened from 36.9 % at baseline to 37.49% in the 12 months assessment and finally improved to 35.80% in the 24 months assessment. Despite the detected improvement in both groups, there was no time ($p \geq 0.073$) or group ($p \geq 0.438$) effect or any statistically significant interaction between group and time ($p \geq 0.439$) (table 5).

Table 5. The results of the fatigue index induced during the 60 seconds of maximum voluntary isometric contraction in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (*p* value)**		
			Time	Time X Group	Group
Fatigue Index Right Quadriceps (%)					
Baseline	- 40.5 ± 18. 3	- 38.16 ± 20.25	0.073	0.823	0.438
12 months	- 40.09 ± 7.18	- 34.51 ± 16.01			
24 months	- 34.22 ± 12.05	- 31.41 ± 16.12			
Fatigue Index Left Quadriceps (%)					
Baseline	- 43.87 ± 13.21	- 36.9 ± 20.02	0.215	0.439	0.440
12 months	- 41.86 ± 14.78	- 37.49 ± 17.83			
24 months	- 35.31 ± 15.59	- 35.80 ± 15.20			

Right peak quadriceps strength progressively reduced in the placebo group by 8.13% from baseline to the first follow up assessment and by 7.07% from the first to the second follow up. In the 24 months follow up right peak quadriceps strength reduced in the placebo group by 14.63%. In the experimental group, the right peak quadriceps strength reduced by 4.01% from baseline to the 12 months assessment and increased by 2.92% from the first to the 24 months assessment. In the 24 months assessment, the right peak quadriceps strength reduced in the experimental group by 1.20%. Left peak quadriceps strength progressively reduced in the placebo group by 3.87% from baseline to the 12 months assessment and by 5.38% from the first to the 24 months assessment. In the 24 months follow up left peak quadriceps strength reduced in the placebo group by 9.05%. In the experimental group left peak quadriceps strength reduced by 1.22% from baseline to the 12 months assessment and increased by 5.39% from the first to the 24 months assessment. In the 24 months assessment, the right peak quadriceps strength increased in the experimental group by 4.09%. Even though the placebo decreased max torque over the years while medication group did not, results revealed only a time effect ($p = 0.001$) between all the time points during the 24 months intervention at both legs. No group effect ($p \geq 0.236$) or interaction ($p \geq 0.293$) detected (table 6).

Table 6. The results of maximum knee extension torque during the 60 seconds of maximum voluntary isometric contraction in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Isometric Max Torque during 60sec of Right Quadriceps at 60º per Lbm (N.m.kg −1)					
Baseline	2.46 ± 0.93	2.49 ± 0.68			
12 months	2.26 ± 0.49	2.39 ± 0.53	**0.001**	0.424	0.305
24 months	2.10 ± 0.99	2.46 ± 0.61			
Isometric Max Torque during 60sec of Left Quadriceps at 60º per Lbm (N.m.kg −1)					
Baseline	2.32 ± 0.83	2.44 ± 0.60			
12 months	2.23 ± 0.62	2.41 ± 0.48	**0.001**	0.293	0.236
24 months	2.11 ± 0.91	2.54 ± 0.63			

All data are mean ± SD Abbreviations: Lbm, Lean body mass

2.5.3 Median Frequency

Median Frequency (MDF) reduction, estimated between two different time-points of the 60 seconds maximum voluntary isometric contraction, (10 secs and 50 secs) (see chapter II methodology section EMG MDF analysis) for the right and left rectus femoris and vastus medialis for all the participants at baseline, 12 and 24 months.

Right rectus femoris reduction in the MDF between 10 and 50 seconds of the MVIC changed in the placebo group from 7.03 hz at baseline to 3.49 hz in the 12 months assessment and finally to 3.83 hz in the 24 months follow up assessment. In the experimental group right rectus femoris reduction in the MDF between 10 and 50 seconds of the MVIC changed from 9.75 hz at baseline to 6.14 hz in the 12 months assessment and finally to 4.88 hz in the 24 months follow up assessment.

During the follow-up period, right rectus femoris MDF reduction did not reveal any statistically significant group effect (p= 0.173) or interaction between group and time (p=0.821). A significant time effect was found between the baseline and the 24 months follow up assessment (p=0.022) (table 7).

Table 7. The results of the reduction in Median Frequency of the right rectus femoris between 10 and 50 secs of the 60 sec of a maximum voluntary isometric contraction in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right_Rectus_Femoris_ Reduction in Median Frequency 10sec – 50sec (Hz)					
Baseline	7.03 ± 6.03	9.75 ± 9.34			
12 months	3.49 ± 6.60	6.14 ± 7.56	**0.022**	0.821	0.173
24 months	3.83 ± 4.34	4.88 ± 7.47			

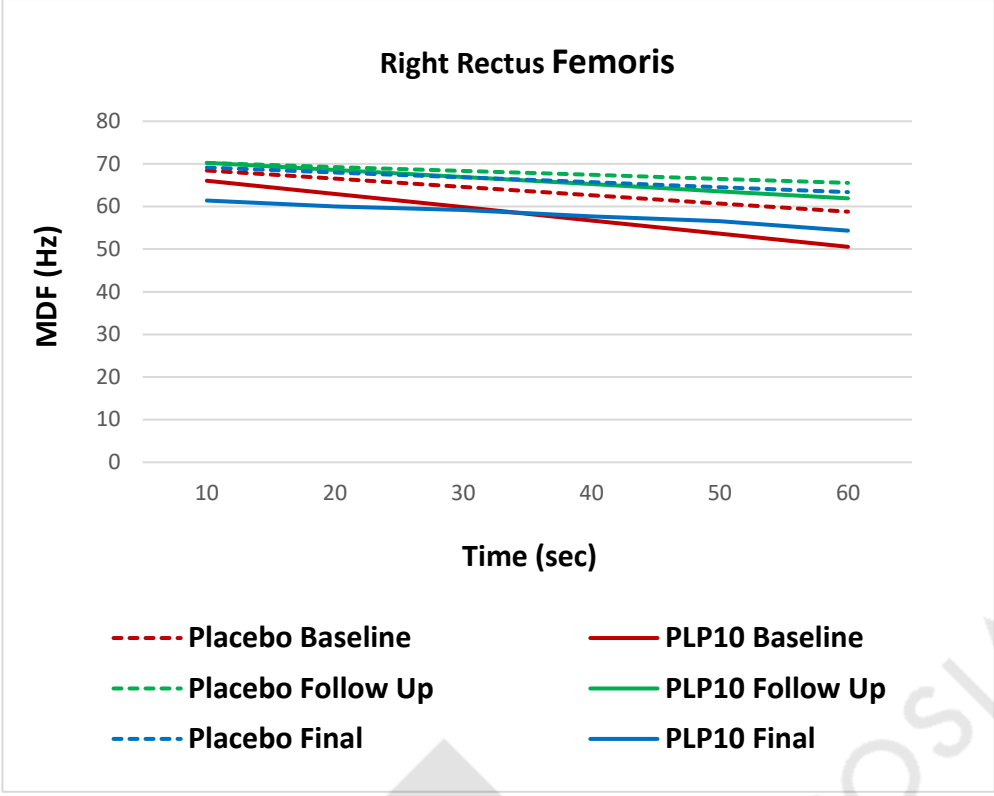

Figure 22. Right rectus Femoris Median Frequency during 60 seconds of maximum voluntary isometric contraction at baseline, 12 months and 24 months follow up assessment

Left rectus femoris reduction in the MDF between 10 and 50 seconds of the MVIC changed in the placebo group from 16.14 hz at baseline to 7.79 hz in the 12 months assessment and finally to 6.52 hz in the 24 months follow up assessment. In the experimental group left rectus femoris reduction in the MDF between 10 and 50 seconds of the MVIC changed from 17.37 hz at baseline to 10.02 hz in the 12 months assessment and finally to 7.25 hz in the 24 months assessment. During the two years assessment, left rectus femoris reduction in the MDF did not reveal any statistically significant group effect (p= 0.603) or interaction between group and time (p=0.953). A significant time effect was found between the baseline and 12 months of assessment and between the baseline and the 24 months assessment (p=0.001) (table 8).

Table 8. The results of the reduction in Median Frequency of the left rectus femoris between 10 and 50 secs of the 60 sec of a maximum voluntary isometric contraction in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left_Rectus_Femoris_ Reduction in Median Frequency 10sec – 50sec (Hz)					
Baseline	16.14 ± 9.19	17.37 ± 15.31	**0.001**	0.953	0.603
12 months	7.79 ± 13.94	10.02 ± 12.51			
24 months	6.52 ± 6.44	7.25 ± 9.01			

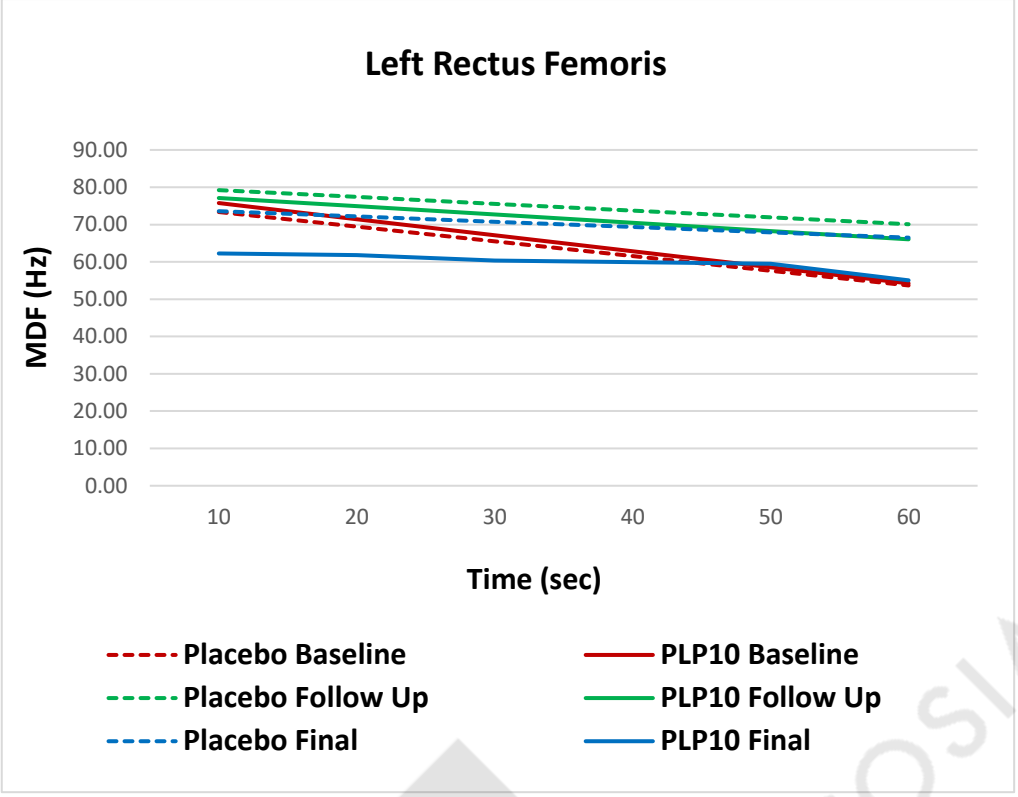

Figure 23. Left rectus Femoris Median Frequency during 60 seconds of maximum voluntary isometric contraction at baseline, 12 months and 24 months follow up assessment

Right vastus medialis reduction in the MDF between 10 and 50 seconds of the MVIC in the placebo group from baseline to the 24 months follow up assessment changed by 64.15%. In the experimental group right vastus medialis reduction in the MDF between 10 and 50 seconds of the MVIC from baseline to the 24 months follow up assessment was less by 60.80 %. During the 24 months assessment right vastus medialis reduction in the MDF did not reveal any statistically significant group effect (p= 0.800) or interaction between group and time (p=0.469). A significant time effect was found between the baseline and 12 months assessment and between the baseline and 24 months assessment (p=0.001) (table 9).

Table 9. The results of the reduction in Median Frequency of the right vastus medialis between 10 and 50 secs of the 60 sec of a maximum voluntary isometric contraction in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right_Vastus_Medialis_ Reduction in Median Frequency 10sec – 50sec (Hz)					
Baseline	7.56 ± 3.65	6.71 ± 6.38			
12 months	2.18 ± 6.69	3.13 ± 6.34	**0.001**	0.469	0.800
24 months	2.71 ± 3.40	2.63 ± 6.30			

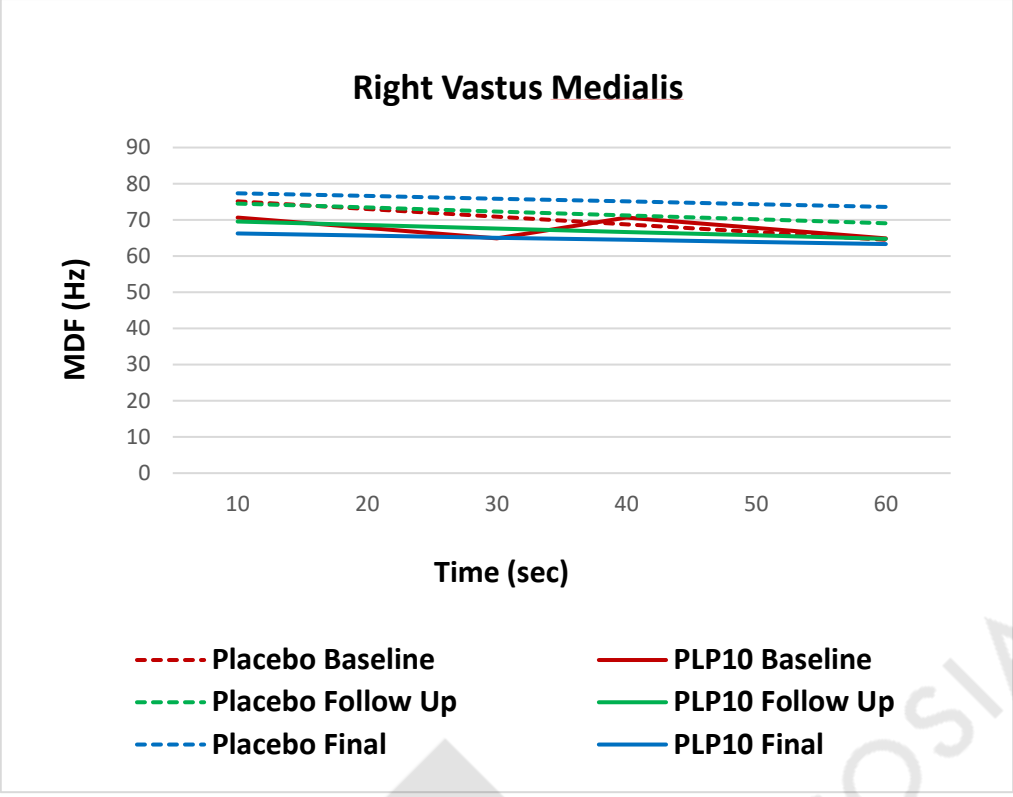

Figure 24. Right Vastus Medialis Median Frequency during 60 seconds of maximum voluntary isometric contraction at baseline, 12 months and 24 months follow up assessment

Left vastus medialis reduction in the MDF between 10 and 50 seconds of the MVIC changed in the placebo group from 9.53 hz at baseline to 2.01 hz in the 12 months assessment and finally to 3.31 hz in the 24 months assessment. In the experimental group left rectus femoris reduction in the MDF between 10 and 50 seconds of the MVIC altered from 7.82 hz at baseline to 6.43 hz in the 12 months assessment and finally to 5.19 hz in the 24 months assessment. During the two years of assessment, left vastus medialis reduction in the MDF did not reveal any statistically significant group effect (p= 0.159) or interaction between group and time (p=0.301). A significant time effect was found between the baseline and 12 months of assessment and between the baseline and 24 months follow up assessment (p=0.001) (table 10).

Table 10. The results of the reduction in Median Frequency of the left vastus medialis between 10 and 50 secs of the 60 sec of a maximum voluntary isometric contraction in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left_Vastus_Medialis_ Reduction in Median Frequency 10sec – 50sec (Hz)					
Baseline	9.53 ± 8.75	7.82 ± 10.32	**0.001**	0.301	0.159
12 months	2.01 ± 7.34	6.43 ± 5.16			
24 months	3.31 ± 3.41	5.19 ± 5.91			

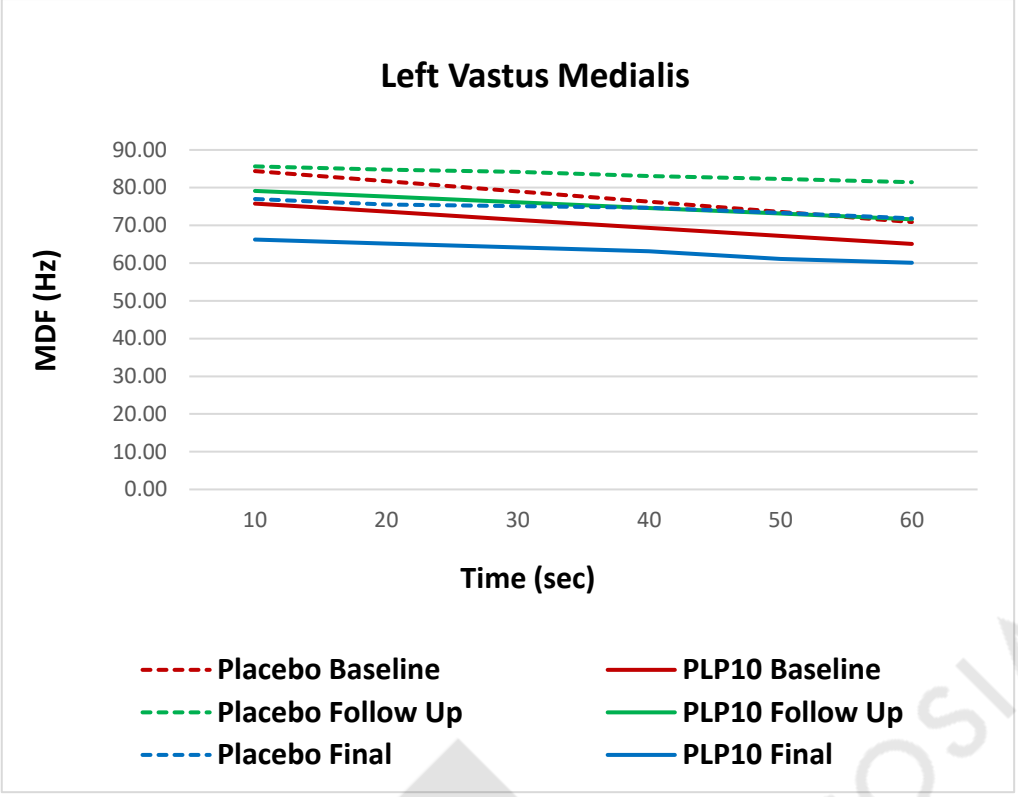

Figure 25. Left Vastus Medialis Median Frequency during 60 seconds of maximum voluntary isometric contraction at baseline, 12 months and 24 months follow up assessment

From baseline to the 12 months follow up assessment, fatigue as revealed by the median frequency difference between 10 and 50 seconds; on the right rectus femoris was less by 50.35% in the placebo group and by 37.02% in the experimental group, on the left rectus femoris was less by 51.73% in the placebo group and by 42.31% in the experimental group, on the right vastus medialis was less by 71.16% in the placebo group and by 53.35% in the experimental group while on the left vastus medialis was less by 78.90% in the placebo group and by 17.77% in the experimental group.

From the 12 months assessment to the 24 months follow up assessment, fatigue as revealed by the median frequency difference between 10 and 50 seconds; on the right rectus femoris was more by 9.74% in the placebo group while for the experimental group was less by 20.52%, on the left rectus femoris was less by 16.30% in the placebo group and by 27.64% in the experimental group, on the right vastus medialis was more by 24.31% in the placebo group while for the experimental group was less by 15.97%, on the left vastus medialis was more by 64.67% in the placebo group while for the experimental group was less by 19.28%,

In total during the 24 months of assessment, fatigue as revealed by the median frequency reduction between 10 and 50 seconds; on the right rectus femoris was less by 45.51% in the

placebo group and by 49.94% in the experimental group, was less on the left rectus femoris by 59.60% in the placebo group and by 58.26% in the experimental group, on the right vastus medialis was less by 64.15% in the placebo group and by 60.80% in the experimental group, on the left vastus medialis was less by 65.26% in the placebo group and by 33.63% in the experimental group.

2.6 Discussion

The primary aim of this chapter is to identify if the medication had any effect on the assessment of muscle function in the upper and lower extremities in 24 months which could provide a subsequently positive effects in the quality of life of PwMS. Additionally, this chapter aimed to investigate if PLP10 supplementation improved a frequent complain of this patient group, namely the muscle fatigue as assessed during a 60s of maximum voluntary isometric contraction in a period of 24 months.

Hand grip strength is an important predictive of poorer health status and an impairment in grip strength is a contributing factor of worsening quality of life in PwMS [128]. The results for handgrip strength showed that both groups improved their strength during the two years assessment with no significant difference between them. The result was unexpected since the subjects are PwMS which is a progressive disease impairing motor function, in this case, handgrip strength. Additionally, PwMS in this study were not exercising during the time of the experiment in order to improve their strength. The explanation for this outcome might be that the disability status of the PwMS was low, patients were increasingly familiar with the procedure over 24 months and the involved muscle groups are small and less affected [202] from the course of the disease. Moreover, motivation could also be a part of the explanation. Last but not least nonspecific effects such as placebo (expectation) could explain the similar differences in both groups since subjects were blind to the intervention the effect of expectation is consider to be the same. The improvement in handgrip strength for both groups was identical and close to the values from baseline. The literature does not identify a clear minimal clinically significant difference for grip strength. However, decrease in strength of 5.0 to 6.5 kg may provide a rough estimate of meaningful changes in grip strength [203]. Additionally, literature suggests that an increase in strength of 5% to 10% is clinically meaningful on several health-related QOL measures in a variety of patient populations [204-207]. When considering these facts, it can be assumed that both groups clinically improved their handgrip strength in this study. However, since both groups improved their strength and literature does not provide a clear difference for test retest clinical important handgrip strength difference, this study prefers to overlook the results and recommends that future studies need to identify a clear clinically meaningful range number which will be used as a gold standard on handgrip strength measurements.

Lower body muscle strength has been defined as an important predictor of ambulatory function [139]. Individuals with MS have significantly reduced muscle strength and the ability to generate maximal force [143] in the lower extremities is reduced compared to healthy controls

[144]. Results in this study showed that maximum isokinetic strength of the left knee extensors increased and both of knee flexors strength decreased over time. However, there was no significant difference between groups or significant interaction between group and time. Although a strength decline was expected, at least in the placebo group the results indicate that both groups increased their extension strength during the period of the 24 months. Meanwhile, the knee flexion strength reduced. The patients during the 3 visits were increasingly more familiar with the tests and this can account for the slight increase in the extension strength between the 3 time points. However, as the corresponding increasing muscle strength is not due to hypertrophy, the most likely mechanism for the increase in muscle torque seem to be neural adaptation with increase recruitment of motor units in these muscles. The decrease in the knee flexion strength can be related to the effects of the pathology. It seems that the knee flexors are more affected by the progression of the disease and their strength deteriorates sooner compared to the knee extensors. Thoumie et al. (2005), concluded that the isokinetic strength of the knee flexor muscles at 60^0/sec is a reliable indicator of gait velocity [115], therefore strength of the flexor muscles can be an important predictor of muscle impairment in PwMS with low disability status such as the present sample as is the first to be affected.

During the 1-minute maximal voluntary isometric contraction of quadriceps, results for fatigue index, did not show any significant improvement over time in any of the two groups. There was the same increase/decrease in both groups over time. Both groups showed greater resistance to fatigue during 24 months. It was reasonable to investigate if both groups set maximum efforts during the 3 visits and whether they experienced fatigue. Indeed, from the torque graphs (fig. 16 -21), it was confirmed that the strength declined during the duration of the 60 seconds voluntary contraction. The subjects of both groups experienced fatigue, and as a result, their torque value reduced over time, indicating that the protocol of this study was successful, at inducing fatigue.

Although knee extension torque values vary during the isometric contraction of 60 seconds, maximum knee extension torque values evaluated during the 3 visits, in order to check if the subjects produced similar initial efforts during the 3 visits. It is normal to experience higher levels of fatigue when the effort is closer to the maximum voluntary contraction. If subjects were working at a lower percentage of maximum at different time-points, they could show different signs of fatigue because of this. Although, max torque during the isometric contraction revealed only a time effect, it was notable that the placebo (control) group decreased their max torque during the 24 months assessment, while the medication group increased their max torque during 24 months. Torque was consistently lower in the control

group in all three time points. This could be the effect of the progression of pathology or could reflect different effort by this group. Considering that the same positive reinforcement was given in both groups the second explanation is less likely. Therefore, despite the reduction in muscle torque was the same between groups during the time of the testing there is still a chance that the compound was effective in slowing down the progression of disease and the associated reduction of muscle torque.

A better indicator of muscles fatigue is the EMG signal. Fatigue of the muscles influences both the amplitude and the frequency properties of EMG signal [153], since during fatigue, further muscle fibers need to be recruited in order to sustain the desired performance. It has been shown that the median frequency of the EMG signal decreases with time during a task that induces fatigue because fibers that are categorized as fast-twitch and rely on anaerobic respiration will decrease in their activity, or could completely stop firing before fibers that are categorized as slow-twitch [181]. In this study, the reduction of Median Frequency between the 10th and the 50th second of the 60 seconds assessment of MVIC evaluated. This reduction is a fatigue measure of the subjects during the maximum isometric effort. The higher the reduction, the greater the fatigue and vice versa. Therefore, the reduction of MDF compared to the 3 visits. PwMS found to have a higher resistance to fatigue (less MDF reduction between 10 and 50 seconds) over the 3 time-points but no significant differences between groups identified. Although less resistance to fatigue was expected during the 24 months period, since MS is a progressive disease impairing motor function and physical activity [208], the results indicate that patients had more resistance to fatigue over the two years of assessment. These results are in agreement with the results of the fatigue index based on the isometric torque. Although the fatigue seems to decrease more in the control group from the 1st to the 3rd measurement the absolute torque produced by this group is lower in each time point. Therefore, the reduction in fatigue probably reflects the different effort of the two groups in the 3 time points.

According to De Luca review (1984) [184] range frequencies are different depending on the fiber type that is being fired. Type I or slow twitch fibers frequency range between 70-125Hz, while Type II or fast twitch fibers range between 126-250Hz. When taking these facts into consideration it can be assumed that PwMS in this study did not recruit as much motor units with type II muscle fibers because MDF ranges below 125 Hz. In addition, literature suggests that Type II muscle fibers are the first to be affected in some neuromuscular diseases [209-212], with Type I muscle fibers following later. This outcome seems to agree with the findings of this study, suggesting that the inability to recruit type II muscle fibers is related to the effects of the pathology.

In conclusion, this chapter acknowledges that PlP10 supplement does not have any effect in handgrip strength, knee extension and flexion strength or fatigue in early MS. Results from this chapter identify that handgrip and knee extensors strength, are not impaired in early MS, in contrast with knee flexion strength which deteriorates sooner compared to the knee extensors and is more affected by the progression of the disease. Additionally, this chapter recommends that future studies need to confirm if PwMS can activate smaller number of motor units with type II muscle fibers compared to healthy controls during maximum voluntary isometric contractions as suggested by the findings of the present study.

CHAPTER 3 GAIT ANALYSIS

3.0 Introduction

Human gait is essential for efficient and safe locomotion. Gait analysis is an examination which provides necessary information regarding human gait. When human gait becomes abnormal, gait analysis provides treatment design and aids intervention [213]. Literature reveals that people select a particular walking speed [214] that minimizes the energy expenditure per unit distance [214]. Any walking speed, other than the specific walking speed, significantly affects kinematic and kinetic gait patterns [215].

A gait analysis isolates the shortest, unique and repeatable task, which defined as a gait cycle, to analyze and quantify how an individual walk. Gait cycle is a sequence of motion occurring from foot contact of one leg to foot contact of the same leg, which can break down into two primary phases, the stance and swing phase [216]. The stance phase represented 60% of the gait cycle and subdivided into double-leg and single-leg stance. The double-leg stance is a phase where both feet are in contact with the ground and occurs at the beginning and the end of the stance phase. Each double-limb support comprises approximately 10% of the gait cycle (total, 20%) under average walking speeds, while the single-limb stance comprises the remaining proportion of stance phase. Double leg stance decreases with increased walking speed and eventually disappears as one begins to run. At slower walking velocities the double-leg support times are longer. The swing phase is a phase where one limb is not weight bearing and represents 40% of a single gait cycle.

Stance and swing phases subdivided into eight different events which exhibited in figure 26. These events are defined as initial contact, loading response (for weight acceptance during stance phase), midstance, terminal stance, pre-swing (during single limb support), initial swing, midswing, and terminal swing (for limb advancement during swing phase) [217].

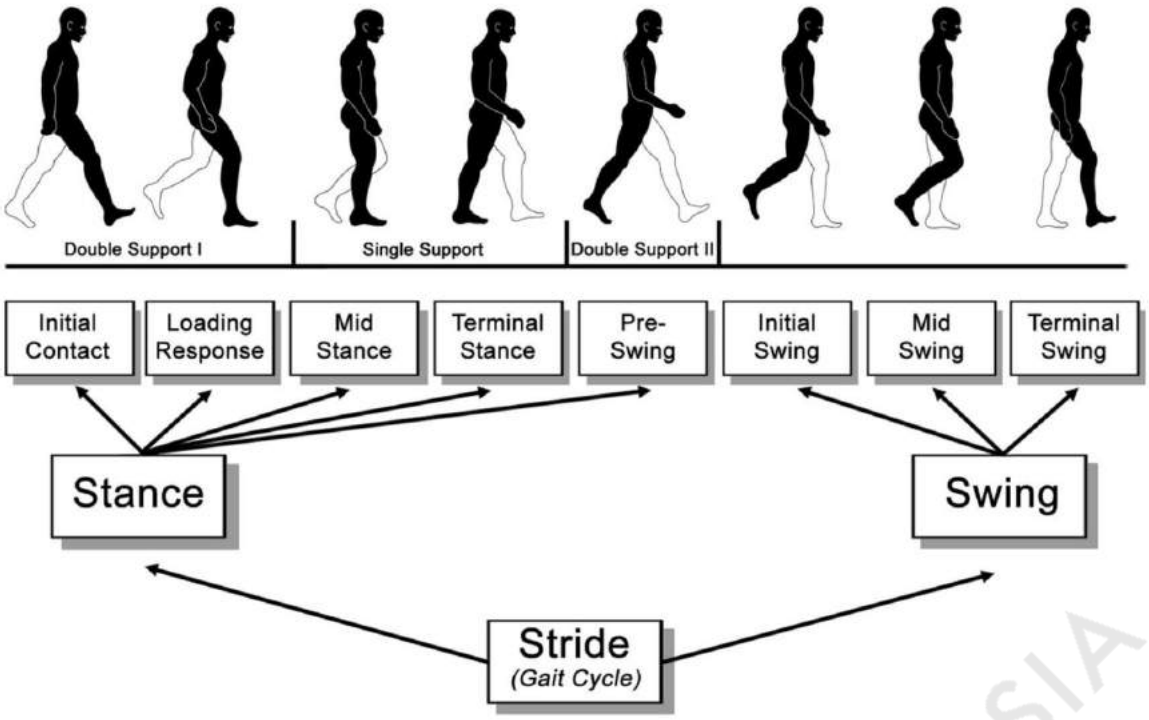

Figure 26. Breakdown of the gait cycle into phases based on the work of Perry and Burnfield (2010) [218]

Distance and time parameters are described as spatiotemporal gait parameters and allows objective reports of walking, such as stride time, step time, stride length, step length, gait velocity, and cadence [217].

Stride time, refers to the time from the initial contact of one foot, to the initial contact of the same foot. Step time refers to the time of the initial contact of one foot to the initial contact of the opposite foot. Stride length refers to the covered distance between the initial contact of one foot to the initial contact of the same foot. Step length reflects the covered distance from the initial contact of one limb to the initial contact of the opposite leg. Gait velocity describes the stride length divided by stride time and is usually expressed as meters per second. Cadence describes the number of steps taken in a unit of time, usually expressed as steps per minute. These characteristics can be monitored with pressure mats, force platforms, and 3D motion analysis camera systems [217].

3.1 Multiple Sclerosis and Gait

Multiple Sclerosis is one of the most common neurological diseases and a growing issue worldwide. At the same time, it is considered to be the leading neurological cause of disability in young adults, associated with impairments physically and psychologically [6]. Difficulty in walking is one of the most commonly reported problems in MS [98], leading up to 93% of PwMS reporting limitations in their walking 10 years following diagnosis. Multiple Sclerosis has significant negative alterations on gait despite the relatively low disability status [219] and presents major personal, social and economic burdens on those living with MS [220]. PwMS display slower speed, reduced stride length [221], prolonged double limb support [222] with fewer, shorter and wider steps [223] compared to their matched healthy controls. As mentioned before people select a specific walking speed [214] that minimizes the energy expenditure per unit distance [214] and any walking speed, other than the particular walking speed, significantly affects kinematic and kinetic gait patterns [215]. PwMS with moderate disability walk slower with shorter step length, alterations which transformed in elevated energetic cost of walking (Cw) [224].

Furthermore, PwMS often experience falls due to negative alterations in balance and mobility [225]. Falls in PwMS related to injuries, activity limitation and further deterioration in mobility levels [226, 227] with a consequent impact on quality of life [228].

3.1.1 Six Minutes walk test (6MWT)

An essential testing protocol which predicts declines in daily activities [101], provides data relative to walking fatigability, walking distance, and functional capacity, is the six minutes walk test [100]. Walking distance often used in clinical population to define the disability status and the disease progression [13, 229]. The 6MWT is a testing procedure which evaluates walking distance and is considered one of the best-characterized measures of walking ability and endurance in PwMS [230, 231].

As shown by previous research, people with MS present lower scores in the 6MWT performance compared to their matched controls [232, 233]. Literature provides information regarding the clinically meaningful changes following intervention in the 6MWT and have reported to be 21.6 m in PwMS [234].

This part of the study aims to examine whether PLP10 supplement increase walking distance in 6MWT in PwMS in the period of 24 months.

3.1.2 Laboratory Gait Analysis

Gait analysis is widely used to classify the mobility of PwMS; however, is mostly limited to functional assessments and use of spatial and temporal measures [235]. In order to provide more detailed and accurate information in the clinical environment [236], advanced motion capture systems are widely used in assessing gait and balance impairment in PwMS affected by subclinical gait impairment [237] or even in PwMS without any clinically observable (qualitative assessment) gait abnormalities [238-241]

As already shown in Parkinson patients [242], the quantification of negative alterations in gait characteristics of PwMS might be the key to understand the progression of disability and a valuable tool to identify factors impairing gait at the very early stage of the disease. Recent studies highlight the importance of identifying factors responsible for the decline in walking distance and the compensation pacing strategies adopted by PwMS [243].

Individuals with Multiple Sclerosis generally walk slower with a shorter stride length and prolonged double support phase [222, 238, 244] compared to their matched healthy controls. Additionally, they adopt a decreased cadence [115, 222], and they exhibit reduced joint motion [245], all of which result in reduced mobility [246]. Another common gait symptom of those living with MS is foot drop, which defined as the inability to hold the foot in ankle dorsiflexion during the swing phase of gait, resulting in an increased possibility for slipping and falling. [247].

The majority of studies which evaluated gait abnormalities have been limited to PwMS with high EDSS score and visible clinical disability. Nevertheless, only a few studies are available, suggesting that mild walking dysfunction may be detected through laboratory-based movement analysis. Three-Dimensional Gait Analysis (3D-GA) can identify walking abnormalities in minimally disabled PwMS [240, 248], providing quantitative measurements of spatiotemporal and kinematic parameters [238, 240]. For instance, in the study of Liparoti et al. (2019) with minimally impaired PwRR-MS (EDSS $\leq$ 2) compared to healthy controls they observed that increased ankle dorsiflexion (during heel off) would be one of the first kinematic parameters to be altered in asymptomatic PwRR-MS [237].

In this study the 3D-GA analysis was evaluated by the Vicon Nexus Motion Capture system (Vicon Motion Systems Ltd., Oxford, UK) which considered as one of the best-characterized systems developed for gait analysis [249]. Identifying changes in gait kinematics and spatiotemporal parameters in early MS may assist in designing gait re-training interventions that aim to increase walking capacity.

This part of the study aims to examine whether the PLP10 intervention supplement improves gait spatiotemporal and kinematic characteristics in PwMS in a period of 24 months.

3.2 Methodology

3.2.1 Six Minutes Walk Text (6MWT)

In the 6MWT participants were instructed to walk as fast and as far as possible without rest or encouragement for 6 minutes [250, 251]. The 6MWT was completed within a single corridor measuring 20 -meters in length, with cones placed on opposite ends, while performing 180° turns around the cones [252, 253] (figure 27).

A multicenter study found no significant effect of the length of straight courses ranging from 50 to 164 ft. [253]. Six-minute walk distance was normalized for every individual by dividing distance covered in meters with leg length in meters. Leg length was measured by the anterior superior iliac spine (ASIS) to the medial malleolus. The sum of both leg lengths was divided by two. The same path of the test attained in every measurement in the period of 24 months.

The required equipment was a countdown timer, two small cones to mark the turnaround points and a chair that could easily be moved along the walking course (in case of emergency, dizziness, fatigue etc.). Patients were instructed to dress comfortably, wear appropriate footwear and avoid eating for at least one hour before the test.

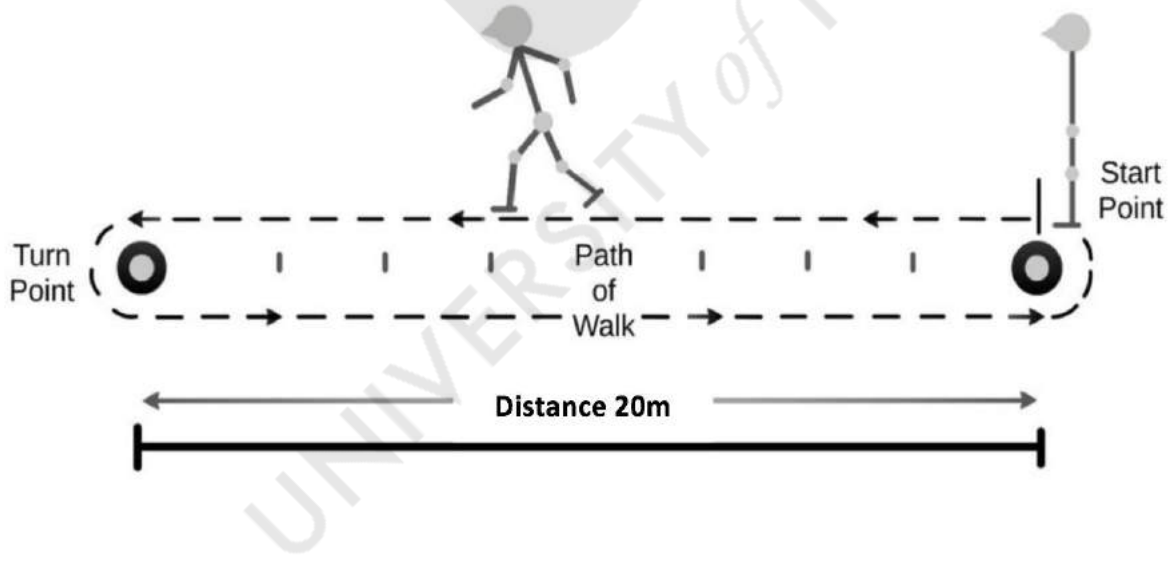

Figure 27. Schematic representation of the 6 -minute walk pathway in this study

Image adapted from Benavent-Caballer (2016) [254]

3.2.2 3D Gait analysis (Spatiotemporal parameters and Kinematics)

A stereophotogrammetric system (Vicon Motion Systems Ltd., Oxford, UK), was used for the acquisition of the lower limb kinematics. Spatiotemporal and kinematic data acquired with eight infrared cameras and a Video Camera (DV1 Bonita 720c) at a sampling rate of 100 Hz using Vicon Nexus 2.8 software.

Initially, the camera masking routine of Vicon system used to black unwanted reflections in the capture space, which remained despite the effort to reduce them as much as possible. Afterwards, a dynamic calibration procedure performed by moving and waving a wand with 5 markers through the capture volume (the area of interest). This step allowed the system to calculate the positions and orientations of all the cameras relative to each other and resulted in accurate 3D reconstructions of the 2D marker trajectories seen by each camera. The act of dynamic calibration involves moving throughout the capture volume, waving the wand so that it passes through as much of the calibration volume as possible allowing each camera to record the wand in several orientations. This calibration repeated at the beginning of each motion capture session. At the end of this calibration, all the cameras had a wand count over 1500 frames and an image error below 0.2 while for the Bonita video camera had an image error below 0.5.

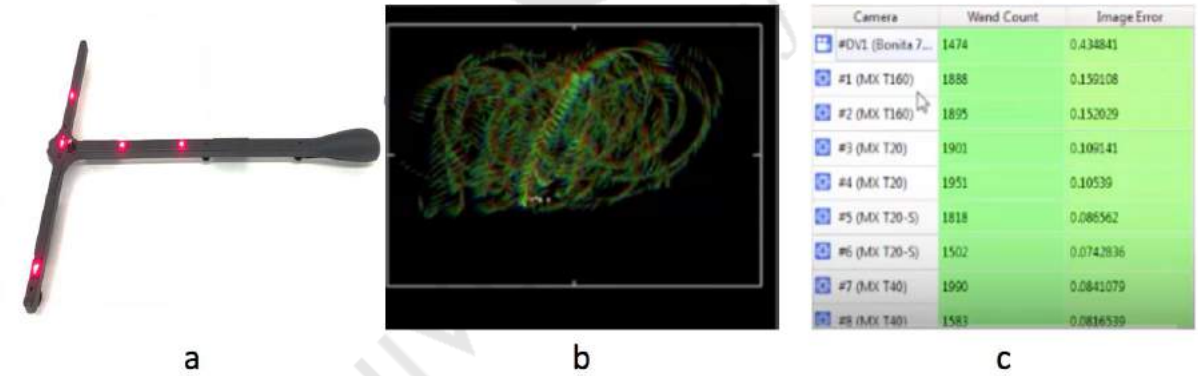

Figure 28. a. Vicon Wand used for the calibration of the infrared cameras, b. Wand movement as detected by one camera during calibration, c. Wand count and image error for each camera after the calibration procedure

The global origin of the volume was set to the same corner of the force plate each time.

Subjects characteristics including height and body weight were recorded (as described in General methods section of Chapter I Anthropometric Characteristics). Knee and ankle joint width (mm) measured in the weight bearing position by Abthroflex Small Bone Anthropometer Sliding Caliper (fig. 29). Leg length (mm) was measured from the anterior superior iliac to the

ankle joint (medial malleoli) by a Abthroflex Anthropometric tape measure (fig. 30). Knee and ankle width, and leg length used in the scaling of the model.

Figure 29. Abthroflex Small Bone Anthropometer Sliding Caliper

Figure 30. Abthroflex Anthropometric tape measure

Subsequently, static calibration performed in which the Vicon software measures the position and orientation of the patient and aligned the global coordinate system to it. At the body of every PwMS twenty-four reflective markers of 14 mm (12 markers for each side) were placed based on the Plug-In-Gait (PiG) lower body functional model with two additional markers in each thigh and shin. Precisely, markers placed in the left and right Anterior Superior Iliac, Posterior Superior Iliac, thigh, knee, tibia, heel, ankle and toe (figure 31).

The model used for capturing the kinematic variables was the PiG LowerBody Ai Functional2 model which has the advantage that the center of rotation of the hip and the axis of rotation (flexion-extension) of the knee are functionally calculated and the placement of markers (for thigh and tibia) is of little importance.

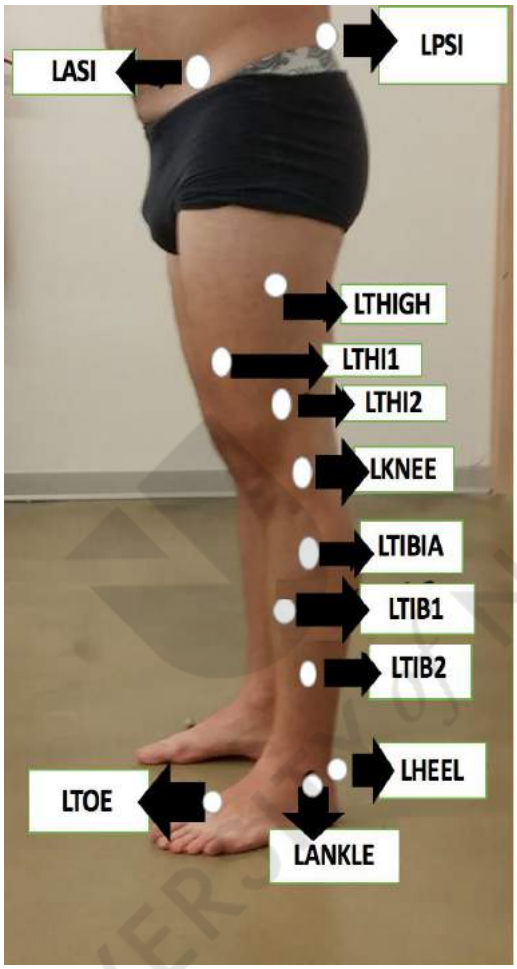

Figure 31. Marker placement on the left lower body part of a patient for PiG LowerBody Ai Functional2 model which used in this study. Abbreviations: LASI, Left Anterior Superior Iliac; LPSI, Posterior Superior Iliac; LTHIGH, Left Thigh, LTHIGH1 & LTHIGH2, two additional markers in the Left Thigh which is the difference from plug-in gait lower body model; LKNEE, Left Knee; LTIBIA, Left Tibia; LTIBIA1 & LTIBIA2, two additional markers in the Left Tibia which is the difference from plug-in gait lower body model; LHEEL, Left Heel; LANKLE, Left Ankle and LTOE, Left Toe

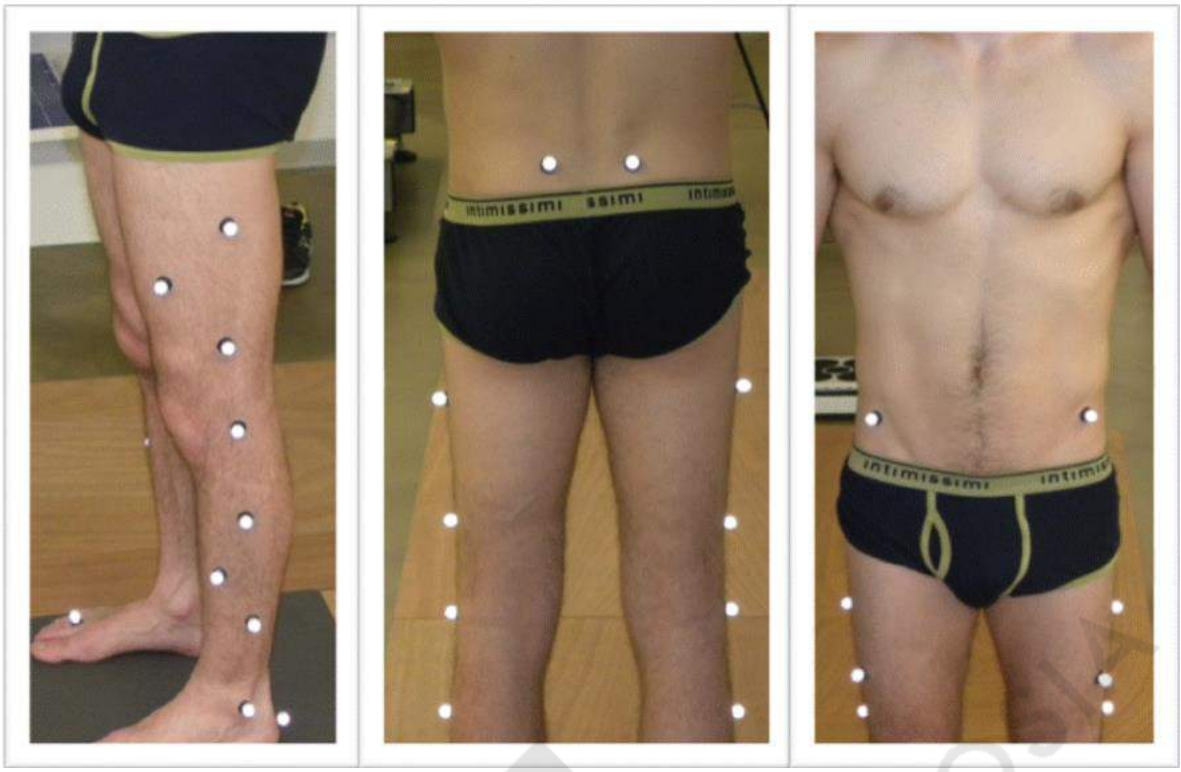

Figure 32. Marker placement on a PwMS in this study

The participants remained motionless for 2-3 seconds in a T pose (arms abducted in 90^{o}) in order to perform the static calibration (figure 33).

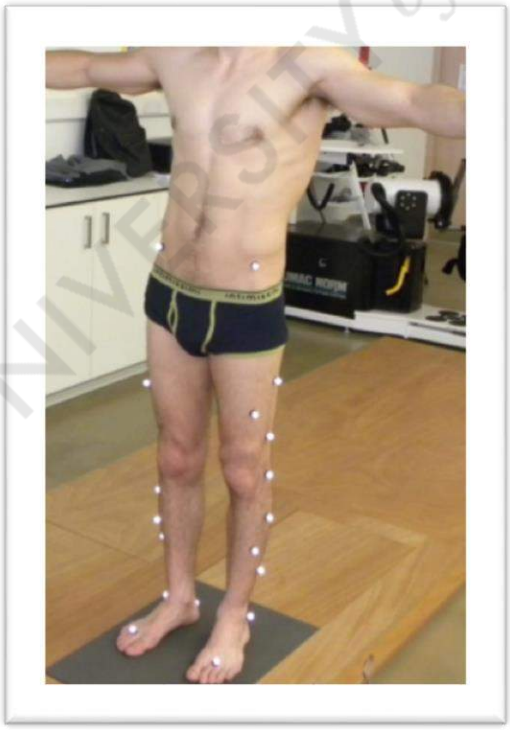

Figure 33. Static Calibration in a T pose from a PwMS in the study

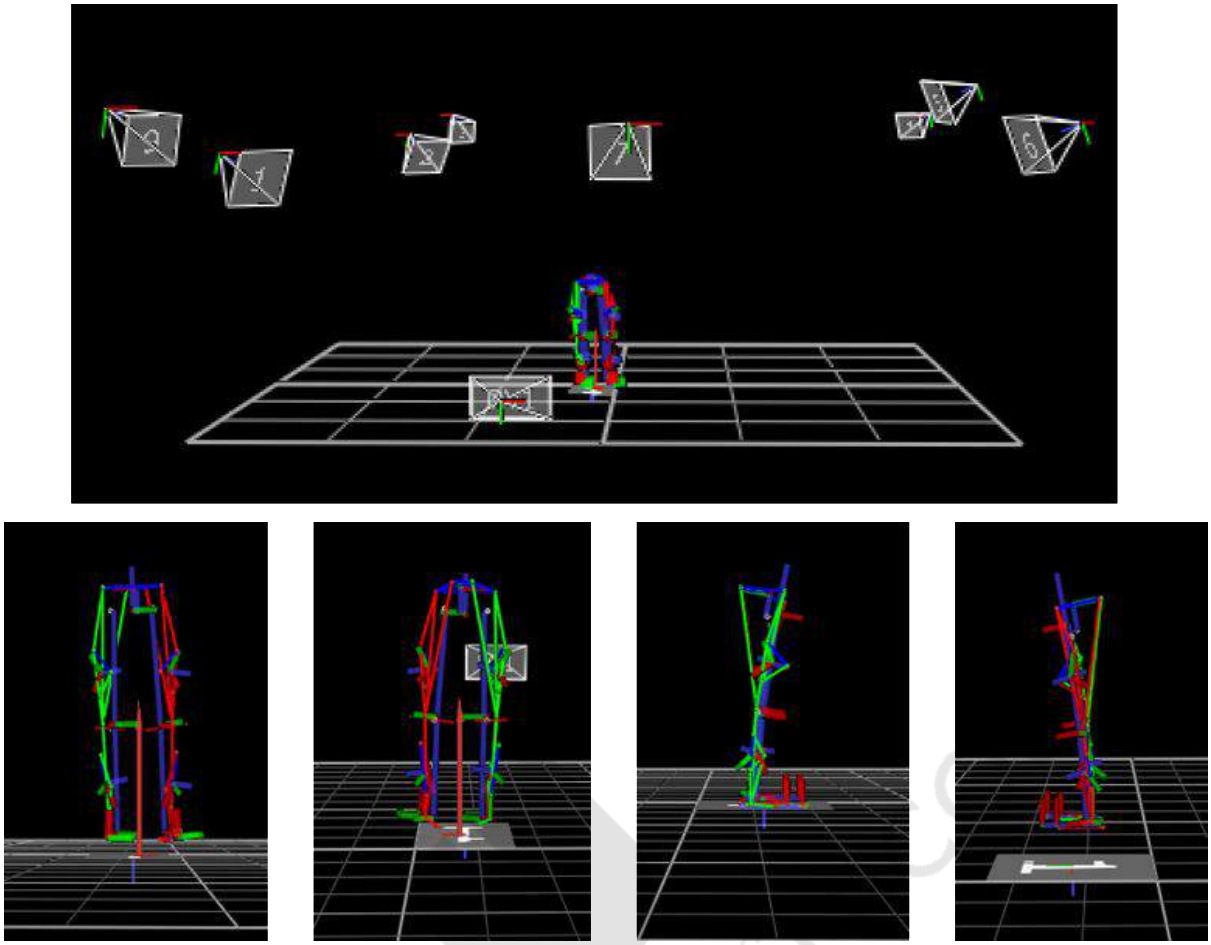

Figure 34. Static Calibration of a PwMS in the study in 3D Perspective view

The static calibration procedure was followed by a dynamic calibration during which the patients were instructed to remain at a fixed point and make reciprocal movements as wide as possible for each joint (hip and knee) in sagittal and frontal planes. In dynamic calibration, every joint center was calculated functionally (figure 35).

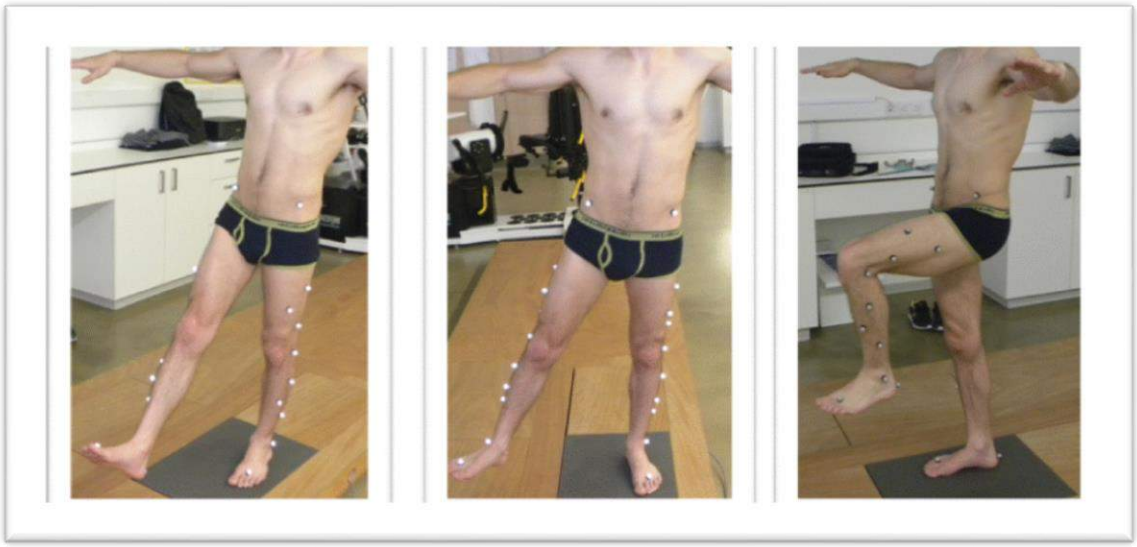

Figure 35. Dynamic Calibration procedure

After the static and dynamic calibration patients were ready to complete their gait evaluation. Each subject performed a series of walking trials at their natural, comfortable self-selected speed. Each subject performed 5 walking trials in a 10 m walkway. All gait trials were performed in barefoot conditions and the participants wore comfortable shorts (women also wore sports bras) (figure 36 and 37).

Figure 36. Photos taken during a gait analysis walking trial of a PwMS in this study

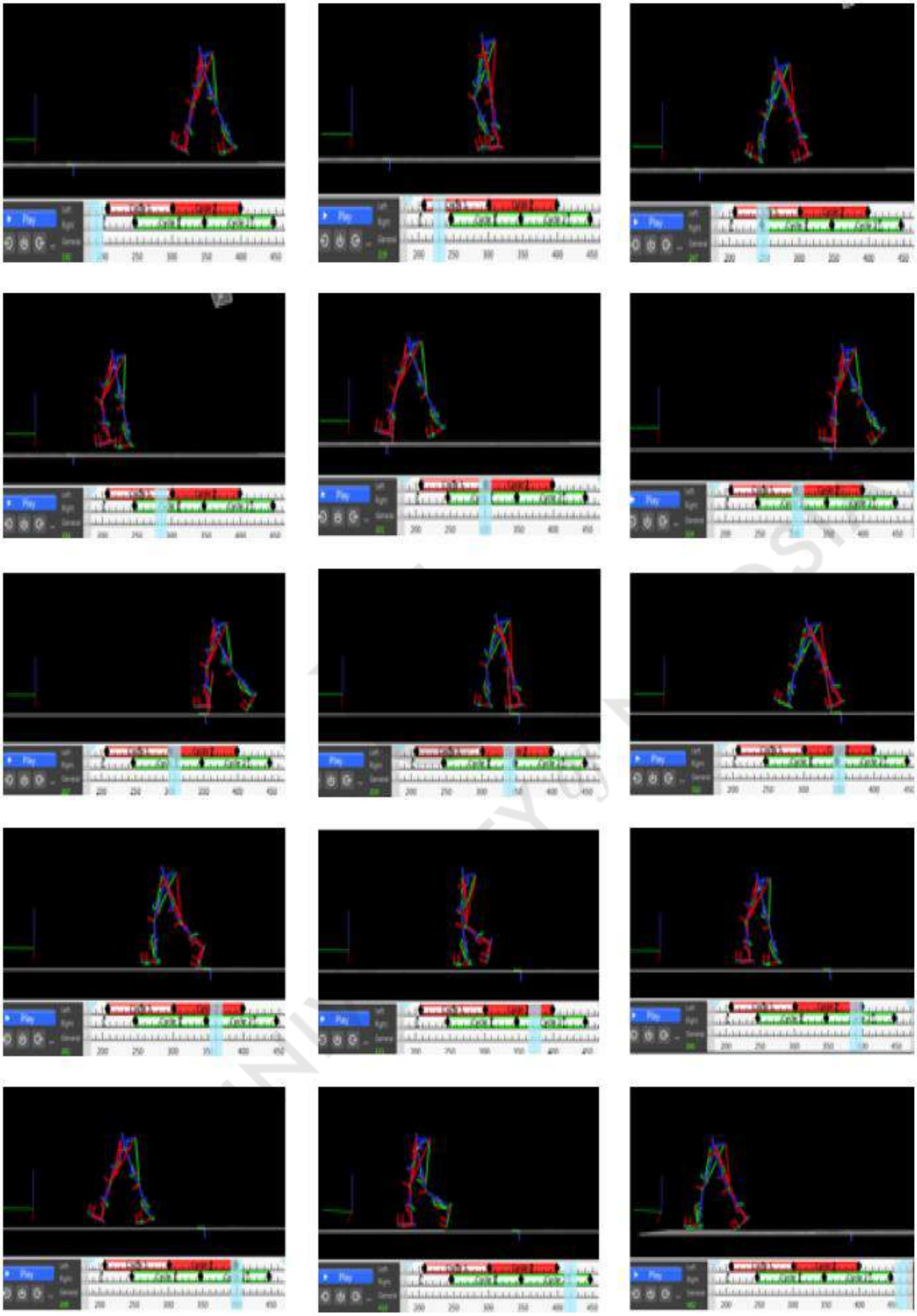

Figure 37. Gait analysis walking trial in 3D Perspective View of a PwMS in this study

All data collection was carried out by the same investigator, experienced in analyzing gait. For every walking trial and each limb, spatiotemporal parameters (walking speed, cadence, stride time, stride length, stance phase, double support, step width etc.) were computed using the Vicon Polygon 4.2 software and then data were averaged for each leg. Some gait parameters were normalized according to Hof's original paper and Pinzone et al. (2016) [255, 256] (table 11) in order to facilitate comparisons between subjects with different anthropometric characteristics.

Table 11. Normalization schemes for different variables. g is the acceleration of gravity (=9.81 m/s2), lo is leg length (in meters) and m is body mass. All quantities normalized via the ND scheme are dimensionless

Variable	**Conventional normalization (CN)**	**Non-dimensional normalization (NDN)**
Velocity	m/s	$\frac{\text{velocity}}{\sqrt{\text{glo}}}$
Cadence	steps/min	$\text{cadence} \times \sqrt{\frac{\text{lo}}{\text{g}}}$
Step Length	m	$\frac{\text{step length}}{\text{lo}}$

Furthermore, kinematic data collected using Vicon Polygon 4.2 software. Data from each leg were time-normalized over one gait cycle and the average values of each the pelvis, hip, knee, and ankle joint angles were calculated over the five trials of each participant. Detailed methodology for deriving joint angles using PiG is available online [257]. Kinematics were calculated for each joint using Euler angles (in the sequence X-YZ). All data expressed in degrees normalized to the 100% of the gait cycle.

Gait Deviation Index (GDI) developed to describe the gait of children with cerebral palsy (CP) was also used to assess the quality of gait [258]. The GDI is computed with kinematic gait data from the pelvis, hip, knee, ankle and foot. It is an index which measures the distance between any chosen gait vector and a gait vector averaged over a placebo group [258]. GDI with a score of 100 indicates a subject without any neurological problem, while a mean score of 85 has been found for children with CP without functional limitations in their walking and a mean score of 55 has been found for children with CP with several mobility problems, using power mobility

aids for longer distances [259]. A GDI of 100 indicates the absence of gait pathology. Every 10 points that the GDI falls below 100 corresponds one standard deviation away from the average unimpaired person.

3.3 Statistical Analysis

Descriptive statistics were used to calculate the mean and standard deviation of spatiotemporal and kinematic variables. Independent sample T-test was used to examine any differences between the two groups at baseline. Paired sample T-test was used to examine any differences between legs. Differences between the groups were tested using a repeated measure (mixed model) ANOVA design with group (control vs experimental) as between subject factor and time (baseline, 12 months and 24 months) as the within subject factor. All analyses were carried out using the Statistical Package for the Social Sciences software (SPSS for Windows, version 19.0, Chicago, Illinois). Data are presented as mean ± SD and the level for statistical significance was set at $p \leq 0.05$.

3.4. Results

All participants were able to perform the gait analysis assessments without injuries and adverse effects. At baseline there were no significant differences in all examined spatiotemporal gait parameters and the GDI between the two groups ($p>0.05$) (table 12).

Table 12. Examined Gait Parameters at Baseline between the 2 groups

Variables	Placebo Group	PLP10 Group	P Value
6MWT (m)	504 ± 131	505 ± 99	0.838
Left Cadence (steps/min)	105.11 ± 13.13	107.22 ± 9.71	0.275
Right Cadence (steps/min)	97.99 ± 3.61	97.79± 4.64	0.985
Left walking speed (m/s)	1.04 ± 0.17	1.17 ± 0.13	0.060
Right walking speed (m/s)	1.06 ± 0.19	1.17 ± 0.14	0.150
Left Double Support (sec)	0.271 ± 0.07	0.238 ± 0.04	0.305
Right Double Support (sec)	0.290 ± 0.08	0.243 ± 0.04	0.072
Left Single Support (sec)	0.436 ± 0.04	0.440 ± 0.04	0.949
Right Single Support (sec)	0.427 ± 0.08	0.432 ± 0.04	0.849
Left Step Length (m)	0.716 ± 0.07	0.755 ± 0.05	0.862
Right Step Length (m)	0.723 ± 0.11	0.750 ± 0.05	0.900
Left Step Time (sec)	0.590 ± 0.07	0.565 ± 0.05	0.084
Right Step Time (sec)	0.577 ± 0.06	0.555 ± 0.05	0.080
Left Step Width (cm)	0.154 ± 0.08	0.129 ± 0.04	0.162
Right Step Width (cm)	0.153 ± 0.07	0.134 ± 0.04	0.272
Left Stride Length (m)	1.40 ± 0.15	1.51 ± 0.1	0.426
Right Stride Length (m)	1.41 ± 0.17	1.50 ± 0.09	0.688
Left Stride Time (sec)	1.16 ± 0.14	1.12 ± 0.09	0.207
Right Stride Time (sec)	1.15 ± 0.14	1.11 ± 0.09	0.133
Left GDI (Gait Deviation Index)	80.88 ± 12.61	78.38 ± 7.07	0.816
Right GDI (Gait Deviation Index)	81.63 ± 9.92	81.80 ± 8.78	0.612

All data are mean ± SD

The placebo group increased the distance in the 6MWT from 504 m at baseline to 523 in the 12 months assessment and then dropped to 494 m in the 24 months follow up assessment. The experimental group showed a progressive increase from 505m at baseline to 546m the 12 months assessment and finally to 551 m the 24 months follow up assessment. During the two years assessment, 6MWT distance reduced by 1.98% in the placebo group and improved by 9.10% in the PLP10 group. The 12 months assessment revealed an improvement in distance by 3.76% in the placebo group and by 7.72% in the experimental group. From 12 months to the 24 months follow up assessment, 6MWT distance decreased by 5.54% in the placebo group and improved by 0.91% in the PLP10 group. Despite the tendency to increase in the experimental group, the walking distance showed no time (p=0.137) or group (p=0.186) effect. There was also no statistically significant interaction between group and time (p=0.337) (table 13).

Table 13. The results of the 6MWT in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Six Minute Walk Test Normalized (m)					
Baseline	504 ± 131	505 ± 99	0.137	0.186	0.337
12 months	523 ± 90	546 ± 73			
24 months	494 ± 79	551 ± 101			

Spatiotemporal parameters

Normalization of cadence by leg length revealed that for the left leg in the placebo group there was a reduction in cadence from 31.15 (steps/min) at baseline to 30.49 (steps/min) in the 12 months assessment and an improvement to 30.59 (steps/min) in the 24 months follow up assessment. In the experimental group there was a progressive improvement in cadence from 31.72 (steps/min) at baseline to 31.80 (steps/min) in the 12 months assessment and finally to 33.97 (steps/min) in the 24 months assessment. In the normalized cadence of the left leg there was no time (p=0.259) or group (p=0.154) effect. There was also no statistically significant interaction between group and time (p=0.099) (table 15). In the placebo group normalized cadence of the right leg progressively improved from 29.03 (steps/min) at baseline to 30.80 (steps/min) in the 12 months assessment and finally to 30.81 (steps/min). In the experimental group normalized right cadence progressively improved from 29.03 (steps/min) at baseline to 32.22 (steps/min) in the 12 months assessment and finally to 32.22 (steps/min) in the 24 months follow up assessment. Results revealed a statistically significant time effect (p=0.001) between the baseline and 24 months follow up assessment and between the 12 months follow up assessment and 24 months follow up assessment, but no statistically significant group effect (p=0.098) or interaction between group and time (p=0.069) revealed (table 15).

Table 14. Percentage change of Left and Right Cadence between baseline and 12 months assessment, 12 months and 24 months follow up assessment, baseline and 24 months follow up assessment

Left Cadence Normalized	**Placebo Group**	**Experimental Group**
Percentage Change Between Baseline & 1st year assessment	↓ 2.11 %	↑ 0.25%
Percentage Change Between 1st year and 2nd year assessment	↑ 0.32%	↑ 6.82%
Percentage Change in two years follow up	↓ 1.59 %	↑ 7.09%
Right Cadence Normalized	**Placebo Group**	**Experimental Group**
Percentage Change Between Baseline & 1st year assessment	↑ 6.09 %	↑ 10.98%
Percentage Change Between 1st year and 2nd year assessment	↑ 0.03%	↑ 4.03%
Percentage Change in two years follow up	↑ 6.09%	↑ 15.43%

The percentage changes in the PLP10 group consistently increase while in the control group decrease or increase marginally. No significant differences detected possibly because the sample size is not large for such a complicated model.

Table 15. The results of the normalized cadence of the left and right leg in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left Cadence Normalized					
Baseline	31.15 ± 3.97	31.72 ± 2.64			
12 months	30.49 ± 2.81	31.80 ± 3.82	0.259	0.099	0.154
24 months	30.59 ± 3.79	33.97 ± 4.47			
Right Cadence Normalized					
Baseline	29.03 ± 0.84	29.03 ± 0.98			
12 months	30.80 ± 0.84	32.22 ± 0.98	**0.001**	0.069	0.098
24 months	30.81 ± 2.77	33.51 ± 3.70			

Normalized walking speed of the left leg revealed that walking speed in the placebo group increased by 4.7% from baseline to the 12 months assessment and decreased by 1.5% from 12 months to the 24 months follow up assessment. During the 24 months assessment normalized walking speed of the left leg improved by 3.08% in the placebo group. In the experimental group normalized walking speed on the left decreased from baseline to the 12 months assessment by 0.7% and improved from 12 months to the 24 months follow up assessment by 7.26%. During the 24 months assessment normalized walking speed of the left leg improved by 6.46%. Results did not reveal any statistically significant time (p=0.097) effect or interaction (p=0.110) between group and time. There was only a statistically significant group effect (p= 0.024). Normalized walking speed of the right leg demonstrated the same outcome as the normalized walking speed of the left leg. During the 24 months assessment, normalized walking speed of the right leg improved by 1.37% in the placebo group and by 7.21% in the experimental group. Results did not reveal any statistically significant time effect (p=149) or

interaction (p=0.140) between group and time. There was only a statistically significant group effect (p= 0.024) (table 16).

Table 16. The results of the normalized walking speed of the left and right leg in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left walking speed Normalized					
Baseline	0.359 ± 0.61	0.402 ± 0.45			
12 months	0.376 ± 0.06	0.399 ± 0.05	0.097	0.110	**0.024**
24 months	0.370 ± 0.06	0.428 ± 0.06			
Right walking speed Normalized					
Baseline	0.364 ± 0.06	0.402 ± 0.04			
12 months	0.38 ± 0.06	0.408 ± 0.05	0.149	0.140	**0.024**
24 months	0.369 ± 0.06	0.431 ± 0.06			

In the placebo group left double support time increased by 0.7% from baseline to the 12 months assessment and then decreased by 2.56% from 12 months to the 24 months assessment. During the 24 months assessment left double support time in the placebo group decreased by 1.84%. The experimental group showed an increase by 3.36% from baseline to the 12 months assessment and then a decrease by 13.82% 12 months to the 24 months assessment. During the 24 months assessment left double support time in the experimental group decreased by 10.92%. There was no time effect (p=0.224) or any statistically significant interaction between group and time (p=0.496), only a statistically significant group effect (p= 0.043). Double support time on the right leg demonstrated the same outcome as the double support time of the left leg. In the two years follow up there was an increase in double support time by 7.93% in the placebo group and a decrease by 10.69% in the experimental group. There was no time effect (p=0.208) or any statistically significant interaction between group and time (p=0.415), only a statistically significant group effect (p= 0.048) revealed (table 17).

Table 17. The results of the double support time of the left and right leg in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left Double Support (sec)					
Baseline	0.271 ± 0.07	0.238 ± 0.04			
12 months	0.273 ± 0.07	0.246 ± 0.06	0.224	0.496	**0.043**
24 months	0.266 ± 0.09	0.212 ± 0.05			
Right Double Support (sec)					
Baseline	0.290 ± 0.08	0.243 ± 0.04			
12 months	0.271 ± 0.06	0.257 ± 0.09	0.208	0.415	**0.048**
24 months	0.267 ± 0.09	0.217 ± 0.05			

Left single support time, progressively increased in the placebo group from 0.436 sec at baseline, to 0.443 sec in the 12 months assessment and finally to 0.452 sec in the 24 months follow up assessment. Meanwhile the experimental group showed a progressively decreased time spend in the left single support from 0.44 sec at baseline to 0.437 sec the 12 months assessment and finally to 0.421 the 24 months assessment. There was no time (p=0.896) or group effect (p=0.3281) between subjects. The tendency in the placebo group to increase and in the experimental group to decrease the time spent in the left single support has a statistically significant interaction (p=0.035) (table 18). During the 24 months assessment, there was an increase in left single support spent time by 3.66% in the placebo group and a decrease by 4.31% in the experimental group.

Although in the right single support, time progressively increased in the placebo group and progressively decreased in the experimental group, there was no statistically significant time (p=0.283) or group effect (p=0.394) between subjects, or any statistically significant interaction between group and time (p=0.132).

Table 18. The results of the single support time of the left and right leg in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction.

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left Single Support (sec)					
Baseline	0.436 ± 0.04	0.440 ± 0.04	0.896	**0.035**	0.328
12 months	0.443 ± 0.03	0.437 ± 0.04			
24 months	0.452 ± 0.04	0.421 ± 0.03			
Right Single Support (sec)					
Baseline	0.427 ± 0.08	0.432 ± 0.04	0.283	0.132	0.394
12 months	0.441 ± 0.03	0.434 ± 0.04			
24 months	0.459 ± 0.04	0.428 ± 0.03			

The time spent on the left leg did not reveal any time (p=0.380) or group effect (p=0.162) neither any statistically significant interaction between group and time (p=0.275) (table 19). The time spent on the right leg revealed a progressively increased time for the placebo group from 0.577 sec at baseline to 0.586 sec in the 12 months assessment and finally to 0.589 in the 24 months follow up assessment. In the experimental group, there was an increased step time from 0.555 sec at baseline to 0.579 sec at the 12 months assessment and a decrease to 0.526 in the 24 months follow up assessment. There was no group effect (p=0.131), but there was a statistically significant time effect (p=0.047) and interaction between group and time (p=0.025) (table 19). In the two years assessment there was an increase in step time of the right leg by 2.07% in the placebo group and a decrease by 5.22% in the experimental group.

Table 19. The results of the step time spent on the left and right leg in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left Step Time (sec)					
Baseline	0.590 ± 0.07	0.565 ± 0.05	0.380	0.275	0.162
12 months	0.596 ± 0.06	0.582 ± 0.1			
24 months	0.599 ± 0.08	0.546 ± 0.06			
Right Step Time (sec)					
Baseline	0.577 ± 0.06	0.555 ± 0.05	**0.047**	**0.025**	0.131
12 months	0.586 ± 0.06	0.579 ± 0.08			
24 months	0.589 ± 0.08	0.526 ± 0.05			

Left stride time progressively increased in the placebo group from 1.16 sec at baseline to 1.17 sec in the 12 months assessment and finally to 1.18 sec in the 24 months follow up assessment. In the experimental group there is an increase in left stride time from 1.12 sec at baseline to 1.14 sec in the 12 months assessment and a decrease to 1.05 sec in the 24 months follow up assessment (table 20). There was no statistically significant time (p=0.148) or group effect (0.073). There was a marginal statistically significant interaction between group and time (p=0.051).

Right stride time progressively increased in the placebo group from 1.15 sec at baseline to 1.16 sec in the 12 months assessment and finally to 1.17 sec in the 24 months follow up assessment. In the experimental group there was an increase in right stride time from 1.11 sec at baseline to 1.13 sec in the 12 months assessment and a decrease to 1.04 sec in the 24 months follow up assessment (table 20). In the two years assessment, in the placebo group there was an increase in right stride time by 1.73% while in the experimental group there was a decrease in right stride time by 6.30%. There was a statistically significant interaction between group and time (p= 0.039) but no time (p=0.196) or group effect (p=0.060).

Table 20. The results of the stride time of the left and right leg in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left Stride Time (sec)					
Baseline	1.16 ± 0.14	1.12 ± 0.09			
12 months	1.17 ± 0.1	1.14 ± 0.16	0.148	**0.051**	0.073
24 months	1.18 ± 0.15	1.05 ± 0.12			
Right Stride Time (sec)					
Baseline	1.15 ± 0.14	1.11 ± 0.09			
12 months	1.16 ± 0.1	1.13 ± 0.13	0.196	**0.039**	0.060
24 months	1.17 ± 0.16	1.04 ± 0.11			

Left step length did not reveal any statistically significant time (p=0.434) or group effect (p=0.112), neither any statistically significant interaction between group and time (p=0.496). The same outcome demonstrated on the right step length. Right step did not reveal any statistically significant time (p=0.660) or group effect (p=0.785), neither any statistically significant interaction between group and time (p=0.083) (table 21).

Table 21. The results of the step length of the left and right leg in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left Step Length Normalized					
Baseline	0.716 ± 0.07	0.755 ± 0.05	0.434	0.496	0.112
12 months	0.733 ± 0.06	0.771 ± 0.10			
24 months	0.732 ± 0.05	0.746 ± 0.07			
Right Step Length Normalized					
Baseline	0.723 ± 0.11	0.750 ± 0.05	0.660	0.785	0.083
12 months	0.716 ± 0.08	0.761 ± 0.04			
24 months	0.712 ± 0.05	0.742 ± 0.07			

Left and right stride length progressively decreased in both groups. Results revealed only a time effect between baseline and 24 months follow up assessment and between 12 months and 24 months follow up assessment (p=0.001) for both legs, but no group effect ($p \geq 0.275$) or time x group interaction ($p \geq 0.109$) demonstrated (table 22).

Table 22. The results of the stride length of the left and right leg in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left Stride Length Normalized					
Baseline	1.40 ± 0.15	1.51 ± 0.10			
12 months	1.27 ± 0.14	1.31 ± 0.17	**0.001**	0.275	0.109
24 months	1.25 ± 0.12	1.28 ± 0.13			
Right Stride Length Normalized					
Baseline	1.41 ± 0.17	1.50 ± 0.09			
12 months	1.26 ± 0.15	1.29 ± 0.13	**0.001**	0.444	0.146
24 months	1.23 ± 0.12	1.28 ± 0.14			

Left step width progressively decreased in the two years follow up for both groups. In the placebo group from 0.154 m at baseline decreased to 0.142 m in the 24 months follow up assessment, while in the experimental group decreased from 0.129 m at baseline to 0.114 m at the 24 months follow up assessment. Results revealed only a time effect (p=0.003) between baseline and 24 months follow up assessment and between 12 months and 24 months follow up assessment, no group effect (p=0.182) or interaction between group and time (p=0.499) (table 23).

Right step width progressively decreased in the two years assessment similar to the left step width. Results reveal only a time effect (p=0.005) between baseline and 24 months follow up assessment and between 12 months and 24 months follow up assessment, no group effect (p=0.781) or interaction between group and time (p=0.341) (table 23).

Table 23. The results of the step width of the left and right leg in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left Step Width Normalized (m)					
Baseline	0.154 ± 0.08	0.129 ± 0.04			
12 months	0.146 ± 0.07	0.114 ± 0.03	**0.003**	0.499	0.182
24 months	0.142 ± 0.07	0.116 ± 0.03			
Right Step Width Normalized (m)					
Baseline	0.153 ± 0.07	0.134 ± 0.04			
12 months	0.144 ± 0.07	0.122 ± 0.04	**0.005**	0.781	0.341
24 months	0.141 ± 0.07	0.122 ± 0.04			

Kinematic Data in the Sagittal Plane

In the placebo group peak plantar flexion of the right leg increased from 12.22 degrees at baseline to 12.65 degrees in the 12 months assessment and decreased to 7.95 degrees in the 24 months follow up assessment. In the experimental group right peak plantar flexion progressively decreased from 15.37 at baseline to 10.28 in the 12 months assessment and finally to 8.72 degrees in the 24 months follow up assessment. In the two years follow up, right peak plantar flexion reduced by 15.22% in the placebo group and by 43.26% in the PLP10 group. Despite the tendency to decrease in the experimental group the peak plantar flexion showed only a time effect ($p=0.023$), indicating significant changes between the baseline and 24 months follow up assessment, but no group effect ($p=0.886$) or interaction between group and time ($p=0.140$) (table 24).

Left peak plantar flexion decreased in both groups during the 3 visits without any statistically significant time ($p=0.063$) or group effect ($p=0.523$), or interaction between group and time ($p=0.509$) (table 24).

Peak dorsi flexion at both legs did not reveal any statistically significant time ($p \geq 0.500$) or group effect ($p \geq 0.619$), neither an interaction between group and time ($p \geq 488$) (table 24).

Table 24. The results of the ankle kinematics in the sagittal plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Peak Plantar Flexion (º)					
Baseline	-0.32 ± 7.21	-12.37 ± 6.90			
12 months	-4.75 ± 10.5	-7.28 ± 7.74	**0.023**	0.140	0.886
24 months	-5.64 ± 8.63	-5.72 ± 4.59			
Left Peak Plantar Flexion (º)					
Baseline	-11.84 ± 7.96	-12.69 ± 5.37			
12 months	-12.31 ± 9.09	-8.19 ± 13.6	0.063	0.509	0.523
24 months	-11.95 ± 7.91	-10.53 ± 6.62			
Right Peak Dorsi Flexion (º)					
Baseline	17.94 ± 6.34	18.55 ± 5.93			
12 months	16.89 ± 6.61	16.01 ± 5.08	0.956	0.488	0.619
24 months	15.97 ± 3.34	15.25 ± 4.00			
Left Peak Dorsi Flexion (º)					
Baseline	14.79 ± 10.69	14.96 ± 8.09			
12 months	14.27 ± 5.03	14.84 ± 9.38	0.500	0.844	0.745
24 months	14.55 ± 5.52	14.65 ± 4.30			

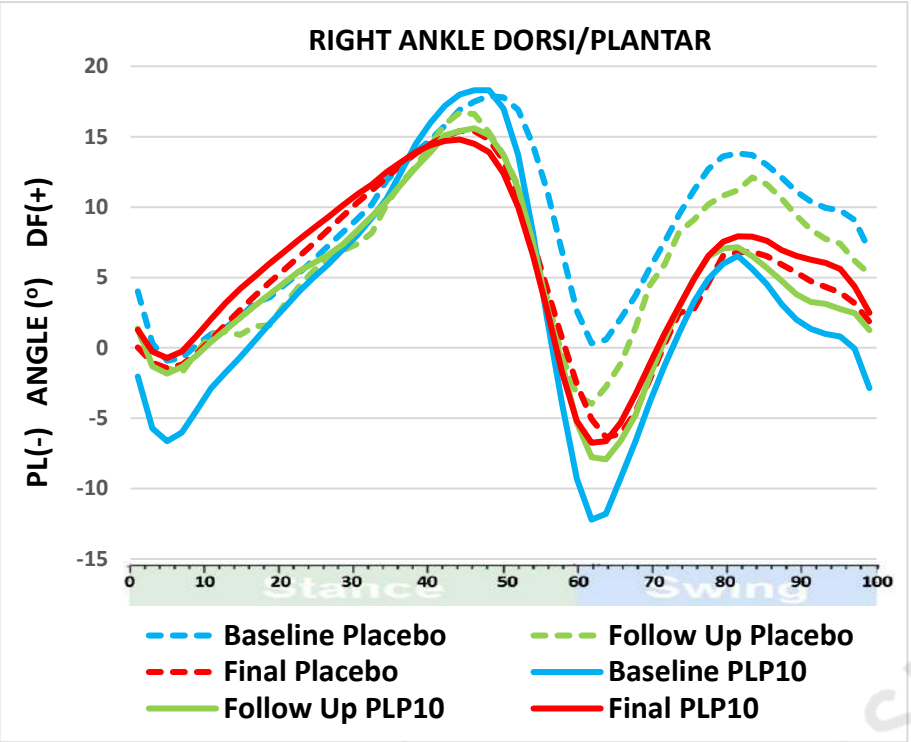

Figure 38. Changing position of right ankle dorsi/plantar flexion angles across the averaged gait cycle in all three time-points for the Placebo and PLP10 group

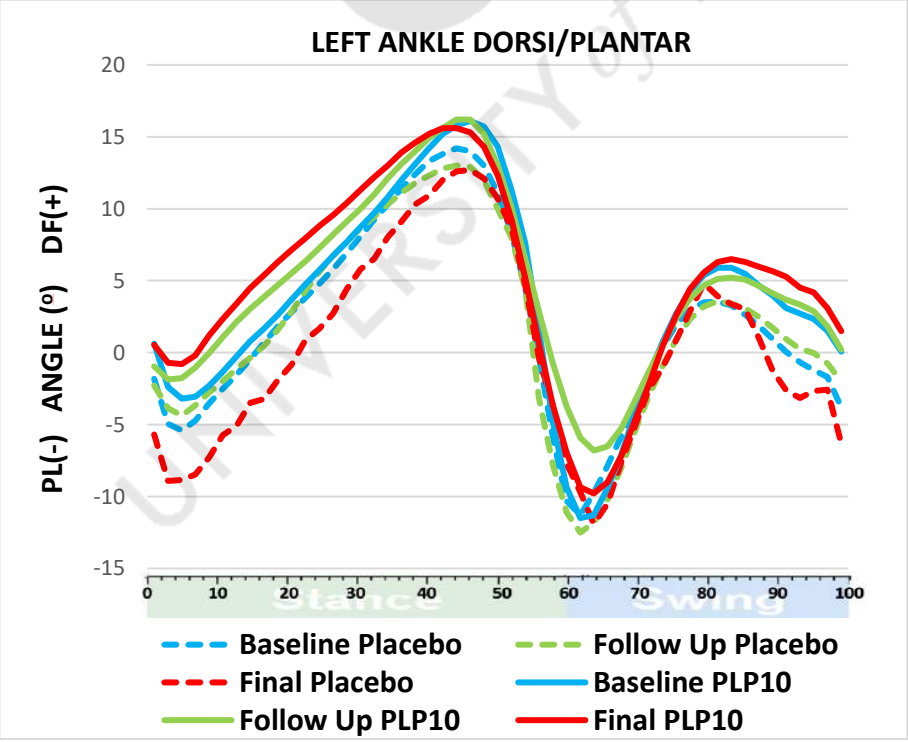

Figure 39. Changing position of left ankle dorsi/plantar flexion angles across the averaged gait cycle in all three time-points for the Placebo and PLP10 group

Hip angles in the sagittal plane did not change statistically significant during the 3 visits (table 25). Results of the right hip flexion angle revealed a statistically significant time effect (p=0.003) between baseline and 12 months follow up assessment in both groups of patients. Results of the left hip flexion angle revealed a statistically significant time effect (p=0.001) indicating significant changes between baseline and 12 months follow up assessment and between baseline and 24 months follow up assessment in both groups of patients (table 25).

Table 25. The results of the hip kinematics in the sagittal plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Hip Peak Extension (º)					
Baseline	-8.10 ± 6.48	-7.72 ± 8.97	0.638	0.526	0.722
12 months	- 8.78 ± 9.41	-9.25 ± 10.05			
24 months	-7.68 ± 7.70	-10.23 ± 9.18			
Left Hip Peak Extension (º)					
Baseline	-3.72 ± 11.12	-5.61 ± 13.81	0.060	0.702	0.444
12 months	- 8.11 ± 8.79	-8.83 ± 9.66			
24 months	- 6.97 ± 6.31	-10.49 ± 9.43			
Right Hip Peak Flexion (º)					
Baseline	35.98 ± 6.57	33.85 ± 7.15	**0.003**	0.813	0.154
12 months	32.43 ± 6.50	29.51 ± 6.30			
24 months	34.06 ± 6.31	30.56 ± 8.83			
Left Hip Peak Flexion (º)					
Baseline	37.23 ± 6.51	35.27 ± 7.36	**0.001**	0.915	0.172
12 months	32.74 ± 6.38	29.75 ± 6.12			
24 months	34.31 ± 5.57	31.59 ± 9.50			

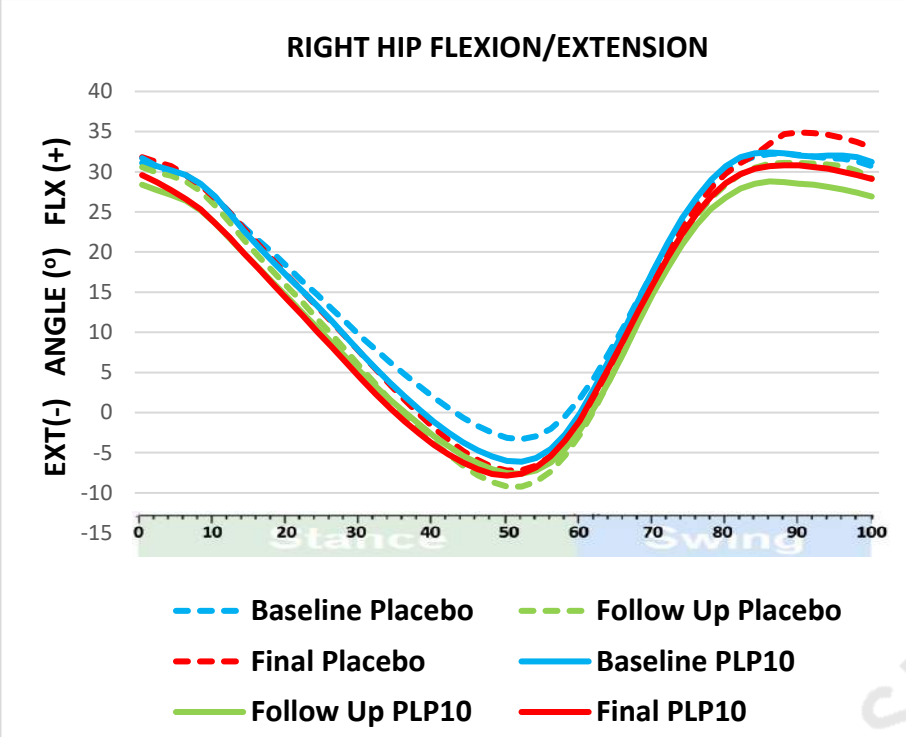

Figure 40. Changing position of the right hip flexion/ extension angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

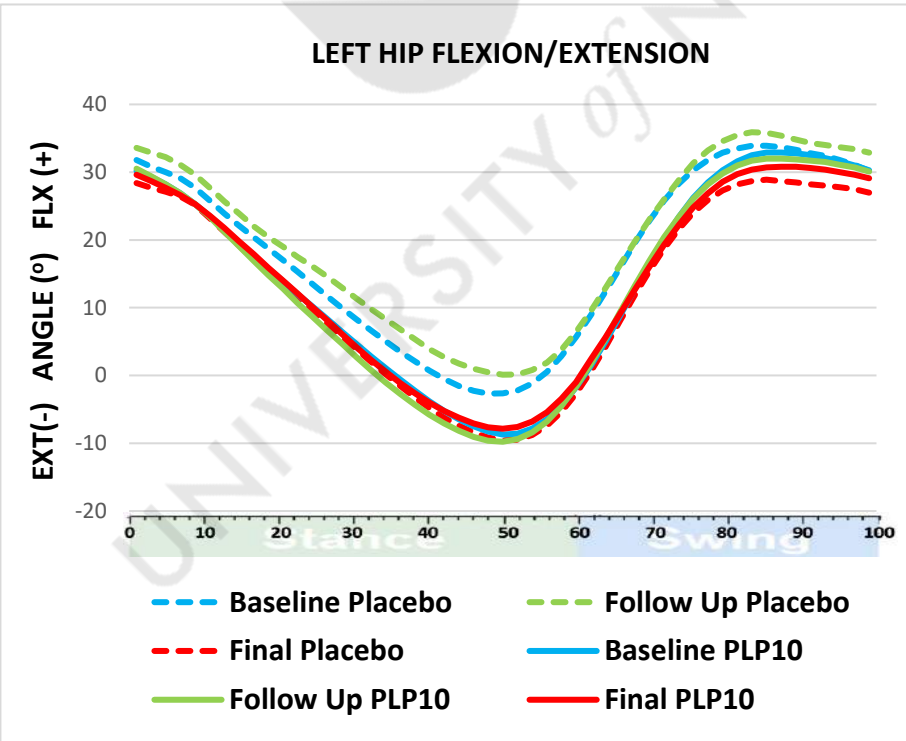

Figure 41. Changing position of the left hip flexion/ extension angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

Peak knee extension angles decreased in both groups during the 24 months follow up. A statistically significant time effect (right; p=0.001, left; p=0.004) was revealed, for the right leg between the baseline and the 12 months follow up assessment and between the baseline and the 24 months assessment while for the left leg time effect was revealed between the baseline and the 24 months assessment. Results did not reveal any statistically significant group effect (right; p=0.197, left; p=0.674) or interaction between group and time (right; p=0.182, left; p=0.570). Left peak knee flexion angle revealed a statistically significant time effect (p=0.034) indicating significant changes in left knee flexion angle between the baseline and the 12 months follow up assessment and between the baseline and the 24 months assessment. Group effect (p=0.471) and interaction between group and time (0.401) were not statistically significant.

Table 26. The results of the knee kinematics in the sagittal plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Knee Peak Extension (º)					
Baseline	-4.53 ± 6.79	-4.28 ± 5.79			
12 months	-1.73 ± 5.07	-0.74 ± 5.82	**0.001**	0.182	0.197
24 months	-2.75 ± 17.75	-1.86 ± 13.51			
Left Knee Peak Extension (º)					
Baseline	-4.11 ± 3.25	-2.92 ± 5.61			
12 months	-2.01 ± 5.46	-1.07 ± 4.62	**0.004**	0.570	0.674
24 months	-0.29 ± 5.77	-0.75 ± 4.18			
Right Knee Peak Flexion (º)					
Baseline	51.43 ± 8.60	51.83 ± 6.85			
12 months	50.27 ± 8.03	50.34 ± 11.89	0.624	0.477	0.617
24 months	49.70 ± 11.62	53.11 ± 8.18			
Left Knee Peak Flexion (º)					
Baseline	50.53 ± 8.70	53.03 ± 6.98			
12 months	48.17 ± 9.79	47.22 ± 12.90	**0.034**	0.401	0.471
24 months	50.40 ± 9.18	53.98 ± 7.36			

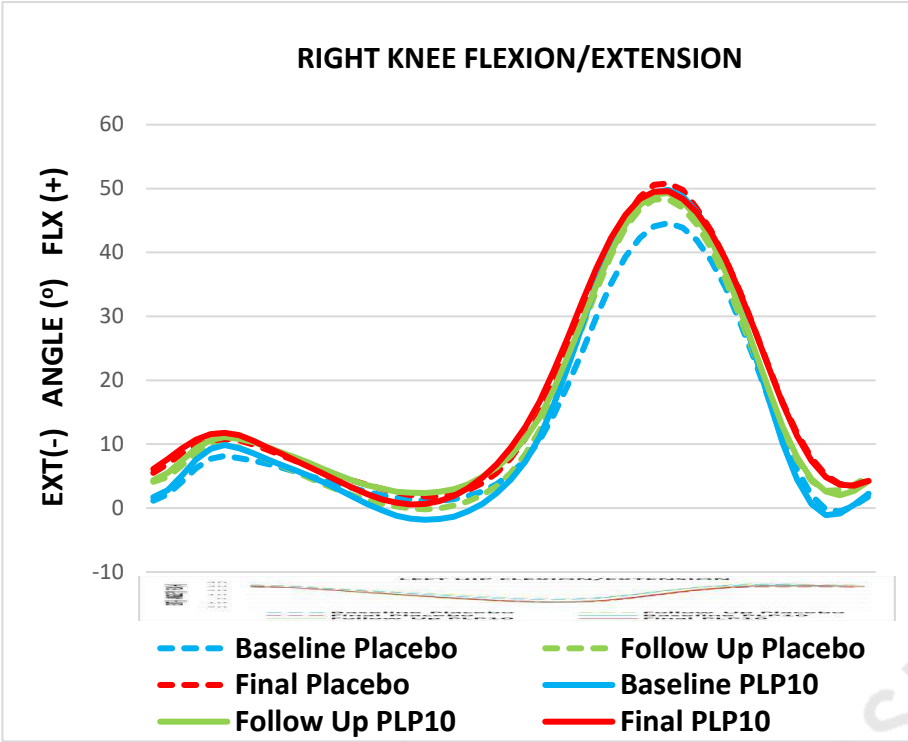

Figure 42. Changing position of the right knee flexion/ extension angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

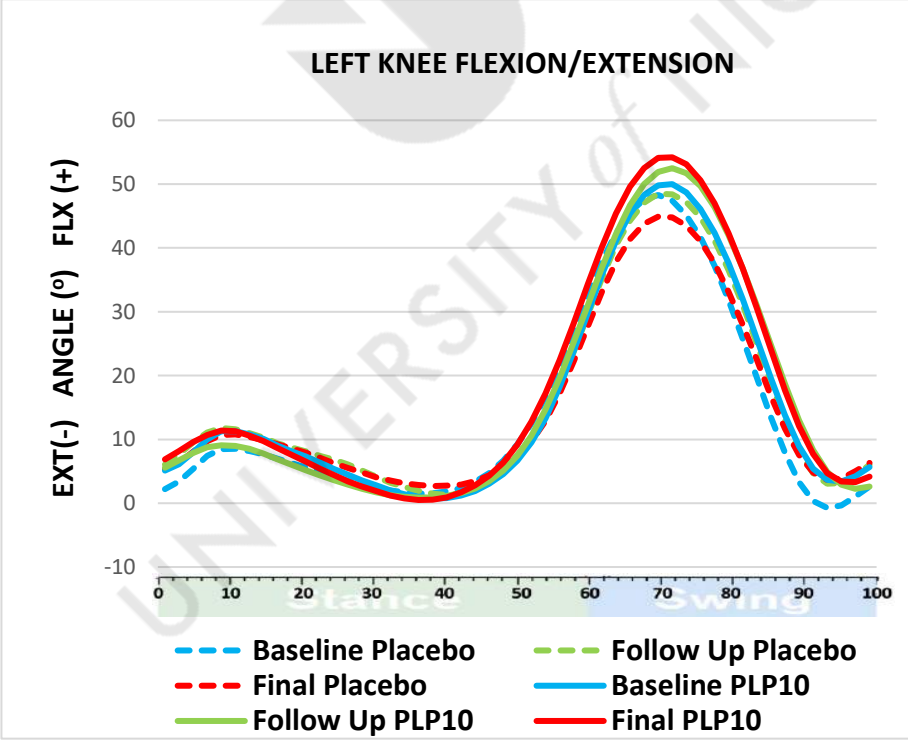

Figure 43. Changing position of the left knee flexion/ extension angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

Peak pelvis posterior tilt angle slightly decreased in both groups during the 3 visits producing a statistically significant time effect (right; p=0.001, left; p=0.002) between baseline and 24 months assessment and between the 12 months and 24 months follow up assessment. No statistically significant group effect (right; p=0.439, left; p=0.392) or interaction between group and time (right; p=0.974, left; p=0.392) was revealed (table 27).

Peak pelvis anterior tilt during the 3 visits showed a statistically significant time effect (right; p=0.001, left; p=0.002) between the 12 months and 24 months follow up assessment. Group effect (right; p=0.284, left; p=0.253) and interaction between group and time (right; p=0.284, left; p=0.811) were not statistically significant. (table 27).

Table 27. The results of the pelvis kinematics in the sagittal plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Pelvis Peak Posterior Tilt (º)					
Baseline	15.17 ± 4.96	14.04 ± 6.50			
12 months	11.66 ± 5.91	10.41 ± 5.85	**0.001**	0.974	0.439
24 months	13.38 ± 5.32	11.82 ± 7.14			
Left Pelvis Peak Posterior Tilt (º)					
Baseline	13.92 ± 4.92	14.18 ± 6.53			
12 months	17.15 ± 5.79	13.34 ± 5.81	**0.002**	0.923	0.392
24 months	11.69 ± 5.45	11.89 ± 7.19			
Right Pelvis Peak Anterior Tilt (º)					
Baseline	16.91 ± 5.17	15.45 ± 6.46			
12 months	13.25 ± 5.74	11.64 ± 5.64	**0.001**	0.795	0.284
24 months	15.22 ± 6.68	11.92 ± 7.07			
Left Pelvis Peak Anterior Tilt (º)					
Baseline	15.49 ± 5.00	11.25 ± 6.40			
12 months	17.36 ± 5.87	13.12 ± 5.60	**0.002**	0.811	0.253
24 months	12.46 ± 6.82	12.57 ± 7.13			

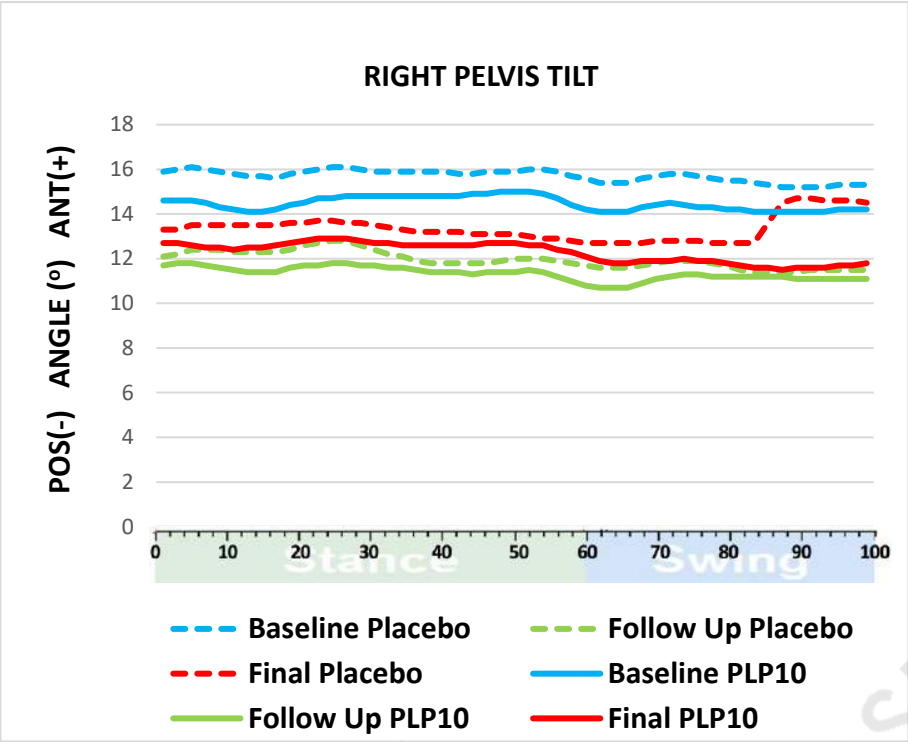

Figure 44. Changing position of the right pelvis tilt angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

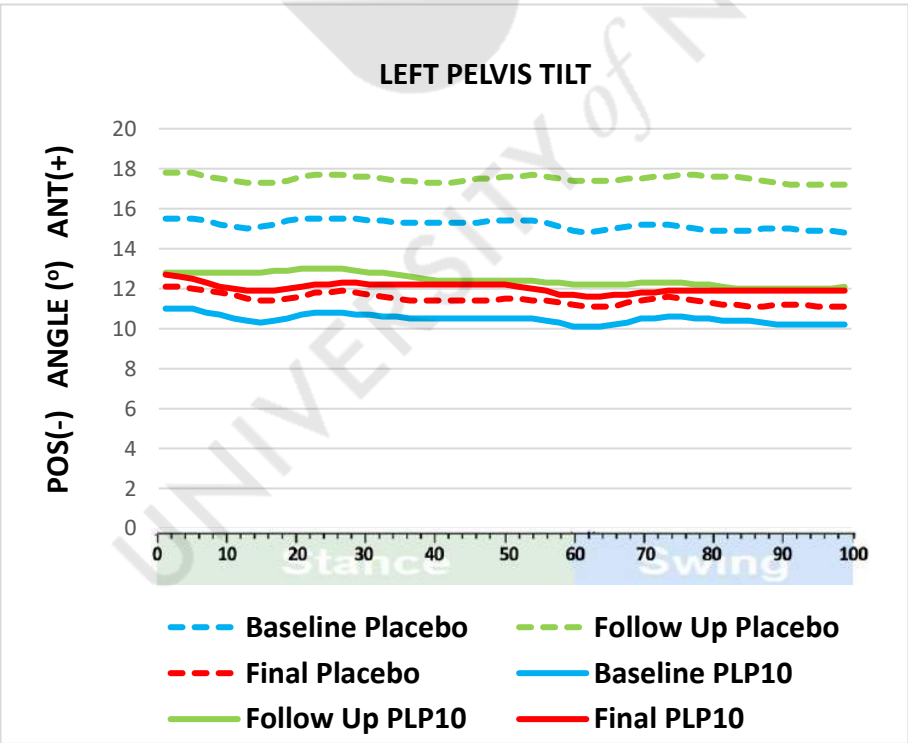

Figure 45. Changing position of the left pelvis tilt angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

Kinematic Data in the Frontal Plane

The results of the ankle kinematics in the frontal plane did not reveal any statistically significant time ($p>0.05$) or group effect ($p>0.05$), neither any statistically significant interaction between group and time ($p>0.05$) during the 24 months assessment (table 28).

Table 28. The results of the ankle kinematics in the frontal plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Ankle Peak Abduction (º)					
Baseline	-5.51 ± 4.07	-5.43 ± 6.36	0.616	0.395	0.755
12 months	-4.37 ± 18.22	-5.13 ± 5.15			
24 months	-3.53 ± 5.54	-3.76 ± 3.21			
Left Ankle Peak Abduction (º)					
Baseline	-5.38 ± 3.10	-4.53 ± 3.11	0.065	0.707	0.864
12 months	-3.79 ± 3.38	-5.54 ± 5.44			
24 months	-5.14 ± 4.54	-5.27 ± 5.15			
Right Ankle Peak Adduction (º)					
Baseline	-0.22 ± 2.68	0.42 ± 4.58	0.146	0.650	0.946
12 months	0.64 ± 9.40	-0.24 ± 4.57			
24 months	2.15 ± 6.96	1.11 ± 2.51			
Left Ankle Peak Adduction (º)					
Baseline	-1.33 ± 3.10	- 0.53 ± 3.11	0.116	0.592	0.344
12 months	- 0.36 ± 3.38	0.34 ± 5.44			
24 months	-0.79 ± 4.54	-0.63 ± 5.15			

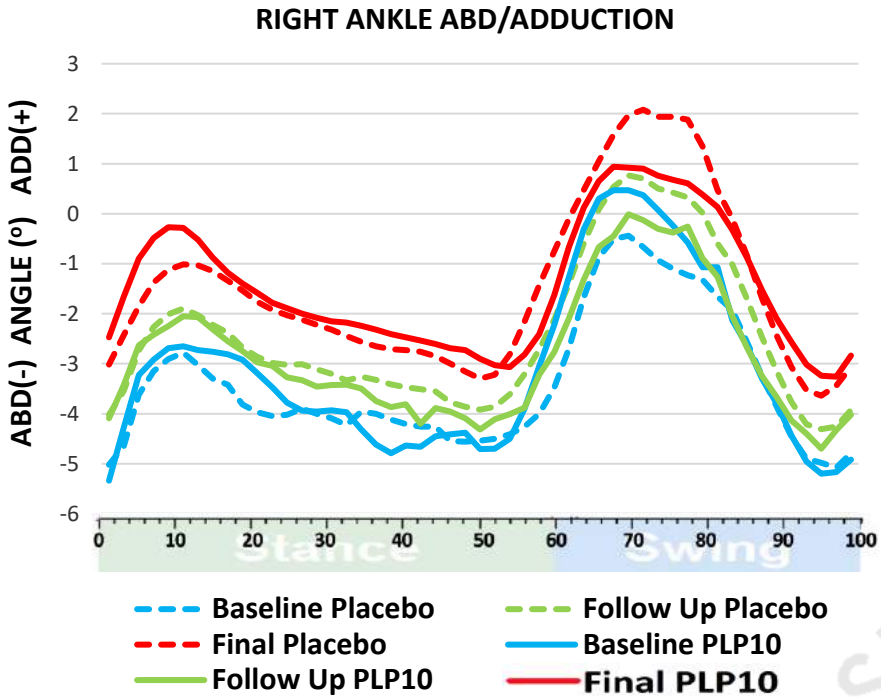

Figure 46. Changing position of the right ankle abduction/adduction angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

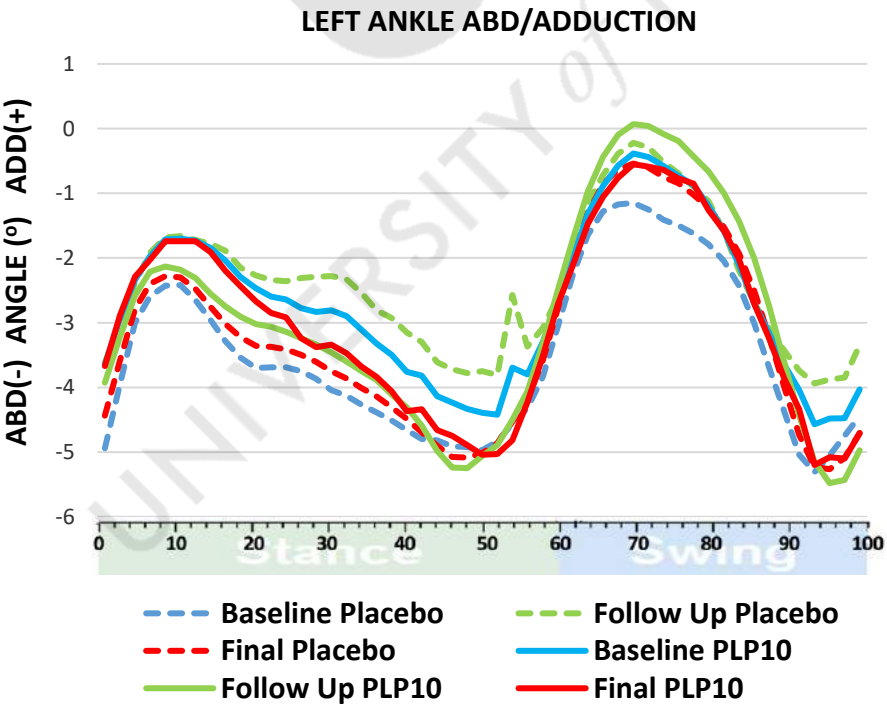

Figure 47. Changing position of the left ankle abduction/adduction angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

The results of the hip kinematics in the frontal plane did not reveal any statistically significant time ($p>0.05$) or group effect ($p>0.05$), neither any statistically significant interaction between group and time ($p>0.05$) during the 24 months assessment (table 29).

Table 29. The results of the hip kinematics in the frontal plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Hip Peak Abduction (º)					
Baseline	-2.12 ± 4.07	-4.09 ± 3.98	0.287	0.945	0.811
12 months	-2.36 ± 3.75	-4.78 ± 3.37			
24 months	-1.82 ± 3.79	-2.80 ± 3.67			
Left Hip Peak Abduction (º)					
Baseline	-2.34 ± 4.97	-5.24 ± 4.67	0.441	0.239	0.241
12 months	-4.81 ± 3.62	-5.32 ± 3.65			
24 months	-4.36 ± 3.66	-4.08 ± 5.02			
Right Hip Peak Adduction (º)					
Baseline	6.38 ± 4.02	6.72 ± 2.97	0.057	0.862	0.768
12 months	7.21 ± 4.30	5.76 ± 3.84			
24 months	8.08 ± 3.60	8.38 ± 4.00			
Left Hip Peak Adduction (º)					
Baseline	6.41 ± 4.47	6.02 ± 3.94	0.271	0.841	0.855
12 months	5.45 ± 3.47	5.82 ± 3.80			
24 months	4.33 ± 3.73	6.56 ± 4.19			

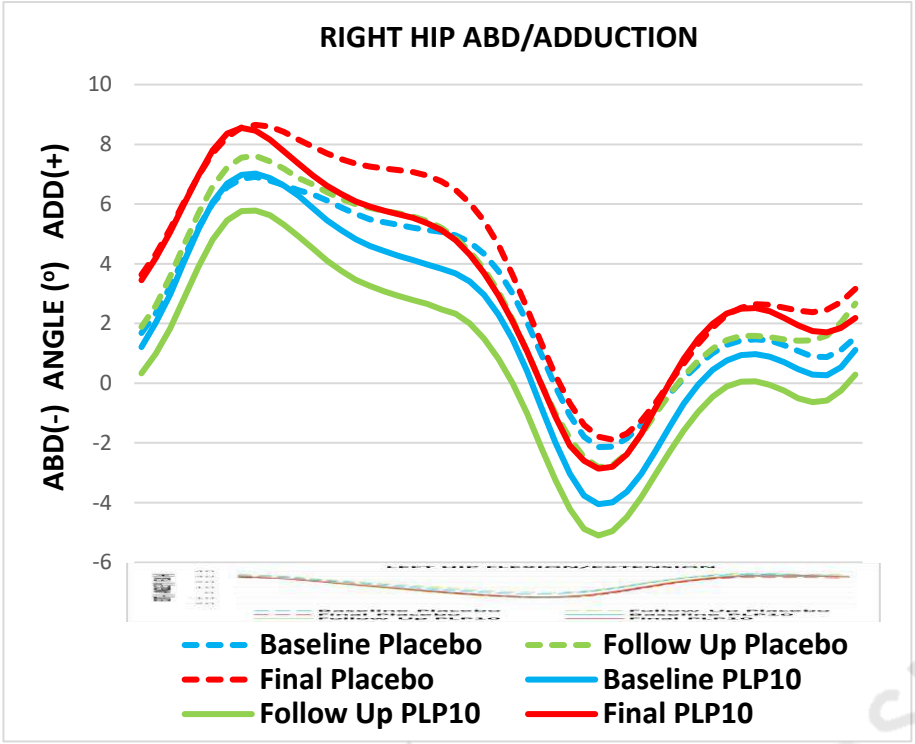

Figure 48. Changing position of the right hip abduction/adduction angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

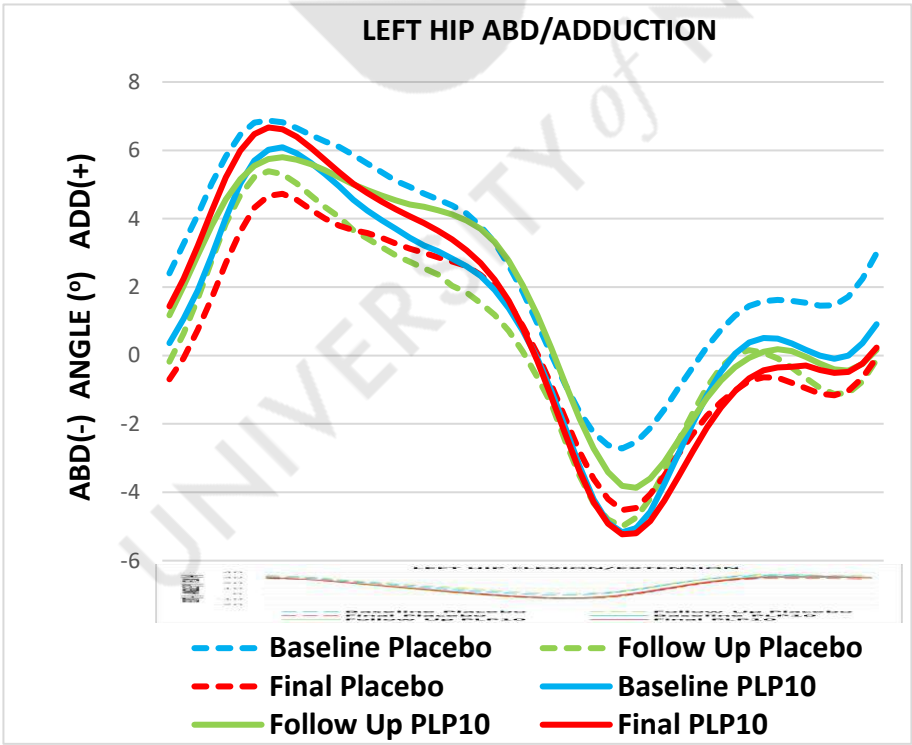

Figure 49. Changing position of the left hip abduction/adduction angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

The results of the knee kinematics in the frontal plane did not reveal any statistically significant time ($p>0.05$) or group effect ($p>0.05$), neither any statistically significant interaction between group and time ($p>0.05$) during the 24 months assessment (table 30).

Table 30. The results of the knee kinematics in the frontal plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Knee Peak Varus (º)					
Baseline	-2.81 ± 8.88	-0.31 ± 7.16	0.178	0.874	0.343
12 months	-2.14 ± 6.09	0.33 ± 6.86			
24 months	-2.56 ± 5.57	-0.28 ± 6.76			
Left Knee Peak Varus (º)					
Baseline	-1.49 ± 8.21	1.89 ± 7.12	0.971	0.286	0.939
12 months	-1.74 ± 5.69	0.83 ± 8.56			
24 months	-0.93 ± 6.80	0.36 ± 6.38			
Right Knee Peak Valgus (º)					
Baseline	-5.48 ± 9.06	-2.62 ± 6.24	0.465	0.685	0.742
12 months	-3.49 ± 6.63	-2.43 ± 4.16			
24 months	-4.52 ± 9.07	-3.83 ± 6.68			
Left Knee Peak Valgus (º)					
Baseline	-7.33 ± 8.88	-9.59 ± 5.17	0.678	0.367	0.969
12 months	-6.28 ± 5.02	-9.77 ± 3.39			
24 months	-9.11 ± 6.16	-9.84 ± 5.16			

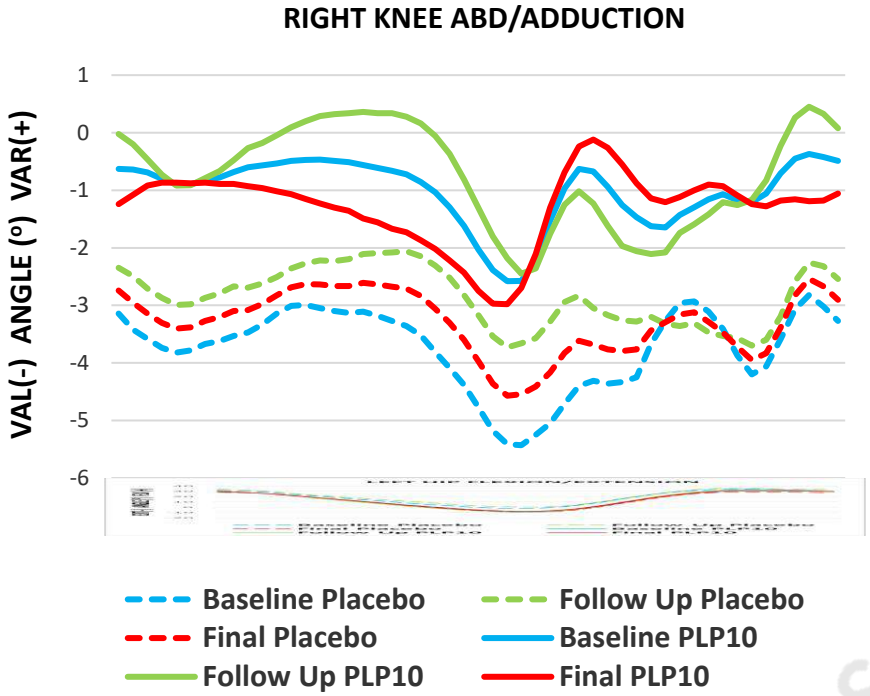

Figure 50. Changing position of the right knee abduction/adduction angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

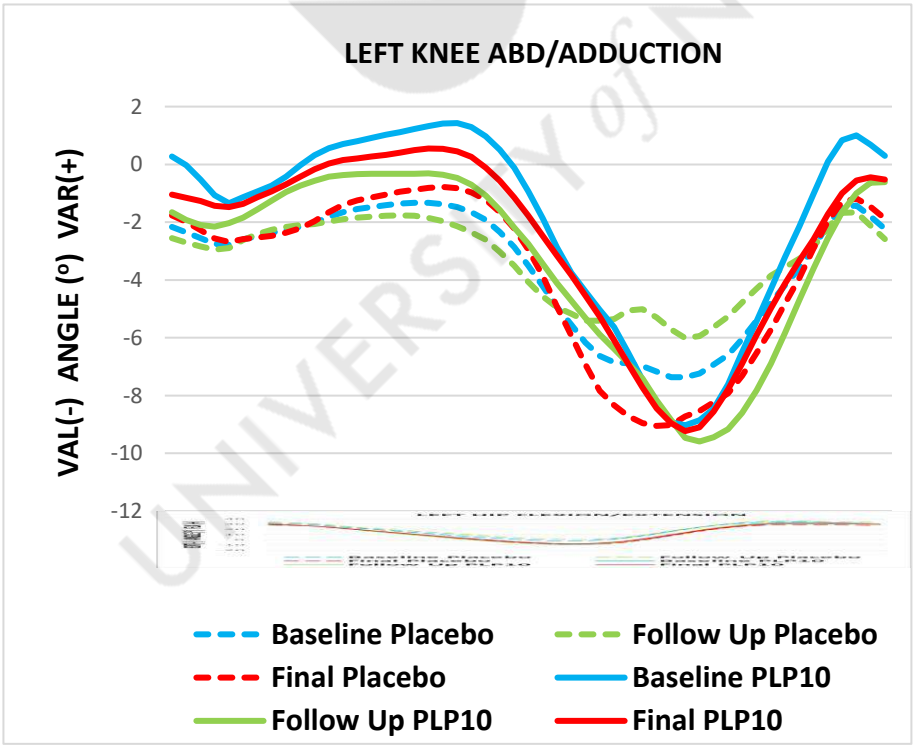

Figure 51. Changing position of the left knee abduction/adduction angles across the averaged gait cycle in all three time-points for the placebo and PLP10 group

The results of the pelvis kinematics in the frontal plane did not reveal any statistically significant time ($p>0.05$) or group effect ($p>0.05$) within-subjects, neither any statistically significant interaction between group and time ($p>0.05$) during the 24 months assessment (table 31).

Table 31. The results of the pelvis kinematics in the frontal plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Pelvis Peak Oblique Down (º)					
Baseline	-4.01 ± 2.81	-4.86 ± 3.00	0.298	0.976	0.801
12 months	-3.78 ± 2.77	-4.23 ± 2.10			
24 months	-3.68 ± 2.32	-4.05 ± 2.85			
Left Pelvis Peak Oblique Down (º)					
Baseline	-2.45 ± 2.74	-3.55 ± 3.26	0.338	0.364	0.146
12 months	-5.63 ± 1.90	-3.18 ± 1.66			
24 months	-3.83 ± 2.99	-4.20 ± 2.95			
Right Pelvis Peak Oblique Up (º)					
Baseline	2.96 ± 2.63	3.87 ± 2.72	0.172	0.613	0.108
12 months	3.96 ± 2.06	3.85 ± 1.94			
24 months	3.91 ± 3.04	4.31 ± 2.83			
Left Pelvis Peak Oblique Up (º)					
Baseline	4.07 ± 2.83	4.76 ± 2.29	0.154	0.598	0.519
12 months	3.91 ± 2.04	4.13 ± 2.24			
24 months	3.19 ± 2.18	4.71 ± 2.65			

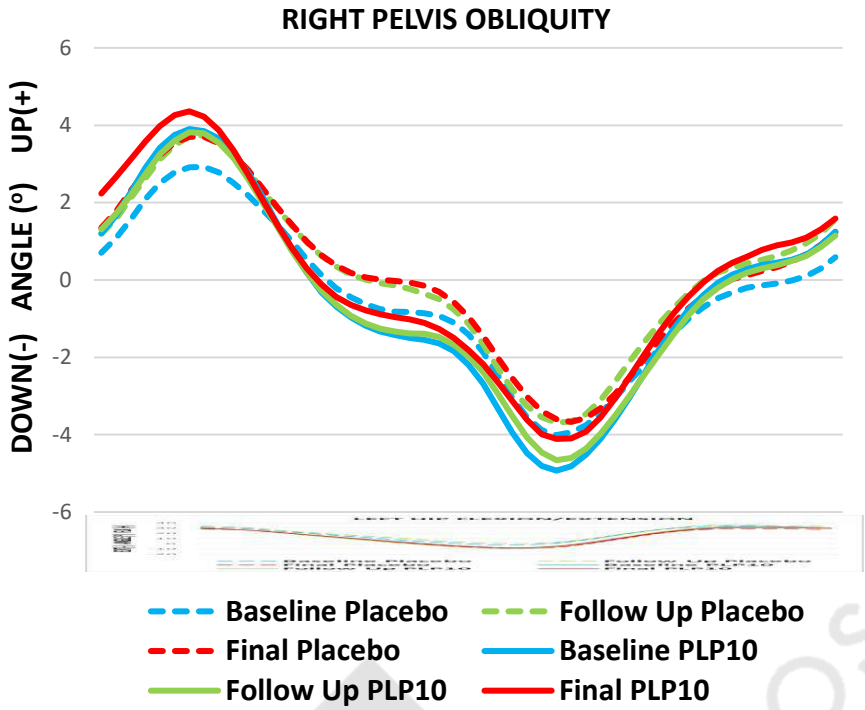

Figure 52. Changing position of the right pelvis obliquity across the averaged gait cycle in all three time-points for the placebo and PLP10 group

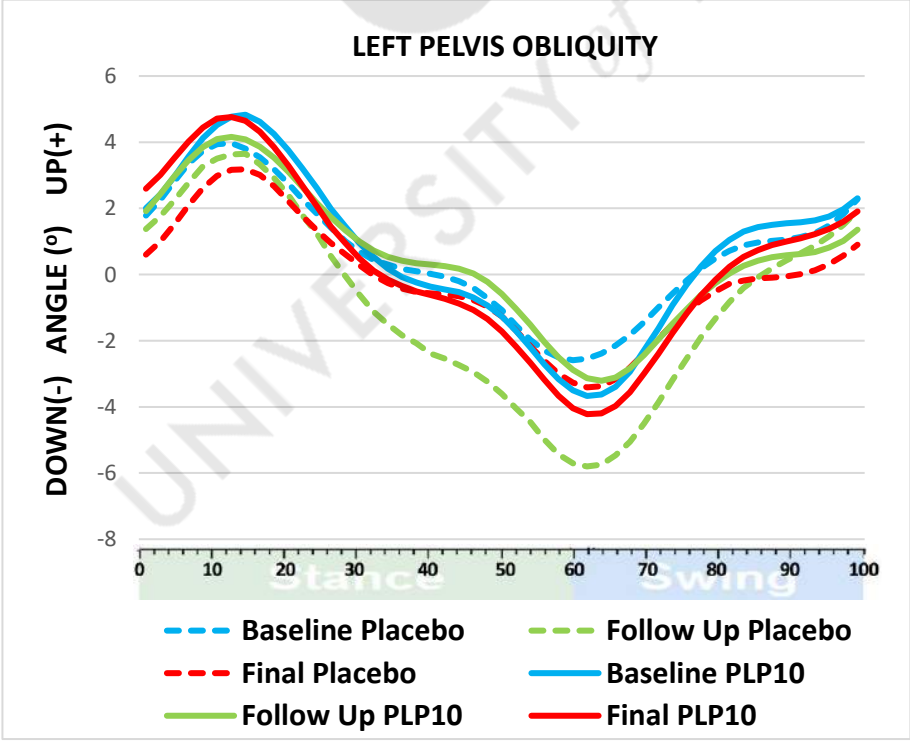

Figure 53. Changing position of the left pelvis obliquity across the averaged gait cycle in all three time-points for the placebo and PLP10 group

Kinematic Data in the Transverse Plane

Results for the left peak ankle eversion angle revealed a statistically significant time effect (p=0.037) indicating significant changes between the baseline and 12 months follow up assessment and between the baseline and 24 months follow up assessment. Group effect (p=0.648) and interaction between group and time (0.451) did not show any statistically significant difference. Results for the left peak ankle inversion angle revealed a statistically significant time effect (p=0.006) indicating significant changes the baseline and 24 months follow up assessment and between the 12 months and 24 months follow up assessment. Group effect (p=0.674) and interaction between group and time (0.304) did not show any statistically significant difference.

Table 32. The results of the ankle kinematics in the transverse plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Peak Right Ankle Eversion (º)					
Baseline	-5.06 ± 14.72	-5.53 ± 23.43			
12 months	-0.36 ± 18.52	-4.81 ± 19.66	0.548	0.641	0.580
24 months	-2.38 ± 26.25	-6.71 ± 12.14			
Peak Left Ankle Eversion (º)					
Baseline	3.63 ± 18.81	5.10 ± 20.03			
12 months	-0.33 ± 15.39	-4.51 ± 19.15	**0.037**	0.451	0.648
24 months	11.14 ± 15.75	-0.20 ± 17.11			
Peak Right Ankle Inversion (º)					
Baseline	19.46 ± 14.28	20.33 ± 22.53			
12 months	16.45 ± 16.66	17.61 ± 16.47	0.114	0.070	0.929
24 months	18.17 ± 21.14	12.39 ± 12.81			
Peak Left Ankle Inversion (º)					
Baseline	23.32 ± 20.13	26.06 ± 17.35			
12 months	19.12 ± 11.19	25.29 ± 17.64	**0.006**	0.304	0.674
24 months	33.63 ± 16.79	25.88 ± 15.67			

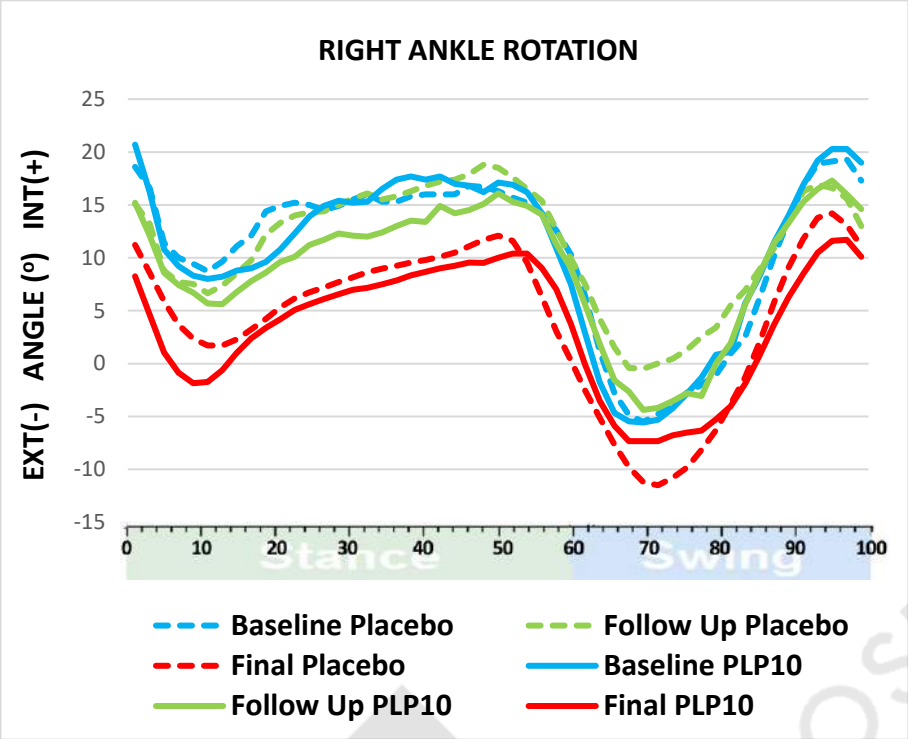

Figure 54. Changing position of the right ankle rotation across the averaged gait cycle in all three time-points for the placebo and PLP10 group

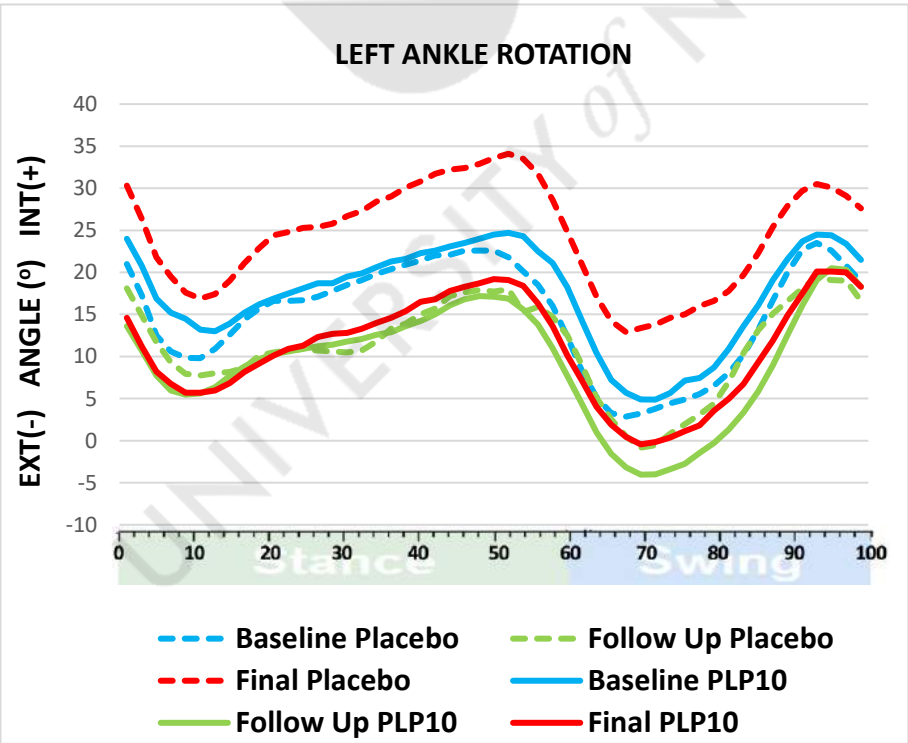

Figure 55. Changing position of the left ankle rotation across the averaged gait cycle in all three time-points for the placebo and PLP10 group

Results for the hip kinematics in the transverse plane did not reveal any statistically significant time effect ($p>0.05$) or group effect ($p>0.05$), neither any statistically significant interaction between group and time ($p>0.05$) during the 24 months assessment (table 33).

Table 33. The results of the hip kinematics in the transverse plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Hip Peak External Rotation (º)					
Baseline	-19.21 ± 18.54	-18.04 ± 10.88	0.187	0.675	0.545
12 months	-17.15 ± 9.91	-18.79 ± 9.50			
24 months	-18.12 ± 16.58	-17.85 ± 10.65			
Left Hip Peak External Rotation (º)					
Baseline	-23.03 ± 17.81	-23.26 ± 9.05	0.842	0.394	0.472
12 months	-24.02 ± 9.08	-23.48 ± 10.79			
24 months	-23.46 ± 7.23	-23.80 ± 8.38			
Right Hip Peak Internal Rotation (º)					
Baseline	-6.13 ± 16.07	-6.42 ± 13.10	0.408	0.771	0.569
12 months	-5.45 ± 13.10	-6.64 ± 9.25			
24 months	-6.01 ± 17.42	-6.30 ± 10.92			
Left Hip Peak Internal Rotation (º)					
Baseline	-8.33 ± 17.49	-13.26 ± 13.16	0.171	0.682	0.810
12 months	-13.32 ± 11.98	-13.12 ± 10.99			
24 months	-11.02 ± 9.63	-13.75 ± 10.91			

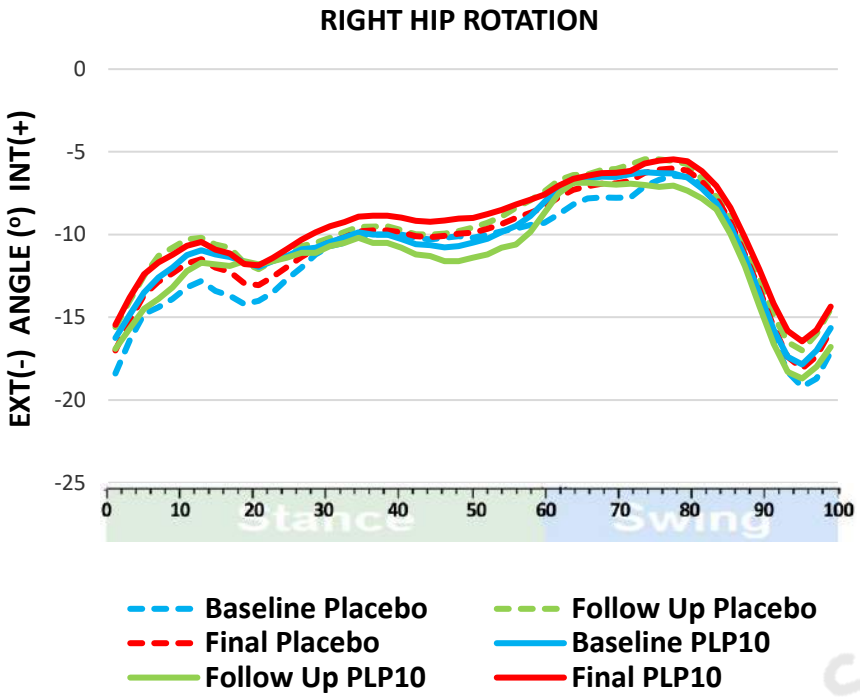

Figure 56. Changing position of the right hip rotation across the averaged gait cycle in all three time-points for the placebo and PLP10 group

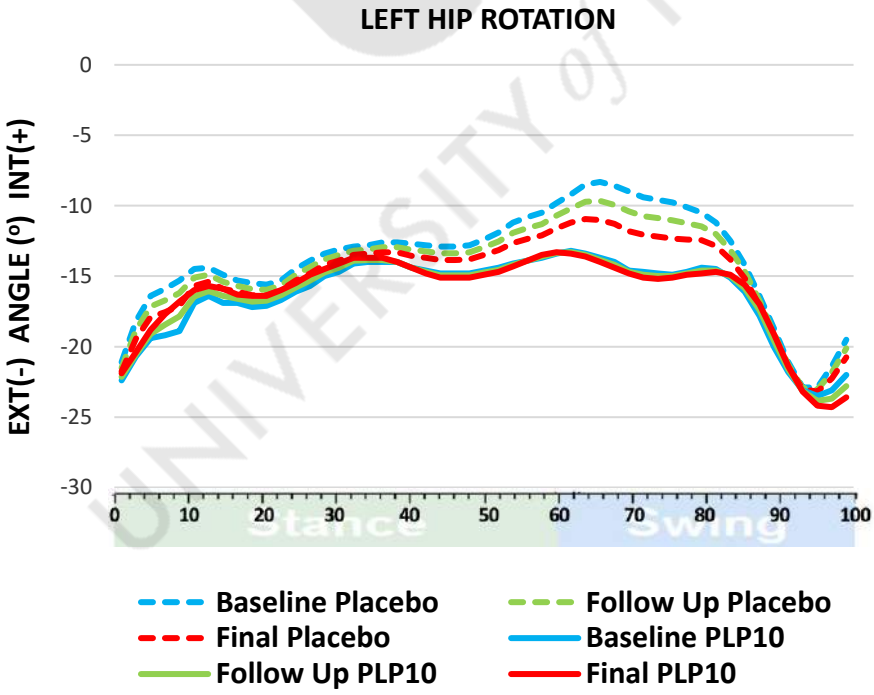

Figure 57. Changing position of the left hip rotation across the averaged gait cycle in all three time-points for the placebo and PLP10 group

Results for the left knee external rotation peak angle revealed a statistically significant time effect (p=0.04) between the baseline and 24 months follow up assessment and between the 12 months follow up and the 24 months follow up assessment. Group effect (p=0.655) or interaction were not significantly different (p=0.629).

Table 34. The results of the knee kinematics in the transverse plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Knee External Rotation (º)					
Baseline	-15.32 ± 26.69	-18.02 ± 19.45			
12 months	- 13.72 ± 10.36	-15.55 ± 12.31	0.211	0.117	0.661
24 months	-15.31 ± 17.75	-13.36 ± 13.51			
Left Knee External Rotation					
Baseline	-13.47 ± 10.01	-9.13 ± 18.09			
12 months	-7.93 ± 11.43	-5.87 ± 15.44	**0.004**	0.629	0.655
24 months	-14.11 ± 13.55	-7.88 ± 12.64			
Right Knee Internal Rotation (º)					
Baseline	-1.57 ± 21.59	-6.62 ± 21.13			
12 months	- 4.71 ± 13.37	-3.91 ± 17.59	0.195	0.834	0.891
24 months	-2.51 ± 26.25	-1.21 ± 12.61			
Left Knee Internal Rotation (º)					
Baseline	-4.77 ± 13.42	-0.58 ± 19.88			
12 months	-1.47 ± 15.61	5.75± 21.97	0.280	0.528	0.843
24 months	-4.24 ± 14.63	2.38 ± 11.71			

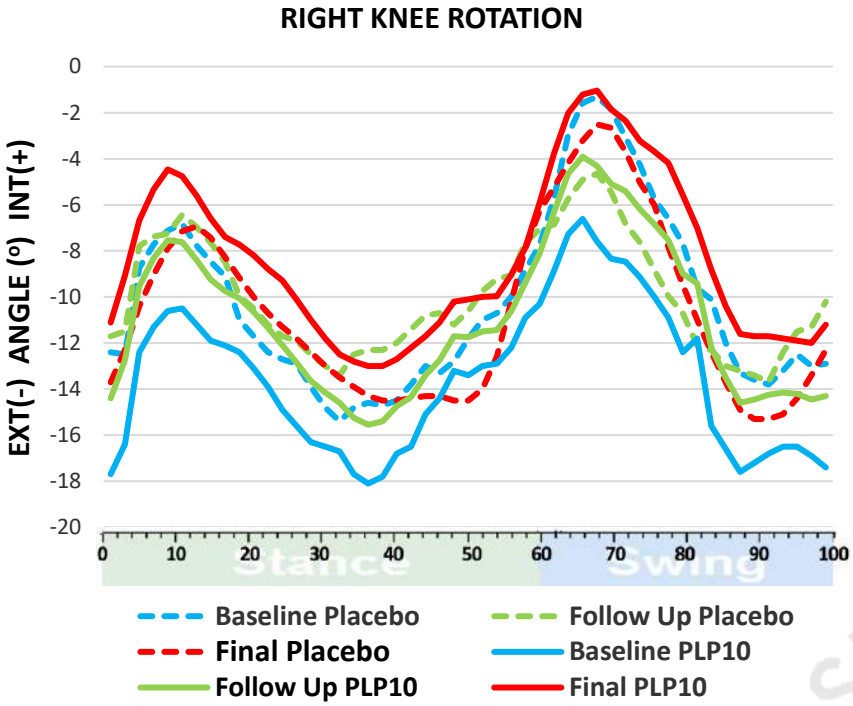

Figure 58. Changing position of the right knee rotation across the averaged gait cycle in all three time-points for the placebo and PLP10 group

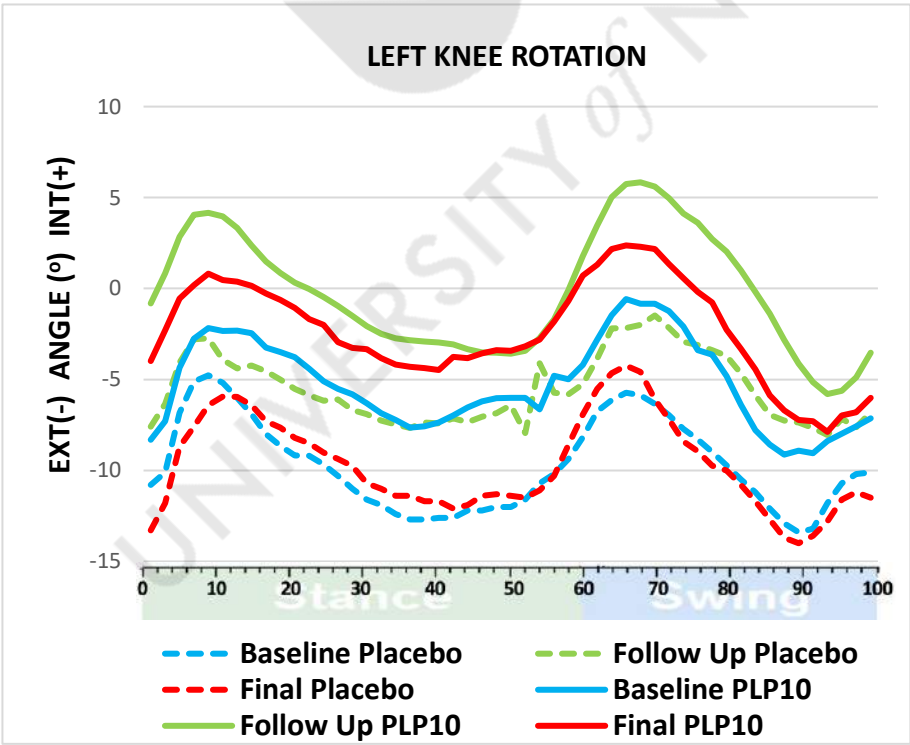

Figure 59. Changing position of the left knee rotation across the averaged gait cycle in all three time-points for the placebo and PLP10 group

Results for the pelvis kinematics in the transverse plane did not reveal any statistically significant time effect (p>0.05) or group effect (p>0.05), neither any statistically significant interaction between group and time (p>0.05) during the 24 months assessment (table 35).

Table 35. The results of the pelvis kinematics in the transverse plane in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Right Pelvis Peak External Rotation (º)					
Baseline	-2.09 ± 3.16	-3.38 ± 2.78	0.075	0.106	0.908
12 months	-4.41 ± 3.45	-3.175 ± 2.90			
24 months	-3.91 ± 3.71	-4.13 ± 4.18			
Left Pelvis Peak External Rotation (º)					
Baseline	-4.81 ± 2.85	-3.74 ± 3.77	0.413	0.615	0.420
12 months	-3.72 ± 3.23	-3.16 ± 2.74			
24 months	-2.55 ± 3.11	-3.82 ± 5.53			
Right Pelvis Peak Internal Rotation					
Baseline	4.55 ± 3.03	4.77 ± 3.44	0.195	0.834	0.891
12 months	3.19 ± 2.95	4.95 ± 2.59			
24 months	3.41 ± 4.23	5.26 ± 5.12			
Left Pelvis Peak Internal Rotation					
Baseline	2.93 ± 2.85	3.94 ± 3.77	0.413	0.615	0.420
12 months	5.59 ± 3.23	3.72 ± 2.74			
24 months	4.18 ± 3.11	5.34 ± 5.53			

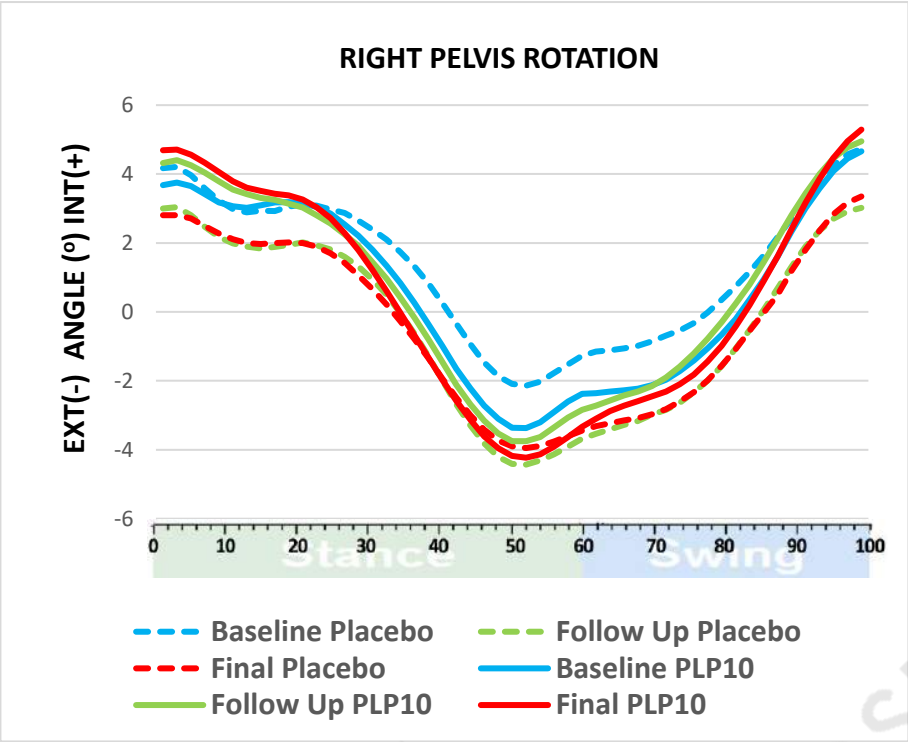

Figure 60. Changing position of the right pelvis rotation across the averaged gait cycle in all three time-points for the placebo and PLP10 group

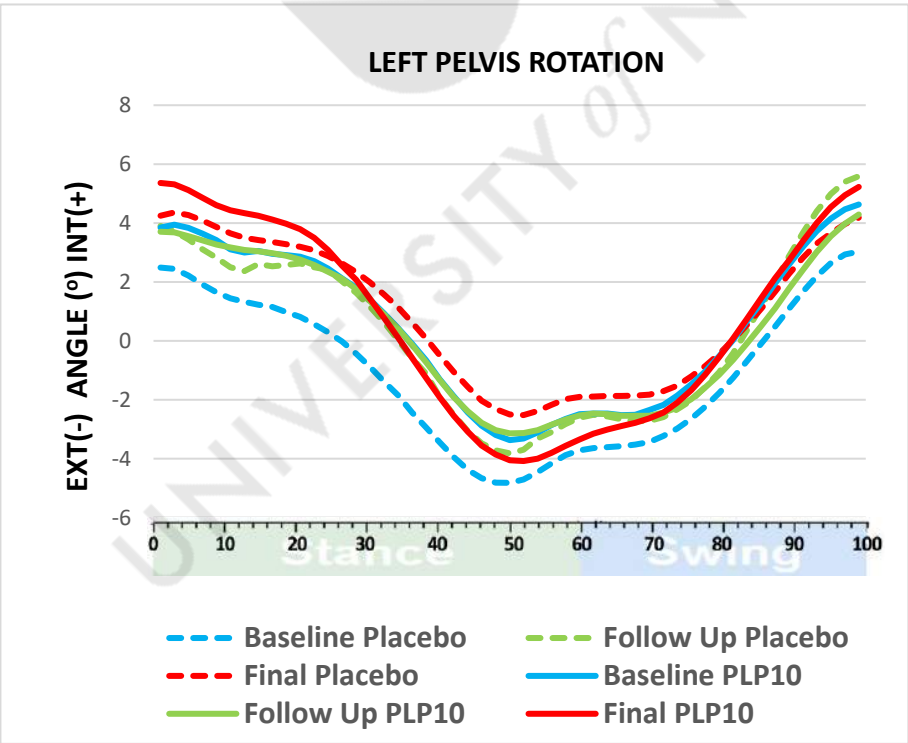

Figure 61. Changing position of the left pelvis rotation across the averaged gait cycle in all three time-points for the placebo and PLP10 group

The gait deviation index (GDI) showed some interesting changes (table 36). Left GDI progressively decreased in the placebo group from 80.88 at baseline to 74.70 in the 12 months assessment and finally to 72.38 in the 24 months follow up assessment. In the experimental group left GDI progressively increased from 78.38 at baseline to 79.20 in the 12 months assessment and finally to 81.04 in the 24 months follow up assessment. In the period of the two years follow up, left GDI decreased by 10.50% in the placebo group while in the experimental group increased by 3.39%. Results reveal a statistically significant interaction between group and time (p=0.001), a statistically significant time effect (p=0.028) between the baseline and the 24 months follow up assessment but no statistically significant group effect (p=0.233).
Right GDI reveal the same results, in the placebo group progressively decreased from 81.63 at baseline to 78.56 in the 12 months assessment and finally to 73.82 in the 24 months follow up assessment. In the experimental right GDI progressively increased from 81.80 at baseline to 82.40 in the 12 months assessment and finally to 85.34 in the 24 months follow up assessment. In the period of the two years right GDI decreased by 9.56% in the placebo group while in the experimental group increased by 4.32%. Results reveal a statistically significant interaction between group and time (p=0.001), but no statistically significant time (p=0.185) or group effect (p=0.075).

Table 36. The results of the Gait Deviation Index in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (p value)**		
			Time	Time X Group	Group
Left Gait Deviation Index					
Baseline	80.88 ± 12.61	78.38 ± 7.07			
12 months	74.70 ± 10.08	79.20 ± 6.82	**0.028**	**0.001**	0.233
24 months	72.38 ± 11.83	81.04 ± 7.87			
Right Gait Deviation Index					
Baseline	81.63 ± 9.92	81.80 ± 8.78			
12 months	78.56 ± 9.63	82.40 ± 7.37	0.185	**0.001**	0.075
24 months	73.82 ± 11.67	85.34 ± 7.76			

3.5 Discussion

The 6-minute walk test (6MWT), generally provides an indirect assessment of walking fatigability, an estimation of maximal walking distance, a measure of functional capacity [100] and a prediction in changes in everyday activities such as habitual walking [101]. The results of this study show no statistically significant difference between the two groups in the 6MWT distance. However, it is important to note that, while the placebo group reduced the distance of 6MWT during the 24 months follow up assessment, the experimental group increased the distance by 46 meters. According to the study of Baert et al. (2014), clinically meaningful changes from the patient's perspective following intervention have reported to be 21.6 m on 6MWT in PwMS [234]. Therefore, the increase in the walking distance in the experimental group although not statistically different from the control group seem to be clinically significant. If this is the case, the medication appears to improve the walking ability in a clinically meaningful size. Perhaps the lack of statistical significance can be attributed to the small sample size.

Spatiotemporal Parameters

One of the primary aims of this chapter was to identify if the medication had any effect on the spatiotemporal parameters of PwMS. Indeed, the medication seems to improve some spatiotemporal parameters, such as the left single support time, the right step time and the stride time of both legs. Furthermore, it is important to recognize that in addition to the obvious effect of the medication in the previous parameters, the PLP10 group presented higher percentages of improvement in cadence and walking speed. This increase in cadence coincides with a corresponding decrease in step time and step length. The increased walking speed concurs with the decrease in time of single and double support.

It seems that the medication had a positive effect in some spatiotemporal parameters such as the single support time, step and stride time in the PLP10 group. Perhaps the lack of the statistical significance in some other parameters (cadence, walking speed) can be attributed to the fact that the PwMS in this study have low disability status and the margin of improvement is small. The magnitude of these changes cannot be compared with the magnitude of changes from other studies. The literature does not provide a clear minimal clinically important difference on spatiotemporal parameters [219]. Additionally, in this study PwMS have low disability and a lot of studies in the literature evaluated PwMS with higher disability [222, 241] which cannot be compared with the data from this study were spatiotemporal parameters of PwMS have small margin for improvement.

The comparison from the data of this study with the data from the studies of Givon et al. (2009) [222] and Linden et al. (2014) [260] (see appendices table 43 and 44), which evaluated PwMS and Healthy controls, indicate that gait cadence, gait velocity, step length, stride length, single and double limb support of the patients in this study are comparable with the values of the healthy subjects in those studies verifying that the subjects of this study have low disability status and minimum gait disturbances. Despite the fact that due to the limited deviation from normal gait there is small room for improvement, there were some promising positive changes in the spatiotemporal parameters.

Kinematic Parameters

Another primary aim of this chapter was to identify if the medication had any effect on the kinematic parameters of PwMS. The outcome from this study reveal that medication had no effect on any of the kinematic parameters in the sagittal, frontal and transverse plane.

Although literature indicates that PwMS exhibit reduced joint motion [245] all of which result in reduced mobility [246], PwMS in this study probably due to the low disability status did not reveal statistically significant different joint motion angles between the 3 visits. Comparing the data from this study with the studies of Linden et al. (2014) [260] and Kwon, J. W et al. (2015) [261] (see appendices table 44 and 45), which evaluated PwMS and Healthy Controls, most of the kinematic joint angle values in this study are similar with the joint values of healthy subjects verifying that PwMS with low disability status present minimum gait disturbances.

The kinematic data in this study focused solely on peak joint motion angles across the averaged gait cycle and also the changing position of the joints angles across the averaged gait cycle in all time points. Researchers in this study assumed that because participants in this study were PwMS which is a progressive disease, peak joint motion angles could provide adequate evidence about the course of the disease or the medication effect but this was not confirmed. Moreover, since PwMS in this study had low disability status and it is already documented that an early symptom of MS is the reduced ankle dorsiflexion during the swing phase [247], ankle dorsiflexion could be evaluated during the swing phase and not just the peak dorsiflexion angle through the averaged gait cycle.

Participants of this study were asked to walk on their preferred self-selected speed. Perhaps it would have been better for PwMS in this study to walk at different speeds during the gait analysis trials since literature provides information which suggest that most kinematic gait parameters change significantly with increased walking speed [262]. The preferred self-

selected speed was chosen as a mean to evaluate subjects in the condition they spent most of their time during activities of daily living (ADLs).

The variation of kinematic measurements is higher in transverse plane, then in frontal and comparatively smaller in the sagittal plane. This is probably an effect of an inherent limitation of the modeling process itself. The error of measurement is increasing from sagittal, frontal to transverse plane. This is because the real articular movements are a combination of rotation and translation, while in modeling they are considered pure rotational movements around the corresponding plane axis. Therefore, the error of measurements affects more the transverse plane, then the frontal plane and then the sagittal plane, as the total range of movement is higher in the sagittal compared to the frontal and transverse plane.

Gait Deviation Index (GDI)

GDI showed some interesting group by time interaction. Specifically, while GDI of the control group decreases between the baseline and the 2 year follow up by about 10% on average across legs, the opposite happens in the PLP10 group. The GDI in the PLP10 group increased by about 4% on average across both legs in the same period. This perhaps shows a significant positive effect of the medication in the gait of the PLP10 group.

The GDI is computed with kinematic gait data from the pelvis, hip, knee, ankle and foot. It is an index which measures the distance between any chosen gait vector and a gait vector averaged over a control group [258]. Although this chapter confirmed a significant correlation between the GDI and the medication effect this was unexpected since GDI depends on kinematic data and most of the kinematic data analysed in this chapter did not reveal statistically significant changes between the placebo and the experimental group. On the other hand, the kinematic data in this study analysed only the detected peak joint angles across the averaged gait cycle and not more clinically meaningful angles during a specific phase of the averaged gait cycle (e.g. heel off, swing or stance phase), something that the provided GDI index depends on (specific phases of the gait cycle). It is possible that if kinematic data obtained at different phases of the averaged gait cycle were analyzed, that would have provided a better understanding on the GDI outcome. Additionally, Vicon doesn't provide enough information about the anthropometric characteristics of the people used for GDI normalization.

3.6 Limitations

Our study used a modified version of the 6MWT with a 20-m walkway and frequent 180^{o} turns, instead of a more standardized 6MWT protocol with longer walking paths. Therefore, the distance, fatigue and effort cannot be compared with other studies. It has been demonstrated that a 6MWT involving walking in a corridor with 180^{o} turns is significantly more demanding in terms of physiological energy cost, when compared with square walking using 90^{o} turns [252]. Our method, however, was consistent throughout the whole study of 24 months and due to the repeated measures design each subject was a control of him/her self.

In this study only the peak joint angles detected across the averaged gait cycle and not during a specific phase of the gait cycle (e.g. heel off, swing or stance phase). It was expected that since the population of this study are PwMS peak angles of the joints during a gait cycle would provide important information about the course of the disease or the medication effect but this was not confirmed. PwMS in this study had low disability status and the detection of peak angles did not reveal something important about the progressive nature of the disease in the early stages. It would be better in the future to identify the joint angles during specific phases of the gait cycle which might provide the opportunity of a better understanding of the results. Finally, the walking speed evaluated in this study was the natural self-selected speed of the subjects. Spatiotemporal and kinematics differences at this speed although clinically important are harder to detect. Higher walking speed are more likely to reveal differences. Future studies should take this into account and assess both the self-selected speed as well as a higher more challenging speed. This will provide an opportunity to detect more subtle differences.

CHAPTER 4 FUNCTIONAL CAPACITY

4.0 Introduction

Functional capacity defined as the ability to perform activities of daily living. The integrated efforts and health of the cardiovascular, pulmonary, and skeletal muscle systems describe an individual's functional capacity [263]. It is well known that adequate functional capacity levels are relevant to perceive good health and quality of life (QOL).

Multiple Sclerosis (MS) is one of the most common neurological diseases and a growing issue worldwide. At the same time, it is considered the leading neurological cause of disability in young adults, associated with impairments physically and psychologically [2, 6].

PwMS present a lower quality of life levels compared to their matched controls [264]. Several components contribute negatively to the QOL of PwMS with a wide range of debilitating symptoms including muscle weakness, extreme fatigue, balance and gait abnormalities, resulting in a significant financial burden on the patient, family, health system and society [108, 220, 265, 266]. PwMS, in order to be able to maintain independence, they must present adequate levels of functional capacity [99].

4.1 Functional Tests

4.1.1 Sit-To-Stand tests

The Sit-to-Stand (STS) test evaluates physical performance [267] and mobility [268]. Sit to stand is considered one of the most mechanically demanding movements within daytime activities [269-271]. A demanding muscle activation is required as individuals need to coordinate a transfer from a horizontal to a vertical position in one movement [272]. Muscle strength [273, 274], balance [275, 276], and synergy of muscle activation [277, 278] required for the realization of the STS movement.

In order to stand up from a chair, an individual has an option of utilizing two different strategies, the momentum transfer strategy and the flexion strategy [271, 279]. For young and healthy people standing up is a fully automated manoeuver, which does not need much mental attention. Discomfort or pain in ankle, knee, hip or back may break this automatism. For individuals with less muscle strength, or reduced proprioception, a failing vestibular system, impaired vision, pain, restrictions in the joints or a combination of these impairments, the momentum transfer strategy becomes less suitable or even impossible.

In the transition phase of the STS transfer, a co-contraction of the bi-articular hamstrings and rectus femoris required. Both hamstrings and rectus femoris are shortening after seat-off, while hip and knee extended.

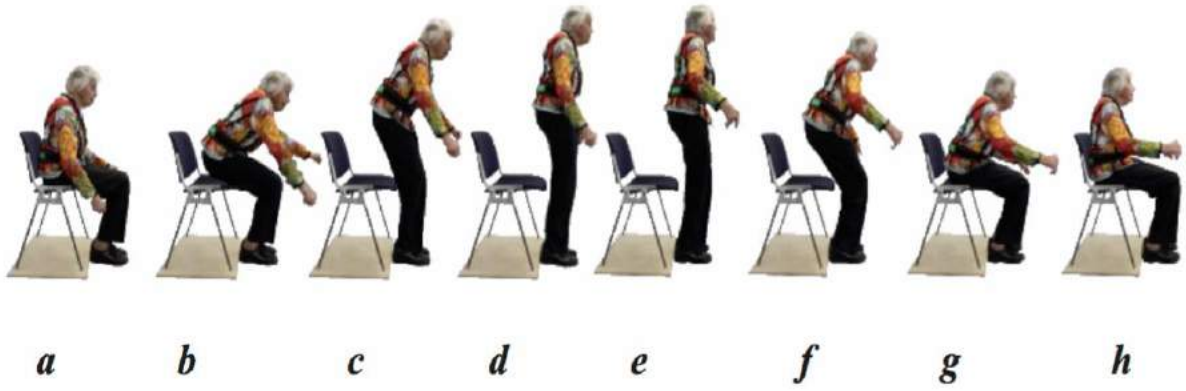

Figure 62. A STS cycle comprised of standing up (a-c), standing including stabilizing (d-e), sitting down (f-g) and sitting (h) [280].

In the horizontal direction, forward rotation of the upper body contributes to the velocity of the COM, whereas, extension of the legs contributes significantly in vertical direction. After seat-off, most muscles are concentrically active, whereas the shortening velocity of the rectus femoris is shallow. Thus hip and knee joints are extended, and a relatively high knee moment delivered to control the ground reaction force in a slightly backward direction [281]. Furthermore, the almost isometrically active rectus femoris transports moment from hip to knee joint, thus pointing the ground reaction force in a backward direction and contributing to the control of balance after seat-off. The stand-to-sit transition is an indicator of control and balance during the eccentric contraction of the extensors.

There are several options for assessing STS performance, including those does not require timing and those that are dependent on timing [7]. In the studies that investigate the relationship between the timed STS tests and physical performance parameters, correlations of the number of repetitions in STS test with the maximum walking speed [282] in 10 seconds STS (10sSTS) test and with the 6MWT distance in 60 seconds STS (60sSTS) test [283] have reported. The sit to stand test measures the functional muscle strength of the lower extremities while performing a primary transfer [284] and has been related to muscle strength as well as balance in PwMS [285]. The present study measured the time taken for five repeated sit-to stands from a standard chair without arm support, and the number of repetition during 30 and seconds. For interventional purposes, a change of >25% regarded as a real change.

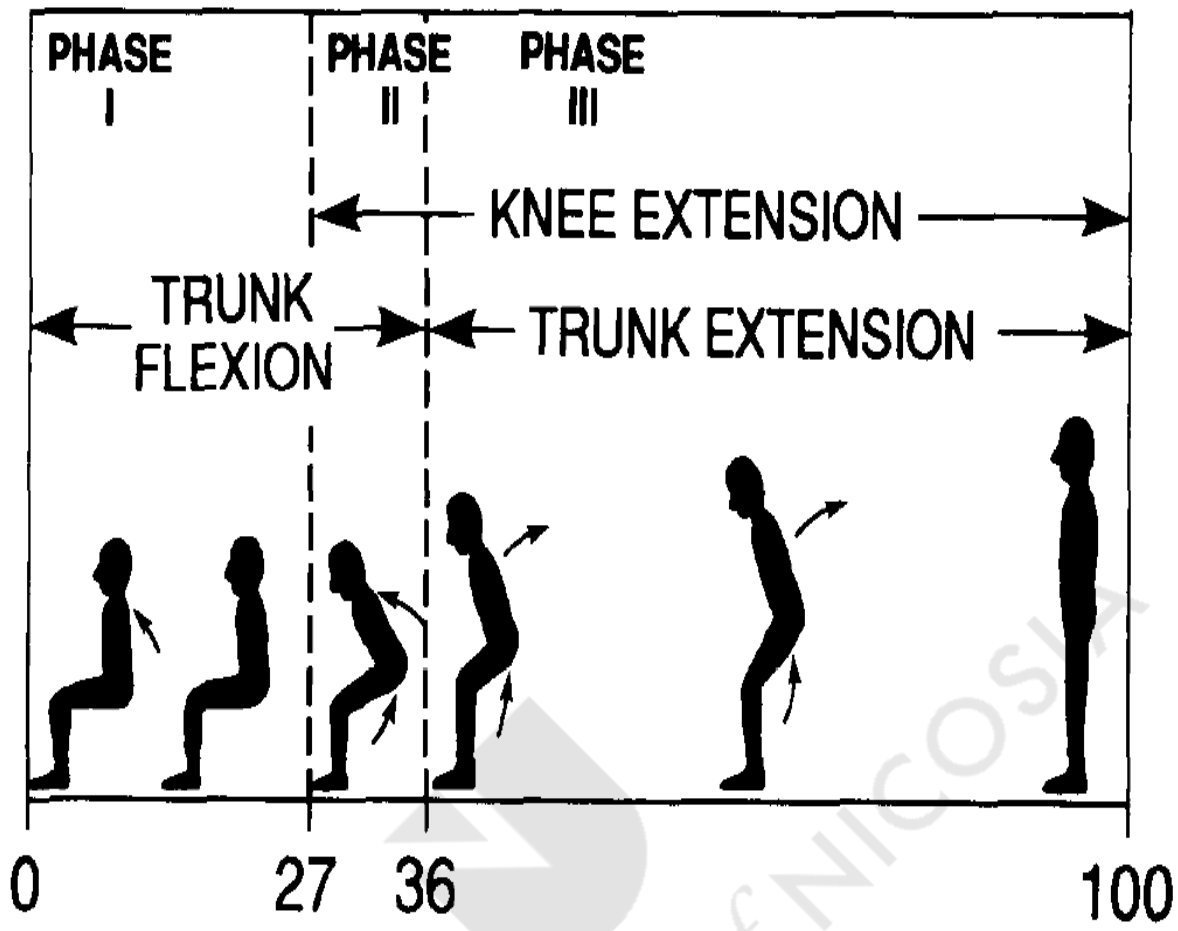

Figure 63. Three phases of sit to stand motion-defined from kinematic data.

Phase 1: weight shift, begins at the first visible trunk flexion and continues until knee extension initiated. Phase 2: transition, begins with the initiation of knee extension and ends with the reversal of trunk flexion to trunk extension. A transition from shifting the weight forward to lifting upwards occurs during this phase. Phase 3: the lift, begins with the reversal of trunk motion to extension. Full-extension to the standing position achieved during this phase. The last visible trunk motion defines the end of the STS motion.

Adapted from Millington et al. (1992) [286]

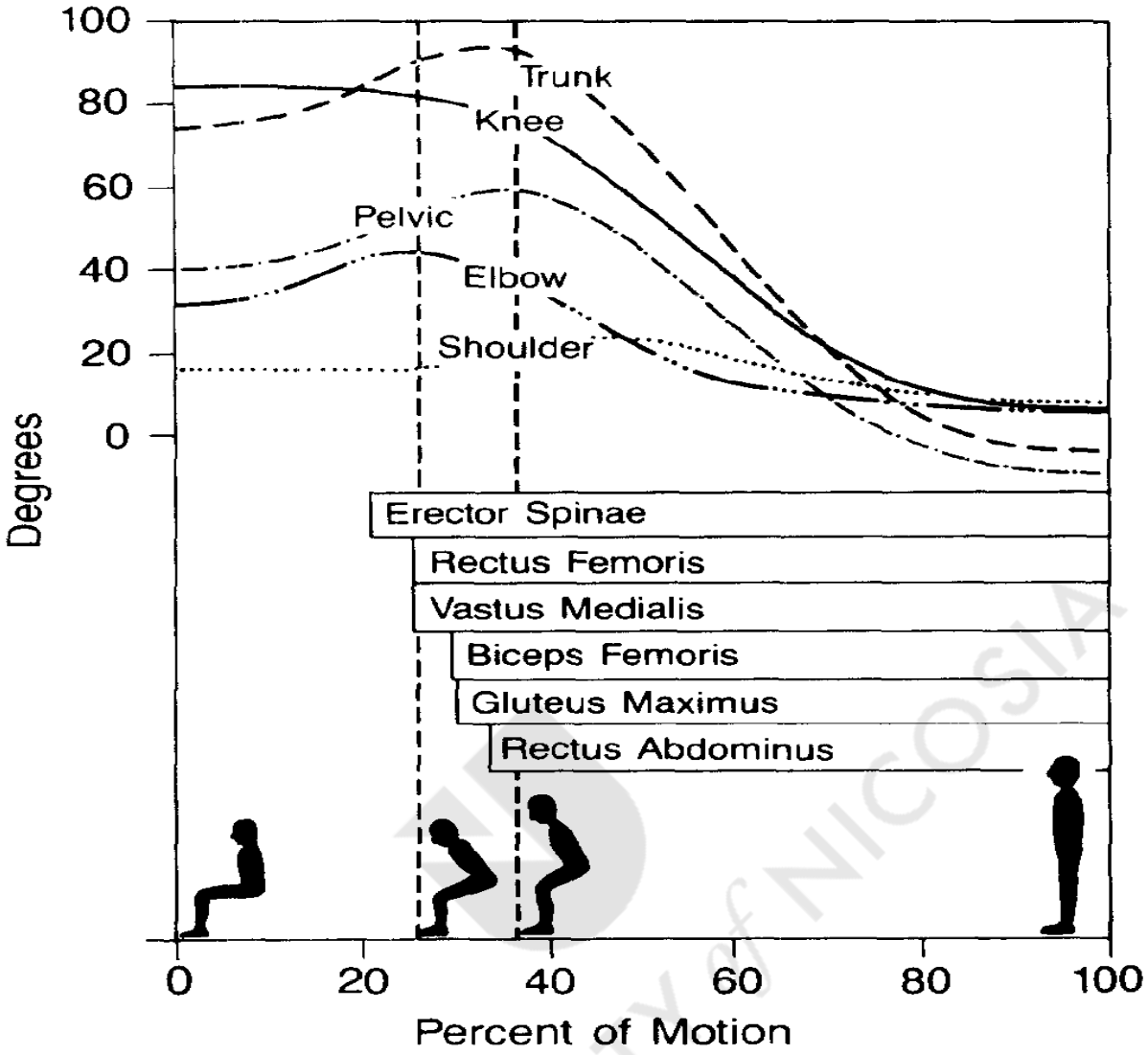

Figure 64. Composite of kinematic and EMG data for healthy elderly subjects

The onset of muscle activity and the figures illustrate body positions during each of the three phases of motion. **Phase 1:** weight shift, is characterized by flexion of the trunk and pelvis, resulting in a forward shift in the center of gravity. The erector spinae act eccentrically to control this motion. At the end of this phase, quadriceps muscle activity begins to prepare for standing. The initiation of trunk flexion could have occurred at many locations, including the head or upper extremity. Identifying a single source of the initial motion may be difficult because deep muscles could initiate the STS motion, and a minimal movement could initiate the action. **Phase 2:** the center of gravity must be controlled strictly while the transition from forward motion to upward motion made. It seems to be accomplished by concentric activity of the quadriceps muscle at the knee, and eccentric activity of biceps femoris at the knee and gluteus maximus at the hip. Peak muscle activity in the erector spinae, quadriceps, hamstrings and gluteus maximus also occurred during this phase. **Phase 3:** trunk extension begins while knee extension continues until full standing reached

Adapted from Millington et al. (1992) [286]

4.1.2 Timed Up and Go test

The Timed Up and Go (TUG) test is a well-established and valid test for PwMS [287], used to assess mobility and balance in PwMS, monitor disease progression and identify potential MS fallers [102, 103]. Furthermore, besides the fact that TUG has an excellent test-retest reliability [288], it is a threshold test for independent living, since it is assessing walking and balance performance in PwMS regardless of the patient's cognitive status [102].

4.1.3 Six minutes walk test

For assessing functional capacity, cardiopulmonary exercise tests commonly used to measure maximal oxygen consumption (VO_2max) directly [289]. However, since most of the daily living related activities do not require maximal effort, the term of functional capacity is also used to express an individual's capacity to perform submaximal activities [263]. Therefore, physical performance tests like the 6-minute walking test (6MWT), shuttle walk test and TUG test, may also be used for assessing functional capacity [290]. The 6MWT is easy to apply and does not require any special equipment, therefore, it is widely used in clinical practice [253]. To date, the 6MWT found to be correlated with the mobility-related function, standing balance, and walking speed [268]. The 6MWT can be used safely and quickly to assess impaired functional capacity in ambulatory patients with MS and provides information that may be a better index of the patient's ability to perform daily activities than the maximum oxygen consumption [291].

Since both the 6MWT and STS tests are considered functional capacity tests, it is hypothesized that the STS tests may be correlated with the 6MWT and therefore a useful alternative for assessing the functional capacity of PwMS, especially as a substitute test for patients with the limited functional capacity.

In the last decade, there have been several studies in non-MS subjects demonstrating a positive impact of EPA and DHA supplementation on skeletal muscle strength and mass [18–20], even though this was not confirmed in other studies [21,22]. A recent study on elderly individuals who used the same supplement with the current study, showed a favorable effect on various functional capacity parameters [292]. Also, omega 3 fatty acids found to reduce inflammation and oxidative stress in the MS population [293]. However, in our knowledge, no evidence exists on the potential effect of omega polyunsaturated fatty acid supplementation on physical performance of MS patients [294-298].

On the other hand, studies have shown that antioxidant vitamins could elicit significant improvements in various physiological (i.e. endothelial function) [299] and exercise

performance parameters [300] which interns could led to improvements in functional capacity and quality of life. Besides, antioxidant vitamins supplementation could reduce oxidative stress levels in MS patients [301]. Still, the effect of a combination of omega 3 and omega 6 polyunsaturated fatty acids with antioxidant vitamins on functional capacity in MS patients remains unexplored.

4.2 Methodology

Anthropometric characteristics, including height and body weight, are shown in the general methods section of Chapter I Anthropometric Characteristics. Total body fat and trunk fat both revealed in the general methods section of chapter I Body Composition Assessment.

The sequence and timing of the different functional tests is depicted in Figure 65. STS 5 was followed by 2 minutes of rest and then STS 30 and STS 60 was obtained. After 3 minutes of rest, TUG was assessed and finally after 2 minutes of rest 6MWT was obtained.

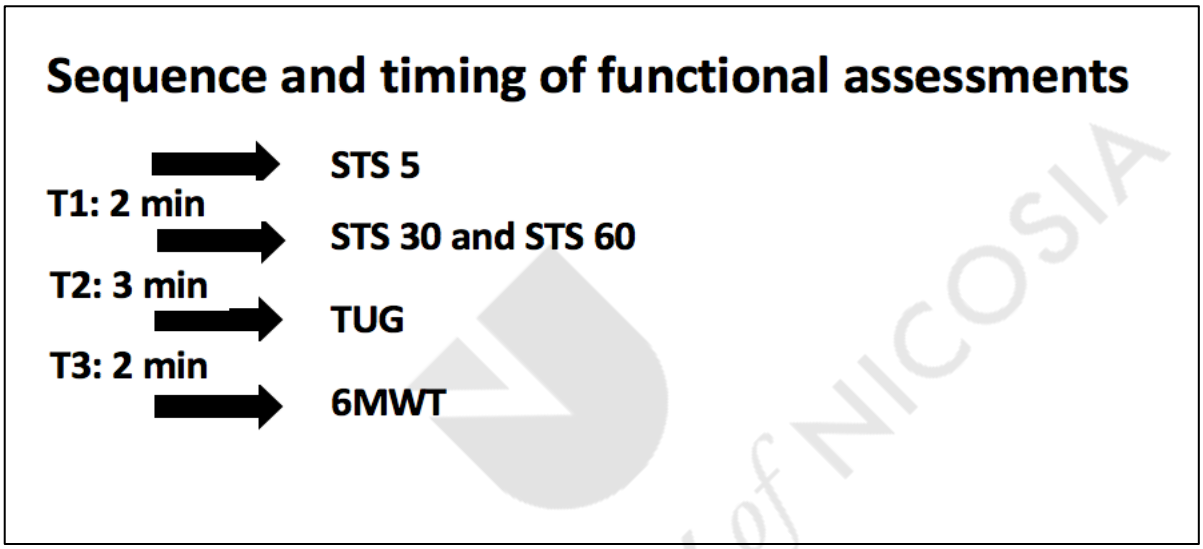

Figure 65. Sequence and timing of functional assessments

4.2.1 Sit to Stand

The Sit-To-Stand-5 Test (STS-5) performed following Moller et al. [285] protocol, on a standard chair (0.43-m height and 0.45-m width). Before to every test session, a standardized instruction given. The examiner explained how the participant should move from a sitting towards a standing position five times. Furthermore, it underlined that the tests should be performed as fast as possible and that two attempts separated by a 3 min break given. Finally, the assessor showed the participant one entire test in order to avoid misunderstandings. No encouragements given during the test. The subjects started the test in a seated position with full weight on the chair, arms folded across their chest and feet being open to the hips (fig. 66). The researcher made sure that the subjects fully extend their trunk and knees before they began the movement to return to sitting. The STS-60 is a similar test that requires the patient to stand up

and sit down to a chair as many times as possible in 60 seconds. The score is the total number of sit to stands cycles within 60 seconds (the number achieved in 30 seconds also recorded also), and it is an index of muscular endurance. The STS time recorded using a stopwatch to the nearest 10th of a second.

Figure 66. Sit to stand phases

Adapted from Madhushri, Priyanka (2017) [302]

4.2.2 Timed-Up-and-Go test

The timed-up and go test (TUG) performed, according to Steffen et al. protocol [303]. The test involves arising from a seated position, walking 3m, turning around, walking back 3m, turning around and sitting back down in the chair. Participants started in a chair with arms, with a tape mark on the floor showing the 3m distance where they were supposed to turn around. Participants were given instructions to perform the task 'as fast as possible, but safely' and they show how to do the task. Stopwatch timing was done according to best practice [303, 304], starting on the word "Go" and ending when the participant's buttocks first made contact with the seat of the chair. A faster time indicates a better functional performance. The TUG time recorded using a stopwatch to the nearest 10th of a second.

Figure 67. Time Up and Go test phases

Adapted from Madhushri, Priyanka (2017) [302]

4.2.3 Six minutes walk test

The six-minutes walk test revealed in the methodology of Chapter III Six Minutes Walk Test (6MWT).

Statistical Analysis

In this study, descriptive statistics used to calculate the mean and standard deviation of the patient's examined variables. Differences between the groups tested using a repeated measure (mixed model) ANOVA design with group (control vs experimental) as between-subject factor and time (baseline, 12 months and 24 months) as the within-subject factor. All analyses carried out using the Statistical Package for the Social Sciences software (SPSS for Windows, version 19.0, Chicago, Illinois). Data presented as mean ± SD and the level for statistical significance set at $p \leq 0.05$.

4.3 Results

It is noteworthy to mention that all participants were able to perform functional assessments without injuries and adverse effects. At baseline, there were no significant differences in all examined anthropometric characteristics and functional parameters between the two groups ($p>0.05$) (table 37).

Table 37. Examined functional parameters at baseline between the 2 groups

Variables	Placebo Group	PLP10 Group	P Value
Weight (kg)	72.78 ± 17.10	70.95 ± 14.63	0.683
Height (cm)	165.79 ± 7.80	166.66 ± 8.22	0.700
Body Fat (%)	28.78 ± 9.16	29.36 ± 7.02	0.815
Abdominal girth (cm)	96.94 ± 18.93	93.61 ± 12.41	0.506
Trunk Fat (%)	33.11 ± 12.94	32.07 ± 9.36	0.961
6MWT (m)	504 ± 131	505 ± 99	0.838
STS 5 (sec)	12.57 ± 3.52	12.37 ± 2.83	0.499
STS 30 (rep)	12.55 ± 2.97	12.44 ± 2.63	0.969
STS 60 (rep)	25 ± 6.10	25.16 ± 5.51	0.800
TUG (sec)	8.90 ± 1.76	8.21 ± 1.47	0.471

All data are mean ± SD

In the placebo group body composition (BC) percentage reduced from 28.98 % at baseline to 27.65 % in the 12 months assessment and then increased to 27.65% in the 24 months follow up assessment. The PLP10 group showed a decrease in BC percentage from 29.36% at baseline

to 29.20 % the 12 months assessment and finally increased to 29.26 % in the 24 months assessment. There was no time effect (p=0.538), group effect (p=0.641) or any statistically significant interaction between group and time (p=0.635) (table 38).

Total body water (TBW) as assessed by the biometrical impedance evaluation progressively increased in the placebo group from 51.68 % at baseline to 53.23 % in the 12 months assessment and finally to 53.27 % in the 24 months follow up assessment. In the PLP10 group, TBW progressively increased from 51.33 % at baseline to 51.89 % the 12 months assessment and finally to 52.57 % the 24 months follow up assessment. There was no time effect (p=0.331), group effect (p=0.603) or any statistically significant interaction between group and time (p=0.723) (table 38). Abdominal girth decreased in the placebo group from 96.94 cm at baseline to 95.16 cm in the 12 months assessment and finally to 94.11 cm in the two years follow up. In the PLP10 group abdominal girth decreased from 93.61 cm at baseline to 92.94 cm in the 12 months assessment and finally increased to 93.16 cm in the 24 months follow up assessment. No statistically significant time effect (p=0.283), group effect (p=0.662) or time x group interaction (p=0.661) was observed (table 38).

Trunk progressively decreased in the placebo group from 33.11% at baseline to 33.06% in the 12 months assessment and finally to 31.37 % in the 24 months follow up assessment. In the PLP10 group trunk fat decreased from 32.07 % at baseline to 31.62 % in the 12 months assessment and finally increased to 31.76 % in the two years follow up. No statistically significant time effect (p=0.774), group effect (p=0.255) or time x group interaction (p=0.421) was observed.

Lean body mass decreased in the placebo group from 21.46 kg at baseline to 19.98 kg in the 12 months assessment and increased to 20.13 kg in the 24 months follow up assessment. In the PLP10 group, lean body mass decreased from 20.40 kg at baseline to 20.06 kg in the 12 months assessment and finally increased to 20.64 kg in the 24 months follow up assessment. Results did not reveal any statistically significant time effect (p=0.320), group effect (p=0.949) or interaction between group and time (p=0.403).

Table 38. The results of body composition, total body water, waist circumference and trunk fat in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	Placebo Group (N= 24)	PLP10 Group (N= 27)	Main Effects and Interactions (p value)		
			Time	Time X Group	Group
Body Composition (%)					
Baseline	28.98 ± 9.16	29.36 ± 7.02			
12 months	27.65 ± 10.44	29.20 ± 8.70	0.538	0.635	0.641
24 months	27.80 ± 8.40	29.26 ± 8.08			
Body Composition TBW (%)					
Baseline	51.68 ± 7.08	51.33 ± 4.92			
12 months	53.23 ± 9.41	51.89 ± 7.99	0.331	0.723	0.603
24 months	53.27 ± 7.69	52.57 ± 6.77			
Waist Circumference (Cm)					
Baseline	96.94 ± 18.93	93.61 ± 12.41			
12 months	95.16 ± 17.05	92.94 ± 13.07	0.283	0.489	0.661
24 months	94.11 ± 15.72	93.16 ± 12.39			
Intra-Abdominal Fat (%)					
Baseline	33.11 ± 12.94	32.07 ± 9.36			
12 months	33.06 ± 12.32	31.62 ± 8.67	0.774	0.421	0.255
24 months	31.37 ± 11.73	31.76 ± 9.50			
Lean Body Mass (kg)					
Baseline	50.55 ± 11.05	50.37 ± 11.76			
12 months	50.34 ± 12.67	49.43 ± 12.56	0.320	0.403	0.949
24 months	50.23 ± 11.01	49.26 ± 11.35			

In regards to the 6MWT, the participants of the placebo group increased the distance from 504 m at baseline to 523 in the 12 months assessment and then dropped to 494 m in the two years follow up. The experimental group showed a progressive increase from 505m at baseline to 546m at the 12 months assessment and finally to 551m at the 24 months follow up assessment. In the 24 months assessment, 6MWT distance was reduced by 1.98% in the placebo group and improved by 9.10% in the PLP10 group. The 12 months assessment revealed an improvement in distance by 3.76% in the placebo group and by 7.72% in the experimental group. From the first 12 months to the 24 months follow up assessment, 6MWT distance decreased by 5.54% in the placebo group and improved by 0.91% in the PLP10 group. Despite the non-statistically significance increase that observed on the walking distance in the experimental group, there was no time (p=0.137) or group (p=0.186) effect in the 6MWT. There was also no statistically significant interaction between group and time (p=0.337) (table 39).

Performance on the STS 5 test appeared to be decreased in both groups after the 24 months intervention period. In particular, the placebo group progressively decreased the STS 5 time from 12.57 sec at baseline to 10.67 sec in the 12 months assessment and finally decreased to 11.52 sec in the 24 months follow up assessment. In the PLP10 group the STS 5 time progressively decreased from 12.37 sec at baseline to 10.33 sec in the 12 months assessment and finally to 9.91 sec in the 24 months follow up assessment (table 39). In the 24 months assessment, STS 5 time improved in the placebo group by 8.35% and by 19.88% in the PLP10 group. There was a statistically significant time effect (p=0.001) between the baseline and the 12 months follow up assessment and between the baseline and 24 months follow up assessment, however, no statistically significant group effect (p= 0.359) while a tendency toward interaction between group and time was observed (p=0.078).

Performance on the STS 30 test appeared to be improved in the placebo group from 12.55 repetitions at baseline to 13.22 repetitions in the 24 months follow up assessment. In the PLP10 group there was an improvement in the number of repetitions from 12.44 at baseline to 15.22 reps in the 24 months follow up assessment (table 39). In the 24 months assessment performance on the STS 30 improved by 5.33% in the placebo group and by 22.34% in the PLP10 group. There was a statistically significant time effect (p=0.001) within-subjects between the baseline and 12 months follow up assessment and between the baseline and 24 months follow up assessment. Additionally, there was a statistically significant interaction between group and time (p=0.040). No statistically significant group effect within-subjects observed (p=0.297).

Performance on the STS 60 test appeared to be improved in the placebo group from 25 reps at baseline to 26.22 repetitions in the 24 months follow up assessment. In the PLP10 group there was an improvement in the number of repetitions from 25.16 at baseline to 30.38 reps in the 24 months follow up assessment. In the two years follow up STS 60 improved by 4.88% in the placebo group and by 20.74% in the PLP10 group. There was a statistically significant time effect ($p=0.001$) within-subjects between the baseline and 12 months follow up assessment and between the baseline and the 24 months follow up assessment and a statistically significant interaction between group and time ($p=0.032$). No statistically significant group effect within-subjects revealed ($p=0.279$).

Performance in the TUG test appeared to be improved in the placebo group from 8.90 secs at baseline to 8.80 secs in the 24 months follow up assessment. In the PLP10 group, TUG time was improved from 8.21 secs at baseline to 7.63 secs in the 24 months follow up assessment. During the 24 months assessment the placebo group improved the TUG time by 1.12% while the PLP10 group improved the TUG time by 7.06%. No statistically significant group effect ($p=0.097$) within-subjects or interaction between group and time was observed ($p=0.411$). Results reveal only a time effect ($p=0.003$) indicating significant changes between the baseline and the 24 months follow up assessment.

Table 39. The results of functional tests in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (*p* value)**		
			Time	Time X Group	Group
Six Minute Walk Test Normalized (m)					
Baseline	504 ± 131	505 ± 99	0.137	0.186	0.337
12 months	523 ± 90	546 ± 73			
24 months	494 ± 79	551 ± 101			
STS5 (sec)					
Baseline	12.57 ± 3.52	12.37 ± 2.83	**0.001**	**0.078**	0.359
12 months	10.67 ± 2.75	10.33 ± 2.26			
24 months	11.52 ± 1.97	9.91 ± 1.72			
STS 30 (rep)					
Baseline	12.55 ± 2.97	12.44 ± 2.63	**0.001**	**0.040**	0.297
12 months	14.66 ± 3.1	15.16 ± 2.66			
24 months	13.22 ± 2.18	15.22 ± 2.39			
STS60 (rep)					
Baseline	25 ± 6.10	25.16 ± 5.51	**0.001**	**0.032**	0.279
12 months	29.55 ± 6.20	30.33 ± 5.12			
24 months	26.22 ± 4.27	30.38 ± 4.86			
TUG (sec)					
Baseline	8.90 ± 1.76	8.21 ± 1.47	**0.003**	0.411	0.097
12 months	8.04 ± 1.94	7.44 ± 1.84			
24 months	8.80 ± 1.61	7.63 ± 1.09			

Abbreviations: STS-5, sit-to-stand test 5-repetitions; STS- 30, sit-to-stand test 30 s; STS- 60, sit-to-stand test 60 s; TUG, timed up and go test

4.4 Discussion

Functional capacity considered to be a highly important factor for quality of life and overall wellbeing in MS patients. The findings of the current study reveal that a 24 months supplementation with omega 3 and omega 6 polyunsaturated fatty acids and antioxidant vitamins could improve the performance in various functional capacity tests such as the sit- to stand tests, while its effects on walking performance appeared to be very promising even though no-statistically significant changes were observed.

As already mentioned in the introduction section of the current chapter, the 6MWT provides an assessment of walking fatigability, maximal walking distance and functional capacity [100] whilst may is useful in terms of prediction of the level of the potential decline in the ability of perform everyday activities such as habitual walking [101]. In this study the performance in the 6MWT (as expressed by the distance covered in meters), appeared to be improved by approximately 46 meters in the experimental group at the end of the 24 months' intervention. According to the study of Baert et al. (2014), clinically meaningful changes from the patient's perspective following intervention reported to be 21.6 m on 6MWT [234]. Therefore, we are plausible to believe that the improvement observed in the 6MWT in the PLP10 group could be considered as clinically significant, even though that the statistical analysis did not reveal significant differences after the intervention period. The fact that only a trend was observed can be attributed to the small sample size as well as due to the significant standard deviation.

PwMS in order to be able to maintain their independence must present adequate levels of functional capacity [99]. The ability to develop STS movements is considered an essential feature for determining the degree of independence and the quality of life of a person [305]. In the hierarchy of disability is indicated that problems in standing up become manifest much later than limitations in walking [306]. The results of the current study revealed that the examined supplement significantly improved the performance of the STS assessments compared to placebo. This outcome is essential taking into account that STS's are considered one of the most mechanically demanding movements within activities of daily living [269-271] due to the high level of muscle activation that it requires, as individuals need to coordinate a transfer from a horizontal to a vertical position in one movement [272]. According to Rodacki et al. (2012) fish oil supplementation causes greater improvement in the STS tests [307] something that is in agreement with the findings of this study. Additionally, Stavrinou et al. (2020) revealed that supplementation of PLP10 for 6 months improved the functional capacity of older adults [292], which is in agreement with the results of the present study in PwMS.

As shown in chapter II, both groups revealed similar knee extensors strength in their isokinetic evaluation during the period of 24 months, but the knee flexors strength reduced. Knee extensor muscles are antigravity muscles operating against an external flexion moment around the knee. Hamstrings are also antigravity muscles counterbalancing an external flexion moment around the hip. However, the hamstrings are supported by the gluteal muscles in their extensor action around the hip while the quadriceps are the sole extensor of the knee. This is probably the reason why the strength of knee flexors is reduced while the strength of the knee extensors is preserved in the 24 months of the experiment [308].

The adequate extensors muscle strength is mainly required for the integration of the STS movement. On the other hand, since both groups display identical extensor strength (table 3), probably the extensor strength is not responsible for the improvement in the STS performance of the experimental group.

Research suggest that supplementation with fish oil may increase muscle mass [298, 309, 310]. In this study although muscle mass was not directly evaluated, body composition and lean body mass didn't change significantly during the two years assessment, resulting in no changes in muscle mass.

DHA is important in regulating neurogenesis, increasing neural synapses and membrane fluidity, protecting neuronal damage [311] and may improve performance in sports where perceptual-motor activity and decision-making are the keys to success (i.e., improvements in complex reaction time and efficiency) [312]. Despite the fact that some parameters were not statistically significant (6MWT, TUG) there was a tendency of greater improvement in the PLP10 group in contrast with the placebo group. The improvement in the experimental group at these parameters which are indicators of motor control, stability and mobility could be due to an improvement in postural control.

The primary aim of this chapter was to identify if the medication had any effect on the functional capacity. Results of this study reveal that the supplement improved the STS performance and thus functional capacity.

4.5 Limitations

In order to complete the STS movement, a variety of muscle groups are needed to be activated during the movement. In this study only the maximum strength of knee extensors and flexors was measured using the isokinetic dynamometer. The muscle activity in trunk (erector spinae, rectus abdominus), ankle (dorsi and plantar flexion), knee (quads and hamstrings) and glutes muscles were not measured using an EMG, therefore the study loss the opportunity to have a better understanding of the results.

Future studies are recommended to apply EMG and Accelerometer sensors when evaluating functional parameters because more sensors in more muscle groups would provide adequate information regarding muscles activity and motor control during the integration of the movement.

Taking all into account, it seems that a supplementation with high dose of omega 3 and omega 6 free fatty acids could be a very promising approach in terms of improving functional capacity in MS patients with low disability scores.

CHAPTER 5 EFFECTS ON QUALITY OF LIFE

5.0 Introduction

According to the literature, PwMS are subjects of lower quality of life (QOL) compared to healthy individuals of the same age [313]. Several factors (including social, physiological and psychological related factors) may contribute negatively to the low levels of the QOL of PwMS. According to the literature, PwMS are suffering from fatigue, depression, poor sleep, visual disturbances, muscle weaknesses, loss of coordination and sensory impairments [109], all of which are significant contributing factors to a lower QOL.

The evaluation of mental and physical health [314] in individuals is commonly assessed by the SF- 36 questionnaire [315], which is consisted by the physical and mental component summary. Both health components, physical as well as mental, are negatively affected by MS [316]. In early MS, QOL known to be influenced by fatigue, sleep quality, depression, cognition and disability are the most known predictors that affects quality of life [317-319]. A recent systematic review reveals that omega 3 fatty acids supplementation could improve QOL in MS patients [293].

Fatigue is an extremely prevalent issue in MS, the most commonly reported symptom and one of the most debilitating, surpassing pain and even physical disability [208]. It is noteworthy to mention that at least the 75% of the MS population exhibit fatigue levels [320]. However, fatigue in MS remains poorly understood, taking under consideration that no gold standard exists by which to measure fatigue. Disease severity, depression and sleep disturbance are significant independent contributors to fatigue in MS, with depression and sleep disturbance appearing as more reliable predictors of fatigue [320]. MS causes sleep fragmentation, and fatigue in MS can partially be explained by poor subjective sleep quality and depression [321]. There is inconsistency in published evidence regarding the potential effect of omega fatty acids on fatigue in MS patients [87, 322], however there is not sufficient clinical indication for applying dietary supplementation as complementary treatment against MS symptomatology.

Daily sleepiness predisposes people to develop severe performance impairments in daily functioning and is a risk factor for potentially life-threatening domestic, occupational, and vehicular accidents [323]. Sleepiness as indicated by elevated ESS scores, is less prevalent and less severe than fatigue but is present in a significant proportion of patients with MS which autonomously may irritate and sustain the presence of fatigue [324]. In relapsing-remitting MS there is a correlation between functional disability, excessive daytime sleepiness and fatigue [325-327]. Sleep disorders merit further attention, given the potential impact on overall health and quality of life [328].

Sleep abnormalities are prevalent among patients diagnosed with MS and remain under-recognized and inadequately addressed. Prevalence is estimated between 42–65%, whilst the wide range of reported rates mainly due to different methodological approaches and type of disorders; sleep abnormalities have reported to be four times higher in MS compared to healthy populations [329-331] exceeding by far rates for other chronically disease patients. Poor sleep quality could harm patients' health and quality of life, contributing even further to the overall disease burden [332, 333]. Poor sleep quality has connected with a reduction is physical function, activities of daily living, psychological health, occupational functions and social desirability in patients with MS [109]. Moreover, sleep disturbances have associated with an increase in perceived fatigue in individuals with MS [334]. A variety of functional tests and questionnaires assessed among years to interpret QOL in MS patients. Sleep disturbance is a prevalent issue in MS population. It is still unknown whether omega polyunsaturated fatty acids could affect sleep quality in MS patients.

Cognitive impairment is prevalent, disabling and poorly managed in persons with MS [335]. It is often associated with depression, unemployment, diminished ability to engage in activities of daily living, deficits in memory, attention, information processing speed and language [336, 337]. These deficits have reported in up to 65% of patients at some point in the course of the disease [338, 339]. The importance of cognitive assessment (with particular involvement of processing speed and memory) from the beginning of MS is emphasized in the study of Moccia et al. (2016), since disability progression and secondary progressive conversion could be predicted in newly diagnosed relapsing remitting MS [340]. The presence and novel of cognitive impairment in PwMS is significantly associated with EDSS, patient age and physical disability, since it exists in all sub-types of MS taking under consideration that the clinical onset and frequency are increased in the progressive forms [341]. Cognitive impairment significantly correlated with physical activity and that some aspects of cognition could predict the functional status in MS [342]. Evidence from several studies supports the efficacy of exercise [343, 344], physical activity [345, 346] and fitness [347, 348] for improving cognition in MS. Moreover, many studies on older adults suggest a beneficial effect of omega fatty acids supplementation in cognitive function [292, 349], however, other studies did not observe such findings [350].

5.1 Methodology

All questionnaires completed using the interview method. The patients' health-related quality of life (HRQOL) levels assessed using the SF-36 questionnaire which evaluates mental and physical health [315]. Fatigue levels assessed using the fatigue severity scale (FSS) [351]. Sleep quality evaluated by the Pittsburgh sleep quality index (PSQI) [352]. Daily sleepiness status assessed by using the Epworth sleepiness scale (ESS) [353]. The patients' depressive symptoms assessed by using the Beck Depression Inventory (BDI) [354]. Finally, cognitive function assessed by the Paced Auditory Serial Addition Test (PASAT), modified with a 3s inter stimulus interval, as an attempt to make the PASAT test more user-friendly [355].

5.1.1 SF36 Health Related Quality of Life questionnaire

The SF36 questionnaire consists from two main dimensions (mental health and physical health) and eight scales or components, *i.e.*, physical function, role physical, body pain, vitality, general health, mental health (not to be confused with the dimension under the same designation), role mental, and social function. The first five of these scales comprise physical health, whereas the last five of these scales constitute the mental health dimension. The scales vitality and general health are part of both the mental health and physical health dimensions. Each of the eight scales is rated 1 to 5, which contribute to the scoring of these scales (see Appendix for more details). The SF36 scores of each of the two dimensions based on mathematical averaging of the scores of five scales [314].

5.1.2 Fatigue Severity Scale (FSS)

The Fatigue Severity Scale (FSS) instructs patients to assign a score of between 1 (completely disagree) and 7 (completely agree) for each of the 9 FSS items. The items designed to rate the extent of fatigue symptoms and their impact on patient functioning (including motivation, exercise, physical functioning, carrying out duties, and interfering with work, family, or social life). Examples of the items include: "exercise brings on my fatigue" and "my fatigue is very debilitating." A higher score indicates a higher degree of fatigue for all items [351]. Scoring of the FSS performed by dividing the sum of the 9 items to produce an FSS total score that ranges from 1 (no fatigue) to 7 (very severe fatigue).

5.1.3 Pittsburgh Sleep Quality Index

Self-reported questionnaires are a standard method used to assess insomnia as sleep data can be collected from multiple nights, they are brief, easy to administer and cost-effective. Several

self-reported questionnaires have been developed to assess aspects of sleep quality and are commonly used to assess insomnia symptoms. These include the Pittsburgh Sleep Quality Index (Pittsburgh questionnaire) [352]. The Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire which assesses sleep quality and disturbances over a 1-week time interval. The section collects information about the previous night's sleep and is completed immediately after awaking. It contains the following items: (1) time went to bed, (2) lights out time, (3) sleep onset latency (SOL) calculated as the minutes from lights out until falling asleep, (4) time of final waking, (5) method/ reason of final waking, (6) number of times the participant woke during the night, wake after sleep onset (WASO), (7) duration of WASO in minutes calculated as the total number of minutes of awake that occurred after sleep onset and before the final awaking, (8) reason(s) for WASO, (9) sleep quality, (10) mood on final awaking, and (11) alertness on final waking. Items 9 to 11 completed on a 0–10 Numerical Rating Scale (NRS).

5.1.4 Beck Depression Inventory

Symptoms of depression screened by the Beck Depression Inventory (BDI–II) that includes 21-question self-reporting multiple choice questions. The patients BDI score was categorized as not depressed (score 0–20) and depressed (over 20) [356-358].

5.1.5 The Epworth sleepiness scale

The Epworth sleepiness scale (ESS) is an 8-item self-report questionnaire used to assess excessive daytime sleepiness over the last week. Participants indicate on a 4-point Likert- type scale (0 = never, 3 = high chance) the likelihood that they will "doze off or fall asleep" in eight different situations. Summation of the 8 responses produces a total score ranging from 0 to 24; with higher scores reflecting greater sleepiness. The cut off score of > 10 has been found to accurately determine excessive day-time sleepiness [359].

5.1.6 Paced Auditory Serial Addition Test (PASAT)

Paced Auditory Serial Addition Test (PASAT), is an auditory test that measures working memory (an aspect of cognitive function). The PASAT presents single digits to the participant in a pre-recorded interval and asks that each new digit be added to the one immediately prior to it [360]. PASAT is part of the Multiple Sclerosis Functional Composite (MSFC), developed to measure impairment and disability in MS [361], and it is widely used in clinical research.

PASAT could assess auditory information processing speed, flexibility, calculation ability and is a sensitive and valid measure to objectively quantify cognitive fatigue in MS [362, 363]. PASAT is a very difficult test even for individuals with high intellectual capability [364]. In order to avoid making the PASAT a negative experience for patients [361] and consequently affecting their performance on other tests, patients well informed about the potential effects of the specific test prior to testing, verified fundamental mathematics ability and agreed to participate.

5.2 Statistical Analysis

In this study, descriptive statistics used to calculate the mean and standard deviation of patient's examined variables. Differences between the groups tested using a repeated measure (mixed model) ANOVA design with group (control vs experimental) as between-subject factor and time (baseline, 12 months and 24 months) as the within-subject factor. All analyses carried out using the Statistical Package for the Social Sciences software (SPSS for Windows, version 19.0, Chicago, Illinois). Data presented as mean ± SD, and the level for statistical significance set at $p \leq 0.05$.

5.3 Results

All participants were able to perform the questionnaires. At baseline, there were no significant differences in all examined parameters between the two groups ($p>0.05$) (table 40).

Table 40. Examined parameters at baseline between the 2 groups

Variables	Placebo Group	PLP10 Group	P Value
SF36 PCS	78.33 ± 28.95	87.05 ± 26.09	0.470
SF36 MCS	73.77 ± 15.02	73.77 ± 20.74	0.715
SF36 TOTAL	82.11 ± 23.59	87.83 ± 24.59	0.616
FSSI	4.70 ± 1.11	4.41 ± 1.43	0.214
PSQI	3.66 ± 3.16	2.55 ± 2.06	0.263
BECK	4.38 ± 6.23	7.55 ± 13.56	0.700
ESS	4.94 ± 5.77	2.72 ± 4.46	0.378
PASAT	68.94 ± 28.76	68.14 ± 30.52	0.715
EDSS	2.36 ± 1.09	2.22 ± 1.08	0.251

All data are mean ± SD. Abbreviations: PCS, physical component score; MCS, mental component score; FSSI, fatigue severity scale index; PSQI, Pittsburgh Sleep Quality Index; Beck, Beck Depression Inventory; ESS, Epworth sleepiness scale; PASAT, Paced Auditory Serial Addition Test; EDSS, Expanded Disability Status Scale.

The SF36 PCS score worsened in the placebo group from 78.33% at baseline to 73.71% in the 12 months assessment and finally improved to 74% in the 24 months follow up assessment. In the PLP10 group the SF36 PCS score worsened from 87.05% at baseline to 77.66% in the 12 months assessment and finally improved to 80.16% in the 24 months follow up assessment (table 41). Results reveal only a statistically significant time effect within-subjects ($p=0.025$) indicating significant changes within groups between the baseline and 24 months follow up

assessment, but no statistically significant group effect (p=0.358) or interaction between group and time detected (p=0.548).

The SF36 MCS score worsened in the placebo group from 73.77% at baseline to 71.37% in the 12 months assessment and finally improved to 73.78% in the 24 months follow up assessment. In the PLP10 group the SF36 PCS score improved from 73.77% at baseline to 76.22% in the 12 months assessment and finally improved to 78.96% in the 24 months follow up assessment. Results didn't reveal any statistically significant time (p=0.395) or group effect (p=0.434) within-subjects, neither any statistically significant interaction between group and time detected (p=0.328).

The SF36 total score worsened in the placebo group from 82.11% at baseline to 74.84% in the 12 months assessment and finally improved to 75.88% in the 24 months follow up assessment. In the PLP10 group, the SF36 total score worsened from 87.83% at baseline to 79.61% in the 12 months assessment and finally improved to 82.61% in the 24 months follow up assessment. Results reveal only a statistically significant time effect within-subjects (p=0.006) indicating significant changes within groups, but no statistically significant group effect (p=0.321) or interaction between group and time detected (p=0.875).

Fatigue severity scale index (FSSI) score worsened in the placebo group from 4.70 at baseline to 4.17 in the 12 months assessment and finally improved to 4.74 in the 24 months follow up assessment. In the PLP10 group FSSI score worsened from 4.41 at baseline to 4.40 in the 12 months assessment and finally improved to 4.54 in the 24 months follow up assessment. Results reveal only a statistically significant time effect within-subjects (p=0.001) indicating significant changes within groups between the baseline and 12 months follow up assessment, and between the 12 months and 24 months follow up assessment, but no statistically significant group effect (p=0.563) or interaction between group and time detected (p=0.843).

Pittsburgh Sleep Quality Index (PSQI) worsened in the placebo group from 3.66 at baseline to 3.44 in the 12 months assessment and finally improved to 3.55 in the 24 months assessment. In the PLP10 group PSQI score remained the same from 2.55 at baseline to 2.55 in the 12 months assessment and finally improved to 2.77 in the 24 months assessment (table 41). Results did not reveal any statistically significant time (p=0.803) or group effect (p=0.094) within-subjects, neither any statistically significant interaction between group and time detected (p=0.803).

Beck Depression Inventory score improved in the placebo group from 4.38 at baseline to 2.83 in the 12 months assessment and finally worsened to 3.50 in the 24 months assessment. In the PLP10 group Beck score progressively improved from 7.55 at baseline to 4.33 in the 12 months

assessment and finally to 3.11 in the 24 months follow up assessment (table 41). Results reveal a statistically significant time effect within-subjects (p=0.017) indicating significant changes within groups between the baseline and the 24 months follow up assessment, but no statistically significant group effect (p=0.604) revealed. Although Beck score improved more than 50% in the experimental group, due to the large standard deviation results didn't show any statistically significant interaction between group and time (p=0.158).

Epworth Sleepiness Scale (ESS) score progressively improved in the placebo group from 4.94 points at baseline to 3.83 points in the 12 months assessment and finally to 2.38 points in the 24 months assessment. In the PLP10 group ESS score worsened from 2.72 points at baseline to 2.77 points in the 12 months assessment and finally improved to 2.05 points in the 24 months assessment (table 41). During the 24 months assessment results reveal only a statistically significant time effect within-subjects (p=0.031) indicating significant changes within groups between the 12 months and 24 months follow up assessment, but no statistically significant group effect (p=0.362) or interaction between group and time detected (p=0.251).

Paced Auditory Serial Addition Test (PASAT) progressively improved in the placebo group from 68.94% at baseline to 75.57% in the 12 months assessment and finally to 77.73% in the 24 months assessment. In the PLP10 group PASAT score progressively improved from 68.14% at baseline to 73.05% in the 12 months assessment and finally to 75.01% in the 24 months assessment (table 41). During the 24 months assessment results reveal only a statistically significant time effect within-subjects (p=0.016) indicating significant changes within groups between the baseline and the 24 months follow up assessment, but no statistically significant group effect (p=0.781) or interaction between group and time detected (p=0.778).

Table 41. The results of questionnaires in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	Placebo Group (N= 24)	PLP10 Group (N= 27)	Main Effects and Interactions (p value)		
			Time	Time X Group	Group
SF36 PCS					
Baseline	78.33 ± 28.95	87.05 ± 26.09			
12 months	73.71 ± 25.23	77.66 ± 15.45	**0.025**	0.548	0.358
24 months	74.00 ± 19.14	80.16 ± 10.68			
SF36 MCS					
Baseline	73.77 ± 15.02	73.77 ± 20.74			
12 months	71.37 ± 18.42	76.22 ± 12.62	0.395	0.328	0.434
24 months	73.38 ± 10.56	78.96 ± 8.11			
SF36 TOTAL					
Baseline	82.11 ± 23.59	87.83 ± 24.59			
12 months	74.84 ± 21.55	79.61 ± 14.45	**0.006**	0.875	0.321
24 months	75.88 ± 14.57	82.61 ± 8.70			
FSSI					
Baseline	4.70 ± 1.11	4.41 ± 1.43			
12 months	4.17 ± 1.24	4.04 ± 1.23	**0.001**	0.843	0.563
24 months	4.74 ± 0.79	4.54 ± 1.06			
PSQI					
Baseline	3.66 ± 3.16	2.55 ± 2.06			
12 months	3.44 ± 2.00	2.55 ± 1.46	0.803	0.803	0.094
24 months	3.55 ± 1.65	2.77 ± 1.11			
BECK					
Baseline	4.38 ± 6.23	7.55 ± 13.56			
12 months	2.83 ± 4.74	4.33 ± 10.48	**0.017**	0.158	0.604
24 months	3.50 ± 4.43	3.11 ± 9.06			
ESS					
Baseline	4.94 ± 5.77	2.72 ± 4.46			
12 months	3.83 ± 5.15	2.77 ± 3.93	**0.031**	0.251	0.362

24 months	2.38 ± 3.1	2.05 ± 3.11			
PASAT					
Baseline	68.94 ± 28.76	68.14 ± 30.52			
12 months	75.57 ± 20.9	73.05 ± 21.03	**0.016**	0.778	0.781
24 months	77.73 ± 15.82	75.01 ± 17.38			

Abbreviations: PCS, physical component score; MCS, mental component score; FSSI, fatigue severity scale index; PSQI, Pittsburgh Sleep Quality Index; Beck, Beck Depression Inventory; ESS, Epworth sleepiness scale; PASAT, Paced Auditory Serial Addition Test.

Expanded Disability Status Scale (EDSS) score progressively increased in both groups in two years follow up. In the placebo group, EDSS score progressively increased from 2.36 points at baseline to 2.41 points in the 12 months assessment and finally to 2.83 points in the 24 months assessment. In the PLP10 group EDSS score increased from 2.22 points at baseline to 2.27 points in the 12 months assessment and finally remained constant to 2.27 points in the 24 months follow up assessment (table 42). During the 24 months assessment, EDSS score increased by 19.91% in the placebo group while in the experimental group increased by 2.25%. In two years follow up, results reveal a statistically significant time effect (p=0.001) between the baseline and 12 months assessment and between the 12 months and the 24 months follow up assessment. Additionally, a statistically significant interaction revealed between group and time (p=0.001) but no statistically significant group effect (p=0.446) detected.

Table 42. The results of Expanded Disability Status Scale in both groups for all three time-points. Results expressed as mean ± SD. Also included the p values for the between (Group) and within (Time) subjects effects as well as their interaction

Variables	**Placebo Group (N= 24)**	**PLP10 Group (N= 27)**	**Main Effects and Interactions (*p* value)**		
			Time	Time X Group	Group
EDSS					
Baseline	2.36 ± 1.09	2.22 ± 1.08			
12 months	2.41 ± 1.10	2.27 ± 1.04	**0.001**	**0.001**	0.446
24 months	2.83 ± 1.20	2.27 ± 1.04			

5.4 Discussion

Several factors (including social, physiological and psychological related factors) may contribute negatively to the QOL in PwMS. According to the literature, PwMS are suffering from fatigue, depression, poor sleep, visual disturbances, muscle weaknesses, loss of coordination and sensory impairments [109], all of which are significant contributing factors to a lower QOL. The primary aim of this chapter is to identify whether the applied intervention had any effect on factors that contribute negatively to the quality of life in PwMS.

The medication had no effect on the physical and mental health evaluated by the SF36 questionnaires. At this point, it is essential to note that PwMS in this study had low disability status, and they presented high scores at baseline, which made the margin of improvement minimal. In two years mark, the PLP10 supplement such as the placebo supplement (due to placebo effect) could maintain QOL in MS patients with low EDSS score.

The medication did not improve any of the examined parameters such as fatigue, sleep quality, depression, daytime sleepiness and cognition in the PLP10 group. It seems that the medication such as the placebo (due to placebo effect) could maintain the subjective assessment scores in PwMS with low disability status. For example, patients in this study were good sleepers (PSQI score was below 4), so the margin of improvement was minimal. Additionally, the cognitive function assessment such as the depression assessment improved (not statistically significant) in both groups during the 24 months intervention, indicating that the placebo effect such as the PLP10 supplement maintain cognition scores and mood levels in low disability status patients. Our findings agree with the findings from the study of Torkildsen et al., (2012) [87], where no beneficial effects on disease activity were detected from omega-3 fatty acids when compared with placebo.

Questionnaires present a subjective assessment approach which many times depends on mood fluctuations and in low disability status groups probably more sensitive tools should be assessed (e.g. more sensitive tool of cognitive function assessment than PASAT etc.). In the future, the effect of a supplement with high dose of omega 3 and omega 6 free fatty acids should be evaluated in PwMS with higher EDSS scores.

The most important outcome of this chapter is that the supplement had a significant effect on the disability status (clinically examined by the treating neurologist). EDSS score increased only by 2.25 % in the PLP10 group during the two years assessment indicating that the supplement could maintain disability levels over the 24 months. This is essential, because if the disability status remains unremitting (constant), the quality of life in PwMS maintain the

same levels, indicating more periods of stability in between relapses, resisting the progressive form of the disease.

Taking all into account, it seems that a supplementation with a high dose of omega 3 and omega 6 free fatty acids could be a promising approach in terms of maintain the same disability status in MS patients with low disability scores.

CHAPTER 6 GENERAL DISCUSSION

The primary aim of the current study was to examine the effects of a 24 months supplementation with a formula containing high dose of omega 3 and omega 6 polyunsaturated fatty acids and antioxidant vitamins on biomechanical and functional capacity parameters in PwMS. Secondary aims were to examine the effects of the above intervention in various quality of life and health parameters using the appropriate methodology.

An assessment of muscle function in the upper and lower extremities and an evaluation of muscle fatigue was obtained pre, 12 month post and 24 month post the intervention period. The results of the present study showed that the PLP10 supplement does not have any effect in handgrip strength, knee extension and flexion strength or fatigue in MS patients with low disability status. Results from this study identify that handgrip and knee extensors strength are not impaired in those patients, in contrast with knee flexion strength which deteriorates sooner compared to the knee extensors and is more affected by the progression of the disease. The decrease in the knee flexion strength can possibly be related to the effects of the pathology. It seems that the knee flexors are more affected by the progression of the disease and their strength deteriorates sooner compared to the knee extensors. As Thoumie et al. (2005) concluded, the isokinetic strength of the knee flexor muscles at 60^0/sec is a strong indicator of gait velocity [115]. This study recommends that the strength of the flexor muscles might be an important predictor of muscle impairment in PwMS with low disability status such as the present sample as is the first to be affected. Additionally, this study observed that literature does not provide a clear difference for test retest clinical important handgrip strength difference, and recommends that future studies need to identify a clear clinically meaningful range number which will be used as a gold standard on handgrip strength measurements for PwMS. Moreover, literature suggests that Type II muscle fibers might be the first to be affected in some neuromuscular diseases [209-212], with Type I muscle fibers following later. This seems to be in agreement with the findings of this study, suggesting that the inability to recruit type II muscle fibers is related to the effects of the pathology but future studies need to provide more evidence.

The results of this study show no statistically significant difference between the placebo and PLP10 group in the 6MWT distance. However, it is important to note that, while the placebo group reduced the distance of 6MWT during the 24 months follow up assessment, the PLP10 group increased the distance by 46 meters. According to the study of Baert et al. (2014), clinically meaningful changes from the patient's perspective following intervention have been reported to be 21.6 m on 6MWT in PwMS [234]. Therefore, the increase in the walking distance in the experimental group although not statistically significant different from the

control group seem to be clinically significant. If this is the case the medication seems to improve the walking ability in a clinically meaningful size. Perhaps the lack of the statistical significance can be attributed to the small sample size.

Another aim of this study was to identify if the supplement had any effect on the spatiotemporal parameters of PwMS. Indeed, the medication seems to improve some spatiotemporal parameters, such as the left single support time, the right step time and the stride time of both legs. Furthermore, it is important to recognize that in addition to the obvious effect of the supplement in the previous parameters, the PLP10 group presented higher percentages of improvement in cadence and walking speed. This increase in cadence coincides with a corresponding decrease in step time and step length. The increased walking speed concurs with the decrease in time of single and double support. Perhaps the lack of the statistical significance in some other parameters (cadence, walking speed) can be attributed to the fact that the PwMS in this study have low disability status and the margin of improvement is small. Despite the fact that due to the limited deviation from normal gait there is small room for improvement, there were some promising positive changes in the spatiotemporal parameters.

An additional aim of this study was to identify if the medication had any effect on the kinematic parameters of PwMS. The outcome from this study reveal that medication had no effect on any of the kinematic parameters in the sagittal, frontal and transverse plane. Although literature indicates that PwMS exhibit reduced joint motion [245] all of which result in reduced mobility [246], PwMS in this study probably due to the low disability status did not reveal statistically significant different joint motion angles between the 24 months intervention. The kinematic data in this study focused solely on peak joint motion angles across the averaged gait cycle and also the changing position of the joints' angles across the averaged gait cycle in all time points. Researchers in this study assumed that since participants in this study were PwMS which is a progressive disease, peak joint motion angles could provide adequate evidence about the course of the disease or the medication effect but this was not confirmed. Participants of this study asked to walk on their preferred self-selected speed. It would be better in the future to identify the joint angles during specific phases of the gait cycle which might provide the opportunity of a better understanding of the results. Finally, the walking speed evaluated in this study was the natural self-selected speed of the subjects. Spatiotemporal and kinematics differences at this speed although clinically important are harder to detect. Higher walking speeds are more likely to reveal differences. Future studies should take this into account and assess both the self-selected speed as well as a higher more challenging speed. This will provide an opportunity to detect more subtle differences.

Gait deviation index showed some interesting group by time interaction. Specifically, while GDI of the control group decreases between the baseline and the 2 year follow up by about 10% on average across legs, the opposite happens in the PLP10 group. The GDI in the PLP10 group increased by about 4% on average across both legs in the same period. This perhaps shows a significant positive effect of the PLP10 supplement in the gait of the PLP10 group.

Functional capacity is considered to be a highly important factor for the quality of life and overall wellbeing in MS patients. The ability to develop STS movements is considered a very important feature for determining the degree of independence and the quality of life of a person [305]. The results of the current study revealed that the examined supplement significantly improved the performance of the STS assessments compared to placebo. This outcome is important taking into account that STS's are considered one of the most mechanically demanding movements within activities of daily living [269-271] due to the high level of muscle activation that it requires, as individuals need to coordinate a transfer from a horizontal to a vertical position in one movement [272]. According to Rodacki et al. (2012) fish oil supplementation causes greater improvement in the STS tests [307] something that is in agreement with the findings of this study. Despite the fact that some parameters were not statistically significant (6MWT, TUG) there was a tendency of greater improvement in the PLP10 group in contrast with the placebo group. There was no effect of the PLP10 supplementation on muscle mass, torque production, fatigue, TUG and STS5 tests. There was an effect of the PLP10 supplementation on the STS30, STS60, 6MWT and GDI. It seems that the supplement PLP10 does not affect muscle parameters, or tests of low duration/ repetitions. On the other hand, the PLP10 supplement appears to improve functional activities of more extensive duration/ repetitions. Therefore, the supplement seems to affect positively tasks and tests of longer duration only. Since this result cannot be attributed to an improvement of muscle stamina (muscle fatigue is similar in both groups) the most likely explanation for such an effect is improvement in central fatigue. Future studies are recommended to apply EMG and accelerometer sensors when evaluating functional parameters because more sensors in more muscle groups would provide adequate information regarding muscles activity and motor control during the integration of the movement.

Although some aspects of the functional capacity improved, this outcome did not reflect in the subjective parameters of the quality of life questionnaires or the mood fluctuations of the patients. At this point it is important to note that PwMS in this study had low disability status and they presented high scores at baseline which made the margin of improvement very small. Interestingly, both the 24 month supplementation with the PLP10 as well as with the placebo

could maintain QOL in MS patients with low EDSS score. The supplement had no significant effect on any of the examined quality of life-related parameters such as fatigue, sleep quality, depression, daytime sleepiness and cognitive function. It seems that the supplement such as the placebo (due to placebo effect) could maintain the subjective assessment scores in PwMS with low disability status. Our findings agree with the findings from the study of Torkildsen et al., (2012) [87], where no beneficial effects on disease activity were detected from omega-3 fatty acids when compared with placebo.

A very promising outcome of this study is that the supplement had a significant effect on the disability status (clinically examined by the treating neurologist). EDSS score increased only by 2.25 % in the PLP10 group during the two years assessment indicating that the supplement could maintain disability levels over the 24 months period. This is very essential, because if the disability status remains unremitting (constant), the quality of life in PwMS maintain the same levels, indicating more periods of stability in between relapses, resisting the progressive form of the disease.

Summarizing, this PhD thesis outcome reveal that the supplementation with a formula containing high dose of omega 3 and omega 6 free fatty acids and antioxidant vitamins is a very promising approach on improving some aspects of functional and biomechanical parameters in people with early MS, however not as a complementary treatment against MS symptomatology.

This thesis recommends future studies to utilize the supplement in people with MS with higher disability status scale in order to observe if the PLP10 supplement has a larger effect on improving some functional capacity and biomechanical aspects of PwMS. Additionally, it would be challenging to observe the combination of the PLP10 supplement with exercise in PwMS with low or higher disability status scale.

References

1. Dilokthornsakul, P., et al., *Multiple sclerosis prevalence in the United States commercially insured population.* Neurology, 2016. **86**(11): p. 1014-21.
2. Aristotelous, P., et al., *Associations between functional capacity, isokinetic leg strength, sleep quality and cognitive function in multiple sclerosis patients: a cross-sectional study.* Postgrad Med, 2019. **131**(7): p. 453-460.
3. Dobson, R. and G. Giovannoni, *Multiple sclerosis - a review.* Eur J Neurol, 2019. **26**(1): p. 27-40.
4. Hickey, W.F., *The pathology of multiple sclerosis: a historical perspective.* Journal of neuroimmunology 98, no. 1, 1999: p. 37-44.
5. Storch, M.K., et al., *Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination.* Ann Neurol, 1998. **43**(4): p. 465-71.
6. Browne, P., et al., *Atlas of Multiple Sclerosis 2013: A growing global problem with widespread inequity.* Neurology, 2014. **83**(11): p. 1022-4.
7. Ebers, G.C., *Environmental factors and multiple sclerosis.* Lancet Neurol, 2008. **7**(3): p. 268-77.
8. Lublin, F.D. and S.C. Reingold, *Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis.* Neurology, 1996. **46**(4): p. 907-11.
9. Confavreux, C. and S. Vukusic, *Natural history of multiple sclerosis: a unifying concept.* Brain, 2006. **129**(Pt 3): p. 606-16.
10. Noseworthy, J.H., et al., *Multiple sclerosis.* N Engl J Med, 2000. **343**(13): p. 938-52.
11. Voskuhl, R.R., *Gender issues and multiple sclerosis.* Curr Neurol Neurosci Rep, 2002. **2**(3): p. 277-86.
12. Polman, C.H., et al., *Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria.* Ann Neurol, 2011. **69**(2): p. 292-302.
13. Kurtzke, J.F., *Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS).* Neurology, 1983. **33**(11): p. 1444-52.
14. Beck, R.W., et al., *Interferon beta-1a for early multiple sclerosis: CHAMPS trial subgroup analyses.* Ann Neurol, 2002. **51**(4): p. 481-90.
15. Jacobs, L.D., et al., *Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG).* Ann Neurol, 1996. **39**(3): p. 285-94.
16. Simon, J.H., et al., *A longitudinal study of brain atrophy in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG).* Neurology, 1999. **53**(1): p. 139-48.
17. *Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. The IFNB Multiple Sclerosis Study Group.* Neurology, 1993. **43**(4): p. 655-61.
18. Paty, D.W. and D.K. Li, *Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. UBC MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group.* Neurology, 1993. **43**(4): p. 662-7.
19. *Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) Study Group.* Lancet, 1998. **352**(9139): p. 1498-504.

20. Group, P.S. and M.S.M.R.I.A.G. the University of British Columbia, *PRISMS-4: Long-term efficacy of interferon-beta-1a in relapsing MS.* Neurology, 2001. **56**(12): p. 1628-36.
21. *Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. The IFNB Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group.* Neurology, 1995. **45**(7): p. 1277-85.
22. Durelli, L., et al., *Every-other-day interferon beta-1b versus once-weekly interferon beta-1a for multiple sclerosis: results of a 2-year prospective randomised multicentre study (INCOMIN).* Lancet, 2002. **359**(9316): p. 1453-60.
23. Bornstein, M.B., et al., *A pilot trial of Cop 1 in exacerbating-remitting multiple sclerosis.* N Engl J Med, 1987. **317**(7): p. 408-14.
24. Comi, G., M. Filippi, and J.S. Wolinsky, *European/Canadian multicenter, double-blind, randomized, placebo-controlled study of the effects of glatiramer acetate on magnetic resonance imaging--measured disease activity and burden in patients with relapsing multiple sclerosis. European/Canadian Glatiramer Acetate Study Group.* Ann Neurol, 2001. **49**(3): p. 290-7.
25. Johnson, K.P., et al., *Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group.* Neurology, 1995. **45**(7): p. 1268-76.
26. Hartung, H.P., et al., *Mitoxantrone in progressive multiple sclerosis: a placebo-controlled, double-blind, randomised, multicentre trial.* Lancet, 2002. **360**(9350): p. 2018-25.
27. Jain, K.K., *Evaluation of mitoxantrone for the treatment of multiple sclerosis.* Expert Opin Investig Drugs, 2000. **9**(5): p. 1139-49.
28. Millefiorini, E., et al., *Randomized placebo-controlled trial of mitoxantrone in relapsing-remitting multiple sclerosis: 24-month clinical and MRI outcome.* J Neurol, 1997. **244**(3): p. 153-9.
29. Swank, R.L., *Multiple sclerosis; a correlation of its incidence with dietary fat.* Am J Med Sci, 1950. **220**(4): p. 421-30.
30. Swank, R.L., et al., *Multiple sclerosis in rural Norway its geographic and occupational incidence in relation to nutrition.* N Engl J Med, 1952. **246**(19): p. 722-8.
31. Thompson, R.H., *A biochemical approach to the problem of multiple sclerosis.* Proc R Soc Med, 1966. **59**(3): p. 269-76.
32. Gurr, M.I., *Editorial: food choice and the control of food intake.* Nutr Res Rev, 1998. **11**(2): p. 169-72.
33. Simopoulos, A.P., *The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases.* Exp Biol Med (Maywood), 2008. **233**(6): p. 674-88.
34. Crawford, M.A., *Fatty-acid ratios in free-living and domestic animals. Possible implications for atheroma.* Lancet, 1968. **1**(7556): p. 1329-33.
35. Crawford, M.A., M.M. Gale, and M.H. Woodford, *Linoleic acid and linolenic acid elongation products in muscle tissue of Sncerus caffer and other ruminant species.* Biochem J, 1969. **115**(1): p. 25-7.
36. Eaton, S.B., et al., *Dietary intake of long-chain polyunsaturated fatty acids during the paleolithic.* World Rev Nutr Diet, 1998. **83**: p. 12-23.
37. Eaton, S.B. and M. Konner, *Paleolithic nutrition. A consideration of its nature and current implications.* N Engl J Med, 1985. **312**(5): p. 283-9.

38. Simopoulos, A.P., *Omega-3 fatty acids in health and disease and in growth and development.* Am J Clin Nutr, 1991. **54**(3): p. 438-63.
39. Simopoulos, A.P., *Evolutionary aspects of omega-3 fatty acids in the food supply.* Prostaglandins Leukot Essent Fatty Acids, 1999. **60**(5-6): p. 421-9.
40. Simopoulos, A.P., *Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases.* Biomed Pharmacother, 2006. **60**(9): p. 502-7.
41. Roncone, M., H. Bartlett, and F. Eperjesi, *Essential fatty acids for dry eye: A review.* Cont Lens Anterior Eye, 2010. **33**(2): p. 49-54; quiz 100.
42. Arshad, Z., et al., *The Sources of Essential Fatty Acids for Allergic and Cancer Patients; a Connection with Insight into Mammalian Target of Rapamycin: A Narrative Review.* Asian Pac J Cancer Prev, 2018. **19**(9): p. 2391-2401.
43. Esparza, M.L., S. Sasaki, and H. Kesteloot, *Nutrition, latitude, and multiple sclerosis mortality: an ecologic study.* Am J Epidemiol, 1995. **142**(7): p. 733-7.
44. Simopoulos, A.P., *Evolutionary aspects of the dietary omega-6:omega-3 fatty acid ratio: medical implications.* World Rev Nutr Diet, 2009. **100**: p. 1-21.
45. Leong, E.M., et al., *Complementary and alternative medicines and dietary interventions in multiple sclerosis: what is being used in South Australia and why?* Complement Ther Med, 2009. **17**(4): p. 216-23.
46. Swank, R.L. and J. Goodwin, *Review of MS patient survival on a Swank low saturated fat diet.* Nutrition, 2003. **19**(2): p. 161-2.
47. Harbige, L.S. and M.K. Sharief, *Polyunsaturated fatty acids in the pathogenesis and treatment of multiple sclerosis.* Br J Nutr, 2007. **98 Suppl 1**: p. S46-53.
48. Wilson, R. and D.R. Tocher, *Lipid and fatty acid composition is altered in plaque tissue from multiple sclerosis brain compared with normal brain white matter.* Lipids, 1991. **26**(1): p. 9-15.
49. Cunnane, S.C., et al., *Essential fatty acid and lipid profiles in plasma and erythrocytes in patients with multiple sclerosis.* Am J Clin Nutr, 1989. **50**(4): p. 801-6.
50. van Meeteren, M.E., et al., *Antioxidants and polyunsaturated fatty acids in multiple sclerosis.* Eur J Clin Nutr, 2005. **59**(12): p. 1347-61.
51. Youdim, K.A., A. Martin, and J.A. Joseph, *Essential fatty acids and the brain: possible health implications.* Int J Dev Neurosci, 2000. **18**(4-5): p. 383-99.
52. Calder, P.C., *Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance.* Biochim Biophys Acta, 2015. **1851**(4): p. 469-84.
53. Calder, P.C., *Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?* Br J Clin Pharmacol, 2013. **75**(3): p. 645-62.
54. Stockard, J.E., et al., *Effect of docosahexaenoic acid content of maternal diet on auditory brainstem conduction times in rat pups.* Dev Neurosci, 2000. **22**(5-6): p. 494-9.
55. Da Boit, M., A.M. Hunter, and S.R. Gray, *Fit with good fat? The role of n-3 polyunsaturated fatty acids on exercise performance.* Metabolism, 2017. **66**: p. 45-54.
56. *Fats and fatty acids in human nutrition. Proceedings of the Joint FAO/WHO Expert Consultation. November 10-14, 2008. Geneva, Switzerland.* Ann Nutr Metab, 2009. **55**(1-3): p. 5-300.
57. Balto, J.M., et al., *Individual and Co-occurring SNAP Risk Factors: Smoking, Nutrition, Alcohol Consumption, and Physical Activity in People with Multiple Sclerosis.* Int J MS Care, 2016. **18**(6): p. 298-304.
58. Gilgun-Sherki, Y., E. Melamed, and D. Offen, *The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy.* J Neurol, 2004. **251**(3): p. 261-8.

59. Monsen, E.R., *Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium, and carotenoids.* J Am Diet Assoc, 2000. **100**(6): p. 637-40.
60. Jiang, Q., *Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy.* Free Radic Biol Med, 2014. **72**: p. 76-90.
61. Butterfield, D.A., et al., *Vitamin E and neurodegenerative disorders associated with oxidative stress.* Nutr Neurosci, 2002. **5**(4): p. 229-39.
62. Vatassery, G.T., *Vitamin E and other endogenous antioxidants in the central nervous system.* Geriatrics, 1998. **53 Suppl 1**: p. S25-7.
63. Huang, H.Y. and L.J. Appel, *Supplementation of diets with alpha-tocopherol reduces serum concentrations of gamma- and delta-tocopherol in humans.* J Nutr, 2003. **133**(10): p. 3137-40.
64. Ohrvall, M., G. Sundlof, and B. Vessby, *Gamma, but not alpha, tocopherol levels in serum are reduced in coronary heart disease patients.* J Intern Med, 1996. **239**(2): p. 111-7.
65. Wright, M.E., et al., *Higher baseline serum concentrations of vitamin E are associated with lower total and cause-specific mortality in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study.* Am J Clin Nutr, 2006. **84**(5): p. 1200-7.
66. Rimm, E.B., et al., *Vitamin E consumption and the risk of coronary heart disease in men.* N Engl J Med, 1993. **328**(20): p. 1450-6.
67. Stampfer, M.J., et al., *Vitamin E consumption and the risk of coronary disease in women.* N Engl J Med, 1993. **328**(20): p. 1444-9.
68. Dong, Y.L., Y.; Shu, Y.; Chen, X.; Hu, J.; Zheng, R.; Ma, D.; Yang, C.; Guan, X, *Link between risk of colorectal cancer and serum vitamin E levels: A meta-analysis of case-control studies.* Medicine (Baltimore), 2017: p. Jul;96(27):e7470.
69. Jimenez-Jimenez, F.J., et al., *Cerebrospinal fluid levels of alpha-tocopherol in patients with multiple sclerosis.* Neurosci Lett, 1998. **249**(1): p. 65-7.
70. Loken-Amsrud, K.I., et al., *Alpha-tocopherol and MRI outcomes in multiple sclerosis--association and prediction.* PLoS One, 2013. **8**(1): p. e54417.
71. Pantzaris, M.C., et al., *A novel oral nutraceutical formula of omega-3 and omega-6 fatty acids with vitamins (PLP10) in relapsing remitting multiple sclerosis: a randomised, double-blind, placebo-controlled proof-of-concept clinical trial.* BMJ Open, 2013. **3**(4).
72. Sommer, A., *Vitamin a deficiency and clinical disease: an historical overview.* J Nutr, 2008. **138**(10): p. 1835-9.
73. Ross, S.A., et al., *Retinoids in embryonal development.* Physiol Rev, 2000. **80**(3): p. 1021-54.
74. Huang, Z., et al., *Role of Vitamin A in the Immune System.* J Clin Med, 2018. **7**(9).
75. Esposito, S., et al., *The role of diet in multiple sclerosis: A review.* Nutr Neurosci, 2018. **21**(6): p. 377-390.
76. Weinstock-Guttman, B., et al., *Low fat dietary intervention with omega-3 fatty acid supplementation in multiple sclerosis patients.* Prostaglandins Leukot Essent Fatty Acids, 2005. **73**(5): p. 397-404.
77. Jelinek, G.A., et al., *Associations of Lifestyle, Medication, and Socio-Demographic Factors with Disability in People with Multiple Sclerosis: An International Cross-Sectional Study.* PLoS One, 2016. **11**(8): p. e0161701.
78. Hoare, S., et al., *Higher intake of omega-3 polyunsaturated fatty acids is associated with a decreased risk of a first clinical diagnosis of central nervous system demyelination: Results from the Ausimmune Study.* Mult Scler, 2016. **22**(7): p. 884-92.

79. Mische, L.J. and E.M. Mowry, *The Evidence for Dietary Interventions and Nutritional Supplements as Treatment Options in Multiple Sclerosis: a Review.* Curr Treat Options Neurol, 2018. **20**(4): p. 8.
80. Hadgkiss, E.J., et al., *The association of diet with quality of life, disability, and relapse rate in an international sample of people with multiple sclerosis.* Nutr Neurosci, 2015. **18**(3): p. 125-36.
81. Bates, D., et al., *A double-blind controlled trial of long chain n-3 polyunsaturated fatty acids in the treatment of multiple sclerosis.* J Neurol Neurosurg Psychiatry, 1989. **52**(1): p. 18-22.
82. Nordvik, I., et al., *Effect of dietary advice and n-3 supplementation in newly diagnosed MS patients.* Acta Neurol Scand, 2000. **102**(3): p. 143-9.
83. Gertsik, L., et al., *Omega-3 fatty acid augmentation of citalopram treatment for patients with major depressive disorder.* J Clin Psychopharmacol, 2012. **32**(1): p. 61-4.
84. Nemets, B., Z. Stahl, and R.H. Belmaker, *Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder.* Am J Psychiatry, 2002. **159**(3): p. 477-9.
85. Peet, M. and D.F. Horrobin, *A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs.* Arch Gen Psychiatry, 2002. **59**(10): p. 913-9.
86. Su, K.P., et al., *Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial.* Eur Neuropsychopharmacol, 2003. **13**(4): p. 267-71.
87. Torkildsen, O., et al., *omega-3 fatty acid treatment in multiple sclerosis (OFAMS Study): a randomized, double-blind, placebo-controlled trial.* Arch Neurol, 2012. **69**(8): p. 1044-51.
88. Ramirez-Ramirez, V., et al., *Efficacy of fish oil on serum of TNF alpha , IL-1 beta , and IL-6 oxidative stress markers in multiple sclerosis treated with interferon beta-1b.* Oxid Med Cell Longev, 2013. **2013**: p. 709493.
89. Baarnhielm, M., T. Olsson, and L. Alfredsson, *Fatty fish intake is associated with decreased occurrence of multiple sclerosis.* Mult Scler, 2014. **20**(6): p. 726-32.
90. Ghadirian, P., et al., *Nutritional factors in the aetiology of multiple sclerosis: a case-control study in Montreal, Canada.* Int J Epidemiol, 1998. **27**(5): p. 845-52.
91. Kampman, M.T., T. Wilsgaard, and S.I. Mellgren, *Outdoor activities and diet in childhood and adolescence relate to MS risk above the Arctic Circle.* J Neurol, 2007. **254**(4): p. 471-7.
92. Cortese, M., et al., *Timing of use of cod liver oil, a vitamin D source, and multiple sclerosis risk: The EnvIMS study.* Mult Scler, 2015. **21**(14): p. 1856-64.
93. Ascherio, A., K.L. Munger, and J.D. Lunemann, *The initiation and prevention of multiple sclerosis.* Nat Rev Neurol, 2012. **8**(11): p. 602-12.
94. Shinto, L., et al., *Omega-3 Fatty Acids for Depression in Multiple Sclerosis: A Randomized Pilot Study.* PLoS One, 2016. **11**(1): p. e0147195.
95. Bjornevik, K., et al., *Polyunsaturated fatty acids and the risk of multiple sclerosis.* Mult Scler, 2017. **23**(14): p. 1830-1838.
96. Zhang, S.M., et al., *Dietary fat in relation to risk of multiple sclerosis among two large cohorts of women.* Am J Epidemiol, 2000. **152**(11): p. 1056-64.
97. Marangoni, F., et al., *Changes of n-3 and n-6 fatty acids in plasma and circulating cells of normal subjects, after prolonged administration of 20:5 (EPA) and 22:6 (DHA) ethyl esters and prolonged washout.* Biochim Biophys Acta, 1993. **1210**(1): p. 55-62.
98. Minden, S.L., et al., *The Sonya Slifka Longitudinal Multiple Sclerosis Study: methods and sample characteristics.* Mult Scler, 2006. **12**(1): p. 24-38.

99. Tinetti, M.E., et al., *Shared risk factors for falls, incontinence, and functional dependence. Unifying the approach to geriatric syndromes.* JAMA, 1995. **273**(17): p. 1348-53.
100. Kieseier, B.C. and C. Pozzilli, *Assessing walking disability in multiple sclerosis.* Mult Scler, 2012. **18**(7): p. 914-24.
101. Pilutti, L.A., et al., *Gait and six-minute walk performance in persons with multiple sclerosis.* J Neurol Sci, 2013. **334**(1-2): p. 72-6.
102. Kalron, A., M. Dolev, and U. Givon, *Further construct validity of the Timed Up-and-Go Test as a measure of ambulation in multiple sclerosis patients.* Eur J Phys Rehabil Med, 2017. **53**(6): p. 841-847.
103. Podsiadlo, D. and S. Richardson, *The timed "Up & Go": a test of basic functional mobility for frail elderly persons.* J Am Geriatr Soc, 1991. **39**(2): p. 142-8.
104. *Committee for medicinal products for human use. Guideline on clinical trials in small populations European Medicines.* Agency, London, UK, 2006: p. 1–10.
105. Giannaki, C.D., et al., *Restless legs syndrome in Multiple Sclerosis patients: a contributing factor for fatigue, impaired functional capacity, and diminished health-related quality of life.* Neurol Res, 2018. **40**(7): p. 586-592.
106. Rolak, L.A., *MS: the basic facts.* Clin Med Res, 2003. **1**(1): p. 61-2.
107. Zwibel, H.L. and J. Smrtka, *Improving quality of life in multiple sclerosis: an unmet need.* Am J Manag Care, 2011. **17 Suppl 5 Improving**: p. S139-45.
108. Naci, H., et al., *Economic burden of multiple sclerosis: a systematic review of the literature.* Pharmacoeconomics, 2010. **28**(5): p. 363-79.
109. Lobentanz, I.S., et al., *Factors influencing quality of life in multiple sclerosis patients: disability, depressive mood, fatigue and sleep quality.* Acta Neurol Scand, 2004. **110**(1): p. 6-13.
110. Armstrong, L.E., et al., *Using isokinetic dynamometry to test ambulatory patients with multiple sclerosis.* Phys Ther, 1983. **63**(8): p. 1274-9.
111. Chung, L.H., et al., *Leg power asymmetry and postural control in women with multiple sclerosis.* Med Sci Sports Exerc, 2008. **40**(10): p. 1717-24.
112. Scott, S.M., et al., *Surface EMG characteristics of people with multiple sclerosis during static contractions of the knee extensors.* Clin Physiol Funct Imaging, 2011. **31**(1): p. 11-7.
113. Hvid, L.G., et al., *Aging impairs the recovery in mechanical muscle function following 4 days of disuse.* Exp Gerontol, 2014. **52**: p. 1-8.
114. Jette, A.M., *Toward a common language for function, disability, and health.* Phys Ther, 2006. **86**(5): p. 726-34.
115. Thoumie, P., et al., *Motor determinants of gait in 100 ambulatory patients with multiple sclerosis.* Mult Scler, 2005. **11**(4): p. 485-91.
116. Yahia, A., et al., *Relationship between muscular strength, gait and postural parameters in multiple sclerosis.* Ann Phys Rehabil Med, 2011. **54**(3): p. 144-55.
117. Kjolhede, T., et al., *Relationship between muscle strength parameters and functional capacity in persons with mild to moderate degree multiple sclerosis.* Mult Scler Relat Disord, 2015. **4**(2): p. 151-8.
118. Bowser, B., et al., *Sit-to-stand biomechanics of individuals with multiple sclerosis.* Clin Biomech (Bristol, Avon), 2015. **30**(8): p. 788-94.
119. Larocca, N.G., *Impact of walking impairment in multiple sclerosis: perspectives of patients and care partners.* Patient, 2011. **4**(3): p. 189-201.
120. Motl, R.W., E. McAuley, and E.M. Snook, *Physical activity and multiple sclerosis: a meta-analysis.* Mult Scler, 2005. **11**(4): p. 459-63.

121. Stuifbergen, A.K., *Physical activity and perceived health status in persons with multiple sclerosis.* J Neurosci Nurs, 1997. **29**(4): p. 238-43.
122. Larson, R.D., et al., *Lower-limb performance disparities: implications for exercise prescription in multiple sclerosis.* J Rehabil Res Dev, 2014. **51**(10): p. 1537-44.
123. Kalron, A., A. Achiron, and Z. Dvir, *Muscular and gait abnormalities in persons with early onset multiple sclerosis.* J Neurol Phys Ther, 2011. **35**(4): p. 164-9.
124. Bohannon, R.W., *Dynamometer measurements of hand-grip strength predict multiple outcomes.* Percept Mot Skills, 2001. **93**(2): p. 323-8.
125. Bohannon, R.W., *Hand-grip dynamometry predicts future outcomes in aging adults.* J Geriatr Phys Ther, 2008. **31**(1): p. 3-10.
126. Morris, J.H., et al., *Predicting health related quality of life 6 months after stroke: the role of anxiety and upper limb dysfunction.* Disabil Rehabil, 2013. **35**(4): p. 291-9.
127. Feys, P., et al., *Validity of the TEMPA for the measurement of upper limb function in multiple sclerosis.* Clin Rehabil, 2002. **16**(2): p. 166-73.
128. Yozbatiran, N., et al., *Motor assessment of upper extremity function and its relation with fatigue, cognitive function and quality of life in multiple sclerosis patients.* J Neurol Sci, 2006. **246**(1-2): p. 117-22.
129. Nowak, D.A. and J. Hermsdorfer, *[Analysis of grip force during object manipulation. Method for the objective measurement of physiological normal and impaired hand function]*. Nervenarzt, 2004. **75**(8): p. 725-33.
130. Krishnan, V. and S. Jaric, *Hand function in multiple sclerosis: force coordination in manipulation tasks.* Clin Neurophysiol, 2008. **119**(10): p. 2274-81.
131. Jorgensen, M., et al., *Muscle strength and power in persons with multiple sclerosis - A systematic review and meta-analysis.* J Neurol Sci, 2017. **376**: p. 225-241.
132. Coleman, C.I., et al., *Impact of mobility impairment on indirect costs and health-related quality of life in multiple sclerosis.* PLoS One, 2013. **8**(1): p. e54756.
133. Blair, S.N. and J.N. Morris, *Healthy hearts--and the universal benefits of being physically active: physical activity and health.* Ann Epidemiol, 2009. **19**(4): p. 253-6.
134. Hu, G., et al., *The effects of physical activity and body mass index on cardiovascular, cancer and all-cause mortality among 47 212 middle-aged Finnish men and women.* Int J Obes (Lond), 2005. **29**(8): p. 894-902.
135. Dalgas, U., et al., *Resistance training improves muscle strength and functional capacity in multiple sclerosis.* Neurology, 2009. **73**(18): p. 1478-84.
136. White, L.J., et al., *Resistance training improves strength and functional capacity in persons with multiple sclerosis.* Mult Scler, 2004. **10**(6): p. 668-74.
137. Broekmans, T., et al., *Effects of long-term resistance training and simultaneous electro-stimulation on muscle strength and functional mobility in multiple sclerosis.* Mult Scler, 2011. **17**(4): p. 468-77.
138. DeBolt, L.S. and J.A. McCubbin, *The effects of home-based resistance exercise on balance, power, and mobility in adults with multiple sclerosis.* Arch Phys Med Rehabil, 2004. **85**(2): p. 290-7.
139. Kim, C.M., J.J. Eng, and M.W. Whittaker, *Level walking and ambulatory capacity in persons with incomplete spinal cord injury: relationship with muscle strength.* Spinal Cord, 2004. **42**(3): p. 156-62.
140. Ostchega, Y., et al., *Isokinetic leg muscle strength in older americans and its relationship to a standardized walk test: data from the national health and nutrition examination survey 1999-2000.* J Am Geriatr Soc, 2004. **52**(6): p. 977-82.
141. Willen, C., et al., *How is walking speed related to muscle strength? A study of healthy persons and persons with late effects of polio.* Arch Phys Med Rehabil, 2004. **85**(12): p. 1923-8.

142. Rudroff, T. and F. Proessl, *Effects of Muscle Function and Limb Loading Asymmetries on Gait and Balance in People With Multiple Sclerosis.* Front Physiol, 2018. **9**: p. 531.
143. Reid, K.F. and R.A. Fielding, *Skeletal muscle power: a critical determinant of physical functioning in older adults.* Exerc Sport Sci Rev, 2012. **40**(1): p. 4-12.
144. Lambert, C.P., R.L. Archer, and W.J. Evans, *Muscle strength and fatigue during isokinetic exercise in individuals with multiple sclerosis.* Med Sci Sports Exerc, 2001. **33**(10): p. 1613-9.
145. Armstrong, L.E., et al., *Using isokinetic dynamometry to test ambulatory patients with multiple sclerosis.* Physical Therapy, 1983. **63**(8): p. 1274-1279.
146. Lambert, C.P., R.L. Archer, and W.J. Evans, *Muscle strength and fatigue during isokinetic exercise in individuals with multiple sclerosis.* Medicine and science in sports and exercise, 2001. **33**(10): p. 1613-1619.
147. Ponichtera, J.A., et al., *Concentric and eccentric isokinetic lower extremity strength in persons with multiple sclerosis.* The Journal of orthopaedic and sports physical therapy, 1992. **16**(3): p. 114-122.
148. Broekmans, T., et al., *Effects of long-term resistance training and simultaneous electrostimulation on muscle strength and functional mobility in multiple sclerosis.* Multiple sclerosis (Houndmills, Basingstoke, England), 2011. **17**(4): p. 468-477.
149. Cantalloube, S., et al., *[Strength, postural and gait changes following rehabilitation in multiple sclerosis: a preliminary study].* Ann Readapt Med Phys, 2006. **49**(4): p. 143-9.
150. Broekmans, T., et al., *The relationship between upper leg muscle strength and walking capacity in persons with multiple sclerosis.* Mult Scler, 2013. **19**(1): p. 112-9.
151. Beck, T.W., et al., *The influence of electrode shift over the innervation zone and normalization on the electromyographic amplitude and mean power frequency versus isometric torque relationships for the vastus medialis muscle.* J Neurosci Methods, 2008. **169**(1): p. 100-8.
152. Shiavi, C.F.a.R., *"Applications in movement and gait analysis,.* in Electromyography: Physiology, Engineering and Noninvasive Applications, R. Merletti and P. Parker, Eds., pp. 381–397, Wiley inter-science, , 2004.
153. Luca, C.J.D., *The use of surface electromyography in biomechanics.* J. of applied biomechanics, 1997. **13**: p. 135–163.
154. Bigland-Ritchie, B., F. Furbush, and J.J. Woods, *Fatigue of intermittent submaximal voluntary contractions: central and peripheral factors.* J Appl Physiol (1985), 1986. **61**(2): p. 421-9.
155. Kamandulis, S., et al., *The decrease in electrically evoked force production is delayed by a previous bout of stretch-shortening cycle exercise.* Acta Physiol (Oxf), 2010. **198**(1): p. 91-8.
156. Westerblad, H. and D.G. Allen, *Cellular mechanisms of skeletal muscle fatigue.* Adv Exp Med Biol, 2003. **538**: p. 563-70; discussion 571.
157. Gandevia, S.C., *Spinal and supraspinal factors in human muscle fatigue.* Physiol Rev, 2001. **81**(4): p. 1725-89.
158. Bilodeau, M., *Central fatigue in continuous and intermittent contractions of triceps brachii.* Muscle Nerve, 2006. **34**(2): p. 205-13.
159. Al-Mulla, M.R., F. Sepulveda, and M. Colley, *A review of non-invasive techniques to detect and predict localised muscle fatigue.* Sensors (Basel), 2011. **11**(4): p. 3545-94.
160. Veer, K. and T. Sharma, *A novel feature extraction for robust EMG pattern recognition.* J Med Eng Technol, 2016. **40**(4): p. 149-54.

161. Oskoei, M.A. and H. Hu, *Support vector machine-based classification scheme for myoelectric control applied to upper limb.* IEEE Trans Biomed Eng, 2008. **55**(8): p. 1956-65.
162. Maffiuletti, N.A., et al., *Rate of force development: physiological and methodological considerations.* Eur J Appl Physiol, 2016. **116**(6): p. 1091-116.
163. Desmedt, J.E. and E. Godaux, *Ballistic contractions in man: characteristic recruitment pattern of single motor units of the tibialis anterior muscle.* J Physiol, 1977. **264**(3): p. 673-93.
164. Duchateau, J. and R.M. Enoka, *Human motor unit recordings: origins and insight into the integrated motor system.* Brain Res, 2011. **1409**: p. 42-61.
165. Buchthal, F. and H. Schmalbruch, *Contraction times and fibre types in intact human muscle.* Acta Physiol Scand, 1970. **79**(4): p. 435-52.
166. Harridge, S.D., et al., *Whole-muscle and single-fibre contractile properties and myosin heavy chain isoforms in humans.* Pflugers Arch, 1996. **432**(5): p. 913-20.
167. Bottinelli, R., et al., *Force-velocity properties of human skeletal muscle fibres: myosin heavy chain isoform and temperature dependence.* J Physiol, 1996. **495 (Pt 2)**: p. 573-86.
168. de Haan, A., et al., *Contractile properties and fatigue of quadriceps muscles in multiple sclerosis.* Muscle Nerve, 2000. **23**(10): p. 1534-41.
169. Garner, D.J. and J.J. Widrick, *Cross-bridge mechanisms of muscle weakness in multiple sclerosis.* Muscle Nerve, 2003. **27**(4): p. 456-64.
170. Ng, A.V., et al., *Functional relationships of central and peripheral muscle alterations in multiple sclerosis.* Muscle Nerve, 2004. **29**(6): p. 843-52.
171. Rice, C.L., T.L. Vollmer, and B. Bigland-Ritchie, *Neuromuscular responses of patients with multiple sclerosis.* Muscle Nerve, 1992. **15**(10): p. 1123-32.
172. Wens, I., et al., *Multiple sclerosis affects skeletal muscle characteristics.* PLoS One, 2014. **9**(9): p. e108158.
173. Burke, R.E., et al., *Physiological types and histochemical profiles in motor units of the cat gastrocnemius.* J Physiol, 1973. **234**(3): p. 723-48.
174. Fuglevand, A.J., V.G. Macefield, and B. Bigland-Ritchie, *Force-frequency and fatigue properties of motor units in muscles that control digits of the human hand.* J Neurophysiol, 1999. **81**(4): p. 1718-29.
175. Thomas, C.K., R.S. Johansson, and B. Bigland-Ritchie, *Attempts to physiologically classify human thenar motor units.* J Neurophysiol, 1991. **65**(6): p. 1501-8.
176. Henneman, E., *Relation between size of neurons and their susceptibility to discharge.* Science, 1957. **126**(3287): p. 1345-7.
177. Henneman, E., G. Somjen, and D.O. Carpenter, *Excitability and inhibitability of motoneurons of different sizes.* J Neurophysiol, 1965. **28**(3): p. 599-620.
178. De Luca, C.J., et al., *Control scheme governing concurrently active human motor units during voluntary contractions.* J Physiol, 1982. **329**: p. 129-42.
179. Monster, A.W. and H. Chan, *Isometric force production by motor units of extensor digitorum communis muscle in man.* J Neurophysiol, 1977. **40**(6): p. 1432-43.
180. Tanji, J. and M. Kato, *Firing rate of individual motor units in voluntary contraction of abductor digiti minimi muscle in man.* Exp Neurol, 1973. **40**(3): p. 771-83.
181. Kupa, E.J., et al., *Effects of muscle fiber type and size on EMG median frequency and conduction velocity.* J Appl Physiol (1985), 1995. **79**(1): p. 23-32.
182. Thongpanja, S., Phinyomark, A., Phukpattaranont, P., & Limsakul, C *Mean and Median Frequency of EMG Signal to Determine Muscle Force based on Time-Dependent Power Spectrum.* Electronics and Electrical Engineering, 19(3), 2013.

183. Winter, D.A.N.Y., *Biomechanics and motor control of human movement* ed. n. Ed. 1990: John Wiley & Sons, Inc., 191-212.
184. De Luca, C.J., *Myoelectrical manifestations of localized muscular fatigue in humans.* Crit Rev Biomed Eng, 1984. **11**(4): p. 251-79.
185. Merletti, R., M. Knaflitz, and C.J. De Luca, *Myoelectric manifestations of fatigue in voluntary and electrically elicited contractions.* J Appl Physiol (1985), 1990. **69**(5): p. 1810-20.
186. Konrad, P., *The ABC of EMG. A Practical Introduction to Kinesiological Electromyography.* 2005.
187. Dorfman, L.J., J.E. Howard, and K.C. McGill, *Motor unit firing rates and firing rate variability in the detection of neuromuscular disorders.* Electroencephalogr Clin Neurophysiol, 1989. **73**(3): p. 215-24.
188. Rosenfalck, A. and S. Andreassen, *Impaired regulation of force and firing pattern of single motor units in patients with spasticity.* J Neurol Neurosurg Psychiatry, 1980. **43**(10): p. 907-16.
189. Zwarts, M.J. and D.F. Stegeman, *Multichannel surface EMG: basic aspects and clinical utility.* Muscle Nerve, 2003. **28**(1): p. 1-17.
190. Andreasen, A.K., et al., *Fatigued patients with multiple sclerosis have impaired central muscle activation.* Mult Scler, 2009. **15**(7): p. 818-27.
191. Johnson, S.L., *The concept of fatigue in multiple sclerosis.* J Neurosci Nurs, 2008. **40**(2): p. 72-7.
192. Sheean, G.L., et al., *An electrophysiological study of the mechanism of fatigue in multiple sclerosis.* Brain, 1997. **120 (Pt 2)**: p. 299-315.
193. Skurvydas, A., et al., *The effect of multiple sclerosis and gender on central and peripheral fatigue during 2-min MVC.* Clin Neurophysiol, 2011. **122**(4): p. 767-76.
194. Hameau, S., et al., *Fatigability in Patients With Multiple Sclerosis During Maximal Concentric Contractions.* Arch Phys Med Rehabil, 2017. **98**(7): p. 1339-1347.
195. Bosnak-Guclu, M., et al., *Comparison of functional exercise capacity, pulmonary function and respiratory muscle strength in patients with multiple sclerosis with different disability levels and healthy controls.* J Rehabil Med, 2012. **44**(1): p. 80-6.
196. Broekmans, T., et al., *The relationship between upper leg muscle strength and walking capacity in persons with multiple sclerosis.* Multiple sclerosis (Houndmills, Basingstoke, England), 2013. **19**(1): p. 112-119.
197. Osternig, L.R., *Isokinetic dynamometry: implications for muscle testing and rehabilitation.* Exerc Sport Sci Rev, 1986. **14**: p. 45-80.
198. Baltzopoulos, V., J.G. Williams, and D.A. Brodie, *Sources of error in isokinetic dynamometry: effects of visual feedback on maximum torque.* J Orthop Sports Phys Ther, 1991. **13**(3): p. 138-42.
199. Hermens, H.J., et al., *Development of recommendations for SEMG sensors and sensor placement procedures.* J Electromyogr Kinesiol, 2000. **10**(5): p. 361-74.
200. Merletti, R. and H. Hermens, *Introduction to the special issue on the SENIAM European Concerted Action.* J Electromyogr Kinesiol, 2000. **10**(5): p. 283-6.
201. ; Available from: https://www.delsys.com/use-emgscripts-fatigue-analysis/.
202. Severijns, D., et al., *Hand grip fatigability in persons with multiple sclerosis according to hand dominance and disease progression.* J Rehabil Med, 2015. **47**(2): p. 154-60.
203. Bohannon, R.W., *Minimal clinically important difference for grip strength: a systematic review.* J Phys Ther Sci, 2019. **31**(1): p. 75-78.
204. Juniper, E.F., et al., *Determining a minimal important change in a disease-specific Quality of Life Questionnaire.* J Clin Epidemiol, 1994. **47**(1): p. 81-7.

205. Kim, J.K., M.G. Park, and S.J. Shin, *What is the minimum clinically important difference in grip strength?* Clin Orthop Relat Res, 2014. **472**(8): p. 2536-41.
206. Osoba, D., et al., *Interpreting the significance of changes in health-related quality-of-life scores.* J Clin Oncol, 1998. **16**(1): p. 139-44.
207. Wyrwich, K.W., et al., *Linking clinical relevance and statistical significance in evaluating intra-individual changes in health-related quality of life.* Med Care, 1999. **37**(5): p. 469-78.
208. Janardhan, V. and R. Bakshi, *Quality of life in patients with multiple sclerosis: the impact of fatigue and depression.* J Neurol Sci, 2002. **205**(1): p. 51-8.
209. Biral, D., et al., *Myosin heavy chain composition of muscle fibers in spinal muscular atrophy.* Muscle Nerve, 1989. **12**(1): p. 43-51.
210. Chali, F., et al., *Long-term exercise-specific neuroprotection in spinal muscular atrophy-like mice.* J Physiol, 2016. **594**(7): p. 1931-52.
211. Schiaffino, S. and C. Reggiani, *Fiber types in mammalian skeletal muscles.* Physiol Rev, 2011. **91**(4): p. 1447-531.
212. Talbot J, M.L., *Skeletal muscle fiber type: Using insights from muscle developmental biology to dissect targets for susceptibility and resistance to muscle disease.* Wiley Interdisciplinary Reviews: Developmental Biology, 2016(5): p. 518-534.
213. Kim, T.Y., et al., *The effects of different gait speeds and lower arm weight on the activities of the latissimus dorsi, gluteus medius, and gluteus maximus muscles.* J Phys Ther Sci, 2013. **25**(11): p. 1483-4.
214. Jordan, K., J.H. Challis, and K.M. Newell, *Walking speed influences on gait cycle variability.* Gait Posture, 2007. **26**(1): p. 128-34.
215. Winiarski, S., J. Pietraszewska, and B. Pietraszewski, *Three-Dimensional Human Gait Pattern: Reference Data for Young, Active Women Walking with Low, Preferred, and High Speeds.* Biomed Res Int, 2019. **2019**: p. 9232430.
216. Winter, D.A., *Biomechanics of normal and pathological gait: implications for understanding human locomotor control.* J Mot Behav, 1989. **21**(4): p. 337-55.
217. Dicharry, J., *Kinematics and kinetics of gait: from lab to clinic.* Clin Sports Med, 2010. **29**(3): p. 347-64.
218. Burnfield, J.P.J.M., *Gait Analysis: Normal and Pathological Function*. 2nd ed. 2010: SLACK Incorporated. 576.
219. Comber, L., R. Galvin, and S. Coote, *Gait deficits in people with multiple sclerosis: A systematic review and meta-analysis.* Gait Posture, 2017. **51**: p. 25-35.
220. Pike, J., et al., *Social and economic burden of walking and mobility problems in multiple sclerosis.* BMC Neurol, 2012. **12**: p. 94.
221. Morris, M.E., et al., *Changes in gait and fatigue from morning to afternoon in people with multiple sclerosis.* J Neurol Neurosurg Psychiatry, 2002. **72**(3): p. 361-5.
222. Givon, U., G. Zeilig, and A. Achiron, *Gait analysis in multiple sclerosis: characterization of temporal-spatial parameters using GAITRite functional ambulation system.* Gait Posture, 2009. **29**(1): p. 138-42.
223. Sosnoff, J.J., B.M. Sandroff, and R.W. Motl, *Quantifying gait abnormalities in persons with multiple sclerosis with minimal disability.* Gait Posture, 2012. **36**(1): p. 154-6.
224. Jeng, B., B.M. Sandroff, and R.W. Motl, *Energetic cost of walking and spasticity in persons with multiple sclerosis with moderate disability.* NeuroRehabilitation, 2018. **43**(4): p. 483-489.
225. Gunn, H., et al., *Risk factors for falls in multiple sclerosis: an observational study.* Mult Scler, 2013. **19**(14): p. 1913-22.
226. Bazelier, M.T., et al., *Risk of fractures in patients with multiple sclerosis: a population-based cohort study.* Neurology, 2012. **78**(24): p. 1967-73.

227. Peterson, E.W., C.C. Cho, and M.L. Finlayson, *Fear of falling and associated activity curtailment among middle aged and older adults with multiple sclerosis.* Mult Scler, 2007. **13**(9): p. 1168-75.
228. Finlayson, M.L. and E.W. Peterson, *Falls, aging, and disability.* Phys Med Rehabil Clin N Am, 2010. **21**(2): p. 357-73.
229. Hohol, M.J., E.J. Orav, and H.L. Weiner, *Disease steps in multiple sclerosis: a simple approach to evaluate disease progression.* Neurology, 1995. **45**(2): p. 251-5.
230. Goldman, M.D., R.A. Marrie, and J.A. Cohen, *Evaluation of the six-minute walk in multiple sclerosis subjects and healthy controls.* Mult Scler, 2008. **14**(3): p. 383-90.
231. Goldman, M.D., R.W. Motl, and R.A. Rudick, *Possible clinical outcome measures for clinical trials in patients with multiple sclerosis.* Ther Adv Neurol Disord, 2010. **3**(4): p. 229-39.
232. Sandroff, B.M., L.A. Pilutti, and R.W. Motl, *Does the six-minute walk test measure walking performance or physical fitness in persons with multiple sclerosis?* NeuroRehabilitation, 2015. **37**(1): p. 149-55.
233. Sandroff, B.M., J.J. Sosnoff, and R.W. Motl, *Physical fitness, walking performance, and gait in multiple sclerosis.* J Neurol Sci, 2013. **328**(1-2): p. 70-6.
234. Baert, I., et al., *Responsiveness and clinically meaningful improvement, according to disability level, of five walking measures after rehabilitation in multiple sclerosis: a European multicenter study.* Neurorehabil Neural Repair, 2014. **28**(7): p. 621-31.
235. Bethoux, F., *Gait disorders in multiple sclerosis.* Continuum (Minneap Minn), 2013. **19**(4 Multiple Sclerosis): p. 1007-22.
236. Benedetti, M.G., et al., *Data management in gait analysis for clinical applications.* Clin Biomech (Bristol, Avon), 1998. **13**(3): p. 204-215.
237. Liparoti, M., et al., *Gait abnormalities in minimally disabled people with Multiple Sclerosis: A 3D-motion analysis study.* Mult Scler Relat Disord, 2019. **29**: p. 100-107.
238. Martin, C.L., et al., *Gait and balance impairment in early multiple sclerosis in the absence of clinical disability.* Mult Scler, 2006. **12**(5): p. 620-8.
239. Nogueira, L.A., et al., *Gait characteristics of multiple sclerosis patients in the absence of clinical disability.* Disabil Rehabil, 2013. **35**(17): p. 1472-8.
240. Benedetti, M.G., et al., *Gait abnormalities in minimally impaired multiple sclerosis patients.* Mult Scler, 1999. **5**(5): p. 363-8.
241. Severini, G., et al., *Evaluation of Clinical Gait Analysis parameters in patients affected by Multiple Sclerosis: Analysis of kinematics.* Clin Biomech (Bristol, Avon), 2017. **45**: p. 1-8.
242. Galna, B., et al., *Progression of gait dysfunction in incident Parkinson's disease: impact of medication and phenotype.* Mov Disord, 2015. **30**(3): p. 359-67.
243. Leone, C., et al., *Prevalence of Walking-Related Motor Fatigue in Persons With Multiple Sclerosis: Decline in Walking Distance Induced by the 6-Minute Walk Test.* Neurorehabil Neural Repair, 2016. **30**(4): p. 373-83.
244. Kragt, J.J., et al., *Clinical impact of 20% worsening on Timed 25-foot Walk and 9-hole Peg Test in multiple sclerosis.* Mult Scler, 2006. **12**(5): p. 594-8.
245. Crenshaw, S.J., et al., *Gait variability in people with multiple sclerosis.* Mult Scler, 2006. **12**(5): p. 613-9.
246. Gijbels, D., et al., *Predicting habitual walking performance in multiple sclerosis: relevance of capacity and self-report measures.* Mult Scler, 2010. **16**(5): p. 618-26.
247. van der Linden, M.L., et al., *Ankle Kinematics and Temporal Gait Characteristics over the Duration of a 6-Minute Walk Test in People with Multiple Sclerosis Who Experience Foot Drop.* Rehabil Res Pract, 2018. **2018**: p. 1260852.

248. Corradini, M.L., et al., *Early recognition of postural disorders in multiple sclerosis through movement analysis: a modeling study.* IEEE Trans Biomed Eng, 1997. **44**(11): p. 1029-38.
249. Cappozzo, A., et al., *Human movement analysis using stereophotogrammetry. Part 1: theoretical background.* Gait Posture, 2005. **21**(2): p. 186-96.
250. Gijbels, D., B.O. Eijnde, and P. Feys, *Comparison of the 2- and 6-minute walk test in multiple sclerosis.* Mult Scler, 2011. **17**(10): p. 1269-72.
251. Singh, S.J., et al., *An official systematic review of the European Respiratory Society/American Thoracic Society: measurement properties of field walking tests in chronic respiratory disease.* Eur Respir J, 2014. **44**(6): p. 1447-78.
252. Sandroff, B.M., et al., *Comparing two conditions of administering the six-minute walk test in people with multiple sclerosis.* Int J MS Care, 2014. **16**(1): p. 48-54.
253. Laboratories, A.T.S.C.o.P.S.f.C.P.F., *ATS statement: guidelines for the six-minute walk test.* Am J Respir Crit Care Med, 2002. **166**(1): p. 111-7.
254. Benavent-Caballer, V., *The effectiveness of exercise interventions and the factors associated with the physical performance in older adults*. 2016. p. 231.
255. A.L, H., *Scaling gait data to body size* Gait Posture, 1996: p. 222-3.
256. Pinzone, O., M.H. Schwartz, and R. Baker, *Comprehensive non-dimensional normalization of gait data.* Gait Posture, 2016. **44**: p. 68-73.
257. *Vicon Motion Systems. Plug-in Gait Reference Guide. Oxford:* https://docs.vicon.com/display/Nexus25/PDF+downloads+for+Vicon+Nexus.
258. Schwartz, M.H. and A. Rozumalski, *The Gait Deviation Index: a new comprehensive index of gait pathology.* Gait Posture, 2008. **28**(3): p. 351-7.
259. Molloy, M., et al., *Further evidence of validity of the Gait Deviation Index.* Gait Posture, 2010. **31**(4): p. 479-82.
260. van der Linden, M.L., et al., *Gait kinematics of people with multiple sclerosis and the acute application of functional electrical stimulation.* Gait Posture, 2014. **39**(4): p. 1092-6.
261. Kwon, J.W., S.M. Son, and N.K. Lee, *Changes of kinematic parameters of lower extremities with gait speed: a 3D motion analysis study.* J Phys Ther Sci, 2015. **27**(2): p. 477-9.
262. Filli, L., et al., *Profiling walking dysfunction in multiple sclerosis: characterisation, classification and progression over time.* Sci Rep, 2018. **8**(1): p. 4984.
263. Arena, R., et al., *Assessment of functional capacity in clinical and research settings: a scientific statement from the American Heart Association Committee on Exercise, Rehabilitation, and Prevention of the Council on Clinical Cardiology and the Council on Cardiovascular Nursing.* Circulation, 2007. **116**(3): p. 329-43.
264. Mitchell, A.J., et al., *Quality of life and its assessment in multiple sclerosis: integrating physical and psychological components of wellbeing.* Lancet Neurol, 2005. **4**(9): p. 556-66.
265. Hoang, P.D., S.C. Gandevia, and R.D. Herbert, *Prevalence of joint contractures and muscle weakness in people with multiple sclerosis.* Disabil Rehabil, 2014. **36**(19): p. 1588-93.
266. Hartung, H.P. and O. Aktas, *Evolution of multiple sclerosis treatment: next generation therapies meet next generation efficacy criteria.* Lancet Neurol, 2011. **10**(4): p. 293-5.
267. Bennell, K., F. Dobson, and R. Hinman, *Measures of physical performance assessments: Self-Paced Walk Test (SPWT), Stair Climb Test (SCT), Six-Minute Walk Test (6MWT), Chair Stand Test (CST), Timed Up & Go (TUG), Sock Test, Lift and Carry Test (LCT), and Car Task.* Arthritis Care Res (Hoboken), 2011. **63 Suppl 11**: p. S350-70.

268. Harada, N.D., V. Chiu, and A.L. Stewart, *Mobility-related function in older adults: assessment with a 6-minute walk test.* Arch Phys Med Rehabil, 1999. **80**(7): p. 837-41.
269. Baer, G.D. and A.M. Ashburn, *Trunk movements in older subjects during sit-to-stand.* Arch Phys Med Rehabil, 1995. **76**(9): p. 844-9.
270. Kralj, A., R.J. Jaeger, and M. Munih, *Analysis of standing up and sitting down in humans: definitions and normative data presentation.* J Biomech, 1990. **23**(11): p. 1123-38.
271. Riley, P.O., et al., *Mechanics of a constrained chair-rise.* J Biomech, 1991. **24**(1): p. 77-85.
272. Seven, Y.B., N.E. Akalan, and C.A. Yucesoy, *Effects of back loading on the biomechanics of sit-to-stand motion in healthy children.* Hum Mov Sci, 2008. **27**(1): p. 65-79.
273. Inkster, L.M., et al., *Leg muscle strength is reduced in Parkinson's disease and relates to the ability to rise from a chair.* Mov Disord, 2003. **18**(2): p. 157-62.
274. Lomaglio, M.J. and J.J. Eng, *Muscle strength and weight-bearing symmetry relate to sit-to-stand performance in individuals with stroke.* Gait Posture, 2005. **22**(2): p. 126-31.
275. Chou, S.W., et al., *Postural control during sit-to stand and gait in stroke patients.* Am J Phys Med Rehabil, 2003. **82**(1): p. 42-7.
276. Gan, S.M., et al., *Psychometric properties of functional balance assessment in children with cerebral palsy.* Neurorehabil Neural Repair, 2008. **22**(6): p. 745-53.
277. Prosser, L.A., et al., *Trunk and hip muscle activity in early walkers with and without cerebral palsy--a frequency analysis.* J Electromyogr Kinesiol, 2010. **20**(5): p. 851-9.
278. Roy, G., et al., *Side difference in the hip and knee joint moments during sit-to-stand and stand-to-sit tasks in individuals with hemiparesis.* Clin Biomech (Bristol, Avon), 2007. **22**(7): p. 795-804.
279. Schenkman, M., et al., *Whole-body movements during rising to standing from sitting.* Phys Ther, 1990. **70**(10): p. 638-48; discussion 648-51.
280. van Lummel, R.C., et al., *Validation of seat-off and seat-on in repeated sit-to-stand movements using a single-body-fixed sensor.* Physiol Meas, 2012. **33**(11): p. 1855-67.
281. Roebroeck, M.E., et al., *Biomechanics and muscular activity during sit-to-stand transfer.* Clin Biomech (Bristol, Avon), 1994. **9**(4): p. 235-44.
282. Bohannon, R.W., *Alternatives for measuring knee extension strength of the elderly at home.* Clin Rehabil, 1998. **12**(5): p. 434-40.
283. Ozatevli S, O.A., Itil O, et al., *Comparison of the Sit-to-Stand Test with 6min walk test in patients with chronic obstructive pulmonary disease.* 2007: p. Resp Med 2007;101:286–93.
284. Csuka, M. and D.J. McCarty, *Simple method for measurement of lower extremity muscle strength.* Am J Med, 1985. **78**(1): p. 77-81.
285. Moller, A.B., et al., *Validity and variability of the 5-repetition sit-to-stand test in patients with multiple sclerosis.* Disabil Rehabil, 2012. **34**(26): p. 2251-8.
286. Millington, P.J., B.M. Myklebust, and G.M. Shambes, *Biomechanical analysis of the sit-to-stand motion in elderly persons.* Arch Phys Med Rehabil, 1992. **73**(7): p. 609-17.
287. Cattaneo, D., A. Regola, and M. Meotti, *Validity of six balance disorders scales in persons with multiple sclerosis.* Disabil Rehabil, 2006. **28**(12): p. 789-95.
288. Learmonth, Y.C., et al., *Reliability and clinical significance of mobility and balance assessments in multiple sclerosis.* Int J Rehabil Res, 2012. **35**(1): p. 69-74.
289. Fleg, J.L., et al., *Assessment of functional capacity in clinical and research applications: An advisory from the Committee on Exercise, Rehabilitation, and*

Prevention, Council on Clinical Cardiology, American Heart Association. Circulation, 2000. **102**(13): p. 1591-7.
290. Noonan, V. and E. Dean, *Submaximal exercise testing: clinical application and interpretation.* Phys Ther, 2000. **80**(8): p. 782-807.
291. Savci, S., et al., *Six-minute walk distance as a measure of functional exercise capacity in multiple sclerosis.* Disabil Rehabil, 2005. **27**(22): p. 1365-71.
292. Stavrinou, P.S., et al., *The Effects of a 6-Month High Dose Omega-3 and Omega-6 Polyunsaturated Fatty Acids and Antioxidant Vitamins Supplementation on Cognitive Function and Functional Capacity in Older Adults with Mild Cognitive Impairment.* Nutrients, 2020. **12**(2).
293. AlAmmar, W.A., et al., *Effect of omega-3 fatty acids and fish oil supplementation on multiple sclerosis: a systematic review.* Nutr Neurosci, 2019: p. 1-11.
294. Krzyminska-Siemaszko, R., et al., *The Effect of a 12-Week Omega-3 Supplementation on Body Composition, Muscle Strength and Physical Performance in Elderly Individuals with Decreased Muscle Mass.* Int J Environ Res Public Health, 2015. **12**(9): p. 10558-74.
295. Lalia, A.Z., et al., *Influence of omega-3 fatty acids on skeletal muscle protein metabolism and mitochondrial bioenergetics in older adults.* Aging (Albany NY), 2017. **9**(4): p. 1096-1129.
296. Rolland, Y.B., P. de S.; Maltais, M.; Guyonnet, S.; Cantet, C.; Andrieu, S.; Vellas, B, *Effect of Long-Term Omega 3 Polyunsaturated Fatty Acid Supplementation with or without Multidomain Lifestyle Intervention on Muscle Strength in Older Adults: Secondary Analysis of the Multidomain Alzheimer Preventive Trial (MAPT).* Nutrients 2019, 11, 1931.
297. Smith, G.I., et al., *Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial.* Am J Clin Nutr, 2011. **93**(2): p. 402-12.
298. Smith, G.I., et al., *Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults.* Am J Clin Nutr, 2015. **102**(1): p. 115-22.
299. Ashor, A.W., et al., *Effect of vitamin C and vitamin E supplementation on endothelial function: a systematic review and meta-analysis of randomised controlled trials.* Br J Nutr, 2015. **113**(8): p. 1182-94.
300. Braakhuis, A.J. and W.G. Hopkins, *Impact of Dietary Antioxidants on Sport Performance: A Review.* Sports Med, 2015. **45**(7): p. 939-55.
301. Elzbieta Dorota Miller., A.D., Joanna Saluk-Bijak., Michal Bijak., *A Review of Various Antioxidant Compounds and their Potential Utility as Complementary Therapy in Multiple Sclerosis.* Nutrients. 2019 Jul; 11(7): 1528.
302. Madhushri, P., *A Model Based Mobility Assessment of Older Adults using Wearable Systems*. 2017.
303. Steffen, T.M., T.A. Hacker, and L. Mollinger, *Age- and gender-related test performance in community-dwelling elderly people: Six-Minute Walk Test, Berg Balance Scale, Timed Up & Go Test, and gait speeds.* Phys Ther, 2002. **82**(2): p. 128-37.
304. Schoppen, T., et al., *The Timed "up and go" test: reliability and validity in persons with unilateral lower limb amputation.* Arch Phys Med Rehabil, 1999. **80**(7): p. 825-8.
305. Cowley, P.M., et al., *Physical fitness predicts functional tasks in individuals with Down syndrome.* Med Sci Sports Exerc, 2010. **42**(2): p. 388-93.
306. Dunlop, D.D., S.L. Hughes, and L.M. Manheim, *Disability in activities of daily living: patterns of change and a hierarchy of disability.* Am J Public Health, 1997. **87**(3): p. 378-83.

307. Rodacki, C.L., et al., *Fish-oil supplementation enhances the effects of strength training in elderly women.* Am J Clin Nutr, 2012. **95**(2): p. 428-36.
308. Tanimura, K., et al., *Accelerated Loss of Antigravity Muscles Is Associated with Mortality in Patients with COPD.* Respiration, 2020. **99**(4): p. 298-306.
309. Logan, S.L. and L.L. Spriet, *Omega-3 Fatty Acid Supplementation for 12 Weeks Increases Resting and Exercise Metabolic Rate in Healthy Community-Dwelling Older Females.* PLoS One, 2015. **10**(12): p. e0144828.
310. Gammone, M.A., et al., *Omega-3 Polyunsaturated Fatty Acids: Benefits and Endpoints in Sport.* Nutrients, 2018. **11**(1).
311. Parletta, N., C.M. Milte, and B.J. Meyer, *Nutritional modulation of cognitive function and mental health.* J Nutr Biochem, 2013. **24**(5): p. 725-43.
312. Shei, R.J., M.R. Lindley, and T.D. Mickleborough, *Omega-3 polyunsaturated fatty acids in the optimization of physical performance.* Mil Med, 2014. **179**(11 Suppl): p. 144-56.
313. Patti, F., et al., *Health-related quality of life and depression in an Italian sample of multiple sclerosis patients.* J Neurol Sci, 2003. **211**(1-2): p. 55-62.
314. McHorney, C.A., J.E. Ware, Jr., and A.E. Raczek, *The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs.* Med Care, 1993. **31**(3): p. 247-63.
315. Ware, J.E., Jr., *SF-36 health survey update.* Spine (Phila Pa 1976), 2000. **25**(24): p. 3130-9.
316. Goksel Karatepe, A., et al., *Quality of life in patients with multiple sclerosis: the impact of depression, fatigue, and disability.* Int J Rehabil Res, 2011. **34**(4): p. 290-8.
317. Ghaem, H. and A. Borhani Haghighi, *The impact of disability, fatigue and sleep quality on the quality of life in multiple sclerosis.* Ann Indian Acad Neurol, 2008. **11**(4): p. 236-41.
318. Gavelova, M., et al., *Importance of an individual's evaluation of functional status for health-related quality of life in patients with multiple sclerosis.* Disabil Health J, 2015. **8**(3): p. 372-9.
319. Nourbakhsh, B., L. Julian, and E. Waubant, *Fatigue and depression predict quality of life in patients with early multiple sclerosis: a longitudinal study.* Eur J Neurol, 2016. **23**(9): p. 1482-6.
320. L., K., *Fatigue is intrinsic to multiple sclerosis (MS) and is the most commonly reported symptom of the disease.* Mult Scler. 2006;12:367–8.
321. Kaynak, H., et al., *Fatigue and sleep disturbance in multiple sclerosis.* Eur J Neurol, 2006. **13**(12): p. 1333-9.
322. Tryfonos, C., et al., *Dietary Supplements on Controlling Multiple Sclerosis Symptoms and Relapses: Current Clinical Evidence and Future Perspectives.* Medicines (Basel), 2019. **6**(3).
323. Sateia, M.J., *International classification of sleep disorders-third edition: highlights and modifications.* Chest, 2014. **146**(5): p. 1387-1394.
324. Popp, R.F., et al., *Daytime sleepiness versus fatigue in patients with multiple sclerosis: A systematic review on the Epworth sleepiness scale as an assessment tool.* Sleep Med Rev, 2017. **32**: p. 95-108.
325. Braga, D.M., et al., *Positive correlation between functional disability, excessive daytime sleepiness, and fatigue in relapsing-remitting multiple sclerosis.* Arq Neuropsiquiatr, 2016. **74**(6): p. 433-8.
326. Al-Dughmi, M. and C.F. Siengsukon, *The relationship between sleep quality and perceived fatigue measured using the Neurological Fatigue Index in people with Multiple Sclerosis.* Neurol Res, 2016. **38**(11): p. 943-949.

327. Attarian, H.P., et al., *The relationship of sleep disturbances and fatigue in multiple sclerosis.* Arch Neurol, 2004. **61**(4): p. 525-8.
328. Brass, S.D., et al., *Sleep disorders in patients with multiple sclerosis.* Sleep Med Rev, 2010. **14**(2): p. 121-9.
329. Bamer, A.M., et al., *Prevalence of sleep problems in individuals with multiple sclerosis.* Mult Scler, 2008. **14**(8): p. 1127-30.
330. Garland, S.N., et al., *Factors Associated with Poor Sleep in Older Adults with Multiple Sclerosis.* Int J Behav Med, 2017. **24**(6): p. 937-945.
331. Boe Lunde, H.M., et al., *Poor sleep in patients with multiple sclerosis.* PLoS One, 2012. **7**(11): p. e49996.
332. Hughes, A.J., K.M. Dunn, and T. Chaffee, *Sleep Disturbance and Cognitive Dysfunction in Multiple Sclerosis: a Systematic Review.* Curr Neurol Neurosci Rep, 2018. **18**(1): p. 2.
333. Amtmann, D., et al., *People with multiple sclerosis report significantly worse symptoms and health related quality of life than the US general population as measured by PROMIS and NeuroQoL outcome measures.* Disabil Health J, 2018. **11**(1): p. 99-107.
334. Strober, L.B., *Fatigue in multiple sclerosis: a look at the role of poor sleep.* Front Neurol, 2015. **6**: p. 21.
335. Benedict, R.H. and R. Zivadinov, *Risk factors for and management of cognitive dysfunction in multiple sclerosis.* Nat Rev Neurol, 2011. **7**(6): p. 332-42.
336. Chiaravalloti, N.D. and J. DeLuca, *Cognitive impairment in multiple sclerosis.* Lancet Neurol, 2008. **7**(12): p. 1139-51.
337. Benedict, R.H., et al., *Predicting quality of life in multiple sclerosis: accounting for physical disability, fatigue, cognition, mood disorder, personality, and behavior change.* J Neurol Sci, 2005. **231**(1-2): p. 29-34.
338. Peyser, J.M., et al., *Guidelines for neuropsychological research in multiple sclerosis.* Arch Neurol, 1990. **47**(1): p. 94-7.
339. Rao, S.M., et al., *Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction.* Neurology, 1991. **41**(5): p. 685-91.
340. Moccia, M., et al., *Cognitive impairment at diagnosis predicts 10-year multiple sclerosis progression.* Mult Scler, 2016. **22**(5): p. 659-67.
341. Ruano, L., et al., *Age and disability drive cognitive impairment in multiple sclerosis across disease subtypes.* Mult Scler, 2017. **23**(9): p. 1258-1267.
342. Kalmar, J.H., et al., *The relationship between cognitive deficits and everyday functional activities in multiple sclerosis.* Neuropsychology, 2008. **22**(4): p. 442-9.
343. Briken, S., et al., *Effects of exercise on fitness and cognition in progressive MS: a randomized, controlled pilot trial.* Mult Scler, 2014. **20**(3): p. 382-90.
344. Leavitt, V.M., et al., *Aerobic exercise increases hippocampal volume and improves memory in multiple sclerosis: preliminary findings.* Neurocase, 2014. **20**(6): p. 695-7.
345. Sandroff, B.M., et al., *Randomized controlled trial of physical activity, cognition, and walking in multiple sclerosis.* J Neurol, 2014. **261**(2): p. 363-72.
346. Motl, R.W., et al., *Physical activity and cognitive function in multiple sclerosis.* J Sport Exerc Psychol, 2011. **33**(5): p. 734-41.
347. Sandroff, B.M. and R.W. Motl, *Fitness and cognitive processing speed in persons with multiple sclerosis: a cross-sectional investigation.* J Clin Exp Neuropsychol, 2012. **34**(10): p. 1041-52.
348. Beier, M., et al., *Improved physical fitness correlates with improved cognition in multiple sclerosis.* Arch Phys Med Rehabil, 2014. **95**(7): p. 1328-34.
349. Dyall, S.C., *Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA.* Front Aging Neurosci, 2015. **7**: p. 52.

350. Burckhardt, M., et al., *Omega-3 fatty acids for the treatment of dementia.* Cochrane Database Syst Rev, 2016. **4**: p. CD009002.
351. Krupp, L.B., et al., *The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus.* Arch Neurol, 1989. **46**(10): p. 1121-3.
352. Buysse, D.J., et al., *The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research.* Psychiatry Res, 1989. **28**(2): p. 193-213.
353. Johns, M.W., *A new method for measuring daytime sleepiness: the Epworth sleepiness scale.* Sleep, 1991. **14**(6): p. 540-5.
354. Beck, A.T., et al., *Screening for major depression disorders in medical inpatients with the Beck Depression Inventory for Primary Care.* Behav Res Ther, 1997. **35**(8): p. 785-91.
355. Tombaugh, T.N., *A comprehensive review of the Paced Auditory Serial Addition Test (PASAT).* Arch Clin Neuropsychol, 2006. **21**(1): p. 53-76.
356. Veauthier, C., et al., *Poor Sleep in Multiple Sclerosis Correlates with Beck Depression Inventory Values, but Not with Polysomnographic Data.* Sleep Disord, 2016. **2016**: p. 8378423.
357. Mohr, D.C., et al., *Identification of Beck Depression Inventory items related to multiple sclerosis.* J Behav Med, 1997. **20**(4): p. 407-14.
358. Solaro, C., et al., *Depressive Symptoms Correlate with Disability and Disease Course in Multiple Sclerosis Patients: An Italian Multi-Center Study Using the Beck Depression Inventory.* PLoS One, 2016. **11**(9): p. e0160261.
359. Chervin, R.D., et al., *Comparison of the results of the Epworth Sleepiness Scale and the Multiple Sleep Latency Test.* J Psychosom Res, 1997. **42**(2): p. 145-55.
360. Cardinal, K.S., et al., *A longitudinal fMRI study of the paced auditory serial addition task.* Mult Scler, 2008. **14**(4): p. 465-71.
361. Fischer, J.S., et al., *The Multiple Sclerosis Functional Composite Measure (MSFC): an integrated approach to MS clinical outcome assessment. National MS Society Clinical Outcomes Assessment Task Force.* Mult Scler, 1999. **5**(4): p. 244-50.
362. Morrow, S.A., H. Rosehart, and A.M. Johnson, *Diagnosis and quantification of cognitive fatigue in multiple sclerosis.* Cogn Behav Neurol, 2015. **28**(1): p. 27-32.
363. Walker, L.A., et al., *Detecting cognitive fatigue in multiple sclerosis: method matters.* J Neurol Sci, 2012. **316**(1-2): p. 86-92.
364. Brooks, J.B., et al., *Paced auditory serial addition test (PASAT): a very difficult test even for individuals with high intellectual capability.* Arq Neuropsiquiatr, 2011. **69**(3): p. 482-4.

Appendices

Table 43. Gait Parameters of MS Population (EDSS: 2.8 ± 0.3, Age: 36.2 ±0.5) and controls Adapted from the study of Givon et al. (2009) [209]

Variable	Mean ± S.E.	
	MS patients	Healthy subjects
Velocity (cm/s)	85.5 ± 3.0	138.6 ± 4.1
Cadence (steps/min)	94.4 ± 2.1	115.2 ± 1.8
Step length R (cm)	46.0 ± 1.1	72.2 ± 1.7
Step length L (cm)	44.6 ± 1.0	72.1 ± 1.7
Step time R (s)	0.67 ± 0.02	0.57 ± 0.02
Step time L (s)	0.65 ± 0.02	0.52 ± 0.02
Double support R (%GC)	24.6 ± 0.67	23.9 ± 0.8
Double support L (%GC)	24.9 ± 0.67	23.6 ± 0.8
Single support R (%GC)	37.3 ± 0.1.0	38.5 ± 0.4
Single support L (%GC)	36.7 ± 0.78	37.7 ± 0.4

Table 44. Means (standard deviation) of the stride parameters and gait kinematics of MS group with foot-drop (age: 49.4 ±7 yrs, height: 1.71 ± 0.08 m) and HC group (age: 49.5 ± 12.1 yrs; height: 1.72 ± 0.07 m) walking at the same speed.
Adapted from the study of Linden et al. (2014) [260]

	MS group (n = 22)	**HC SSWS (n = 11)**
Walking speed (m/s)	0.74 (0.20)	1.45 (0.18)
Stride length (m)	0.92 (0.16)	1.40 (0.10)
Cadence (steps/min)	96 (15)	124 (11)
Double support (s)	0.37 (0.13)	0.19 (0.05)
Dorsiflexion at IC(º)	- 4.6 (5.6)	3.2 (1.6)
Peak plantar flexion (º)	-8.3 (6.8)	-16.0 (4.2)
Peak dorsiflexion in swing (º)	2.4 (6.8)	5.8 (1.1)
Peak knee extension (º)	-1.9 (6.7)	0.8 (5.9)
Peak knee flexion in swing (º)	42.6 (11.7)	59.7 (6.0)
Peak hip flexion (º)	27.6 (6.8)	35.8 (8.8)
Peak hip extension (º)	-6.2 (5.7)	-9.1 (7.3)
Hip range of motion (º)	33.8 (6.6)	44.9 (4.0)
Peak pelvic obliquity in swing (º)	2.6 (2.6)	2.3 (1.5)

IC: initial contact; SSWS: self-selected walking speed

Table 45. Kinematics on lower extremity joint (°) at normal speed of 40 healthy subjects

Adapted from the study of Kwon, J. W et al. (2015) [261]

Joint	Plane	Angle	Normal Speed
Hip Joint	Sagittal	Flexion peak	28.6 ± 5.20
		Extension peak	12.9 ± 5.82
	Frontal	Abduction peak	4.15 ± 4.05
		Adduction peak	8.62 ± 3.21
	Transverse	External rotation peak	16.0 ±12.4
		Internal rotation peak	8.20 ±11.3
Knee joint	Sagittal	Flexion peak	54.3 ± 6.83
		Extension peak	1.90 ± 4.56
	Frontal	Abduction peak	15.8 ± 10.7
		Adduction peak	−2.35 ± 4.45
	Transverse	External rotation peak	7.70 ± 5.74
		Internal rotation peak	3.58 ± 8.58
Ankle joint	Sagittal	Dorsiflexion peak	12.7 ± 3.26
		Plantarflexion peak	12.9 ± 9.11
	Frontal	Abduction peak	4.95 ± 2.77
		Adduction peak	− 0.90 ± 1.99
	Transverse	External rotation peak	- 4.59 ± 12.4
		Internal rotation peak	25.6 ± 10.2

Taylor & Francis
Taylor & Francis Group

Postgraduate Medicine

ISSN: 0032-5481 (Print) 1941-9260 (Online) Journal homepage: https://www.tandfonline.com/loi/ipgm20

Associations between functional capacity, isokinetic leg strength, sleep quality and cognitive function in multiple sclerosis patients: a cross-sectional study

Panayiotis Aristotelous, Manos Stefanakis, Marios Pantzaris, Constantinos Pattichis, Georgios M Hadjigeorgiou & Christoforos D Giannaki

To cite this article: Panayiotis Aristotelous, Manos Stefanakis, Marios Pantzaris, Constantinos Pattichis, Georgios M Hadjigeorgiou & Christoforos D Giannaki (2019): Associations between functional capacity, isokinetic leg strength, sleep quality and cognitive function in multiple sclerosis patients: a cross-sectional study, Postgraduate Medicine, DOI: 10.1080/00325481.2019.1662271

To link to this article: https://doi.org/10.1080/00325481.2019.1662271

Accepted author version posted online: 30 Aug 2019.
Published online: 01 Oct 2019.

POSTGRADUATE MEDICINE
https://doi.org/10.1080/00325481.2019.1662271

Taylor & Francis
Taylor & Francis Group

CLINICAL FOCUS: NEUROLOGICAL & PSYCHIATRIC DISORDERS
ORIGINAL RESEARCH

Associations between functional capacity, isokinetic leg strength, sleep quality and cognitive function in multiple sclerosis patients: a cross-sectional study

Panayiotis Aristotelous[a], Manos Stefanakis[a], Marios Pantzaris[b], Constantinos Pattichis[c], Georgios M Hadjigeorgiou[d] and Christoforos D Giannaki[a]

[a]Department of Life and Health Sciences, University of Nicosia, Nicosia, Cyprus; [b]The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; [c]Department of Computer Sciences, University of Cyprus, Nicosia, Cyprus; [d]Medical School, University of Cyprus, Nicosia, Cyprus

ABSTRACT

Objectives: Recent evidence suggests an association between functional capacity and cognitive function, at least in older adults. The aim of this cross-sectional study was to examine the association between cognitive function, functional capacity, isokinetic leg strength, health-related quality of life (HRQOL), sleep quality, body fat, handgrip strength, and fatigue among a sample of MS patients.

Methods: Fifty-one relapsing-remitting MS patients (age: 38.4 ± 7.1 yrs; 30 females) were recruited and agreed to participate in this study. Cognitive function was assessed by the Paced Auditory Serial Addition Test (PASAT). Functional capacity was examined using various functional tests commonly used in MS patients. Maximal voluntary unilateral leg strength was assessed using isokinetic dynamometer. Isometric handgrip strength was assessed by a dynamometer. Total body and visceral fat levels were assessed via bioelectrical impedance analyzers. Finally, the patients' HRQOL, sleep quality, and fatigue levels were evaluated using specific questionnaires.

Results: A significant association was found between the PASAT score and the performance score in various functional capacity tests ($p < 0.050$). On the other hand, a weak but statistically significant association was found between the PASAT score and isokinetic strength of knee extensors ($r = 0.319$, $p = 0.022$) and knee flexors ($r = 0.354$ $p = 0.011$). Poor sleep quality was associated with lower performance in all the functional capacity tests examined ($p < 0.05$) whilst was negatively associated with the PASAT score ($r = -0.334$, $p = 0.017$). The multivariate regression analysis revealed that the performance on the TUG test was a significant predictor of cognitive function.

Conclusion: Based on the results of this study, functional capacity was found to be associated with both impaired cognitive performance and low HRQOL in MS patients. In addition, an association between sleep quality and cognitive performance was revealed, confirming existing literature. Functional capacity as assessed by the TUG test emerged as the best predictor of cognitive function.

ARTICLE HISTORY
Received 29 March 2019
Accepted 27 August 2019

KEYWORDS
Cognition; sleep; isokinetic dynamometry; quality of life

Introduction

Multiple sclerosis (MS) is a chronic, progressive inflammatory and neurodegenerative disease of the Central Nervous System (CNS) characterized by demyelination and axon loss resulting in a variety of motor symptoms and disabilities [1]. It is noteworthy that MS is one of the most common neurological diseases and a growing issue worldwide, whilst it is considered to be the leading neurological cause of disability in young adults, associated with both psychological and physical/functional impairments [2].

According to the literature, cognitive impairment is present in 40–65% of the MS patients [3], whilst it is reported to be a predictor of long-term disability in the current population [4]. Despite the fact that rates of cognitive impairment are very high among the MS population, little is known about the natural history of cognitive impairment in MS. The interpretation of available data is complicated due to different disease subtype, stage, duration, or disability level [5].

According to the literature, many factors have been reported to be associated with worse cognitive impairment the MS patients including brain magnetic resonance imaging lesion volumes [6] and atrophy [3], sleep [7], depression, anxiety, and fatigue [3]. Interestingly, recent data indicate an association between functional capacity and physical fitness levels and cognition in non-MS populations, and especially in the elderly [8]. In the MS patients, cognitive impairment is present from the clinical onset of the disease, regardless of MS subtypes, and its severity seems to be more related to patients' age and physical disability [9].Although it seems that some aspects of cognition could predict the functional status in MS [10], in our knowledge, there are inconsistent data regarding the correlation between cognition, functional capacity and fitness levels in MS patients.

The MS patients are subjects of lower health-related quality of life (HRQOL) levels compared to healthy individuals of the same age [11].Several factors (including social, physiological, and psychological) contribute negatively to the low HRQOL of MS patients. According to the literature, MS patients are suffering from fatigue, depression, poor sleep, visual disturbances, muscle weaknesses,

CONTACT Georgios M Hadjigeorgiou hadjigeorgiou.georgios@ucy.ac.cy Medical School, University of Cyprus, Nicosia, Cyprus

loss of coordination, and sensory impairments [12], all of which are significant contributing factors to a lower HRQOL.

In addition, it seems that lower functional capacity levels of the MS patients contribute to the lowerHRQOL. For instance, balance and gait abnormalities, muscle weakness, physical-related fatigue, and other functional impairments and deficits are well documented as major factors which affect the HRQOL of MS patients [13]. Adequate functional capacity is required for MS patients in order to be able to independently perform activities of daily living. In particular, MS patients in order to be able to function independently in daily activities such as walking or stair climbing must present adequate levels of lower extremity muscle strength [14].

Although non-pharmacological therapies (e.g., cognitive rehabilitation) have shown some positive effects on cognition, these approaches generally focus on strategies that are compensatory rather than restorative [15]. As research has only just recently started focusing on evaluating cognitive function in MS, there is a need for a more comprehensive understanding of how other secondary symptoms of the disease interact with cognitive function and whether or not they can predict an individual's susceptibility to cognitive fatigue.

The aim of this cross-sectional study was to examine the association between cognitive function (information processing speed) and functional capacity, isokinetic strength of knee flexors and knee extensors and various factors of HRQOLand fatigue among a sample of MS patients.

Materials and methods

Participants

Fifty-one relapsing-remitting MS patients (age: 38.4 ± 7.1 yrs; 30 females) were recruited from the neurology clinics of the Cyprus institute of Neurology and Genetics and agreed to participate in this study. This study was conducted between September 2015 and March 2016. MS was diagnosed according to the revised McDonald criteria [16], whilst the disability level was assessed by the expanded disability status scale (EDSS) [17]. Inclusion criteria for this study were: patients with relapsing-remitting MS, MRI showing lesions consistent with MS and at least one documented clinical relapse within the 18 months before enrollment and with an EDSS score below 5.0. Exclusion criteria included severely impaired visual function, severe psychiatric disorder, severe arthritis of the knee or hips, pregnancy and other neurological or vestibular disorders that would prevent the patient from completing the functional examination. All patients were given a full explanation of the purpose and procedures of the study and gave their written consent. Ethical approval was obtained by the national ethics committee.

Anthropometry and body composition assessment

Anthropometric characteristics including height and body weight were measured using a standing stadiometer (Seca model 720, Germany) and an analogue scale (Secamodel 755, Germany) respectively. Body mass index (BMI) was calculated as body weight divided by height squared. Total body fat and trunk fat were both assessed by bioelectrical impedance analyzers (Bodystat, Quadscan 4000 and Tanita AB 140 Viscan, respectively). The later analyzer provided also abdominal girth data of the patients in cm.

Questionnaires

All questionnaires were completed using the interview method. The patients' health-related quality of life (HRQOL) levels were assessed using the SF-36 questionnaire which evaluates mental and physical health [18]. Fatigue levels were assessed using the fatigue severity scale (FSS) [19]. Finally, sleep quality was evaluated by the Pittsburgh sleep quality index (PSQI) [20].

Cognitive function was assessed by the Paced Auditory Serial Addition Test (PASAT), modified with a 3s interstimulus interval, as an attempt to make the PASAT test more user-friendly [21]. PASAT could assess auditory information processing speed, flexibility, calculation ability and has been shown to be a sensitive and valid measure to objectively quantify cognitive fatigue in MS [22,23]. PASAT is a very difficult test even for individuals with high intellectual capability [24]. In order to avoid making the PASAT a negative experience for patients [25] and consequently affecting their performance on other tests, patients were well informed about the potential effects of the specific test prior to testing, verified fundamental mathematics ability and agreed to participate.

Functional capacity assessment

The patients' functional capacity was assessed using various tests, commonly used in the MS population. In particular, the patients performed two sit-to-stand tests (STS-5 and STS-60) [26], the six- minute walk test [27] and the timed up and go test (TUG) [28].The STS-5 requires from the patients to perform five sit-to-stand cycles on a standard chair (0.43-m height and 0.45-m width) as fast as possible measured in seconds and can be used as an indicator of the patient's lower extremities strength [26].Patients were not permitted to use their upper extremities and armrests. The STS time was recorded using a stopwatch to the nearest 10th of a second. The STS-60 is a similar test that requires the patients to stand up and sit down to a chair as many times as possible in 60 s. The score is the total number of sit to stand cycles within 60 s (the number achieved in 30 s was recorded also) and it is an index of muscular endurance. The six-minute walk test requires from the patients to cover as much distance as they can within a six-minute period (the covered distance was recorded in meters) [27]. The TUG test requires from the patients to rise from a chair, walk to a line on the floor 3 m away, turn around, walk back to the chair and sit down again [28]. A faster time indicates a better functional performance.

Handgrip strength assessment

Maximal handgrip strength (kg) of the right hand was measured with a portable handgrip dynamometer (Takei, Tokyo, Japan). Participants were seated in a standard chair without armrests (seat height 0.43 m, seat width 0.45 m). They were instructed to

sit in the middle of the chair, with their back straight, with the shoulder in neutral position and elbow in 90° flexion position holding the dynamometer, not touching the trunk. The handle was adjusted to the participant's hand size. Three maximal effort trials lasting 4 to 5 s interspersed with 60-s rests were performed and the highest value was retained for the analysis.

Isokinetic dynamometry

Maximal voluntary strength of the right leg was assessed using an isokinetic dynamometer (model 770, CSMI Humac Norm, MA, USA) which allowed recording of instantaneous isokinetic torque. Prior to isokinetic dynamometer testing, the patients performed a five-minute warm-up at a low intensity on a cycle ergometer followed by 5 consecutive isokinetic repetitions (50% of peak effort). Strength tests were performed on an adjustable chair (backward inclined 5°) in a seated position. The alignment between the knee joint and the dynamometer rotational axis was adjusted to correspond to the femoral condyle axis and the lever arm was secured 10 cm above the ankle. The upper leg (thigh), hip and shoulders were secured to the equipment with safety straps [29]. The range of motion was set at 0–90° (0°corresponding to full knee extension).Before each test, the gravity compensation procedure was performed according to the manufacturer's instructions. Following three submaximal knee isokinetic trial contractions and 1-min rest period, bilateral isokinetic (concentric/concentric) flexion and extension of the knee at 60°/s were performed five times. Patients had periods of rest between the sessions and verbal encouragement was standardized. The highest of five isokinetic extension and flexion torques (Nm) were selected as the peak dynamic torque[isokinetic peak torque knee extensors (IPT-KE); isokinetic peak torque knee flexors (IPT-KF)].

Statistical analysis

In this study, descriptive statistics were used to calculate the mean and standard deviation of patient's characteristics. Simple linear regression was used to examine the relationship between the examined variables and the cognitive, functional and mental status of the patients. Variables which proved significant predictors were used in a Multiple linear regression analysis (stepwise method) to determine the parameters that affect the variability in PASAT, SF-36 PCS, and SF-36 MCS scores. All analyses were carried out using the Statistical Package for the Social Sciences software (SPSS for Windows, version 19.0, Chicago, Illinois). Data are presented as mean ± SD and the level for statistical significance was set at $p \leq 0.05$.

Results

All participants were able to perform the functional testing and assessments without injuries and adverse effects. The patients' characteristics, anthropometric and body composition data are presented in Table 1. Pool data about functional capacity, handgrip strength and isokinetic strength of knee extensors and flexors are presented in Table 2. Cognitive performance, health-related quality of life, fatigue, and sleep quality data are presented in Table 3.

Table 1. Patient's anthropometric characteristics and body composition.

Variables	
Number of Subjects	51
Female/Male	30/21
Age (yr)	38.4 ± 7.1
Weight (kg)	71.8 ± 15.7
Height (cm)	166.2 ± 7.9
BMI	25.8 ± 5.1
Body fat (%)	29.2 ± 8.1
Trunk fat (%)	32.5 ± 10.4
EDSS score	2.38 ± 1.04
	Median: 2.0
	Range: 3.0

All data are mean ± SD. Abbreviations: BMI, body mass index; EDSS, expanded disability status scale score.

Table 2. Functional performance, handgrip strength and isokinetic strength of knee extensors and flexors.

Functional performance	
STS-5 (sec)	12.4 ± 2.9
STS-30 (rep)	12.4 ± 2.5
STS-60 (rep)	24.8 ± 5.1
TUG (sec)	8.7 ± 1.6
6MWT (m)	437.1 ± 100.8
Isokinetic strength of knee extensors and flexors	
IPT-KF (N·m)	66.2 ± 28.7
IPT-KE (N·m)	103.2 ± 41.4
Isometric handgrip strength	
Right hand (kg)	28.8 ± 9.9

All data are mean ± SD. Abbreviations: STS-5, sit-to-stand test 5-repetitions; STS-60, sit-to-stand test 60 s; TUG, timed up and go test; IPT-KE, isokinetic peak torque knee extensors; IPT-KF, isokinetic peak torque knee flexors.

Table 3. Cognitive performance, health-related quality of life, fatigue and sleep quality.

PASAT	67.8 ± 28.9
MCS	70.5 ± 19.1
PCS	85.0 ± 25.6
PSQI	3.7 ± 3.6
FSSI	4.6 ± 1.2

All data are mean ± SD. Abbreviations: PASAT, Paced Auditory Serial Addition Test; MCS, mental component score; PCS, physical component score; PSQI, Pittsburgh Sleep Quality Index; FSSI, fatigue severity scale index.

Linear regression showed that PASAT score was associated with the performance score in various functional capacity tests such as the TUG ($R = 0.473$, $R^2 = 0.223$, $p = 0.001$), the six-minutes walk test ($R = 0.412$, $R^2 = 0.170$, $p = 0.003$), the PSQI ($R = 0.334$, $R^2 = 0.111$, $p = 0.017$) and the STS-5 test ($R = 0.315$, $R^2 = 0.099$, $p = 0.025$) (Table 4). In addition, weak but significant associations were found between the PASAT score and isokinetic strength of knee extensors ($R = 0.319$, $R^2 = 0.102$, $p = 0.022$ for the right leg and $R = 0.267$, $R^2 = 0.071$, $p = 0.050$ for the left leg) and knee flexors ($R = 0.354$, $R^2 = 0.126$, $p = 0.011$ for the right leg and $R = 0.317$, $R^2 = 0.101$, $p = 0.023$ for the left leg) (Table 4). In contrast, PASAT score was not predicted by any of the body composition related-parameters or with age, HRQOL, and fatigue index ($p > 0.050$).

The physical component summary (PCS) of the HRQOL questionnaire was significantly predicted by the performance score in the 6MWT ($R = 0.323$, $R^2 = 0.104$, $p = 0.021$), STS-5 ($R = 0.458$, $R^2 = 0.209$, $p = 0.001$), STS-60 ($R = 0.457$, $R^2 = 0.209$, $p = 0.001$), TUG ($R = 0.398$, $R^2 = 0.158$, $p = 0.004$) and FSS index ($R = 0.561$,

Table 4. Linear regression between the variables examined and PASAT score.

	Constant	Coefficient	R	R^2	P Value
TUG	141.86	− 8.504	0.473	0.223	0.001
6MWT	16.005	0.119	0.412	0.170	0.003
PSQI	77.797	−2.672	0.334	0.111	0.017
STS 5	106.176	−3.080	0.315	0.099	0.025
IPT-KE(R Leg)	44.775	0.223	0.319	0.102	0.022
IPT-KE(L Leg)	47.818	0.201	0.267	0.071	0.050
IPT-KF(R Leg)	44.140	0.358	0.354	0.126	0.011
IPT-KF(L Leg)	42.681	0.386	0.317	0.101	0.023
Abdominal girth	74.894	−0.074	0.038	0.001	0.793
Fat (%)	81.395	−0.463	0.129	0.017	0.368
Visceral fat	78.994	−0.343	0.123	0.015	0.389
Age	86.506	−0.485	0.118	0.014	0.409
SF36 TOTAL	63.478	0.050	0.040	0.002	0.783
PCS	64.998	0.033	0.030	0.001	0.836
MCS	45.146	0.322	0.211	0.045	0.137
IHS (R side)	57.983	0.406	0.143	0.020	0.339
IHS (L side)	59.199	0.364	0.123	0.015	0.412
ESSS	86.601	−7.874	0.284	0.081	0.043
FSSI	89.913	−0.527	0.199	0.04	0.162

Abbreviations:PASAT, Paced Auditory Serial Addition Test; MCS, mental component score; PCS, physical component score; 6MWT, six-minutes walk test; PSQI, Pittsburgh Sleep Quality Index; FSSI, fatigue severity scale index; IPT-KE, isokinetic peak torque knee extensors; IPT-KF, isokinetic peak torque knee flexors; IHS, isometric handgrip strength; ESSS, expanded disability status scale score.

Table 5. Linear regression between the variables examined and PCS score.

	Constant	Coefficient	R	R^2	P Value
6MWT	49.033	0.082	0.323	0.104	0.021
STS5	134.423	−3.971	0.458	0.209	0.001
STS60	28.737	2.263	0.457	0.209	0.001
TUG	140.199	−6.342	0.398	0.158	0.004
FSS	140.156	−1.318	0.561	0.314	0.001
IPT-KE(R Leg)	61.640	0.226	0.365	0.133	0.008
IPT-KE (L Leg)	59.449	0.256	0.384	0.147	0.005
IPT-KF (R Leg)	61.720	0.351	0.393	0.154	0.004
IPT-KF (L Leg)	60.738	0.372	0.345	0.119	0.013
PSQI	87.521	−0.677	0.095	0.009	0.505
EDSS	105.370	−8.550	0.348	0.121	0.012
HIS (R side)	61.686	0.763	0.289	0.083	0.049
HIS (L side)	61.313	0.777	0.282	0.079	0.055

Abbreviations: PSQI, Pittsburg Sleep Quality Index; 6MWT, six-minute walk test; STS-5, sit-to-stand test 5-repetitions; TUG, timed up and go test; IHS, isometric handgrip strength; FSS, fatigue severity scale; EDSS, expanded disability status scale; IPT-KE, isokinetic peak torque knee extensors; IPT-KF, isokinetic peak torque knee flexors.

R^2 = 0.314, p = 0.001) (Table 5). Isokinetic strength of knee extensors (R = 0.365, R^2 = 0.133, p = 0.008 for the right leg and R = 0.384, R^2 = 0.147, p = 0.005 for the left leg) and knee flexors (R = 0.393, R^2 = 0.154, p = 0.004 for the right leg and R = 0.345, R^2 = 0.119, p = 0.013 for the left leg) were also found significant predictors of PCS of HRQOL (Table 5).

MCS of the HRQOL questionnaire score was significantly predicted by STS-60 (R = 0.315, R^2 = 0.099, p = 0.025), 6MWT (R = 0.329, R^2 = 0.108, p = 0.018), handgrip strength (R = 0.335, R^2 = 0.113, p = 0.021 for both the right hand and the left hand), isokinetic strength of knee extensors (R = 0.316, R^2 = 0.100, p = 0.024 for the right leg and R = 0.358, R^2 = 0.128, p = 0.010 for the left leg) isokinetic strength of knee flexors (R = 0.379, R^2 = 0.143, p = 0.006 for the right leg and R = 0.378, R^2 = 0.143, p = 0.006 for the left leg) STS-5 (R = 0.305, R^2 = 0.093, p = 0.029), TUG (R = 0.283, R^2 = 0.080, p = 0.044), FSS index (R = 0.769, R^2 = 0.592, p = 0.001) and PSQI (R = 0.472, R^2 = 0.222, p = 0.001) (Table 6).

Table 6. Linear regression between the variables examined and MCS score.

	Constant	Coefficient	R	R^2	P Value
STS60	41.848	1.154	0.315	0.099	0.025
6MWT	43.376	0.062	0.329	0.108	0.018
IHS(R side)	50.873	0.653	0.335	0.113	0.021
IHS(L side)	50.126	0.680	0.335	0.112	0.021
IPT-KE (R Leg)	55.580	0.145	0.316	0.100	0.024
IPT-KE (L Leg)	52.905	0.177	0.358	0.128	0.010
IPT-KF (R Leg)	53.912	0.251	0.379	0.143	0.006
IPT-KF (L Leg)	50.831	0.302	0.378	0.143	0.006
STS5	94.990	−1.964	0.305	0.093	0.029
TUG	99.678	−3.347	0.283	0.080	0.044
FSS Index	126.580	−12.054	0.769	0.592	0.001
PSQI	79.783	−2.479	0.472	0.222	0.001
EDSS	86.536	−6.711	0.369	0.136	0.008

Abbreviations: PSQI, Pittsburg Sleep Quality Index; 6MWT, six-minute walk test; STS-5, sit-to-stand test 5-repetitions; TUG, timed up and go test; IHS, isometric handgrip strength; FSS, fatigue severity scale; EDSS, expanded disability status scale; IPT-KE, isokinetic peak torque knee extensors; IPT-KF, isokinetic peak torque knee flexors.

PSQI, six-minute walk test, STS-5, STS-60, TUG, IHS, FSS, EDSS, IPT-KE and IPT-KF, body composition variables, age and gender were included as independent variables in the linear multiple regression analysis to identify the factors associated with cognitive function as indicated by the performance in the PASAT questionnaire, and physical and mental function as indicated by the PCS and MCS of HRQOL. In relation to the PASAT score the only significant predictor that remained was TUG (R = 0.473, R^2 = 0.223, p = 0.001) (Figure 1). All the other variables were rejected by the model. Two different models remained significant for PCS. The first included only the FSS (R = 0.561, R^2 = 0.314, p = 0.001) as significant predictor while the second included FSS and age (R = 0.643, R^2 = 0.414, p = 0.001) (Table 7). In addition, two models explained the variability of the MCS. The first included only FSS (R = 0.769, R^2 = 0.592, p = 0.001) (Figure 2) as a significant predictor and the second included FSS and TBW (R = 0.790, R^2 = 0.624, p = 0.001) (Table 8).

Discussion

The findings from the current study reveal an association between cognitive function (information processing speed) as expressed by the PASAT score and functional capacity and sleep quality among this sample of MS patients. The most significant predictor of cognitive function (information processing speed) was the TUG test. However, we should note that the findings of the current study should be treated with caution as the design of the study and the statistical analysis used does not necessarily imply a causal relationship between the examined variables. In addition, the functional tests used in the current study were found to predict only a small percentage of PASAT scoring, thus probably, there are other factors that influence both PASAT and functional capacity in the MS patients.

Cognitive impairment is associated with deficits in memory, attention, information processing speed, and language among patients with MS [30]. These deficits have been reported in up to 65% of the patients at some point in the course of the disease [31,32].In the current study,45% of relapsing remitting MS patients were classified as cognitively impaired.

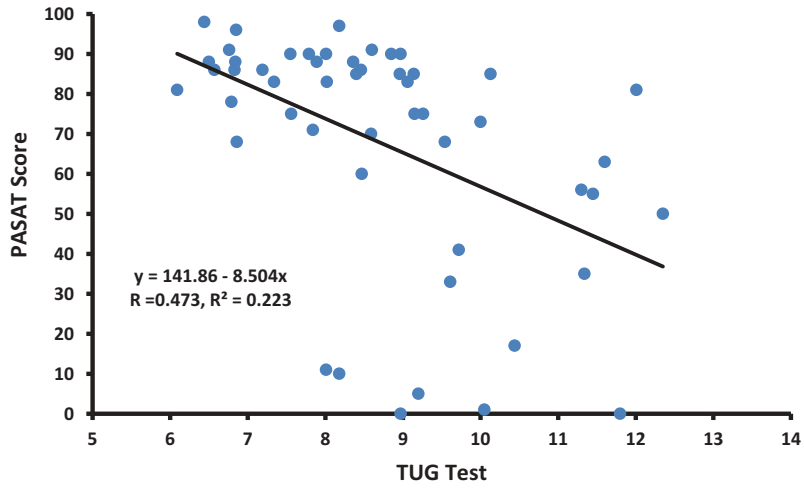

Figure 1. Multiple linear regression revealed TUG as the only significant predictor of PASAT score.

Table 7. Multiple linear regression models for the PCS score.

	Constant	Coefficients	R	R^2	P Value
Model 1 (FSS)	140.156	−1.318	0.561	0.314	0.001
Model 2 (FSS, Age)	179.108	−1.176 (FSS) -1.167 (Age)	0.643	0.414	0.001

Perceived cognitive impairment represents a significant problem among MS patients and can impact individuals' expectations for how they carry out daily work, personal and family activities [33].

Although improved physical fitness and exercise appears to correlate positively with executive function scores [34,35], a positive effect of physical activity on cognition in persons with MS is equivocal because this relation is confounded by factors that may have influenced the outcomes [36]. Physical activity could be a feasible approach to protect or improve cognition, but a strong scientific evidence for its effectiveness in this population is lacking. According to the literature, the potential mechanisms by which increased functional capacity and physical fitness could affect cognition include increased blood flow to the brain, improved cardio metabolic health, reduction of depressive symptoms, improved sleep, and reduction in chronic inflammation levels [37].

TUG test is considered a reliable and practical test in assessing functional mobility, cognition and gait speed in MS patients [38]. TUG time is associated with performance on global cognition, executive function, and memory tests in non-MS population [39]. Lower extremity strength, muscle balance, and endurance in MS patients usually investigated by STS-5 and STS-60 [26] which are considered reliable, low cost, and easy to implement. In the current study cognition was found to be associated with the performance in various functional capacity tests, more specific with STS5, STS-60, 6MWT, and TUG tests, results that despite the equivocal effect of functional capacity to cognition seems to be reliable. In addition, associations were found also between the PASAT score and the performance in leg strength as assessed by the isokinetic dynamometer.

Table 8. Multiple linear regression models for the MCS score.

	Constant	Coefficients	R	R^2	P Value
Model 1 (FSS)	126.620	−1.340	0.769	0.592	0.001
Model 2 (FSS, TBW)	86.558	−1.178 (FSS) 0.649 (TBW)	0.790	0.624	0.001

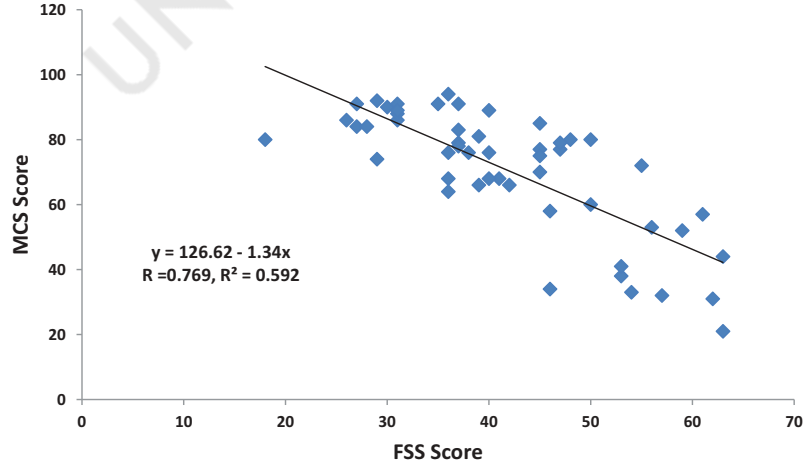

Figure 2. Multiple linear regression revealed FSS as a significant predictor of MCS score.

In this study, it was revealed that TUG test is the best predictor for cognitive function confirming data on non-MS elderly individuals [40]. TUG measures balance and physical mobility, which capture basic transfer skills [28]. The TUG test could be useful in terms of early prognosis of cognitive decline in MS; however, more research is needed in order to extract safer conclusions on this issue.

Limitations in hand and arm function are present in more than 50% of MS patients including patients with low disease severity [41].Hand grip strength is often used to evaluate upper limb impairments [42]. Grip strength deficit is an debilitating factor in performing various activities of daily living such as drinking, eating and writing [42,43], which can affect aspects of quality of life and cause consequent dependence. However, most studies in MS are testing lower extremity muscle strength because it seems that relatively to healthy population MS patients are weaker in lower extremity muscles than in upper extremity strength [44] and MS patients in order to be able to function alone in daily activities (i.e. walking) require to have good lower extremity strength. However, upper extremity strength may become important in later stages of the disease where the patient is required to use walking aid.

To our knowledge, no study has examined the association between handgrip strength and cognitive function in MS patients. The findings of the current study revealed that handgrip strength was not associated with cognitive performance, whilst it was found to be associated with both the physical and mental component of the HRQOL.

Research on elderly populations showed that both aerobic and resistance exercise training improves cognitive function [45,46]; however, we should note that the effects of exercise training on cognition in MS patients are still conflicting [47]. Data from a recent study failed to reveal the positive effect of aerobic exercise in cognition [48], whilst in contrast, data from another recent study revealed cognitive improvement following aerobic exercise programs [49].

The findings of the current study reveal an association between 6MWT and cognition and an association between lower extremity muscle strength and cognition. The above findings indicate an association between various aspects of physical functionality and cognition in the MS patients. Kalmar's et al. (2008), reported also an association between cognitive function and functional capacity in MS patients [10], in line with the findings of the current study. However, we should note that both studies could not clarify whether the cognitive deficits lead to functional capacity impairment or vice versa.

Both physical and mental components of HRQOL are negatively affected by MS [50]. Depression, fatigue, and disability are some factors which are reported to independently reduce HRQOL in MS patients [51]. Additionally, cognitive impairment has been shown to negatively affect HRQOL independent of physical disability [52]. The findings of the current study did not reveal an association between HRQOL and PASAT score but an association with functional capacity, fatigue, handgrip strength, and lower extremity muscle strength.

Additionally, only the mental component summary was found to be associated with sleep quality. Sleep abnormalities are common among individuals with multiple sclerosis (MS) [53].Poor sleep quality has been connected with significant impairments in physical function, ability to perform activities of daily living, psychological and mental health, occupational functions, and social attractiveness in patients with MS [12,53]. To the best of our knowledge, only a few studies examined how sleep difficulties may predict an individual's objective or subjective level of cognitive fatigue. In a recent systematic review, Hughes and colleagues reported an association between sleep disturbances and cognitive function in MS [7]. Sater and colleagues demonstrated that sleep efficiency positively correlated with performance on objective measures of executive function and information processing speed [54]. In addition, self-reported sleep quality is independently related to perceived but not objective cognitive impairment, particularly impairments in planning, organization and prospective memory [55].Braley and colleagues revealed that total sleep time is associated with attention, processing speed, and verbal memory [56], while Shahrbanian et al. (2015) revealed that sleep correlated with global cognitive function [57]. In line with the existing literature, findings from the current study reveal a significant association between sleep quality and cognitive function among this sample of MS patients.

Fatigue is an extremely prevalent issue in MS and one of the most distressing complaints that MS patients may experience over their lifetime [58].No clear definition exists in the literature regarding this complaint which is usually perceived as a 'lack of energy', 'lack of motivation', 'tiredness', 'exhaustion' or 'subjective lack of mental or cognitive energy'. Most importantly, fatigue can drastically impact patients' HRQOL and may result in serious socioeconomic difficulties, namely loss of employment [59].

Regarding the association between cognitive function and fatigue, results are inconsistent. While some studies found a correlation between self-reported fatigue and each of information processing speed [60,61], attention [62,63] and memory [63] domains, others failed to detect such a relationship [64–66]. An inconsistency also exists among the available literature regarding the effects of cognitive behavioral therapies in MS fatigue [67]. Although cognitive behavioral therapies seem to have positive effects on MS fatigue [67], the onset and duration of effects varied across the studies.

Findings from this study reveal that fatigue is associated with HRQOL but not with PASAT score. HRQOL as mentioned above is affected by disability status, physical ability, and functional capacity. Additionally, fatigue is well known for its multifactorial nature. Patients in the current study have mild to moderate disability status whilst the mean FSS score was 4.1 which indicates 'borderline fatigue'. As a result, fatigue was not associated with PASAT score but at later stages of the disease in which the disability status will be worse and fatigue will become more dominant the possibility to be associated with cognition will probably increase [60,68].

According to the literature, there is evidence that in non-MS population obesity and cognitive function are associated. Intentional weight loss among obese and overweight individuals is associated with improvements in cognitive performance across different cognitive domains [69], while in healthy older adults elevated visceral fat levels have been associated with negative effects on cognition, and brain structural abnormalities [70]. In the current study, cognitive function was not associated with any of the body composition related-parameters examined.

The current study has some limitations which have to be pointed out. Factors which can contribute significantly to cognitive impairment in MS patients such as cognitive reserve and premorbid general intelligence status were not accounted for this study. In addition, cognitive function was assessed by the Paced Auditory Serial Addition Test (PASAT). PASAT evaluates different aspects of cognition at the same time such as reaction time, processing speed and remembering information, forcing MS patients to work under challenging conditions. Although PASAT is considered a stressful and difficult test for MS patients, future studies could use other tools for the assessment of cognitive function which can cover more aspects of cognition such as the Montreal Cognitive Assessment (MoCA), the Addenbrooke's Cognitive Examination-Revised (ACE-R), the Brief International Cognitive Assessment for MS (BICAMS) and the symbol-digit modalities test (SMDT). Moreover, we should note that because the participants of this study were relapsing remitting MS patients with low EDSS status, the results might not automatically apply to other MS subtypes or in MS patients with higher EDSS score. In addition, the sample size of this study is relatively small; therefore, future clinical trials with larger sample size are required in order to draw safer conclusions.

Conclusion

In conclusion, the current study shows an association between cognitive function, functional capacity and sleep quality in MS patients. Performance on the TUG test emerged as the best predictor for cognitive function among this sample of MS patients. It is still unknown whether improvement or maintenance of adequate levels of functional capacity could sustain cognitive performance or vice versa. On the other hand, the association between sleep quality and cognitive performance revealed in the present study confirms existing literature. Functional capacity should be considered as a potential predictor for impaired cognitive performance in the MS patients.

Funding

This paper was not funded.

Declaration of financial/other relationships

Authors and peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Declaration of interest

No potential conflict of interest was reported by the authors.

ORCID

Christoforos D Giannaki http://orcid.org/0000-0003-0029-1189

References

1. Dilokthornsakul P, Valuck RJ, Nair KV, et al. Multiple sclerosis prevalence in the United States commercially insured population. Neurology. 2016;86(11):1014–1021.
2. Browne P, Chandraratna D, Angood C, et al. Atlas of Multiple Sclerosis 2013: a growing global problem with widespread inequity. Neurology. 2014;83(11):1022–1024.
3. Jongen PJ, Ter Horst AT, Brands AM. Cognitive impairment in multiple sclerosis. Minerva Med. 2012;103(2):73–96.
4. Moccia M, Lanzillo R, Palladino R, et al. Cognitive impairment at diagnosis predicts 10-year multiple sclerosis progression. Mult Scler. 2016;22(5):659–667.
5. Patti F. Cognitive impairment in multiple sclerosis. Mult Scler. 2009;15(1):2–8.
6. Vollmer T, Huynh L, Kelley C, et al. Relationship between brain volume loss and cognitive outcomes among patients with multiple sclerosis: a systematic literature review. Neurol Sci. 2016;37(2):165–179.
7. Hughes AJ, Dunn KM, Chaffee T. Sleep disturbance and cognitive dysfunction in multiple sclerosis: a systematic review. Curr Neurol Neurosci Rep. 2018;18(1):2.
8. Yang M, Guo Y, Gong J, et al. Relationships between functional fitness and cognitive impairment in Chinese community-dwelling older adults: a cross-sectional study. BMJ Open. 2018;8(5):e020695.
9. Ruano L, Portaccio E, Goretti B, et al. Age and disability drive cognitive impairment in multiple sclerosis across disease subtypes. Mult Scler. 2017;23(9):1258–1267.
10. Kalmar JH, Gaudino EA, Moore NB, et al. The relationship between cognitive deficits and everyday functional activities in multiple sclerosis. Neuropsychology. 2008;22(4):442–449.
11. Patti F, Cacopardo M, Palermo F, et al. Health-related quality of life and depression in an Italian sample of multiple sclerosis patients. J Neurol Sci. 2003;211(1–2):55–62.
12. Lobentanz IS, Asenbaum S, Vass K, et al. Factors influencing quality of life in multiple sclerosis patients: disability, depressive mood, fatigue and sleep quality. Acta Neurol Scand. 2004;110(1):6–13.
13. Hoang PD, Gandevia SC, Herbert RD. Prevalence of joint contractures and muscle weakness in people with multiple sclerosis. Disabil Rehabil. 2014;36(19):1588–1593.
14. Tinetti ME, Inouye SK, Gill TM, et al. Shared risk factors for falls, incontinence, and functional dependence. Unifying the approach to geriatric syndromes. JAMA. 1995;273(17):1348–1353.
15. Goverover Y, Chiaravalloti ND, O'Brien AR, et al. Evidenced-based cognitive rehabilitation for persons with multiple sclerosis: an updated review of the literature from 2007 to 2016. Arch Phys Med Rehabil. 2018;99(2):390–407.
16. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011;69(2):292–302.
17. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale. EDSS Neurology. 1983;33 (11):1444–1452.
18. Ware JE Jr. SF-36 health survey update. Spine (Phila Pa 1976). 2000;25(24):3130–3139.
19. Krupp LB, LaRocca NG, Muir-Nash J, et al. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol. 1989;46(10):1121–1123.
20. Buysse DJ, Reynolds CF 3rd, Monk TH, et al. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193–213.
21. Tombaugh TN. A comprehensive review of the paced auditory serial addition test (PASAT). Arch Clin Neuropsychol. 2006;21(1):53–76.
22. Morrow SA, Rosehart H, Johnson AM. Diagnosis and quantification of cognitive fatigue in multiple sclerosis. Cogn Behav Neurol. 2015;28(1):27–32.
23. Walker LA, Berard JA, Berrigan LI, et al. Detecting cognitive fatigue in multiple sclerosis: method matters. J Neurol Sci. 2012;316 (1–2):86–92.
24. Brooks JB, Giraud VO, Saleh YJ, et al. Paced auditory serial addition test (PASAT): a very difficult test even for individuals with high intellectual capability. Arq Neuropsiquiatr. 2011;69(3):482–484.
25. Holdwick DJ Jr., Wingenfeld SA. The subjective experience of PASAT testing. Does the PASAT induce negative mood? Arch Clin Neuropsychol. 1999;14(3):273–284.

26. Moller AB, Bibby BM, Skjerbaek AG, et al. Validity and variability of the 5-repetition sit-to-stand test in patients with multiple sclerosis. Disabil Rehabil. 2012;34(26):2251–2258.
27. Gijbels D, Eijnde BO, Feys P. Comparison of the 2- and 6-minute walk test in multiple sclerosis. Mult Scler. 2011;17(10):1269–1272.
28. Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc. 1991;39(2):142–148.
29. Broekmans T, Gijbels D, Eijnde BO, et al. The relationship between upper leg muscle strength and walking capacity in persons with multiple sclerosis. Mult Scler. 2013;19(1):112–119.
30. Johnen A, Landmeyer NC, Burkner PC, et al. Distinct cognitive impairments in different disease courses of multiple sclerosis-A systematic review and meta-analysis. Neurosci Biobehav Rev. 2017;83:568–578.
31. Peyser JM, Rao SM, LaRocca NG, et al. Guidelines for neuropsychological research in multiple sclerosis. Arch Neurol. 1990;47(1):94–97.
32. Rao SM, Leo GJ, Bernardin L, et al. Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. Neurology. 1991;41(5):685–691.
33. Samartzis L, Gavala E, Zoukos Y, et al. Perceived cognitive decline in multiple sclerosis impacts quality of life independently of depression. Rehabil Res Pract. 2014;2014:128751.
34. Beier M, Bombardier CH, Hartoonian N, et al. Improved physical fitness correlates with improved cognition in multiple sclerosis. Arch Phys Med Rehabil. 2014;95(7):1328–1334.
35. Briken S, Gold SM, Patra S, et al. Effects of exercise on fitness and cognition in progressive MS: a randomized, controlled pilot trial. Mult Scler. 2014;20(3):382–390.
36. Morrison JD, Mayer L. Physical activity and cognitive function in adults with multiple sclerosis: an integrative review. Disabil Rehabil. 2017;39(19):1909–1920.
37. Gallaway PJ, Miyake H, Buchowski MS, et al. Physical activity: a viable way to reduce the risks of mild cognitive impairment, alzheimer's disease, and vascular dementia in older adults. Brain Sci. 2017;7:2.
38. Allali G, Laidet M, Assal F, et al. Adapted timed up and go: a rapid clinical test to assess gait and cognition in multiple sclerosis. Eur Neurol. 2012;67(2):116–120.
39. Donoghue OA, Horgan NF, Savva GM, et al. Association between timed up-and-go and memory, executive function, and processing speed. J Am Geriatr Soc. 2012;60(9):1681–1686.
40. Katsumata Y, Todoriki H, Yasura S, et al. Timed up and go test predicts cognitive decline in healthy adults aged 80 and older in Okinawa: keys to optimal cognitive aging (KOCOA) project. J Am Geriatr Soc. 2011;59(11):2188–2189.
41. Johansson S, Ytterberg C, Claesson IM, et al. High concurrent presence of disability in multiple sclerosis. Associations with perceived health. J Neurol. 2007;254(6):767–773.
42. Krishnan V, Jaric S. Hand function in multiple sclerosis: force coordination in manipulation tasks. Clin Neurophysiol. 2008;119(10):2274–2281.
43. Nowak DA, Hermsdorfer J. [Analysis of grip force during object manipulation. Method for the objective measurement of physiological normal and impaired hand function]. Nervenarzt. 2004;75(8):725–733.
44. Schwid SR, Thornton CA, Pandya S, et al. Quantitative assessment of motor fatigue and strength in MS. Neurology. 1999;53(4):743–750.
45. Northey JM, Cherbuin N, Pumpa KL, et al. Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis. Br J Sports Med. 2018;52(3):154–160.
46. Gheysen F, Poppe L, DeSmet A, et al. Physical activity to improve cognition in older adults: can physical activity programs enriched with cognitive challenges enhance the effects? A systematic review and meta-analysis. Int J Behav Nutr Phys Act. 2018;15(1):63.
47. Sandroff BM, Motl RW, Scudder MR, et al. Systematic, evidence-based review of exercise, physical activity, and physical fitness effects on cognition in persons with multiple sclerosis. Neuropsychol Rev. 2016;26(3):271–294.
48. Baquet L, Hasselmann H, Patra S, et al. Short-term interval aerobic exercise training does not improve memory functioning in relapsing-remitting multiple sclerosis-a randomized controlled trial. PeerJ. 2018;6:e6037.
49. Zimmer P, Bloch W, Schenk A, et al. High-intensity interval exercise improves cognitive performance and reduces matrix metalloproteinases-2 serum levels in persons with multiple sclerosis: A randomized controlled trial. Mult Scler. 2018;24(12):1635–1644.
50. Goksel Karatepe A, Kaya T, Gunaydn R, et al. Quality of life in patients with multiple sclerosis: the impact of depression, fatigue, and disability. Int J Rehabi Res. 2011;34(4):290–298.
51. Nourbakhsh B, Julian L, Waubant E. Fatigue and depression predict quality of life in patients with early multiple sclerosis: a longitudinal study. Eur J Neurol. 2016;23(9):1482–1486.
52. Langdon DW. Cognition in multiple sclerosis. Curr Opin Neurol. 2011;24(3):244–249.
53. Sakkas GK, Giannaki CD, Karatzaferi C, et al. Sleep abnormalities in multiple sclerosis. Curr Treat Options Neurol. 2019;21(1):4.
54. Sater RA, Gudesblatt M, Kresa-Reahl K, et al. The relationship between objective parameters of sleep and measures of fatigue, depression, and cognition in multiple sclerosis. Mult Scler J Exp Transl Clin. 2015;1:2055217315577828.
55. Hughes AJ, Parmenter BA, Haselkorn JK, et al. Sleep and its associations with perceived and objective cognitive impairment in individuals with multiple sclerosis. J Sleep Res. 2017;26(4):428–435.
56. Braley TJ, Kratz AL, Kaplish N, et al. Sleep and cognitive function in multiple sclerosis. Sleep. 2016;39(8):1525–1533.
57. Shahrbanian S, Duquette P, Kuspinar A, et al. Contribution of symptom clusters to multiple sclerosis consequences. Qual Life Res. 2015;24(3):617–629.
58. Freal JE, Kraft GH, Coryell JK. Symptomatic fatigue in multiple sclerosis. Arch Phys Med Rehabil. 1984;65(3):135–138.
59. Ayache SS, Chalah MA, Kumpfel T, et al. Multiple sclerosis fatigue, its neural correlates, and its modulation with tDCS. Fortschr Neurol Psychiatr. 2017;85(5):260–269.
60. Andreasen AK, Spliid PE, Andersen H, et al. Fatigue and processing speed are related in multiple sclerosis. Eur J Neurol. 2010;17(2):212–218.
61. Neumann M, Sterr A, Claros-Salinas D, et al. Modulation of alertness by sustained cognitive demand in MS as surrogate measure of fatigue and fatigability. J Neurol Sci. 2014;340(1–2):178–182.
62. Hanken K, Eling P, Hildebrandt H. Is there a cognitive signature for MS-related fatigue? Mult Scler. 2015;21(4):376–381.
63. Pokryszko-Dragan A, Zagrajek M, Slotwinski K, et al. Event-related potentials and cognitive performance in multiple sclerosis patients with fatigue. Neurol Sci. 2016;37(9):1545–1556.
64. Bailey A, Channon S, Beaumont JG. The relationship between subjective fatigue and cognitive fatigue in advanced multiple sclerosis. Mult Scler. 2007;13(1):73–80.
65. Paul RH, Beatty WW, Schneider R, et al. Cognitive and physical fatigue in multiple sclerosis: relations between self-report and objective performance. Appl Neuropsychol. 1998;5(3):143–148.
66. Sandry J, Genova HM, Dobryakova E, et al. Subjective cognitive fatigue in multiple sclerosis depends on task length. Front Neurol. 2014;5:214.
67. Chalah MA, Ayache SS. Cognitive behavioral therapies and multiple sclerosis fatigue: A review of literature. J Clin Neurosci. 2018;52:1–4.
68. Cavallari M, Palotai M, Glanz BI, et al. Fatigue predicts disease worsening in relapsing-remitting multiple sclerosis patients. Mult Scler. 2016;22(14):1841–1849.
69. Veronese N, Facchini S, Stubbs B, et al. Weight loss is associated with improvements in cognitive function among overweight and obese people: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2017;72:87–94.
70. Isaac V, Sim S, Zheng H, et al. Adverse associations between visceral adiposity, brain structure, and cognitive performance in Healthy Elderly. Front Aging Neurosci. 2011;3:12.