CATÓLICA
ESCOLA SUPERIOR DE BIOTECNOLOGIA
PORTO

Gait characteristics and Electromyographic Activity in people with Diabetes in early stages of the International Working Group on Diabetic Foot Classification Risk

by
Maria Miguel Lopes Viseu de Carvalho

November, 2018

CATÓLICA
ESCOLA SUPERIOR DE BIOTECNOLOGIA
PORTO

Gait characteristics and Electromyographic Activity in people with Diabetes in early stages of the International Working Group on Diabetic Foot Classification Risk

Thesis presented to *Escola Superior de Biotecnologia* of the *Universidade Católica Portuguesa* to fulfill the requirements of Master of Science degree in Biomedical Engineering

by
Maria Miguel Lopes Viseu de Carvalho

Place: LABIOMEP-UP

LABORATÓRIO DE BIOMECÂNICA DO PORTO
U.PORTO

Supervision:
Supervisor- Prof. Dr. Maria António Castro
Co-Supervisor- Prof. Dr. João Paulo Vilas-Boas

November, 2018

Resumo

Diabetes mellitus é uma doença metabólica e crónica grave, caracterizada por altos níveis glicémicos. Com a contínua expansão epidémica, a diabetes não é considerada apenas uma crise de saúde pública, mas uma catástrofe social global, responsável por uma elevada taxa de mortalidade e morbidade mundial.

Mais de metade dos pacientes com diabetes há mais de 20 anos desenvolve neuropatia periférica diabética, uma das complicações mais comum e dispendiosa da diabetes. A neuropatia periférica diabética tem sido associada a alterações biomecânicas na marcha, principalmente no membro inferior. De facto, com a alteração do padrão de marcha como uma característica comum desta doença, a análise de marcha de sujeitos diabéticos com neuropatia periférica tem sido objeto de vários estudos.

A avaliação da marcha visa aumentar o conhecimento e compreensão da marcha humana, seja como por si só um objetivo, ou de forma a melhorar o diagnóstico e tratamento médico no futuro. Em pacientes diabéticos com neuropatia periférica, a análise tridimensional de movimento permite o estudo e identificação de estratégias de movimento ou musculosqueléticas e pode ser útil para detetar, numa fase inicial, a influência da neuropatia periférica na marcha e no desempenho dos membros inferiores, fornecendo informações decisivas sobre a evolução, características e consequências prejudiciais desta doença.

Este estudo teve como objetivo a caracterização da marcha e investigação de desvios na atividade eletromiográfica de sujeitos diabéticos em fases iniciais do Sistema de Classificação de Risco do Grupo de Trabalho Internacional em Pé Diabético (the International Working Group on Diabetic Foot (IWGDF) Risk Classification System). Os dados foram gerados a partir de dois grupos: diabéticos sem neuropatia periférica (nível 0 do IWGDF) e diabéticos com neuropatia periférica, com doença arterial e/ou deformidade do pé (nível 2 do IWGDF). O estudo foi realizado no Laboratório de Biomecânica do Porto, Universidade do Porto (LABIOMEP-UP), através de um sistema de captura de movimento, sistema tridimensional de análise de marcha e quatro plataformas de força. A atividade eletromiográfica dos músculos *gastrocnemius medialis* e *tibialis anterior* de ambas as pernas foi avaliada ao longo do ciclo de marcha, em sincronia com o sistema de captura de movimento.

Os participantes com neuropatia periférica apresentaram um conjunto de alterações evidentes na marcha, incluindo uma velocidade de marcha significativamente inferior, passos mais curtos, menor cadência, restrição da mobilidade articular dos membros inferiores e alteração dos padrões eletromiográficos dos músculos dos membros inferiores. O grupo neuropata apresentou também valores de força de reação do solo inferiores em amplitude ao longo do ciclo de marcha e posteriores no tempo, o que poderá contribuir para as diferenças observadas na velocidade e cadência de marcha.

No geral, os resultados deste estudo destacam as diferenças biomecânicas na marcha de pessoas com diabetes classificadas em grupos de risco distintos. Destaca também a importância da análise de

marcha no fornecimento de conhecimento preciso e confiável das características de marcha e estratégias compensatórias adaptadas num dado instante, e também ao longo do tempo, como fator de prevenção, diagnóstico e inovação para a população diabética.

No entanto, trabalho futuro é necessário de forma a avaliar os distúrbios da marcha na neuropatia periférica diabética, principalmente na marcha sob condições de 'vida real' em ambientes mais desafiadores, dinâmica inversa e momentos articulares. Estudos adicionais para identificar as alterações do padrão de atividade muscular e esclarecer os fatores específicos relacionados à neuropatia periférica diabética são também necessários.

Palavras-chave: Diabetes, Neuropatia Periférica Diabética, Marcha, Eletromiografia, Biomecânica.

Abstract

Diabetes mellitus is a serious metabolic and chronic disease, characterized by high blood glucose levels. As the epidemic continues to expand, diabetes is not only considered a health crisis, but a global societal catastrophe, responsible for great mortality and morbidity worldwide. More than half of patients who have diabetes for more than 20 years develop diabetic peripheral neuropathy, one of the most common and costly complication of diabetes. Diabetic peripheral neuropathy has been associated with biomechanical alterations in gait, particularly at the lower limb. Indeed, with alteration of walking patterns has a common characteristic of this disease, the gait analysis of diabetic patients with peripheral neuropathy has been the subject of several studies.

Assessment of gait aims to increase the knowledge and understanding of the human gait, either as an end in itself or in order to improve medical diagnosis or treatment in the future. In diabetic patients with peripheral neuropathy, three-dimensional movement analysis allows the study and identification of movements and musculoskeletal strategies and may be useful to detect, in an early stage, the influence of peripheral neuropathy on walking and lower limbs performance, providing decisive information on the evolution, characteristics and detrimental consequences of this disease.

The aim of this study was to characterize the gait and investigate electromyographic activity deviations of people with diabetes in early stages of the International Working Group on Diabetic Foot (IWGDF) Risk Classification System. Data were generated from two groups: diabetic subjects with no peripheral neuropathy (level 0 of the IWGDF) and diabetic subjects with peripheral neuropathy, with arterial disease and/or a foot deformity (level 2 of the IWGDF). The study was conducted in Porto Biomechanics Laboratory, University of Porto (LABIOMEP-UP) by means of a Motion Capture system (MoCap), 3D-instrumented gait analysis system, and four force platforms. The electromyography activity of the *gastrocnemius medialis* and *tibialis anterior* muscles for both legs was monitored throughout the gait cycle, in synchrony with the MoCap system.

A range of gait alterations were evident in participants with peripheral neuropathy including significantly slower gait speed, shorter steps, lower cadence, restriction of lower-limb joint mobility and altered electromyographic patterns of the lower limb muscles. This group also showed a trend to produce less ground reaction force in amplitude through the gait cycle and later in time, which could contribute for the differences observed in speed and cadence.

Overall, the results of this study highlights the biomechanical differences in gait of people with diabetes classified in distinct risk groups. It's also shown the importance of gait analysis to provide accurate and reliable knowledge of gait characteristics and compensatory strategies adopted at a given time, and also over time, as a factor for prevention, diagnosis and innovation for the diabetic population.

Future research is necessary to evaluate gait disorders in diabetic peripheral neuropathy, particularly in terms of gait under 'real-life' conditions in more challenging environments, inverse dynamics and net

joint moments. Further studies to identify the muscle activity pattern alterations, and clarify the specific factors related to diabetic peripheral neuropathy are also required.

Keywords: Diabetes, Diabetic Peripheral Neuropathy, Gait, Electromyography, Biomechanics.

Acknowledgements

I would like to express my sincere gratitude and appreciation:

To my supervisor Prof. Dr.Maria António Castro, and co-supervisor Prof. Dr. João Paulo Vilas-Boas for the opportunity to work on this topic, for their guidance, help and recommendations during the development of this dissertation and the critical evaluation of this manuscript.

To Eng. Pedro Fonseca, LABIOMEP-UP technician, for all the company, suggestions, discussions and to share some of his knowledge and experience.

To all the participants in the data collection, that kindly contributed to this study.

To my family and friends for their support and encouragement.

Contents

List of Figures

List of Tables

List of Abbreviations

3D	Three-dimensional
ADA	American Diabetes Association
BMI	Body Mass Index
BW	Body weight
CMRR	Common-mode Rejection Ratio
DM	Diabetes Mellitus
DPN	Diabetes Peripheral Neuropathy
EMG	Electromyography
FA	Anterior-posterior ground reaction force component
FM	Medio-lateral ground reaction force component
FPG	Fasting Plasma Glucose
FZ	Vertical ground reaction force component
GDM	Gestational Diabetes Mellitus
GM	Gastrocnemius Medialis
GRF	Ground Reaction Forces
HbA1C	Glycated Hemoglobin Test
HS	Heel strike
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IWGDF	International Working Group on Diabetic Foot
L	Left
LABIOMEP-UP	Porto Biomechanics Laboratory, University of Porto
MID	Midstance
MNSI	Michigan Neuropathy Screening Instrument
MoCap	Motion Capture
MVC	Maximal Voluntary Contraction
OGTT	Oral Glucose Tolerance Test
PG	Plasma Glucose
R	Right
SENIAM	Surface EMG for a Non-Invasive Assessment of Muscle
TA	Tibialis Anterior
TKEO	Teager-Kaiser Energy Operator
TO	Toe-off
WHO	World Health Organization

Chapter 1

Overview of the Thesis

Motivation and Objectives

Contribution of the Thesis

Structure of the Dissertation

Overview of the thesis

Analysis of the human gait begun several centuries ago, and over the past decades an interest in obtaining in-depth knowledge of gait mechanisms and function has increased dramatically. Progress in measuring technologies has given rise to the development of more powerful and efficient devices and techniques, which allow an objective and reliable analysis of different gait characteristics. Three-dimensional (3D) instrumented gait analysis is a crucial method that provides comprehensive data on normal and pathological gait, measuring time-distance variables (spatiotemporal data), the movements of the limb's joints and segments (kinematics), forces, joint's moments and power (kinetics) and activity of the muscles during the gait cycle.

In the clinical field, 3D-gait analysis provides accurate and reliable knowledge of gait characteristics at a given time, and more importantly, over time. Therefore, with gait disorders affecting a high percentage of the world's population and as key problems in neurodegenerative diseases, gait analysis may be valuable for early diagnosis, prevention of their complications, explanation and evaluation of the functional limitation level, follow up evaluation over time, and monitoring the effects of rehabilitation intervention and treatment of some pathologies.

Neuropathy is a common and costly complication of diabetes, which is expected to lead to biomechanical disorders, particularly at the lower-limb. Several studies have analysed gait characteristics of diabetic peripheral neuropathy individuals, suggesting that this pathology significantly alters the walking pattern. For this reason, the assessment of gait in diabetic peripheral neuropathic individuals may be useful to detect, in an early stage, the impact on walking and lower limbs performance, in order to quantify the degree of gait deviation from normal, stratify the severity of pathology, document the changes in gait patterns and evaluate treatment interventions.

Motivation and Objectives

Diabetes mellitus is a serious metabolic and chronic disorder and one of the most common causes of mortality and morbidity worldwide, not only due to the disease itself, but also due to its long-term complications. One of the many consequences of diabetes, and the most common, is the onset of diabetic peripheral neuropathy.

Diabetic peripheral neuropathy affects the peripheral nerve function, generating significant deficits in sensitivity and proprioception and several other impairments in muscle strength, power and endurance and, consequently, in the range of motion and in motor control, especially in the lower limbs. These significant disabilities can lead to reduced functional capacity, development of foot ulcers, which may progress to lower limb-amputation, altered gait and lower limb muscle activity patterns and impaired balance, with great impact in patients' mobility and quality of life.

Diabetic neuropathy is frequently unnoticed and underreported, with up to 50% of the patients with no symptoms/asymptomatic. Therefore, gait analysis may be a priceless tool for the identification of early signs that allow the discrimination of diabetic stages, and can help in the prevention of worst complications, in the follow up evaluation of the pathology over time and in the adoption of the most appropriate and effective treatment or intervention for each individual. The insight of the effect of diabetic peripheral neuropathy on the biomechanical aspects of human locomotion is clinically important, being nowadays subject of many research projects. Indeed, various studies have demonstrated the detrimental effect of diabetic peripheral neuropathy in gait characteristics and patterns and lower limb muscle activity.

The purpose of the present study was to characterize the gait and investigate electrical muscle activity deviations during the entire gait cycle of people with diabetes in early stages according to the International Working Group on Diabetic Foot (IWGDF) Risk Classification System. Diabetic subjects with no peripheral neuropathy (level 0 of the IWGDF) and diabetic subjects with peripheral neuropathy, with arterial disease and/or a foot deformity (level 2 of the IWGDF), were therefore the aim of this study. The focus and novelty of the research presented in this thesis lies with the combinations of different parameters for analysis. Spatiotemporal gait parameters, kinematics of the lower limb, the three components of ground reaction forces and electromyographic variables were assessed to determine whether any restricted dimension is contributing to the gait changes observed in people with diabetes peripheral neuropathy.

Contribution of the Thesis

The results emerged from this thesis will be soon presented in:

- 8th Portuguese Congress of Biomechanics (CNB2019) (future oral presentation)

Structure of the Dissertation

Apart from this introduction, the following thesis contains nine more chapters. Chapter 2 is an introduction to the thematic of diabetes mellitus and diabetes peripheral neuropathy. Chapter 3 presents a review of the literature focused on clinical gait analysis and diabetic peripheral neuropathic gait alterations. In Chapter 4, the patient recruitment and methodology implemented are described. Chapter 5 presents the results obtained from all the work developed. In Chapter 6, the results obtained are described and discussed in detail and compared with the literature review. In Chapter 7, the major conclusions are summarized and some future work is presented and summed up in Chapter 8.

Chapter 2

Classification of Diabetes Mellitus

Diagnostic Criteria for Diabetes Mellitus

Diabetic Peripheral Neuropathy

Classification of Diabetes Mellitus

Diabetes mellitus is a group of serious chronic and metabolic disorders characterized by high blood glucose levels, resulting from the body's inability to produce insulin or resistance to insulin action, or both (1,2). Insulin is a hormone synthesized by beta cells (β-cells) of the pancreas in response to various stimulus such as glucose, sulphonylureas, and arginine (3). However, the increments in plasma glucose levels caused by glucose entrance into the circulation from food absorption and digestion, is the major determinant (3,4).

Insulin promotes the absorption of glucose from the bloodstream into the liver, fat tissue and skeletal muscle cells, regulating the metabolism of carbohydrates, fat and protein and, therefore, one of its main roles is to help regulate the blood sugar (5). So the maintenance of blood glucose concentrations within a narrow range requires an adequate number of pancreatic β-cells capable to produce insulin that respond properly to blood glucose concentrations, maintaining a balance between insulin secretion and insulin action (6).

Insufficient insulin secretion (absolute or relative) can occur in association with destruction of pancreatic β-cells or due to dysfunction within the pancreatic β-cells themselves. Besides the decreased in insulin supply, decreased insulin sensitivity can contribute to relative insufficient insulin action (7–11). In either case, the progressive loss of β-cell mass (8,9) and/or function (10), as a result of many different pathways driven by genetic and environmental factors, leads to insufficiency production and/or action of insulin (7,11) that manifests clinically as hyperglycemia, the hallmark of diabetes.

The classification of diabetes is complex and has been the subject of much consultation, debate and revision stretching over many decades. The current World Health Organization (WHO) classification of diabetes, subdivides this group of conditions into four clinically distinct types: type 1 diabetes, type 2 diabetes, gestational diabetes and a group of other less common specific types of diabetes (2,11,12).

Type 1 diabetes, previously known as “insulin-dependent diabetes” or “juvenile-onset diabetes”, accounts for 5-10% of all cases of diabetes (11,13). It is an autoimmune regulated disease, resulting in targeted destruction of the pancreatic β-cells, usually leading to a complete lack of insulin production (11,13). Multiple genetic predispositions, autoimmune and environmental factors (11,14,15), family history and certain viral infections are responsible for this destructive process (13). Yet they are still poorly defined and not fully understood (11,13,16). Type 1 diabetes often presents with symptoms like polydipsia (excessive thirst), polyphagia (excessive hunger), and polyuria (excessive quantity of urine), the classic trio of symptoms associated with the disease onset (17,18). Long-term insulin therapy is essential for the health and survival of patients with type 1 diabetes (19).

Type 2 diabetes is considered the most common form of diabetes (12,18,20), accounting for 90-95% of cases globally (2,11,18,21). This type is characterized by disorders of insulin secretion caused by pancreatic β-cell dysfunction, and disorders of insulin resistance in target organs (22–24), either of which

may be the predominant feature (21,24). The risk of developing diabetes type 2 is associated with increased age (13,22), race or ethnicity, family history and past history of gestational diabetes, (13,25) and is inevitably linked to changes towards an unhealthy western lifestyle-style, obesity, lack of physical activity, poor diet and nutrition, smoking and overconsumption of alcohol (22–26). Often, this form of diabetes is associated with multiple genetic defects, however this factor is quite complex and not fully understood (11,15,23).

In the majority of type 2 diabetes cases, hyperglycemia develops gradually and at earlier stages is not severe enough to provoke the classic symptoms of diabetes, so is usually asymptomatic and undiagnosed for many years, with a true onset time difficult to determine (12,27).

Initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive (27,28), so a healthy lifestyle which includes healthy diet, physical activity, smoking cessation and maintenance of a healthy body weight is the essential for type 2 diabetes treatment (28–30). If a healthy lifestyle is not sufficient to control blood glucose levels, a range of anti-diabetic therapies are now available (28).

Like type 2 diabetes, gestational diabetes mellitus (GDM) is characterized by a state of hyperglycemia due to insulin resistance and an inability of β-cells to compensate with a sufficient increase in insulin secretion (31,32). This usually affects pregnant women during the second and third trimesters of gestation (11,30), with insulin resistance increasing gradually until delivery when, in nearly all cases, it rapidly ceases (30,32,33). However, the affected women and their babies remain at a high risk of developing type 2 diabetes in the future (30,32,34).

GDM and type 2 diabetes have many risk factors in common and they share the same genetic susceptibility (32). Obesity, excessive gestational weight gain (32–37), poor nutrition, absence or lack of physical activity (33,37), previous delivery of a macrocosmic infant (newborn with an excessive birth weight) (32,36,37) and family history of diabetes (32,37) are risk factors for GDM. Preventive behaviors and also the behavior during pregnancy, such as regular moderate physical exercise programs (30,33,36), healthy diets (30,32,33), maintenance of a normal body weight (33), are associated with lower incidence of GDM and less maternal weight gain (32,33,36).

The group of other specific types of diabetes are less common and can be classified as genetic, exocrine pancreatic, endocrine, drug-induced diabetes, uncommon manifestations of autoimmune diabetes, and genetic syndromes associated with diabetes (2,20,38). Several forms of diabetes are associated with monogenic defects in pancreatic β-cell function (2,39,40) and, more unusual, with genetically abnormalities relevant to mechanism of insulin action (2). Diabetes can also occur in individuals that suffer from pancreatic diseases, genetic syndromes (like Down syndrome, Klinefelter syndrome, and Turner syndrome) and viral infections (41,42). Certain drugs can also trigger the onset of diabetes, either temporarily or permanently (41–43), like several hormones that can antagonized insulin action (41,42). Several immunologic diseases, with a different pathogenesis from that which leads to type 1 diabetes,

are associated with rare manifestations of diabetes, like insulin autoimmune syndrome, a disease due to anti-insulin receptor antibodies (41,42).

Diagnostic Criteria for Diabetes Mellitus

Classification of diabetes at the time of diagnosis is crucial for determining therapy. Some individuals cannot be precisely classified as having type 1 or type 2 diabetes, as the traditional paradigm that type 2 occurs only in adults and type 1 only in children and adolescents is no longer accurate, since they may occur in all age-groups (21,44). However, in most cases of type 1 diabetes, blood glucose cut points are not required for diagnosis, since this form has a sufficiently characteristic clinical onset, with acute elevations in glucose concentrations accompanied by symptoms. In contrast, in type 2 diabetes hyperglycemia develops gradually, with slowly rising glucose levels over time, and at early stages is usually asymptomatic, so specific glucose values to distinguish pathologic glucose concentrations in non-diabetic population is mandatory for diagnosis (45).

In 1997 and 2003, the Expert Committee on Diagnosis and Classification of Diabetes Mellitus recognized an intermediate group of individuals whose glucose levels do not meet criteria for diabetes, yet are higher than those considered normal. These people were defined as having prediabetes, intermediate form between diabetes and normoglycemia, and the diagnosis can be established by impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (46). Individuals with IFG and/or IGT have a relatively high risk for future development of type 2 diabetes, but should not be classified as diseases in their own right but rather risk factors for diabetes (47).

Diabetes and its pre-states may be diagnosed on plasma glucose criteria, either the fasting plasma glucose (FPG), or the 2-h plasma glucose (2-h PG) value during a 75-g oral glucose tolerance test (OGTT), or glycated hemoglobin test (A1C criteria) (11,45,48).

A fasting glucose test measures glucose in a blood sample taken while the patient is in a fasting state for at least 8 hours (48). The threshold of a FPG of 126 mg/dL (7.0 mmol/L) has been used to diagnose diabetes (46,48). The OGTT is used to determine whether the body has difficulty metabolizing intake of sugar. Diabetes is diagnosed by measuring glucose in a blood sample taken while the patient is in a fasting state, and 2 hours after a 75g oral load of glucose dissolved in water has been taken (48). A 2-hour oral glucose tolerance test above 200 mg/dL (11.1 mmol/L) has been used to diagnose diabetes (46,48). The OGTT diagnoses more patients than the FPG, still the OGTT test is more expensive, complex and less reproducible (46,48).

The measurement of glycated hemoglobin (HbA1c) was recommended and adopted as an optional test by American Diabetes Association (ADA) in 2010 (49), and the WHO in 2011 (50) as a means to diagnose diabetes. To measure the glycated hemoglobin level, end product of non-enzymatic glycation

of the most prevalent protein in blood (51), a blood sample is taken without requiring an overnight fast (52).

The HbA1c test reflects the average plasma glucose levels over the previous 2 to 3 months (50,52), with a cut point of ≥6.5% (48 mmol/mol) to identify undiagnosed diabetes (46,48). With greater preanalytical stability and less variability than glucose determinations (48,53,54) the HbA1c test is now globally standardized and routinely used in primary care setting (48,52,55). Despite of providing a badly needed, clinically practical indicator of the risk for diabetes, the A1c has a greater cost, limited availability in certain regions of the developing world (48,52,54), and the results may not have a good correlation to the average glucose in certain individuals (55–59). The diagnostic tests need to be practical and cost effective, and both FPG and OGTT are suitable tests. However, measuring A1c is now the preferred screening test for clinical settings as it is easier and more acceptable to patients (48,50,54).

Table 1. Current WHO recommendations for the diagnostic criteria for diabetes and intermediate hyperglycemia (12).

	Diagnostic cut points
Diabetes Mellitus: Fasting plasma glucose **or** 2-h oral glucose tolerance test **or** HbA1c	 ≥ 126 mg/dl (7.0 mmol/L) ≥200 mg/dl (11.1 mmol/L) ≥6.5% (48 mmol/mol)
Impaired Fasting Glucose (IFG): Fasting plasma glucose **and** 2-h oral glucose tolerance test	 <126 mg/dl (7.0 mmol/L) ≥ 140mg/dl and <200mg/dl (7.8 mmol/L and 11.1 mmol/L)
Impaired Glucose Tolerance (IGT): Fasting plasma glucose **and** 2-h oral glucose tolerance test	 110 mg/dl to 125mg/dl (6.1 to 6.9 mmol/L) < 140mg/dl (7.8 mmol/L)
Gestational Diabetes (GDM): **One or more of the following:** Fasting plasma glucose 2-h oral glucose tolerance test	 92-125 mg/dl (5.1-6.9 mmol/L) 153-199 mg/dl (8.5-11.0 mmol/L)

The diagnosis of both prediabetes and diabetes can be easily established, but unless there is a clear clinical diagnosis (patient in hyperglycemic crisis, classic symptoms of hyperglycemia and a random plasma glucose ≥200 mg/dL), a second test, the same or a different test using a new blood sample, is required for confirmation (48,59,60). Ultimately, an easy access to basic diagnostics for diabetes and a well-established set of criteria to screen the high-risk population, allows for earlier diagnosis and intervention, with potential reductions in future complications.

Diabetic Peripheral Neuropathy

Diabetes is a metabolic disorder recognized as an important cause of premature death and disability, associated with microvascular and macrovascular complications, several neuromusculoskeletal impairments, physical disability and lower health-related quality of life.

The prevalence of diabetes is constantly increasing worldwide at an alarming rate. According to WHO, in 2014 an estimated 422 million people globally were suffering from this condition, compared to the 108 million in 1980 (61,62). The global prevalence of diabetes has nearly quadrupled over these 35 years, from 4.7% in 1980 to 8.5% in 2014. In 2015, an estimated 1.6 million deaths were directly caused by diabetes and the WHO projects that this disease will be the seventh leading cause of death worldwide in 2030 (61,62). Additionally, in Portugal, the Annual Report of the National Diabetes Centre estimated that in 2015 the prevalence of diabetes in the Portuguese population, aged between 20 and 79 years (7.7 million individuals), was 13.3%, this means that more than 1 million individuals in this age group has diabetes (63). Comparing this value with the results from 2009, where diabetes prevalence was 11.7%, it can be seen an increase of 1.6 % in the rate of diabetes in Portugal between 2009 and 2015. Diabetes plays a significant role in the causes of death in Portugal, accounting for 4.0% of deaths in 2015 (63). This global rise in prevalence has been compounded by population stagnation linked to increase aging, and reflects an increase in associated risk factors for diabetes (62).

The human and economic costs of diabetes and its complications are enormous. This epidemic imposes a large economic burden on the global health-care system and the wider global economy, with substantial economic cost on patients, their families, health systems, and national economies because of direct costs of treatment and indirect costs associated with productivity loss and premature mortality (61,64). Efforts to reduce the global health and economic burden of diabetes should emphasize prevention of diabetes or delaying its onset. Recent advances in knowledge, therapies, and technology, improved healthy behaviors and diets at the population level, early detection and management of high-risk individuals have enhanced the ability to help. However, much of the disability and cost associated with diabetes are related to the care of acute and chronic complications (65,66).

Chronic complications involve long-term macrovascular (involving large vessels, such as arteries and veins) and microvascular (involving small blood vessels, such as capillaries) damage. Microvessels, the basic functional unit of the cardiovascular system, differ from macrovessels in both their architecture and cellular components. In contrast to macrovessels supplying blood to organs, microvessels have specific duties in regulating blood pressure and proper nutrients delivery (67). Chronic hyperglycemia plays a major role in long-term damage and dysfunction of small and large blood vessels, through many metabolic and structural derangements (68–72). Generally, the injurious effects of hyperglycemia are separated into macrovascular complications (ischemic heart disease, peripheral vascular disease and cerebrovascular disease) and microvascular complications that include renal system damage (nephropathy), eye damage (retinopathy) and nervous system damage (neuropathy) (69,70,72).

Diabetic neuropathies are the most prevalent chronic complications of diabetes. This heterogeneous group of conditions is a common complication of diabetes type 1 and type 2 (65) and a great source of morbidity and mortality (73,74). The term diabetic neuropathy includes a spectrum of clinical syndromes with differing anatomic distributions, clinical courses, and underlying pathogenic mechanisms. This life threatening complication affects both sensory, autonomic and motor neurons of the peripheral nervous system, which is to say that nearly every type of nerve fiber and every organ system that relies on innervation for function is vulnerable and consequently subject to pathology (74,75). Among the various forms of diabetic neuropathy, diabetic peripheral neuropathy (DPN) and diabetic autonomic neuropathies are by far the most studied (76).

The most typical form of diabetic neuropathy encountered is the diabetic peripheral neuropathy. A symmetrical, length-dependent sensorimotor polyneuropathy which is attributed to metabolic and microvessels alterations due to hyperglycemia and concomitant cardiovascular risk covariates, accounting for about 75% of the diabetic neuropathies (77). An internationally agreed simple definition of DPN for clinical practice is “the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes” (78). The exact cause that provokes this destructive process is unknown and has remained subject of research. DPN can result from multiple different biochemical perturbations, with chronic hyperglycemia being the major contributor to the development of these metabolic events (79,80). Additional studies have implicated poor glycemic control (81,82), duration of diabetes, obesity, smoking, presence of microalbuminuria (elevated albumin excretion rates), presence of cardiovascular diseases and dyslipidemia (abnormal amount of lipids in the blood) as risk factors for the development of DPN (79,81,83).

The prevalence of diabetic peripheral neuropathy increases with age and disease duration (84). In newly diagnosed patients with diabetes the prevalence of DPN is estimated to be 8% and greater than 50% in patients with long-standing disease, tending to be more common in patients with type 2 diabetes mellitus than those with type 1 (65,85). However, it remains impossible to precisely dictate the true prevalence of DPN, because the diagnosis criteria vary, epidemiological studies are limited to a sample that receives medical care, and there exists a high proportion of undiagnosed diabetes mellitus globally.

Symptoms of distal symmetric polyneuropathy may be either ‘negative’, such as loss of feeling, or ‘positive’, including pain and paresthesia (abnormal sensations), according to the class of sensory fibers involved. In this neuropathic process, signs and symptoms start and remain more pronounced in the most distal extremities, such as the lower limbs (the fingers and toes). Once lower limbs are affected the increased duration or intensity of diabetes, may move the symptoms to a more proximal position, with sensory loss following in a symmetrical pattern described as a ‘glove and stocking’ distribution. The most common early symptoms are the positive symptoms and are induced by the involvement of small fibers (79,80,84,86,87). Painful symptoms such as burning, lancing (stabbing), shooting (electric shock-like), tingling (‘pins and needles’ or paresthesia) (79,80,84,86,88), contact pain (allodynia) (84,88), are present in varying combinations in around a third of patients with DPN (80,87,89), and tend to be worse at night (86–88). Neuropathic pain is the first symptom that prompts patients to seek medical care and

has a significant negative effect on daily activities, psychosocial impairment and health-related quality of life (88,89). The involvement of large fibers are relative to the negative symptoms, and may cause sensory loss, numbness, tingling without pain, and inability to feel, identify, or manipulate smaller objects. Patients can gradually lose the capacity to judge temperature, sense painful or threatening stimuli, can have reduce or absent ankle and knee reflexes and the loss of innervation can also lead to atrophy of essential pedal muscles, resulting in deformities (e.g. hammertoes) (79,80,84,86,87).

A combination of neuropathy with extrinsic factors, such as ill-fitting footwear or a foreign body in shoe, or intrinsic factors, such as loss of protective sensation, high foot pressures, or plantar callus, can lead to painless trauma and development of diabetic foot ulcers (80,86), which is responsible for 85% of lower-extremity amputations in diabetes patients (90). DPN has been identified as one of the leading causes of diabetic foot ulcer and amputations, but at least half of all foot ulcers and many amputations are preventable if minor problems are diagnosed and treated in time (90,91).

The diagnosis of DPN is principally a clinical one. In a patient with diabetes a combination of typical symptomatology and symmetrical distal sensory or classic signs in the absence of symptoms is highly suggestive of DPN and may not require additional evaluation. Whereas 10-20% of patients may experience troublesome symptoms, up to 50% may be asymptomatic, so a diagnosis may only be made on careful, regular and clinical examination or, in some cases, when the patient presents a painless foot ulcer (91,92). The combined use of appropriate tools and clinical examination has been show to provide greater than 87% accuracy in the detection of DPN (92,93).

The diagnosis requires an accurate history of sensory and motor symptoms, signs and clinical examination (80,85,87). Assessments should follow the typical DPN pattern, starting distally on both sides and move proximally until sensory threshold is identified. A number of methods can be used for detecting diabetic peripheral neuropathy. Clinical examination includes either temperature or pinprick sensation (small-fiber function) and vibration sensation using a 128-Hz tuning fork, proprioception, ankle reflexes and an annual 10-g monofilament testing to assess for feet at risk for ulceration and amputation (large-fiber function) (76,79,92,93). Several questionnaires with physical examination have been developed and validated to assist clinicians in the diagnosis of DPN. One example frequently used to screen DPN is the Michigan Neuropathy Screening Instrument (MNSI), a simple non-invasive clinical test that assess symptoms and signs of DPN and can be used in the primary care setting (85,91,94–96). This test includes two separate assessments, a 15-item self-administered questionnaire that is scored by summing abnormal responses, and a lower extremity examination that consists of inspection and evaluation of appearance, ulceration, ankle reflexes, vibration perception at the great toe, and a monofilament examination. The questionnaire consists of 15 questions about sensation, general asthenia, and peripheral vascular disease. A positive response on 7 or more of the questions is diagnostic of diabetic peripheral neuropathy (95–97). The questionnaire is followed by the lower extremity examination, where an MNSI score ≥2.5 represents the presence of neuropathy (95–97) with a high specificity (~95%) and sensitivity (~80%) (97). Additional nerve testing may be required; this would include nerve conduction studies that unfortunately are time-consuming and costly (95,96).

International guidelines and parameters that allow to distinguish diabetic peripheral neuropathy stages are therefore essential in order to prevent feet injuries and amputation, improve the diagnosis, follow-up of patients and treatment, while preserving the quality of life and reduce the social and economic cost associated.

Diabetic peripheral neuropathy has no known cure, so the goals of treatment are to prevent and slow the progression of the disease, relieve pain and manage complications (80,85,98). There remains a lack of treatment options that effectively target the natural history of DPN or, reverse DPN once established. Improved glycemic control has been shown to improve nerve function in patients with diabetes (85,98). Intensive glycemic control has also been suggested to reduce the risk of developing diabetic peripheral neuropathy in type 1 diabetes (99–101), however in type 2 diabetes the relationship between intensive glycemic control and prevention of neuropathy is not as strong (85,102,103). Keeping blood pressure under control and maintaining a healthy weight and lifestyle are also other important factors that allow to delay or even prevent disease. Several pharmacologic approaches are available for diabetes-related nerve pain, however a disease-modifying treatments other than optimize glycemic control await a more complete understanding of the pathogenic mechanisms responsible for the diabetic peripheral neuropathy and the development of promising treatments (104,105).

Chapter 3

Clinical Gait Analysis

DPN Gait Alterations

Clinical Gait Analysis

Human gait is the most daily activity for humans and consists of a voluntary and cyclical natural form of vertical locomotion, characterized by an alternating and progressive action of the lower limbs. Basically, human gait can be defined as the result of two capacities: balance, essential to maintain upright posture, and locomotion, an indispensable successive coordination of movements. There are also other factors that are involved in the gait process, such as the musculoskeletal system, the muscle tone, the sensory systems, the vestibular system and the sensorimotor system (106,107).

Normal walking is the end-product of a healthy neuromuscular system, which requires both sensory feedback from the plantar surface and muscular output with cautious balancing of eccentric and concentric muscle contractions to execute the desired action. Balance and locomotion require the central integration of afferent information arising from three peripheral sensory systems: the vestibular organs, visual apparatus, and somatosensory receptors throughout the body. In addition, the ability to keep the body in the upright position and adjust to external destabilizing forces involves an intricate/ adequate and accurate sequence of motor and sensory feedback mechanisms (106,107). An intact central and peripheral nervous system to initiate and control the complex series of events in gait, adequate muscle strength, and bones and joints moving in full range are the essential factors for normal locomotion (106).

Gait is the forward propulsion of the body's center of gravity through a series of movements that requires a periodic movement of each foot from one position of support to the next. The two main phases of the gait cycle are the stance phase, in which the foot is in contact with the surface, and the swing phase, where the foot is no longer in contact with the ground and the leg is swinging through in preparation for the next foot strike. One gait cycle is measured from heel strike to heel strike of the same foot (108–111).

The stance phase comprises about 60% of the gait cycle and is subdivided into five events: heel strike (initiates the gait cycle and represents the point at which the body's center of gravity is at its lowest position), foot-flat (time when the plantar surface of the foot touches the ground), midstance (swinging foot passes the stance foot and the body's center of gravity is at its highest position), heel-off (the heel loses contact with the ground and push off is initiated via the triceps surae muscle, which plantar flex the ankle) and toe-off (terminates the stance phase as the foot leaves the ground). The swing phase covers the remaining 40% of the gait cycle. This phase is subdivided into three periods: acceleration (the foot leaves the ground and the subject activates the hip flexor muscles to accelerate the leg forward), midswing (the foot passes directly beneath the body's center of gravity, coincidental with midstance for the other foot), and deceleration (muscles slow the leg and stabilize the foot in preparation for the next heel strike) (108–111).

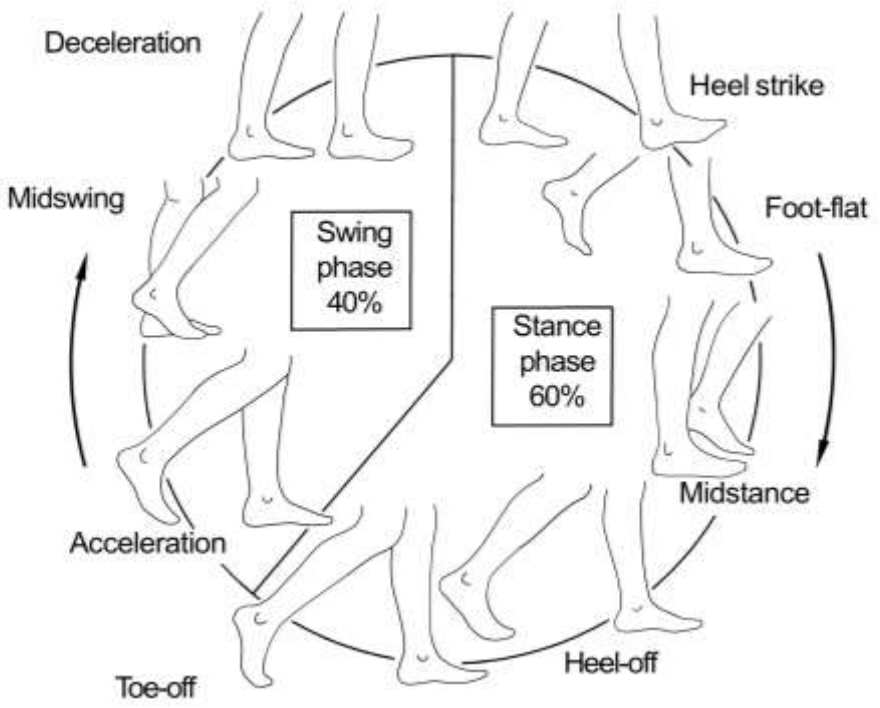


Figure 1. Traditional nomenclature for describing main events of the cyclic nature of human gait (*Adapted from:* (108)).

Stability in stance phase, toe clearance in swing phase, swing phase pre-positioning, adequate step length, and a good mechanical and metabolic efficiency are the prerequisites of normal gait (106,107).

Locomotion involves also a combination of voluntary eccentric and concentric muscular contractions of the lower limbs and the rest of the body, including trunk and arms, in order to resist gravity and achieve forward propulsion of the human skeleton. Most of the major muscle groups are active at or around heel strike and toe-off, at the beginning and end of the stance and swing phases of the cycle (108–111).

Upon heel strike there is an eccentric contraction of the *tibialis anterior* muscle. Just after the heel strike, the *rectus femoris* has to work in eccentric conditions, in order to stabilize the joints and to allow the extension of the knee and flexion of the hip. During midstance and midswing most muscles are inactive, with the exception of *gluteus medius* and *triceps surae* during stance, and *tibialis anterior* during swing. During midstance, *gluteus medius* acts as a hip abductor to stabilize the pelvis and the *gastro-soleus* complex contracts eccentrically, followed by a concentric contraction in terminal stance. During midswing the *tibialis anterior* provides active dorsiflexion, preventing the toes from dragging on the ground. The swing phase involves concentric contractions of the *tibialis anterior* for adequate ground clearance. The *hamstrings* and quadriceps muscles also play an important role in gait, as do the proprioceptive feedback mechanisms within the body, in order to maintain both static and dynamic balance (108–111).

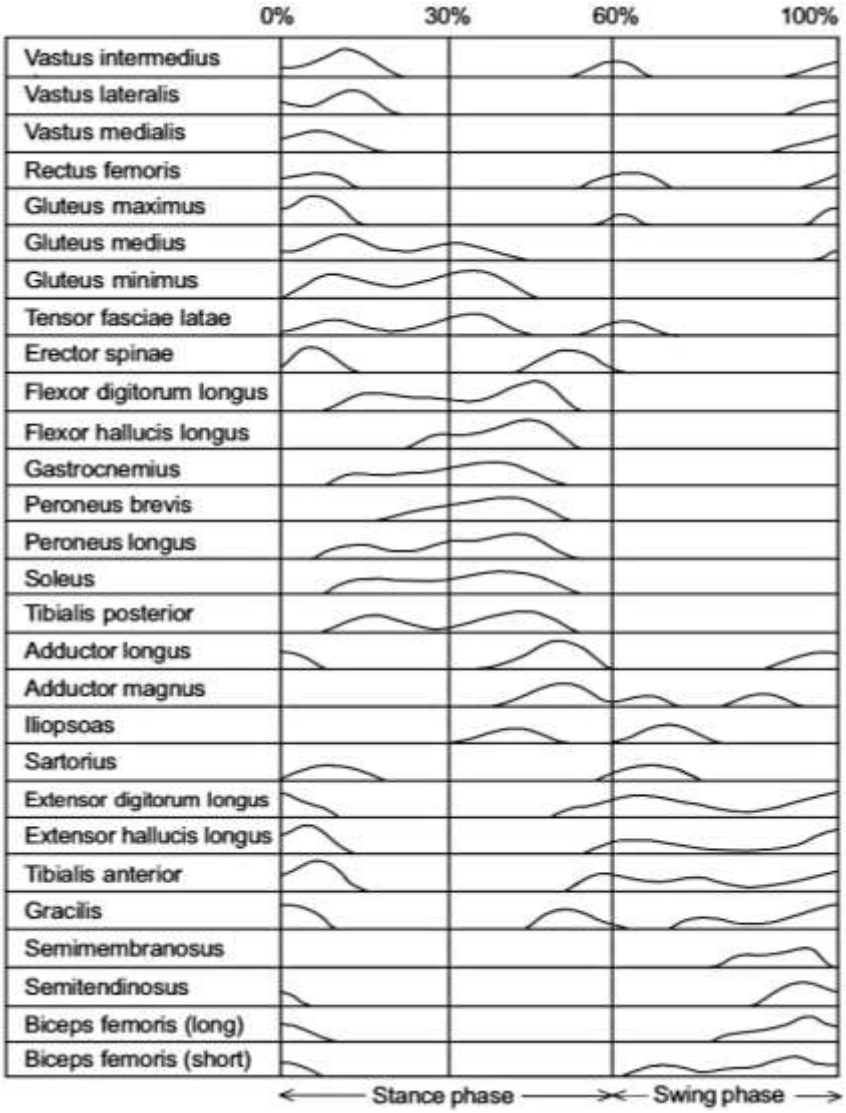


Figure 2. Normal electric muscle activity for 28 of the major muscles in the lower extremities plotted as a function of the gait cycle (*Adapted from:* (108)).

All phases of the gait cycle are influenced by several kinematic, kinetic, neuromuscular and anthropometric variables, which can be identify as characteristics of the gait pattern. The biomechanical analysis of the gait involves an interaction between these variables, and reflect the interest of understanding possible changes or problems in gait (106,107).

Kinetics enables the study of external and internal forces involved in movement. Internal forces and moments arise from muscular action, ligaments, friction between muscles and joints, among other factors. The external forces represent mainly the force of reaction of the ground to the weight and action of the individual and the resistance opposed by the air to its advance. During gait, the direction and intensity of the ground reaction force (GRF) allows to know the mechanic request to which the muscles and joints of the lower limbs are exposed, one of the most significant indicators of the mechanical stresses generated during gait, and can be obtained through the use of force platforms (106,107,110).

Force platforms are considered as a basic, but essentially important tool for gait analysis that are capable of measuring the three components of the GRF. Therefore, GRF can be divided into vertical (the weight of the body and how it progresses over the supporting limb), anterior-posterior (the accelerating and braking forces) and medio-lateral (the force acting from side to side) components. The vertical component measurements are by far the most quoted in the literature (110,112).

The vertical component results from the acceleration of the upward and downward movement of the body center of mass during gait. In the initial phase of gait, the center of mass moves from its lowest position to the highest, making the vertical GRF value higher than body weight. After this rapid ascent, there is a slowing of the center of mass velocity, resulting in a deceleration and reduced vertical GRF to a value lower than the body weight. From this lower point, the body center of mass drops vertically, causing an increase in the downward acceleration, which leads to a further increase of the vertical GRF. When approaching the lowest position, the center of mass decelerates, reducing once again the vertical force (110,112).

In addition to the vertical component, friction between the foot and the ground gives rise to an anterior-posterior force, which reflects the accelerations of the body center of mass in this two directions. In the initial phase of gait, the heel is in contact with the ground and the body decelerates, resulting in a posterior shear force and increased anterior-posterior GRF value. After this period, this force decreased due to the little acceleration of the center of mass in the anterior direction. Following this, the heel lifts and the foot is pushed down and back into the ground by the action of muscles that accelerate the body in the anterior direction, producing a maximum anterior component of the GRF that propels the body forwards. There is still a third component of the GRF in the medio-lateral direction. In the initial phase of gait there is a lateral thrust during loading, during which the foot is working as a mobile adaptor. After the initial loading, the forces push in a medial direction as the body moves over the stance limb. During the final push off stage, small lateral forces are often seen. The medio-lateral component is the most variable of the three GRF components (110).

Each component of the GRF can be divided into three sections, remarkable points in the force-time curve that allow its characterization as depicted in figure 3. The anterior-posterior component is divided in a posterior shear force at heel strike (FA1), a cross-over point, sometimes defined as the point of midstance (FA2) and an anterior component of GRF that propels the body forwards (FA3). The medio-lateral is divided in a maximum lateral force (FM1), a first peak value of maximum medial force (FM2) and a second peak value of maximal medial force (FM3). The vertical component is split in a first peak where the foot strikes the ground (FZ1), the cross-over point (FZ2) and a second peak relative to the amount of vertical propulsive force (FZ3). Therefore, each section may be related to functional events during foot contact, giving important information about the overall functioning of lower limb (106,107,110,112).

Categorizing the GRF into the different body planes provides important information about the loading, propulsion and the stability of the body during gait, giving important information about the overall functioning of lower limb.

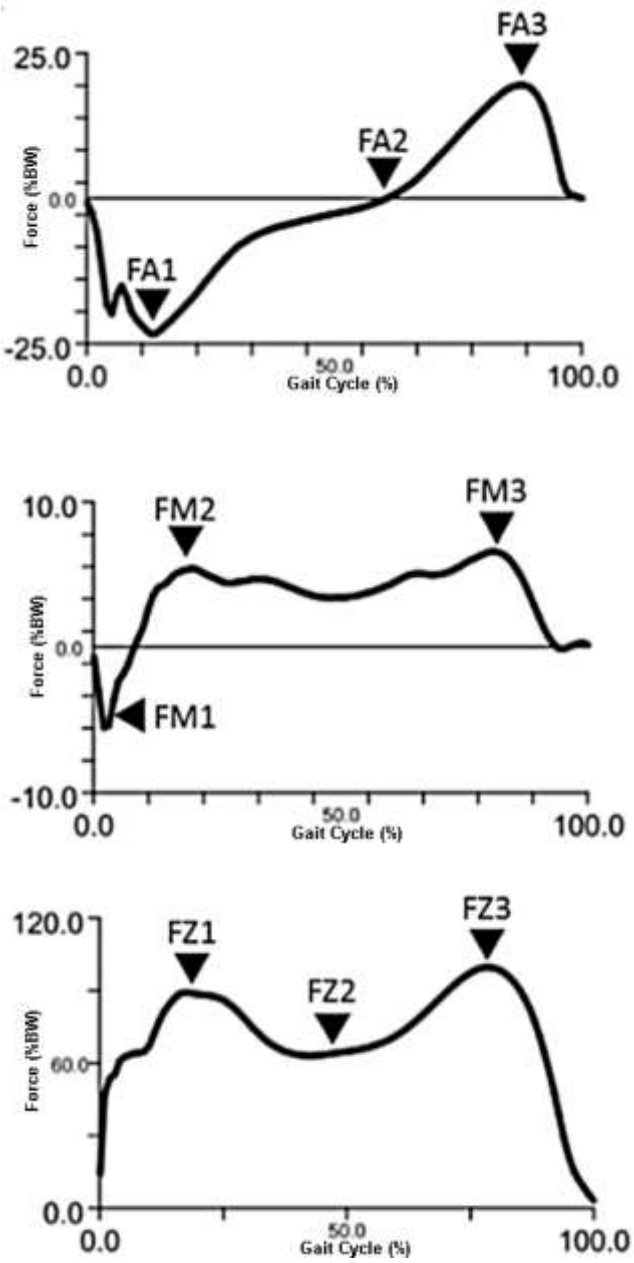


Figure 3. Ground reaction forces events descriptors (*Adapted from:* (110)).

Kinematics consists of evaluating motion without considering the forces involved. The integration of three-dimensional motion measurement using multi-camera systems (use of two or more cameras, at least two cameras are needed) has been successfully developed to track human body parts and perform dynamic analysis of their physical behaviors during gait. To accurately and effectively quantify the gait kinematics the following aspects need to be considered, such as camera positioning, camera speed, sampling frequency and shutter speed, synchronization of all cameras, calibration of image space, data capture, digitizing and transformation, data filtering and anatomical models and markers sets (108).

Most commercial kinematic systems use a 3D calibration object, which is viewed by all cameras. The most common and reliable way is to use a static frame to define the origin, or zero position, and the direction of axis. Additionally, a wand is moved dynamically through the volume of the cameras, in order to create a calibrated analysis volume. Reflective markers are placed on various anatomical landmarks to represent body segments and identify their positions and orientations. Markers can be singular to represent a joint, and are placed on lateral and medial aspects of joints on anatomical landmarks at the proximal and distal ends of the segment. Or can be in the form of clusters which are positioned on the body segment, in order to improve the segment tracking accuracy. These markers are generally made of a retroreflective material, to reflect the light from synchronized infra-red-light-emitting diodes mounted around the camera back to the camera lens.

Kinematic systems suffer from measurement errors, depending to a large extent on the field of view of the cameras, and the errors inherent in measuring the position of the markers, due to considerable movement that may take place between a skin markers and the underlying bone, often referred to as soft-tissue artifacts. Kinematic systems are used in gait analysis to record the position and orientation of the body segments, the angles of the joints and the corresponding linear and angular velocities and accelerations. When this system, is combined with a force platform, kinetic system, the capacity and potential of the combined system to dynamic gait evaluation is greater than that of the sum of its component parts (108–110,112).

With the prospect of gaining some insight into the musculoskeletal system during gait, electromyography (EMG) has been important for understanding the behavior and biomechanical functioning of this system. EMG is the measurement of the electrical activity of a contracting muscle - the muscle action potential. The contraction of skeletal muscles results from the chemical variation induced by the nervous system in the membrane of the muscle fibers, and these potential variations can be measured using EMG.

One important application of the EMG is the precise detection of motor events, such as the determination of the exact onset and offset time of a muscle contraction. The onset/offset timing pattern of muscle in the gait cycle, indicates at what time portion within a cycle the muscle is on and off. This pattern allows to calculate how long a muscle needs to turn on and how long it stays on to perform a gait cycle, thus providing a good overview of the neuromuscular activity pattern in normal and pathological walking (106,115).

Still, due to the hypothetical characteristics of the EMG signal, the precise detection of the onset and offset is a challenging task. To overcome this difficulty, the Teager-Kaiser energy operator (TKEO) can be used to robustly detect the onset and offset time of muscle activity. The TKEO measures instantaneous energy changes of signals composed of a single time-varying frequency. The calculated energy is derived from instantaneous amplitude and frequency of the signal, improving the ability to analyse muscle activity as depolarization of the muscle cell membrane during contraction produces rapid fluctuations in signal's amplitude and frequency (106,113,114).

Another important EMG parameter is the time to peak, that represents the duration from the beginning of contraction (muscle onset) to the peak amplitude value. This parameter addresses timing characteristics within the EMG signal and in ratio to other biomechanical signals or movement events, such as gait cycle (110,111,115).

The amplitude data of an EMG signal is, from a clinical and practical point of view, the most important and useful way to analyse how much is the muscle active and if is more or less active between patients. Although, EMG amplitudes are strongly influenced by the given detection conditions, electrodes sites, subjects and even day measures of the same muscle site. One solution to overcome the influence of the given condition is the Maximal Voluntary Contraction (MVC) test. The MVC test allows the normalization to a reference contraction value, eliminating the influence of the given condition and rescaling from microvolt to a percent of a reference value (115).

The use of EMG in gait analysis is a complex activity because many variables influence it. There still exist difficulties in understanding the relationship between the EMG signal and the physiological processes that give rise to it. However, the monitoring of the electrical activity of the excitable membranes of muscle cells is extremely important in assessing whether a given movement is activating the correct muscles, the gross innervation input of a selected muscle for a given task or whether the EMG activity is consistent with the kinematic and kinetic activity of the individual (115).

Dynamic gait evaluation is a useful tool that allows the examination of the intrinsic and extrinsic factors affecting an individual's ability to walk, its valuable for early diagnosis, explanation and evaluation of the functional limitation level and follow up evaluation over time.

To perform a detailed assessment of a patient with a walking disorder, 3D-instrumented gait analysis synchronized with force platforms and EMG have been used as a routine part of patient management to objectively quantify changes in the biomechanics of walking, together with the history and physical examination of the patient. Therefore, a fully equipped gait analysis allows the study and identification of movement adaptations, musculoskeletal mechanisms and strategies developed to compensate for some pathological conditions of the human body (106–108,112,116), providing decisive information on the evolution, characteristics and detrimental consequences of some pathologies.

DPN Gait Alterations

Spatiotemporal, Kinematics and Kinetics Parameters

DPN and gait are connected through shared physiology and anatomy of the lower limbs. DPN generally starts with sensory nerve damage and advances with the motor involvement, muscle weakness and atrophy, and physical dysfunction (76,77,79,92,104).

Whilst gait relies on intact sensory function for feedback to enable continual refinements and adjustments during walking, DPN impairs this sensory function. Significant deficits in vibration sensation, tactile sense and proprioception, and the loss of muscle strength, power and endurance, synergistically contribute to reduce functional capacity, altered gait and impaired balance in patients with DPN (76,79,116). The neuromuscular damage implied by peripheral neuropathy may thereby result in altered lower extremity biomechanics, such as modified walking speed and gait pattern or instability during walking. These biomechanical changes resulting from DPN contributes to the pathogenesis and development of foot ulcers and increases the risk of falling (76,77,79,116,117). So the identification of gait deviations, the factors that influence falls such as postural control deficit and gait instability and associated clinical parameters are particularly important for DPN subjects, as might help in the prevention and diagnosis of future development of ulcer and falls.

Gait analysis of diabetic peripheral neuropathy patients has received an increasing interest. An association between diabetic peripheral neuropathy and postural instability and uncoordinated gait was initially identified by Cavanagh et al. (118). In a study of type 1 diabetes patients with and without peripheral neuropathy, they reported that subjects with DPN were 15 times more likely to report an injury (fall, fracture, sprained ankle, or cuts and bruises) during walking or standing than the control group of patients with diabetes but no peripheral neuropathy (DM). They showed that the control of gait and posture is a clinically significant problem in patients with DPN and that diabetic peripheral neuropathy could represent the mechanism for gait abnormalities and greater risk of falls (118).

Since then, several studies have shown the strong evidence that diabetic individuals with peripheral neuropathy demonstrate a relative deficit in their ability to maintain posture, even when normal function of the other sensory organs is present (119–124). However, the deficit is greater when visual (119–121,123), vestibular (123) or somatosensory (123) cues are absent or degraded. The detrimental effect of distal symmetrical sensory neuropathy on postural stability and balance aggravate the risk of falls in such patients of all age and gender groups (119–124).

In addition to the relation between DPN, postural instability and increased risk of fall, several authors have found an association between diabetic peripheral neuropathy and gait abnormalities. A number of spatiotemporal gait parameters are different in subjects with diabetic peripheral neuropathy, such as smaller step length (125–130) and stride length (131,132), reduced duration of single support (125,128), higher duration of double support (128,129,133,134), decreased gait velocity (125–129,131–

133,135,136), lowered cadence (126,128), increased step time and step time variability (127,130), and greater gait variability (126,133), compared with control healthy subjects (125–129,131–137) or/and diabetic subjects without peripheral neuropathy (128,130,132,134–137).

There are few studies that evaluate and identify specific kinematics changes in subjects with diabetic peripheral neuropathy (131,132,135,137,138). Restriction of joint mobility is well documented in diabetic peripheral neuropathy (131,132,135,137,138). Reduced ankle range of motion (131,132,137), and reduced range of motion at the knee in both flexion and extension (132,135), are some of the main kinematics findings for DPN. However, the impact of DPN on the range of motion at the hip is still unclear, with some authors finding an increase (138), others a decrease (132) in the hip range of motion.

Differences in kinetic patterns with modified GRF and joint moments of force were also recognized in DPN individuals (116,134,135,137,139–142). GRF seem to differ in patients with DPN at either the initial contact or toe-off stages of gait (116,134,135,137,139–142). Although, there still exists considerable disparity among the findings.

Mueller et al. (131) revealed that diabetic peripheral neuropathic patients demonstrate reduced ankle mobility and decreased ankle plantar-flexor strength compared with age-matched controls. The authors believed that these changes contributed to a consequent reduction in the dynamic function during walking, resulting in slower walking velocity, shorter stride length, less ankle motion, lower peak ankle moments and power. The DPN group subjects had greater hip moments and power than ankle, opposite to the pattern observed in the control group. With this finding, Mueller et al. (131) suggested that the DPN group needs to compensate the loss of function in foot and ankle muscle by the proximal musculature of the hips. DPN subjects use the hip joint as a mechanism of forward progression of the body instead of using the ankle, by pulling their legs forward using hip flexor muscle (hip strategy) rather than pushing the legs forward using plantar-flexor muscles (ankle strategy). These findings support the hypothesis that ankle plantar-flexor peak torque and mobility, rather than walking velocity, sensory loss, or other complications of diabetes peripheral neuropathy, were the fundamental factors contributing to changes in gait pattern (131).

Katoulis et al. (135) observed alterations in some gait parameters during walking in subjects with DPN (with and without a history of foot ulceration) when compared to healthy control and diabetic non-neuropathic group. The DPN group shown significantly slower gait speed, smaller maximum knee joint angle in the sagittal plane during stance phase, and smaller maximum value of the vertical and anterior-posterior component of GRF. The authors suggested that a significant reduction in gait speed may be related to the proprioceptive deficit present in neuropathic patients. Additionally, they reported that the alterations observed in the knee joint angle and GRF could be associated with the differences in walking speed, since changes in a fundamental gait parameter, such as gait speed, could produce modifications in the overall pattern of movement. Furthermore, Katoulis et al. (135) concluded that neuropathy leads to a disturbance of foot mechanics during walking that could facilitate foot injuries, thus contributing to frequent foot ulceration. Similar alterations were found by Sawacha et al. (132) based on both diabetic gait and postural alterations, through 3D gait analysis. Trunk and lower limb joint mobility, in static and

dynamic states, were significantly reduced in diabetics either with or without peripheral neuropathy on each anatomical plane. However, in DPN subjects, lower ranges of motion were registered. Over the entire stance phase were observed significant differences on each joint, except for the right and left trunk rotation and the knee flexion-extension during the initial contact, the hip abduction-adduction between the loading response and the pre-swing, and the ankle abduction–adduction during the first 10% of the stance phase. In spatiotemporal parameters analysis, DPN subjects displayed significant differences almost for each variable when compared to control. Reduced walking velocity, stride length and longer stance phase duration were some of the main findings. However, when compared these parameters with the diabetic non-neuropathic group, no significant differences were observed. Sawacha et al. (132) showed marked alterations in the motion pattern in diabetic patients, suggesting that gait abnormalities are already significant without clinical evidence of peripheral neuropathy.

Saura et al. (137) observed also a reduced range of movement of the ankle in the sagittal plane, regardless of the presence or absence of peripheral neuropathy. However, unlike Katoulis et al. (135), Saura et al. (137) reported a statistically higher first and second vertical GRF peaks during walking in the neuropathic group when compared with the diabetic non-neuropathic and control groups. The authors suggested that this overload is probably related to the alterations in the intrinsic musculature of the foot, which has dynamic and static alterations overloading the forefoot (137).

Uccioli et al. (139) described the three GRF components for total foot and for three subareas of interest (heel, metatarsal area, big toe) during stance phase in diabetic patients with or without peripheral neuropathy. Under the heel and big toe areas the same observations were made in patients with peripheral neuropathy, such as significant reduction in the peak value in the vertical and anterior-posterior GRF. The authors suggested that this changes could be related to peripheral neuropathy and to the severe deformities of the toes (claw toe), typical at the most advanced stage of DPN. In the metatarsal area, Uccioli et al. (139) reported a statistically significant increase in the vertical component in patients with peripheral neuropathy, and in the medio-lateral component also, but only in subjects with previous neuropathic ulcers. With the increased tangential forces recorded at the metatarsal area, these authors suggested that they may have a role in the greater risk of foot ulcer recurrence (139).

Sacco et al. (134) described the gait of peripheral neuropathic diabetic patients using dynamic and temporal parameters as gait indicators. Temporal data indicated a tendency for longer double and single stance phase times in neuropathic patients in comparison to the control group. The authors also observed significant differences in the intermediate minimum vertical force, with DPN patients showing a tendency to present a lower value than other groups (diabetic group without peripheral neuropathy, and control group). Sacco et al. (134) demonstrated that this deficiency in the intermediate minimum vertical force may indicate a possible alteration in the locomotor capacity to reduce loads after heel strike, a common factor associated with pathological movements.

The presence of peripheral neuropathy sensory loss is one particular concern related to changes in gait characteristics of DPN subjects, although another yet to be considered is the neuropathic pain associated to DPN. To that extent, Lalli et al. (130) has assessed painful diabetic neuropathy and its

impact in gait. The authors of this study reported that diabetic patients with DPN and neuropathic pain had greater variance in gait for both step length and step velocity when compared to DPN subjects without neuropathic pain and control subjects. Still, these differences were not observed when compared to diabetic non-neuropathic patients. Besides that, the results of this study suggested that the presence of neuropathic pain impacts upon gait stability, leading to an increased number of falls with subsequent hospitalizations for sustained injuries, as well as a greater fear of falling (130).

Few authors have attempted to evaluate gait under a real life environment, to correctly analyse and understand individuals gait and fall risk. Altered spatiotemporal gait parameters were reported by Menz et al. (126). They observed reduced gait speed, cadence and step length and fewer acceleration patterns at the head and pelvis in subjects with diabetic peripheral neuropathy in comparison to healthy, age-matched controls. These effects were greater on an irregular surface than on level surface. Menz et al. (126) also found impaired peripheral sensation, lower-limb reaction time and balance control in DPN group, which may help to explain the high rate of falls in this group and the lack of confidence when they walk in an irregular/unfamiliar floor or surface. These authors suggested that, with sensory-motor function deficits, individuals with DPN have a more conservative gait, with less potential for instability, as a protective adaptation to perform a well-established movement patterns, with a constantly need to carefully monitor and continually adapt the basic stepping pattern (126). Similarly, Paul et al. (128) also observed that individuals with DPN have a more conservative gait pattern, possibly due to a overloading of the sensory-motor system, when investigating the effect of a secondary motor or cognitive task on their gait. For all gait variables, these authors observed a statistically significant difference between people with diabetes and no peripheral neuropathy and people with diabetes and diabetic peripheral neuropathy. Subjects with DPN presented a slower walking velocity and smaller steps when compared with those with DM. Paul et al. (128) reported that the addition of either cognitive or motor tasks negatively affected the gait parameters for both groups, with greater effect in the DPN group. Additionally, these authors suggested that subjects with DPN use their attentional capacity to maintain their gait, thus leaving less capacity to divide and divert their attention for other simultaneous cognitive tasks (128).

Richardson et al. (127) also showed that environmental factors, such as a challenging environment in which either walking surface conditions or lighting intensity was manipulated, have a significant effect on all spatiotemporal gait parameters in diabetic peripheral neuropathic subjects. Reduced step length and speed and increased step width, step-width variability, step width to step length ratio and step-time variability were observed. Therefore, diabetes peripheral neuropathy associated gait dysfunctions are highlighted and sensitively detected in challenging environments (126–128).

Although several studies have suggested that altered gait patterns of diabetic persons are largely related to peripheral neuropathy, similar abnormalities have been detected in diabetic patients free of peripheral neuropathy. Acquisition of new knowledge into gait pattern alterations that occur in diabetic non-neuropathic individuals is crucial for the development of strategies that effectively prevent mobility impairment in early stages diabetes.

Petrofsky et al. (143) studied the gait changes in people with type 2 diabetes mellitus but no peripheral neuropathy. In this study, subjects with diabetes walked significantly slower and used more steps to complete the linear walking compared to healthy controls. In addition, the velocity of gait was also significantly lower and the steps wider during turns in the diabetic group. Petrofsky et al. (143) demonstrated that there is indication of gait impairment independent of muscle strength and sensory loss in the foot, abnormalities associated to DPN. Similarly, Yavuzer et al. (116) reported that diabetic patients free of peripheral neuropathy had significant gait deviations. In this study, the DM group, but not the diabetic peripheral neuropathic group, revealed a slower walking velocity, shorter steps, longer double support time, limited knee and ankle mobility in the sagittal plane, lower peak ankle plantar flexion moment and power than the control group. Identical kinetic and kinematic gait deviations have been reported in patients with diabetes as a result of peripheral neuropathy, as stated above. So, Yavuzer et al. (116) suggested that the abnormalities observed in gait parameters of diabetic patients may be a compensatory strategy adopted to improve stability or to maintain balance rather than a direct result of peripheral neuropathy.

Dynamic EMG

In addition to gait problems, some studies have reported muscle deficits in diabetic peripheral neuropathic individuals. Up to now, the main findings from the study of electrical muscle activity during gait in this population, are a delayed activation of the *gastrocnemius* (138,144), *soleus*, *peroneus brevis* and *peroneus longus* muscles, and mostly importantly, of the *tibialis anterior* muscle (dorsiflexor muscle), that may be responsible for the increase impact over the forefoot during the flat foot phase in gait (145). However, some studies have observed a premature activation of such muscles, *soleus*, *gastrocnemius medialis* in normal conditions (146), and *vastus lateralis* and *tibialis anterior* with an increased gait cadence (138). Findings relative to the cessation times have also been done. DPN individuals demonstrated a significantly later cessation of the *soleus*, *gastrocnemius* (138,146) and *tibialis anterior* (146,147).

Kwon et al. (146) when comparing the average onset and cessation times, as a percentage of gait cycle, of lower extremity muscle activity, observed a significantly premature activation and a delay average cessation of the *soleus*, *gastrocnemius medialis*, and *medial hamstring muscles* in subjects with DPN. These authors suggested that the premature activation of *soleus* and *gastrocnemius medialis* muscles may contribute to early forefoot contact with the ground, decreased shock absorption at heel strike, increased impulse at the forefoot, and prolonged duration of forefoot loading during walking (146). They also observed a prolonged activity of the *tibialis anterior* muscle, with a significantly later cessation time in this group. Additionally, the activity pattern in this study demonstrated co-contractions of agonist and antagonist muscles during stance phase at the ankle and knee joints in the DPN group, suggesting that may be related to an adaptive walking strategy, to employ a safer and more stable gait, that compensates for the diminished sensory information (146).

Neuropathic ulceration is the most prevalent type of long-term chronic injury for diabetic subjects, so the presence of foot ulceration may be an indicator of the progression of diabetic peripheral neuropathy. In this sense, Akashi et al. (144) investigated the influence of diabetic peripheral neuropathy and previous history of plantar ulcers on EMG of the thigh and calf. Delayed in the time of peak occurrence in the *vastus lateralis* at the beginning of the stance phase and *gastrocnemius lateralis* during push-off phase were observed for the ulcerated group in comparison to controls (control subjects and diabetic neuropathic group without any history of foot ulceration). The authors proposed that this delay demonstrate that individuals with DPN and previous history of foot ulcers have a motor deficit. The mechanisms of load and shock attenuation in the moment of heel contact may be weakened due to the *vastus lateralis* delay, and during the push off phase of gait the *gastrocnemius lateralis* activation delay can significantly alter the propulsion function, compromising their ability to walk (144). Contradicting some studies (145,146), Akashi et al. (144) showed no differences in the *tibialis anterior* time of peak activation or magnitude, among groups.

To understand the neuropathic effect in the generation and control of movements in DPN individuals, Savelberg et al. (147) and Gomes et al. (138) evaluated the influence of increase gait cadence on muscle activation patterns, a situation that demands greater strength and neuromuscular control. Savelberg et al. (147) reported that timing of activation of muscles involved in braking the forward velocity after heel strike, was susceptible to both diabetes mellitus with and without peripheral neuropathy, and walking velocity (preferred and standard velocity). In both gait velocities, cessation of activity of *tibialis anterior* and mono articular knee-joint extensors (*vastus medialis*) was significantly delayed in both diabetic groups when compared to healthy controls. Increased gait velocity caused different adaptations of timing of muscle activation, with total relative duration of *gluteus maximus* shorter and duration of *tibialis anterior* activity increased in the DPN group than in diabetic non-neuropathic and control group. The authors suggested that the extended activity of ankle-joint dorsal flexors (*tibialis anterior*) and of mono articular knee-joint extensors (*vastus medialis*) may be related to an increased effort to control the forward displacement (147).

Additionally, Gomes et al. (138) observed similar EMG activation patterns between groups (control and DPN group) for all muscles (*vastus lateralis*, *gastrocnemius medialis*, *peroneus longus* and *tibialis anterior*) in each phase (stance and swing phase) and cadence, with the exception of *gastrocnemius medialis* muscle. The authors observed a delay in the activation of *gastrocnemius medialis* during the stance phase in DPN individuals regardless of the cadence, and a longer delay in the swing phase when walking at the imposed cadence compared to the self-selected. Between groups there were not observed significant differences in the *vastus lateralis*, *peroneus longus* and *tibialis anterior*, although statistical difference between cadences in each group for the *vastus lateralis* and *tibialis anterior* was found. Premature activation was observed for these muscles in both groups at the stance phase during the imposed cadence (138). With these findings, Gomes et al. (138) concluded that, when challenged with an increased gait cadence, diabetic peripheral neuropathic individuals can't generate the expected and proper knee and ankle motor responses to the mechanical task demand.

As seen in the chapter of gait abnormalities, diabetes may impact gait mechanisms before onset of peripheral neuropathy (116,143). Aiming to fill a potentially gap in the research literature Sawacha et al. (148) investigated muscle activity deviations during the gait cycle in the early stages of diabetes, when neuropathy is absent. At initial contact and loading response, these authors observed an early peak of *rectus femoris* activity in both diabetic groups, with and without peripheral neuropathy. In the diabetic non-neuropathic group was also found a delay of *gastrocnemius lateralis* activity during midstance, and a delay of *rectus femoris* and *gluteus medius* activity during terminal swing (148). Sawacha et al. (148) demonstrated that important muscle activity abnormalities are present in diabetic subjects free of peripheral neuropathy, indicating that changes in foot muscles occur before changes in nerve function can be detected, so they may not be directly related to peripheral neuropathy.

Although there are clear evidences that muscle activation of the lower limbs is affected in diabetic gait, studies are still very limited and still scarce. Results in the literature are controversial, inconsistency exists about whether muscle activation in patients with diabetes peripheral neuropathy is delayed or prolonged.

The literature describes a variety of alterations in gait and electrical muscular activity associated with diabetic peripheral neuropathy, although the nature and extent of these remains undefined due to the limitations in study design. A tendency towards increased variability in gait parameters such as spatiotemporal, joint angles and GRF has been documented, in addition to lower-extremity muscles deficits during gait cycle.

Chapter 4

Methodology

Methodology

Participants/Patient Recruitment

Sixteen diabetic patients were recruited from the patients attending the outpatient clinic of the São João Hospital Center (Porto, Portugal). All diabetic patients were classified in the levels zero or two of the 2015 International Working Group on Diabetic Foot (IWGDF) Risk Classification System and distributed in two groups: diabetic subjects with no peripheral neuropathy (level 0-DM group) and diabetic subjects with peripheral neuropathy, and with arterial disease and/or a foot deformity (level 2-DPN group).

Fifteen control subjects were recruited to the dynamic EMG part of the study. This group consisted on healthy subjects, with no history of diabetes mellitus and over 50 years of age.

Inclusion and Exclusion Criteria

The study was conducted at LABIOMEP - Porto University Biomechanics Laboratory - after approval of the Ethics Committee of São João Hospital Center, where it was recorded with the number CEs 213-16. Inclusion in either of the diabetes groups required a diagnosis of type 1 or type 2 diabetes, which was established from participant's individual medical case notes. Only the subjects that read and signed an informed consent, agreeing to participate in the verbally and written explained procedures/steps were included in the study.

Exclusion criteria was based on medical history. Subjects were excluded from the study in the presence of any disturbance that might affect gait abilities like an orthopedic, neurological or visual impairment or other, including current injury, pain, active ulceration or previous amputation.

Eligibility criteria for the additional control group in the EMG part of the study were healthy individuals over 50 years of age, no history of diabetes, absence of neurological diseases and orthopedic problems, and ability to ambulate independently without pain or the use of an assistive device.

Assessment for DPN/Neuropathy Diagnosis

Neuropathy was diagnosed in the outpatient clinic of the São João Hospital Center. The diagnosis was based on medical assessment and included monofilament test, vibration test with biosthiometer, deep tendon reflexes and autonomic testing in addition to glycated hemoglobin HbA1c, transcutaneous oxygen pressure and ankle-brachial index.

When the subjects arrived to the LABIOMEP-UP, and before data collection, the Michigan Neuropathy Screening Instrument (MNSI), previously validated to the Portuguese population (149), was also performed.

Experimental Procedures- Gait Analysis and Dynamic EMG

Three-dimensional computerized gait analysis was performed to all diabetic participants (DM and DPN group) to assess the movement characteristics such as joint angular kinematics and descriptors of the gait cycle, termed spatiotemporal parameters. Data was captured with a 12-camera Qualisys motion capture (MoCap) system (Qualisys AB, Sweden). A full-body marker setup based in the IOR model (150,151), comprising sixty-five reflective markers, was used.

The reflective markers were placed at the thorax, head and upper limbs as follows: headband with four markers (anteriorly over the temple and aligned with the lateral commissure of the eye and posterior over the occipital bone at the same level as the anterior ones on the frontal and sagittal plane), on the acromial border of the right and left scapula, on the 7th cervical vertebrae, on the jugular insertion/ notch of the sternum, on the xiphoid process of the sternum, on the lateral and medial epicondyle of the humerus of both arms, on the radio-styloid process and on the ulna styloid process of both arms, and on the lateral portion of the 2th and 5th metacarpal head of both hands. The markers of the lower limbs were placed: on each side of the anterior superior iliac spine, and also on the posterior superior iliac spine, on each trochanter, on the lateral and medial epicondyle of the femur of both legs, on the proximal tip of the head of the fibula and on the most anterior portion of the tibia tuberosity for both knees.Tracking markers (4 marker clusters) were also placed over the thighs and shanks in order to improve the segment tracking accuracy.

In this study a multi-segmented model of the foot was implemented following the Oxford Model: on the lateral and medial prominence of the lateral malleolus of both ankles, on each proximal, medial and distal end of the posterior aspect of calcaneus (superior, medial and inferior heel) of both feet, on the lateral and medial aspect of the calcaneus of both feet, on the dorsal aspect of the base of the 1st metatarsal, on the lateral aspect of the head of the 1st metatarsal, on the lateral aspect of the base and on the head of the 5th metatarsal, on the lateral aspect of the head of the 2nd metatarsal and on the lateral aspect of the head of the hallux, all for both feet. The exact placement of anatomical and tracking markers is depicted in figure 4, 5 and 6.

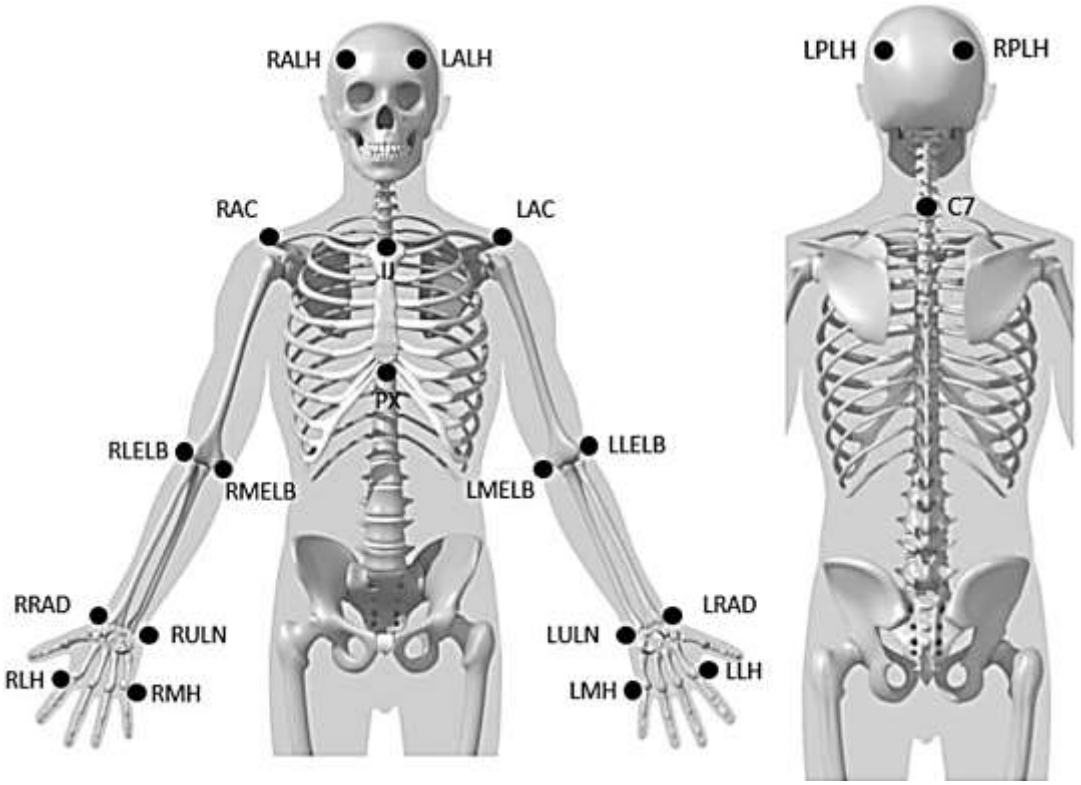


Figure 4. Thorax, head and upper limbs markers.

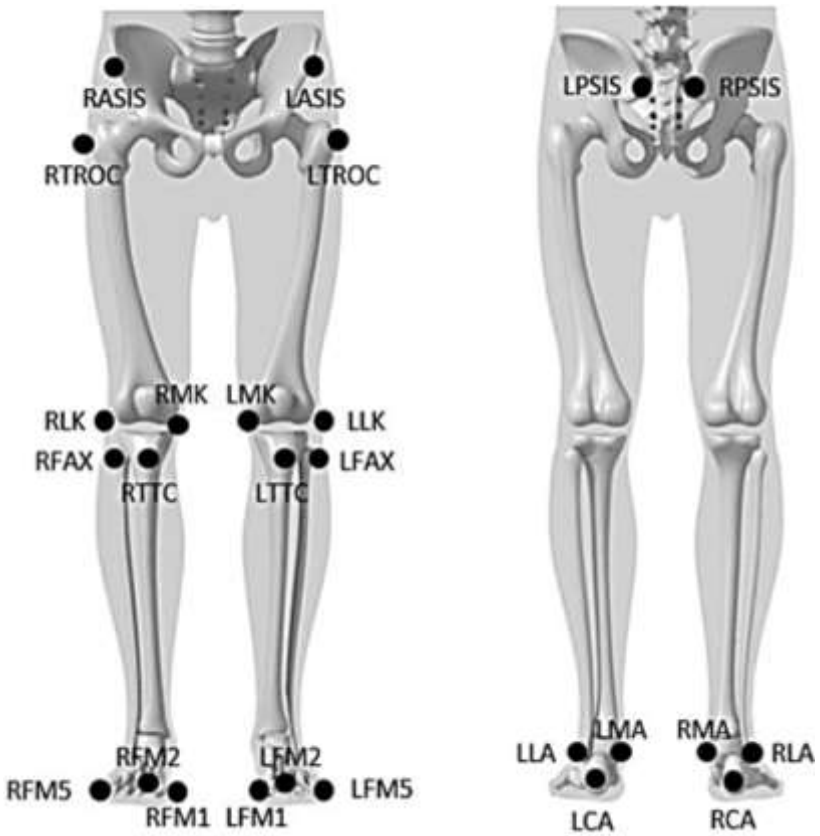


Figure 5. Lower limb markers.

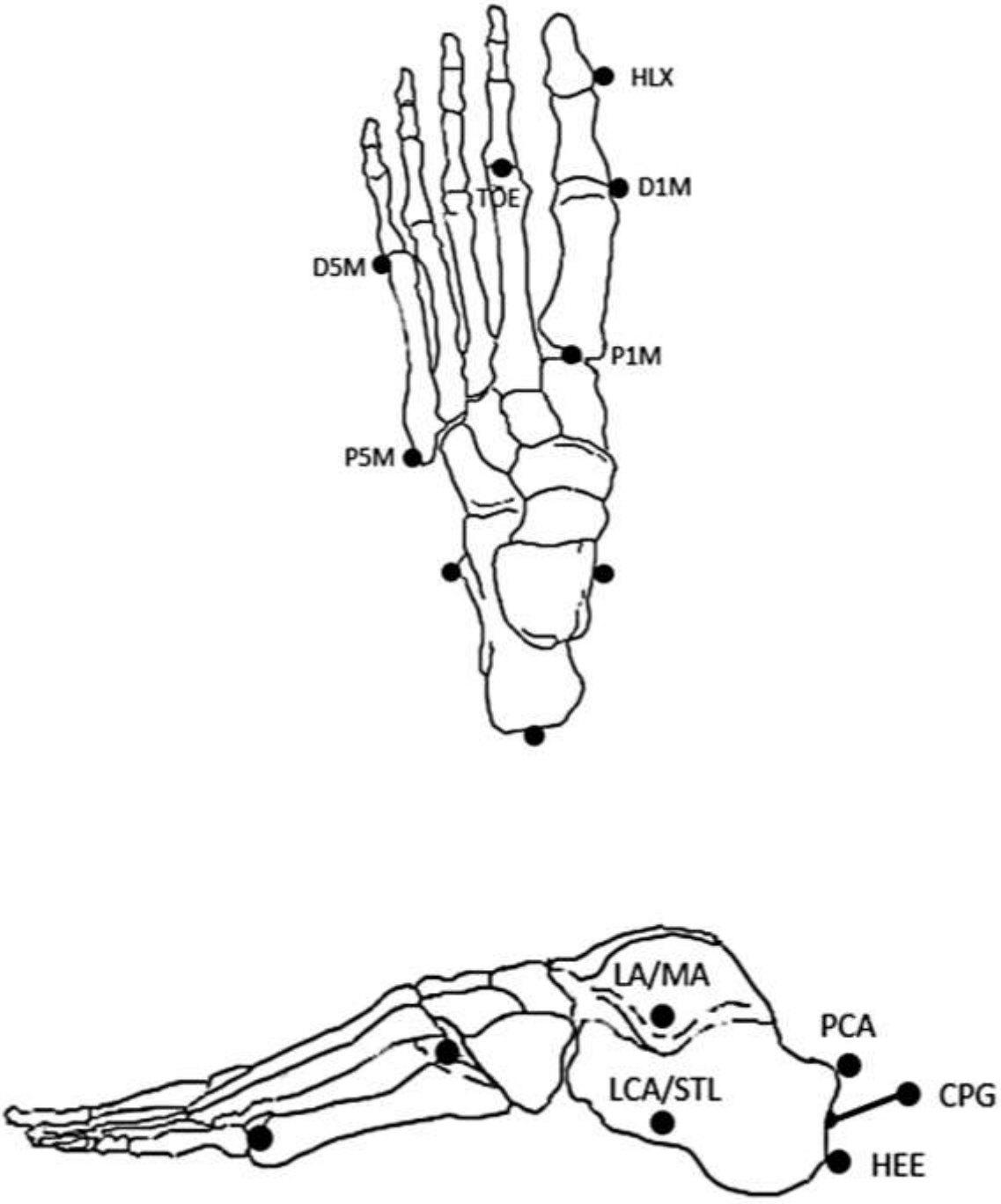


Figure 6. Multisegmented foot: Oxford model.

Kinematic data were collected in a previously calibrated volume, with a calibration error bellow 0.6 mm at a 200 Hz sampling frequency.

Ground reaction forces were measured by four Bertec (Bertec Corporation, USA) force platforms (2 platforms of 40x60 cm and 2 platforms of 60x90 cm) recording at a 2000 Hz sampling rate, in synchrony with the MoCap system. The arrangement of force platforms allowed for the measurement of three consecutive steps, as depicted in figure 7.

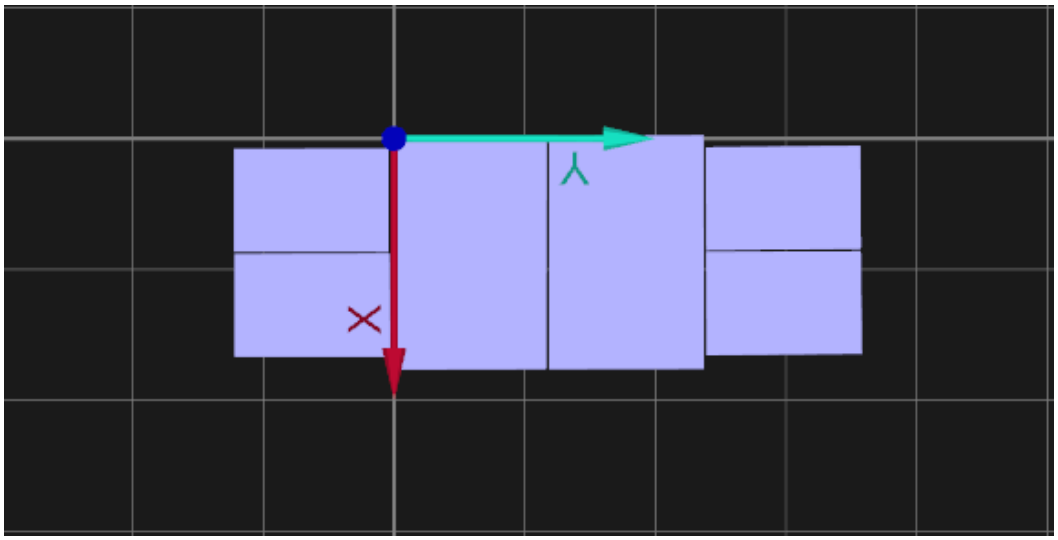


Figure 7. LABIOMEP-UP arrangement of six Bertec force platforms.

The electromyography activity of the *gastrocnemius medialis* (GM) and *tibialis anterior* (TA) muscles for both legs was monitored throughout the gait cycle of all diabetic subjects (DM and DPN group) and of the additional control group (CG). These muscles were selected due to their essential role in gait progression (GM), as well as knee and ankle impact attenuation (TA), and because of their agonist/antagonist relationship. Additionally, because of the lower subcutaneous body fat-associated impedance, the EMG activity of these muscles is frequently reported in the literature.

EMG was measured with the Delsys Trigno™ Wireless EMG System (Boston, MA, USA). These sensors use four rectangular (5x1 mm) embedded electrodes with an inter-electrode distance of 10 mm, a signal resolution of 16-bit and a common-mode rejection ratio (CMRR) >80 dB. Additionally, the sensors include an on-board band-pass filter, limiting the signal bandwidth to 20-450 Hz. The EMG data was recorded in synchrony with the MoCap system, with a sampling frequency of 2000 Hz.

Previous to any EMG data recording, the skin was prepared and the locations of EMG electrodes were chosen in agreement with the recommendations of the project Surface EMG for a Non-Invasive Assessment of Muscle (SENIAM) (152). Skin preparation consisted of shaving, if necessary, and a light scrubbing with alcohol. The electrodes were place on the belly of the muscle of interest, with the long axis of the electrode positioned parallel to the muscle fibers. In TA the electrode was placed at 1/3 on

the line between the tip of the fibula and the tip of the medial malleolus, and in the GM in the most prominent bulge of the muscle. The placement of electrodes of GM and TA is depicted in appendices figure 16 and 17, respectively.

Double-sided adhesive tape was used to attach the electrodes to the skin, and an elastic band to secure the electrodes avoiding accidental sensors falls.

Before data acquisition, all participants were instructed to walk barefoot at a self-selected and comfortable pace across a 10-meter walkway, which allowed them to adapt to the laboratory environment and reproduce their daily gait. In order to standardize the gait initiation, a starting line was established so that the participant had to perform two gait cycles before reaching the force platforms. All subjects wore tight-fit shorts and t-shirt, exposing the lower extremity for attachment of reflective markers and EMG electrodes. No other constraints were placed over the participants.

The procedure was repeated ten times to generate sufficient data to obtain a mean value for each parameter being measured. If more than one foot was in contact with the force platform or if a clear targeting behavior was perceived by the researchers, the trial was discarded and a new one was performed without notifying the participant. Trials in which all the markers were clearly and possible to identify by the system and with only one foot in contact with each force platform, were defined as valid.

After gait trials were recorded, a maximum voluntary contraction test was performed for each individual, in order to normalize the EMG data. Three maximum isometric voluntary contractions against static resistance were performed for each analysed muscle separately. For each muscle, a specific test position was performed, during which a 3-second activation of EMG was obtained. A 30- to 60-second rest period in between each MVC was provided.

To obtain the maximum voluntary contraction of the TA, individuals were instructed to seat and the leg was supported above the ankle joint with the ankle joint in dorsiflexion and the foot in inversion without extension of the great toe. While seated, maximum resistance was applied by the examiner against the medial side, dorsal surface of the foot in the direction of plantar flexion of the ankle joint and eversion of the foot. The MVC of GM was performed standing. Participants were instructed to perform plantar flexion of the foot with emphasis on pulling the heel upward more than pushing the forefoot downward. The examiner held the subject's shoulders to apply pressure against the forefoot and the calcaneus. An example of MVC technique is illustrated in appendices figure 18 and 19.

Prior to the start of the trials, the procedures were explained to the subjects, and they signed an informed consent form approved by Ethics Committee of São João Hospital Center. The procedures lasted about 60 minutes for each subject, therefore if any subject showed or verbalized any sign of fatigue an additional rest period was given during the data collection.

Data Processing

The recorded motion data was pre-processed with the Qualisys Track Manager (Qualisys AB, Sweden) software, and the resulting data exported to C-Motion's Visual3D (C-Motion, USA) for further analysis. The marker's trajectories were then filtered with a 6-Hz low-pass Butterworth filter and gait events (heel strike and toe-off) were automatically identified with the software's proprietary routine.

GRF were filtered with a 50-Hz low-pass Butterworth filter in order to reduce some high-frequency parasitic signals encountered in the data. After this, the events of the anterior-posterior, medio-lateral and vertical forces were characterized. The zero-crossing instant of the anterior-posterior force was identified as the midstance gait event.

The raw EMG data was filtered using a Butterworth band-pass filter with a cut-off frequency at 20 Hz and 500 Hz, and then full-wave rectified. To obtain a representative envelope of the EMG activity, a 10 Hz low-pass filter was used. The onset and offset of the muscle activity was determined by the Teager-Kaiser energy operator method. The EMG data obtained during gait and MVC test were treated the same way.

To ensure the validity of the computer derived GRF and EMG variables, each trace was visually inspected to ensure that any movement artifacts or any other interference was not identified incorrectly as a force interference or muscle activity signal.

Spatiotemporal gait parameters, such as cycle time, speed, statures per second, stride length, stride width, stance time, step length and time, strides and steps per minute, swing time, and right initial and terminal double limb support time were statically compared between subjects with DPN and diabetic non-neuropathic subjects. Between these groups, was also compared the anterior-posterior, medio-lateral and vertical GRF normalized to body weight and the time of occurrence normalized to the stance duration. The kinematics of each lower limb (pelvis, hip, knee and ankle angle) was analysed at the three major gait moments, initial contact (heel strike), midstance and pre-swing (toe-off), and compared between the diabetic groups.

From the EMG linear envelope signal was calculated the duration of muscle activity relative to cycle time (activation time), the mean amplitude value during activation, and the time to peak occurrence. These variables characterize the muscle activity of GM and TA of the three groups (DM, DPN and control group). With the exception of temporal variables, all other EMG variables were normalized to the MVC.

Statistical Analysis

In this cross-sectional study, appropriate summary statistics were applied in the descriptive analysis of the sample. Categorical variables (gender, type of diabetes, groups) were described using absolute (n) and relative (%) frequencies. To analyse the association between categorical variables the Chi-Square test of independence (*) was used. When the expected frequency of any cell from the contingency table related to the association analysis of two categorical variables was less than 5, Fisher's exact test (**) (if the two variables have two categories) or the Chi-Square exact test (***) (if at least one of the variables has more than two categories).

The continuous variables of the study were described using the mean and standard deviation or median, minimum and maximum, depending on whether the distribution of these variables is normal or non-normal, respectively. Before data inferential analysis, normality of distribution was explored. The t-test and the Mann-Whitney test were used to test hypotheses in two independent samples relative to continuous variables with normal and non-normal distribution, respectively. The One-Way ANOVA and Kruskal-Wallis test were used to test hypotheses in more than two independent samples concerning continuous variables with normal or non-normal distribution, respectively.

A significance level of 0.05 (5%) was set for all hypothesis tests. The analysis of data was performed using the statistical analysis program IBM SPSS® Statistics v.24.0 (IBM Corporation, New York, USA).

Chapter 5

Results

Results

Socio-Demographic Characterization

The following data is presented for both diabetic groups, DM group-diabetic subjects with no peripheral neuropathy (n=9; 56%) and DPN group-diabetic subjects with peripheral neuropathy and arterial disease and/or foot deformity (n=7; 44%). From the analysis of table 2, it can be seen that the majority of the participants in the study were male, n=12 (75%). Additionally, regarding the diabetic group, no significant differences in terms of percentage were observed regarding the gender (DM: female-33%, male-67%; DPN: female-14%, male-86%; *p*=0.585). In DM group, the majority of subjects have type 1 diabetes, n=5 (56%), and in the DPN group the majority have type 2 diabetes, n=6 (86%), this difference was not statistically significant (*p*=0.524).

Table 2. Socio-demographic characterization of diabetic participants of both groups 0 and 2 IWGDF risk (DM and DPN group).

	Total (n=16)		**Group**				
			DM (0-no peripheral neuropathy) (n=9; 56%)		**DPN (2-peripheral neuropathy with arterial disease and/or a foot deformity) (n=7; 44%)**		
	n	%	n	%	n	%	*p*
Gender							0.585**
Female	4	25%	3	33%	1	14%	
Male	12	75%	6	67%	6	86%	
Diabetes Type							0.524**
Type 1	6	38%	5	56%	1	14%	
Type 2	10	62%	4	44%	6	86%	

*Chi-Square Test of Independence; **Fisher's Exact Test; ***Chi-Square Exact Test.

In table 3 is presented the distribution of age, height, weight and body mass index (BMI) of all diabetic participants. With a mean age of 53 (± 20) years, significant differences were found in relation to the level of neuropathy, with the DPN group with older participants, mean age of 67 (± 11) years, and the DM group with younger participants, 42 (± 18) years old (p=0.004). The mean height was 1.67 (± 0.10) m and the mean weight is 75.92 (± 14.29) kg, and no differences were found between both diabetic groups (p_{height}=0.917; p_{weight}=0.055). As for BMI, the DPN group presented significant higher values (30.17 ± 2.84 kg/m^2), when compared to the DM group (24.77 ± 2.71 kg/m^2) (p=0.002).
As expected, lower values for age, weight, BMI were found in the diabetic group without peripheral neuropathy, when compared to the DPN group (table 3).

Table 3. Distribution of age (in years), height (m), weight (kg) and BMI (kg/m2) of diabetic participants, DM and DPN group.

			Group				
	Total (n=16)		**DM (n=9; 56%)**		**DPN (n=7; 44%)**		
	Mean	(sd)	Mean	(sd)	Mean	(sd)	*p*||
Age (years)	53	(20)	42	(18)	67	(11)	***0.004***
Height (m)	1.67	(0.10)	1.67	(0.12)	1.67	(0.08)	0.917
Weight (kg)	75.92	(14.29)	69.96	(15.45)	83.59	(8.41)	0.055
BMI (kg/m^2)	27.13	(3.84)	24.77	(2.71)	30.17	(2.84)	***0.002***

sd: Standard deviation; *p*=Percentile; || T-test. Bold *p* values are significant with p<0.05.

Spatiotemporal Gait Parameters

Regarding the spatiotemporal data collected, this study included 9 (56%) participants in the DM group, and 7 (44%) in the DPN group. The spatiotemporal gait parameters, summarized in table 4, showed significant differences between the groups. DM participants presented a faster gait (p=0.002) with higher cadence (left: p=0.029), greater stride (p=0.002), and step length (right: p=0.001; left p=0.005), and less stride width (p=0.011) when compared to the DPN group. To better compare speed between subject's, a variable that allows the normalization of the speed as it corresponds to the average speed divided by the subject height, statures per second, was also analysed.

Table 4. Spatiotemporal gait parameters of both diabetic groups, DM and DPN.

	Group						
	DM (n=9; 56%)			DPN (n=7; 44%)			
	Mean	(sd)		Mean	(sd)		*p*
Cycle Time (s)	1.06	(0.12)		1.21	(0.15)		***0.044***||
Speed (m/s)	1.21	(0.10)		0.89	(0.18)		***0.002***||
Statures per second	0.73	(0.08)		0.54	(0.12)		***0.002***||
Stride Length (m)	1.27	(0.13)		1.03	(0.12)		***0.002***||
Stride Width (m)	0.12	(0.02)		0.15	(0.02)		***0.011***||
Right Cycle Time (s)	1.06	(0.12)		1.21	(0.14)		***0.045***||
Right Stance Time (s)	0.64	(0.08)		0.77	(0.12)		***0.021***||
Right Step Length (m)	0.65	(0.07)		0.51	(0.06)		***0.001***||
Right Step Time (s)	0.53	(0.06)		0.60	(0.07)		0.066||
Right Stride Length (m)	1.27	(0.13)		1.04	(0.13)		***0.003***||
Right Strides p/min	57.23	(6.23)		50.67	(5.83)		***0.050***||
Right Swing Time (s)	0.42	(0.04)		0.44	(0.03)		0.406||
Left Cycle Time (s)	1.06	(0.12)		1.21	(0.14)		***0.040***||
Left Stance Time (s)	0.65	(0.10)		0.76	(0.11)		0.059||
Left Step Length (m)	0.63	(0.06)		0.53	(0.06)		***0.005***||
Left Step Time (s)	0.53	(0.06)		0.61	(0.07)		***0.026***||
Left Stride Length (m)	1.28	(0.12)		1.04	(0.13)		***0.002***||
Left Steps p/min	114.34	(12.09)		99.72	(11.69)		***0.029***||
Left Strides p/min	57.26	(6.18)		50.39	(5.77)		***0.039***||
Left Swing Time (s)	0.41	(0.04)		0.45	(0.04)		0.078||
	Median	min	max	Median	min	max	*p*
Double Limb Support (DLSP) Time (s)	0.41	0.36	0.48	0.45	0.38	0.51	0.023§
Right Initial DLSP (s)	0.10	0.08	0.23	0.15	0.11	0.23	***0.031***§
Right Steps p/min	113.54	3.25	123.08	98.12	85.66	116.54	0.252§
Right Terminal DLSP (s)	0.11	0.08	0.15	0.14	0.10	0.22	***0.031***§

sd: Standard deviation; min: Minimum; max: Maximum; *p*: Percentile; || T-test; § Mann-Whitney Test.
Bold *p* values are significant with $p<0.05$.

Kinematics

Regarding the kinematic data collected, this study included 9 (56%) participants in the DM group, and 7 (44%) in the DPN group. A series of graphs illustrating the results of the joint angle of the right and left lower limb from the DM and DPN group are reported in figure 8 and 9, respectively.

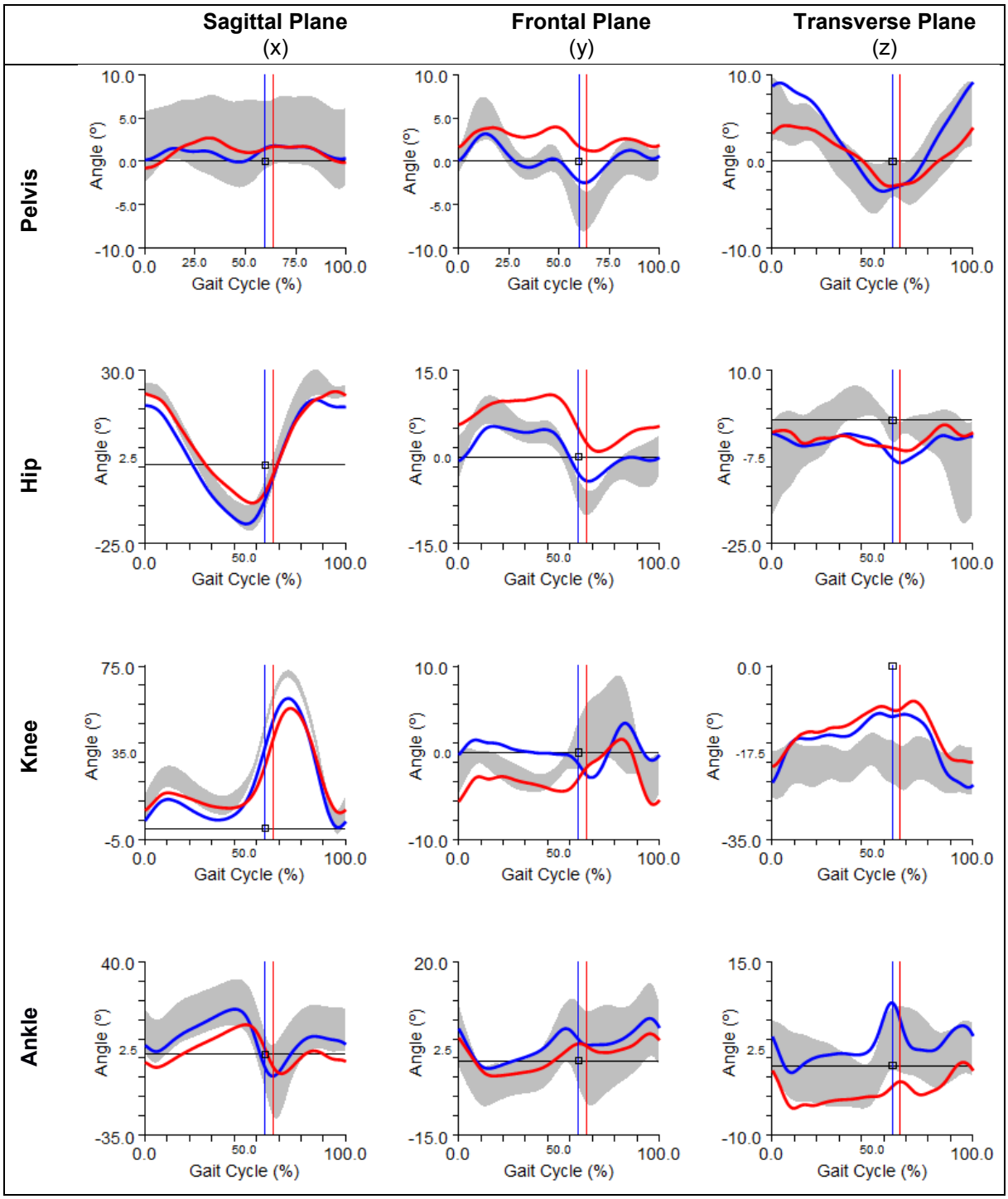


Figure 8. Gait analysis joint kinematic results for the right lower limb of all diabetic subjects' in the sagittal, frontal and transverse plane.

Legend: Mean joint angles of the DM and DPN. The DM group is depicted in blue and DPN group in red. Reference data is depicted in gray. Vertical axis represents joint angulation in degrees. Horizontal axis represents the duration of a gait cycle expressed as a percentage. The vertical line represents the moment of toe-off.

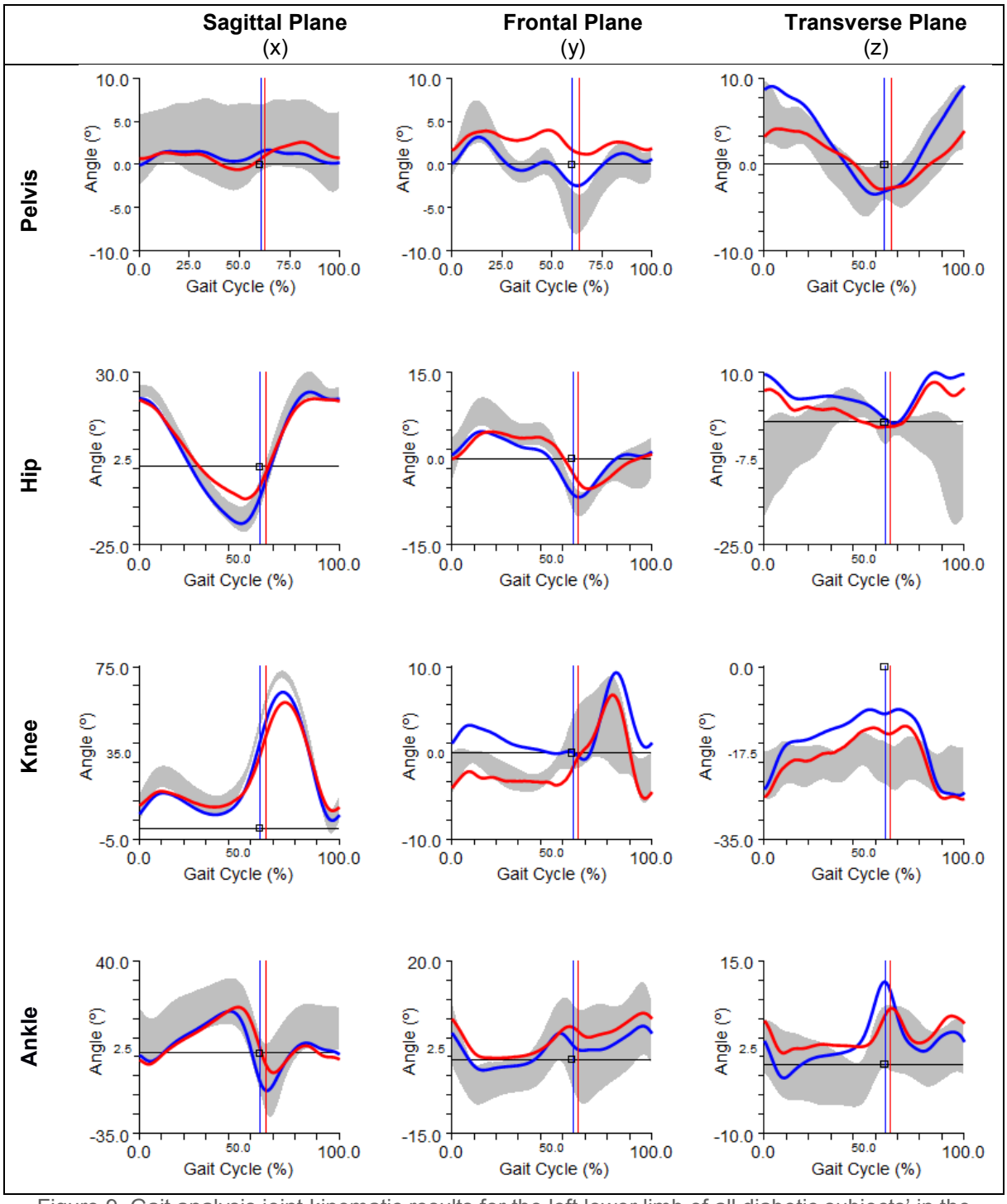


Figure 9. Gait analysis joint kinematic results for the left lower limb of all diabetic subjects' in the sagittal, frontal and transverse plane.

Legend: Mean joint angles of the DM and DPN. The DM group is depicted in blue and DPN group in red. Reference data is depicted in gray. Vertical axis represents joint angulation in degrees. Horizontal axis represents the duration of a gait cycle expressed as a percentage. The vertical line represents the moment of toe-off.

The pelvis, hip, knee and ankle angle of each lower limb (right and left) were analysed in three major gait events of the stance phase, heel strike (table 5 and 6), midstance (table 7 and 8) and toe-off (table 9 and 10). In each body segment (pelvis, hip, knee and ankle) is described the movement in the sagittal (x), frontal (y) and transverse (z) plane. For each plane the first movement is in the positive direction and the second in the negative, for example, for the pelvis in the sagittal plane, positive values correspond to the pelvic retroversion and negative values to the pelvic anteversion. In order to better understand the movement in each body segment, a description of the direction of movement for gait analysis is depicted in appendices (Table 19).

Knee, hip and pelvis are the joints where most differences between the diabetic groups can be observed in all three gait events. During the initial contact (heel strike) a tendency for the DPN group to walk with knee valgus and greater hip adduction is observed (right: *p*knee=0.019 *p*hip=0.023; left: *p*knee=0.013). Still, on the right lower limb, the pelvis showed a reduced pelvic rotation to the left, and on the left lower limb a greater pelvic elevation to the right (right: *p*pelvis=0.003; left: *p*pelvis=0.035).

Table 5. Distribution of pelvis, hip, knee and ankle angle at the event right heel strike (RHS) of all subjects in each group.

RHS	Group						
	DM (n=9; 56%)			DPN (n=7; 44%)			
	Median	min	max	Median	min	max	*p*
Pelvis (º)							
x (retroversion/anteversion)	0.61	-3.23	3.09	-0.50	-4.05	3.66	0.681§
y (right/left elevation)	-0.22	-5.07	6.30	1.37	-1.89	13.16	0.606§
z (left/right rotation)	8.50	4.97	13.63	5.02	-0.69	5.73	***0.003§***
Hip (º)							
x (flexion/extension)	17.77	12.01	26.49	24.14	12.47	33.74	0.174§
y (adduction/abduction)	0.22	-7.43	11.40	5.25	0.32	8.58	***0.023§***
z (internal/external rotation)	-1.03	-11.86	7.79	0.60	-12.92	3.89	0.408§
	Mean	(sd)		Mean	(sd)		*p*
Knee (º)							
x (flexion/extension)	3.74	(4.32)		8.92	(5.67)		0.056||
y (varus/valgus)	-0.23	(4.27)		-5.49	(3.43)		***0.019||***
z (internal/external rotation)	-22.32	(7.91)		-20.91	(2.43)		0.659||
	Median	min	max	Median	min	max	*p*
Ankle (º)							
x (dorsiflexion/plantar flexion)	1.33	-5.28	53.34	-0.09	-14.17	6.21	0.408§
y (inversion/eversion)	6.80	1.28	11.61	6.46	1.35	9.77	0.536§
z (pronation/supination)	1.74	-6.05	13.93	-0.23	-8.49	1.98	0.174§

sd: Standard deviation; min: Minimum; max: Maximum; *p*: Percentile; || T-test; § Mann-Whitney Test.
Bold *p* values are significant with $p<0.05$.

Table 6. Distribution of pelvis, hip, knee and ankle angle at the event left heel strike (LHS) of all subjects in each group.

LHS	Group				
	DM (n=9; 56%)		DPN (n=7; 44%)		
	Mean	(sd)	Mean	(sd)	*p*||
Pelvis (º)					
x (retroversion/anteversion)	0.24	(1.96)	0.53	(2.58)	0.804
y (right/left elevation)	0.33	(2.16)	3.19	(2.75)	***0.035***
z (left/right rotation)	-3.04	(3.97)	-2.21	(2.23)	0.631
Hip (º)					
x (flexion/extension)	21.40	(7.52)	22.53	(8.17)	0.778
y (adduction/abduction)	0.66	(3.26)	1.07	(4.31)	0.832
z (internal/external rotation)	8.50	(13.40)	6.76	(10.69)	0.783
Knee (º)					
x (flexion/extension)	6.60	(6.32)	12.02	(6.63)	0.118
y (varus/valgus)	0.91	(3.63)	-4.28	(3.62)	***0.013***
z (internal/external rotation)	-24.65	(13.41)	-26.05	(8.22)	0.813
Ankle (º)					
x (dorsiflexion/plantar flexion)	-0.59	(3.80)	-2.43	(3.52)	0.339
y (inversion/eversion)	5.92	(5.01)	8.16	(2.83)	0.311
z (pronation/supination)	3.55	(3.38)	5.52	(4.86)	0.353

sd: Standard deviation; *p*: Percentile; || T-test. Bold *p* values are significant with $p<0.05$.

During midstance, knee joint position in DPN subjects is particularly in valgus and greater flexion, and a larger hip flexion and adduction is also observed (right: *p*knee=0.046, *p*knee=0.020, *p*hip=0.024, *p*hip=0.016; left: *p*knee=0.030). The pelvis showed an increase elevation to the right (right: *p*pelvis=0.042).

Table 7. Distribution of pelvis, hip, knee and ankle angle at the event right midstance (RMID) of all subjects in each group.

RMID	Group						
	DM (n=9; 56%)			DPN (n=7; 44%)			
	Median	min	max	Median	min	max	*p*
Pelvis (º)							
x (retroversion/anteversion)	1.16	-2.13	3.77	2.16	-3.10	8.55	0.408§
y (right/left elevation)	-0.91	-2.35	3.62	0.97	-0.65	8.77	***0.042§***
z (left/right rotation)	1.35	-3.41	8.39	1.66	-1.29	4.19	0.681§
	Mean	(sd)		Mean	(sd)		*p*
Hip (º)							
x (flexion/extension)	-9.58	(3.45)		-3.78	(6.99)		***0.024***||
y (adduction/abduction)	4.39	(3.84)		9.95	(4.67)		***0.016***||
z (internal/external rotation)	-2.97	(5.78)		-5.11	(3.73)		0.317||
Knee (º)							
x (flexion/extension)	4.90	(4.56)		10.79	(4.66)		***0.046***||
y (varus/valgus)	0.23	(3.91)		-4.50	(2.65)		***0.020***||
z (internal/external rotation)	-13.29	(5.87)		-9.99	(6.87)		0.252||
	Median	min	max	Median	min	max	*p*
Ankle (º)							
x (dorsiflexion/plantar flexion)	11.65	8.08	63.67	11.91	-6.87	14.54	0.606§
y (inversion/eversion)	0.88	-2.89	5.00	1.25	-9.16	4.00	0.918§
z (pronation/supination)	2.29	-4.19	10.19	-4.10	-9.02	2.71	0.114§

sd: Standard deviation; min: Minimum; max: Maximum; *p*: Percentile; || T-test; § Mann-Whitney Test.
Bold *p* values are significant with $p<0.05$.

Table 8. Distribution of pelvis, hip, knee and ankle angle at the event left midstance (LMID) of all subjects in each group.

LMID	Group				
	DM (n=9; 56%)		DPN (n=7; 44%)		
	Mean	(sd)	Mean	(sd)	*p*||
Pelvis (º)					
x (retroversion/anteversion)	1.08	(2.22)	0.52	(2.66)	0.648
y (right/left elevation)	0.67	(3.06)	0.95	(0.95)	0.805
z (left/right rotation)	3.35	(2.80)	1.03	(2.53)	0.108
Hip (º)					
x (flexion/extension)	-7.44	(4.67)	-3.29	(6.75)	0.167
y (adduction/abduction)	2.51	(2.43)	5.61	(4.73)	0.110
z (internal/external rotation)	5.64	(10.36)	0.61	(8.73)	0.321
Knee (º)					
x (flexion/extension)	7.68	(5.34)	12.74	(7.55)	0.138
y (varus/valgus)	1.06	(3.60)	-3.57	(4.09)	***0.030***
z (internal/external rotation)	-13.72	(10.90)	-13.61	(9.01)	0.983
Ankle (º)					
x (dorsiflexion/plantar flexion)	12.80	(3.59)	13.42	(4.34)	0.759
y (inversion/eversion)	-0.18	(3.57)	2.07	(3.25)	0.215
z (pronation/supination)	2.05	(3.90)	1.08	(3.37)	0.609

sd: Standard deviation: *p*: Percentile; || T-test. Bold *p* values are significant with $p<0.05$.

In pre-swing phase (toe-off) is observed significantly differences between the diabetic groups in the pelvis, hip and ankle joint. The DPN group is characterized by less hip extension and greater adduction in this moment (right: *p*hip=0.004, *p*hip=0.020; left: *p*hip=0.035). Ankle also tends to leave the floor with less plantar flexion and in a more supinated position (right: *p*ankle=0.002; left: *p*ankle=0.048). Relatively, to the pelvis joint on the right lower limb is observed an increase pelvic elevation to the right, and on the left lower limb a reduced pelvic rotation to the left (right: *p*pelvis=0.020; left: *p*pelvis=0.020).

Table 9. Distribution of pelvis, hip, knee and ankle angle at the event right toe-off (RTO) of all subjects in each group.

RTO	Group				
	DM (n=9; 56%)		DPN (n=7; 44%)		
	Mean	(sd)	Mean	(sd)	*p*||
Pelvis (º)					
x (retroversion/anteversion)	1.55	(1.93)	1.03	(1.41)	0.561
y (right/left elevation)	-2.08	(1.38)	1.13	(3.36)	***0.020***
z (left/right rotation)	-3.16	(4.00)	-2.98	(2.68)	0.924
Hip (º)					
x (flexion/extension)	-10.82	(3.36)	-2.81	(5.98)	***0.004***
y (adduction/abduction)	-2.93	(2.89)	1.07	(3.21)	***0.020***
z (internal/external rotation)	-7.96	(5.38)	-6.51	(5.73)	0.610
Knee (º)					
x (flexion/extension)	38.26	(6.12)	41.29	(5.84)	0.333
y (varus/valgus)	-1.53	(3.89)	-1.13	(4.82)	0.855
z (internal/external rotation)	-9.59	(8.64)	-8.86	(5.49)	0.848
Ankle (º)					
x (dorsiflexion/plantar flexion)	-4.58	(17.18)	-2.60	(7.50)	0.782
y (inversion/eversion)	3.58	(3.65)	3.69	(4.05)	0.953
z (pronation/supination)	7.41	(5.69)	-2.93	(4.95)	***0.002***

sd: Standard deviation; *p*: Percentile; || T-test. Bold *p* values are significant with $p<0.05$.

Table 10. Distribution of pelvis, hip, knee and ankle angle at the event left toe-off (LTO) of all subjects in each group.

LTO	Group						p
	DM (n=9; 56%)			DPN (n=7; 44%)			
	Mean	(sd)		Mean	(sd)		p
Pelvis (º)							
x (retroversion/anteversion)	1.15	(1.85)		1.21	(3.10)		0.967||
y (right/left elevation)	2.80	(3.47)		4.08	(4.08)		0.506||
z (left/right rotation)	8.23	(2.82)		4.50	(2.82)		***0.020*||**
Hip (º)							
x (flexion/extension)	-8.22	(3.83)		-1.07	(8.17)		***0.035*||**
y (adduction/abduction)	-5.64	(3.59)		-3.36	(5.02)		0.306||
z (internal/external rotation)	-0.06	(7.86)		-0.73	(10.31)		0.885||
	Median	min	max	Median	min	max	p
Knee (º)							
x (flexion/extension)	39.57	-9.79	50.37	41.77	37.55	58.79	0.408§
y (varus/valgus)	-3.16	-4.00	8.75	-0.64	-5.89	8.25	0.758§
z (internal/external rotation)	-12.85	-17.43	6.78	-11.42	-25.65	-5.71	0.837§
	Mean	(sd)		Mean	(sd)		p
Ankle (º)							
x (dorsiflexion/plantar flexion)	-12.34	(6.62)		-5.13	(6.55)		***0.048*||**
y (inversion/eversion)	3.13	(5.95)		6.22	(3.59)		0.248||
z (pronation/supination)	11.79	(5.82)		7.92	(5.23)		0.191||

sd: Standard deviation; min: Minimum; max: Maximum; *p*: Percentile; || T-test; § Mann-Whitney Test.
Bold *p* values are significant with $p<0.05$.

Kinetics

Regarding the GRF data, this study included 9 (60%) participants in the DM group, and 6 (40%) in the DPN group. One subject of the DPN group was excluded due to lack of sufficient data for the statistical analysis. The anterior-posterior, medio-lateral and vertical GRF of each lower limb were normalized to body weight (BW) and their time of occurrence normalized to the stance duration. The temporal evolution of the antero-posterior, medio-lateral and vertical GRF over the gait cycle of the DM and DPN right and left lower limb is reported in figure 10 and 11, respectively.

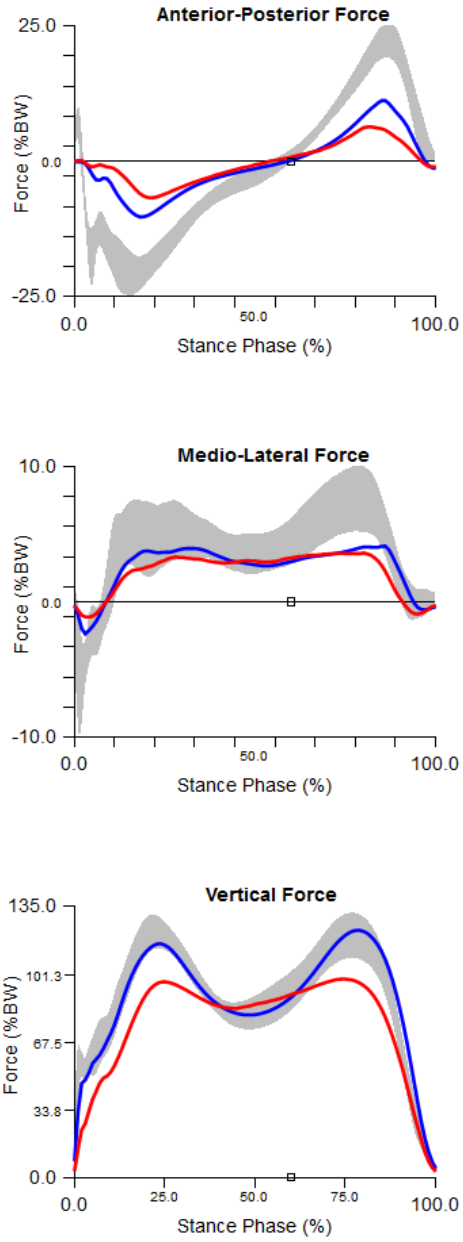


Figure 10. Averaged temporal evolution of the anterior-posterior, medio-lateral and vertical GRF of the right lower limb for all class of diabetic patients over the stance phase.

Legend: DM group is depicted in blue and DPN group in red. Reference data is depicted in gray. Vertical axis represents the ground reaction force normalized to body weight and expressed as a percentage. Horizontal axis represents the duration of stance phase, expressed as a percentage.

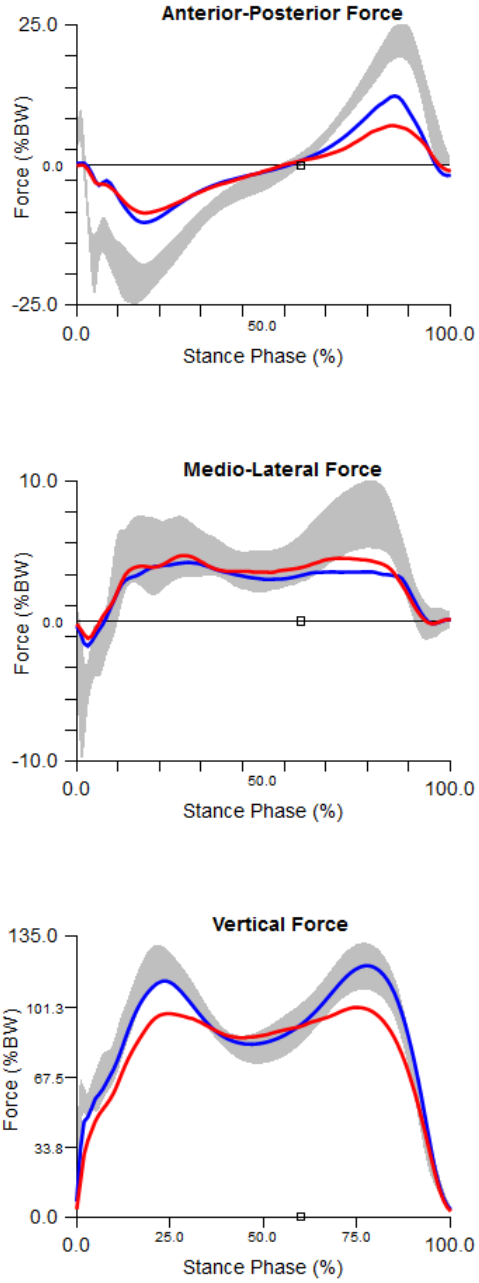


Figure 11. Averaged temporal evolution of the anterior-posterior, medio-lateral and vertical GRF of the left lower limb for all class of diabetic patients over the stance phase.

Legend: DM group is depicted in blue and DPN group in red. Reference data is depicted in gray. Vertical axis represents the ground reaction force normalized to body weight and expressed as a percentage. Horizontal axis represents the duration of stance phase, expressed as a percentage.

For statistical analysis each component of the GRF was divided into three sections, remarkable points in the force-time curve that allow its characterization (anterior-posterior: FA1, FA2, FA3; medio-lateral: FM1, FM2, FM3; vertical: FZ1, FZ2, FZ3). Each force descriptor GRF were compared (table 11) as well as their moment of occurrence in the cycle (table 12) for right and left lower limb of all diabetic subjects.

Statistical differences were found for most of GRF amplitude variables (right: *p*RFA1=0.038, *p*RFA3=0.006, *p*RFZ3=0.021; left: *p*LFA3=0.001, *p*LFZ3=0.032), with exception of the first anterior-posterior GRF peak for the left leg and the second anterior-posterior, all the medio-lateral, and the first and second vertical GRF for both legs.

Table 11. Distribution of the amplitude of anterior-posterior, medio-lateral and vertical ground reaction forces normalized to body weight for right and left lower limb of all subjects in each group.

Amplitude Data	Group				
	DM (n=9; 60%)		DPN (n=6; 40%)		
	Mean	(sd)	Mean	(sd)	*p*||
RFA (%BW)					
RFA1	-10.93	(2.93)	-7.57	(2.47)	***0.038***
RFA2	0.06	(0.07)	0.05	(0.02)	0.889
RFA3	12.76	(2.18)	8.45	(2.89)	***0.006***
RFM (%BW)					
RFM1	-2.62	(1.16)	-1.67	(0.93)	0.120
RFM2	4.55	(1.25)	3.93	(0.48)	0.198
RFM3	5.03	(1.33)	5.03	(0.78)	0.998
RFZ (%BW)					
RFZ1	116.16	(13.41)	105.97	(9.31)	0.131
RFZ2	78.89	(9.61)	83.42	(5.31)	0.315
RFZ3	124.42	(13.53)	108.14	(8.27)	***0.021***
LFA (%BW)					
LFA1	-11.17	(3.76)	-9.63	(3.43)	0.434
LFA2	0.09	(0.05)	0.06	(0.02)	0.144
LFA3	13.88	(2.62)	8.47	(1.86)	***0.001***
LFM (%BW)					
LFM1	-1.92	(1.19)	-1.53	(0.64)	0.476
LFM2	4.88	(1.65)	5.21	(1.98)	0.734
LFM3	4.74	(1.47)	5.75	(2.46)	0.335
LFZ (%BW)					
LFZ1	115.60	(14.83)	106.41	(9.41)	0.203
LFZ2	81.29	(9.06)	83.80	(4.18)	0.540
LFZ3	123.15	(13.89)	108.60	(5.83)	***0.032***

sd: Standard deviation; *p*: Percentile; || T-test. Bold *p* values are significant with $p<0.05$

Additionally, few statistical differences were found for the GRF time of occurrence variables, only the first anterior-posterior and third medio-lateral GRF time for the right leg and the third vertical GRF time for the left leg presented differences between both diabetic groups (right: *p*RFA1=0.002, *p*RFM3=0.003; left: *p*LFZ3=0.008).

Table 12. Distribution of anterior-posterior, medio-lateral and vertical ground reaction forces temporal data for right and left lower limb of all subjects in each group.

Temporal Data	Group						
	DM (n=9; 60%)			DPN (n=6; 40%)			
	Mean	(sd)		Mean	(sd)		*p*
RFA (s)							
RFA1	17.48	(1.33)		20.53	(1.76)		***0.002*|**
RFA2	57.10	(4.94)		55.78	(4.24)		0.602|
RFA3	85.57	(1.76)		83.40	(2.60)		0.074|
RFM (s)							
RFM1	3.72	(0.94)		3.90	(1.74)		0.804|
RFM2	25.18	(5.37)		25.43	(3.00)		0.920||
RFM3	81.84	(3.50)		76.07	(2.22)		***0.003*||**
	Median	min	max	Median	min	max	*p*
RFZ (s)							
RFZ1	23.60	21.34	24.41	23.75	21.82	33.66	0.388§
RFZ2	49.08	39.07	49.95	45.13	41.78	52.05	0.224§
RFZ3	77.60	76.52	80.02	76.37	52.05	79.58	0.066§
LFA (s)							
LFA1	17.04	12.05	18.61	17.46	14.44	19.23	0.955§
LFA2	56.37	47.35	59.11	55.98	44.31	64.29	1.000§
LFA3	85.36	83.38	87.62	82.86	73.41	88.56	0.328§
LFM (s)							
LFM1	2.98	1.96	3.86	3.35	2.40	4.69	0.181§
LFM2	21.64	14.38	31.37	22.43	18.64	30.68	0.864§
LFM3	76.76	60.17	85.45	72.17	65.67	80.48	0.388§
LFZ (s)							
LFZ1	22.85	20.98	27.73	24.67	19.51	31.11	0.607§
LFZ2	47.28	38.52	49.56	43.75	35.56	53.50	0.328§
LFZ3	77.55	74.68	79.79	74.06	61.18	77.77	***0.008§***

sd: Standard deviation; min: Minimum; max: Maximum; *p*: Percentile; || T-test; § Mann-Whitney Test.
Bold *p* values are significant with $p<0.05$.

Electromyography

Regarding the electromyographic data collected this study included 7 (26%) participants in the DM group, 5 (18%) in the DPN group and 15 (56%) in the additional control group (CG). Due to technical problems, two subjects from each group of diabetics (DM and DPN) were not evaluated for muscular electrical activity during gait (table 13 and 14).

From the analysis of table 13, it can be noticed that the majority of the participants in the study were male, n=21 (81%), although there were no significant differences regarding gender between groups (*p*=0.503).

Table 13. Socio-demographic characterization of DM, DPN and CG groups.

	Total (n=27)		**Group**						
			DM (n=7; 26%)		**DPN (n=5; 18%)**		**CG (n=15, 56%)**		
	n	%	n	%	n	%	n	%	*p*
Gender									0.503***
Female	6	19%	3	43%	1	20%	2	13%	
Male	21	81%	4	57%	4	80%	13	87%	
Diabetes Type									0.524**
Type 1	5	42%	4	57%	1	20%	-	-	
Type 2	7	58%	3	43%	4	80%	-	-	

*Chi-Square Test of Independence; **Fisher's Exact Test; ***Chi-Square Exact Test.

Regarding the age, weight and BMI, significant differences were found between the three groups (*p*age=0.005; *p*weight=0.027; *p*BMI<0.001), with the DPN group with older, 67 (55-85) years, and heavier participants (83.59 ± 8.41 kg) (table 14).

Table 14. Distribution of age (in years), height (m), weight (kg) and BMI (kg/m2) of the DM, DPN and CG groups.

	Total (n=27)		**Group**						
			DM (n=7; 26%)		**DPN (n=5; 18%)**		**CG (n=15, 56%)**		
	Median	(min-max)	Median	(min-max)	median	(min-max)	median	(min-max)	*p*£
Age (years)	55	(17-85)	41	(17-64)	67	(55-85)	52	(43-62)	***0.005***
	Mean	(sd)	Mean	(sd)	Mean	(sd)	Mean	(sd)	*p*#
Height (m)	1.68	(0.08)	1.67	(0.12)	1.67	(0.08)	1.69	(0.06)	0.779
Weight (kg)	72.17	(13.24)	69.96	(15.45)	83.59	(8.41)	68.18	(11.12)	***0.027***
BMI (kg/m²)	25.50	(3.73)	24.77	(2.71)	30.17	(2.84)	23.76	(2.76)	***<0.001***

sd: Standard deviation; min-Minimum; Max-Maximum; *p*=Percentile; # One-Way ANOVA Test; £ Kruskall-Wallis Test. Bold *p* values are significant with *p*<0.05.

Table 15 presents the results of EMG analysis of the GM muscle for both right and left leg of the DM, DPN and CG groups.

The activation time and mean amplitude of the GM muscle were similar between groups. Additionally, no significant differences were recorded for the electromyographic parameters of the GM muscle, with the exception of the time to peak of the right GM when normalized to the activation time (p=0.016).

Table 15. Electromyographic parameters for the *Gastrocnemius Medialis*.

Gastrocnemius Medialis	**Group**						
	DM (n=7; 26%)		**DPN (n=5; 18%)**		**Control (n=15; 56%)**		
	Mean	(sd)	Mean	(sd)	Mean	(sd)	*p*#
Activation time L (s)	0.48	(0.09)	0.55	(0.13)	0.51	(0.09)	0.456
Activation time R (s)	0.46	(0.09)	0.51	(0.15)	0.53	(0.09)	0.367
Activation time (**norm1**) L (%)	45.08	6.42	45.52	10.04	48.88	7.57	0.496
Activation time (**norm1**) R (%)	43.72	6.09	41.86	12.03	50.77	8.55	0.084
Average Amplitude L (**norm2**) (%MVC)	22.75	(13.38)	22.85	(7.70)	23.99	(7.13)	0.945
Average Amplitude R (**norm2**) (%MVC)	24.86	(11.00)	24.02	(12.39)	24.19	(11.07)	0.989
Time 2 Peak L (s)	0.37	(0.09)	0.37	(0.13)	0.36	(0.07)	0.957
Time 2 Peak R (s)	0.35	(0.09)	0.35	(0.12)	0.40	(0.09)	0.336
Time 2 Peak (**norm3**) L (%)	76.96	(6.41)	65.40	(10.82)	71.72	(10.16)	0.136
Time 2 Peak (**norm3**) R (%)	71.05	(6.48)	63.80	(7.17)	75.01	(7.11)	***0.016***

sd: Standard deviation; *p*: Percentile; # One-way ANOVA Test; R: Right; L: Left. Bold *p* values are significant with $p<0.05$; **norm1**- normalized to gait cycle time; **norm2**- normalized to the maximum voluntary contraction; **norm3**- normalized to the activation time of the muscle.

To identify between which groups a significance level was reached for the parameter time to peak muscle activity (**norm3** %), the Bonferroni post-hoc test was applied (table 16).

Table 16. Bonferroni post hoc test result for *Gastrocnemius Medialis* right time to peak normalized.

Dependent Variable: *Gastrocnemius Medialis* Time2Peak (**norm3**) R (%) Bonferroni Test			
(I) Group	(J) Group	Mean Difference (I-J)	*p*
DM	DPN	7.29	0.265
	Control	-3.95	0.681
DPN	DM	-7.25	0.265
	Control	-11.20*	***0.014***
Control	DM	3.95	0.681
	DPN	11.20*	***0.014***

p: Percentile; R: Right. Bold *p* values are significant with $p<0.05$.

Regarding the Bonferroni post-hoc test (table 16), it can be concluded that the right GM muscle demonstrated a significant difference in the time to peak muscle activity when normalized to the activation time, only between the DPN and CG (p=0.014). In electromyographic terms, the DPN group with a time to peak value of 63.80% (± 7.17) and the CG with 75.01% (± 7.11), it means that during the activation of the right GM, the DPN group reached the peak of activity earlier than the control group. No significant differences were observed when compared to the DM group.

In order to analyse the time of activation and cessation of the muscle along the gait cycle, the EMG profile for the GM muscle was obtained. Figure 12 and 13 presents the ensemble-averaged EMG profiles of the right and left lower extremity GM, respectively, during walking in subjects with DM, subjects with DPN and control subjects.

For the right lower limb (figure 12), the GM muscle presented a delayed activation during the initial stance phase for the DPN group.

The activity of this muscle ceased approximately at the same time of gait cycle for all groups.

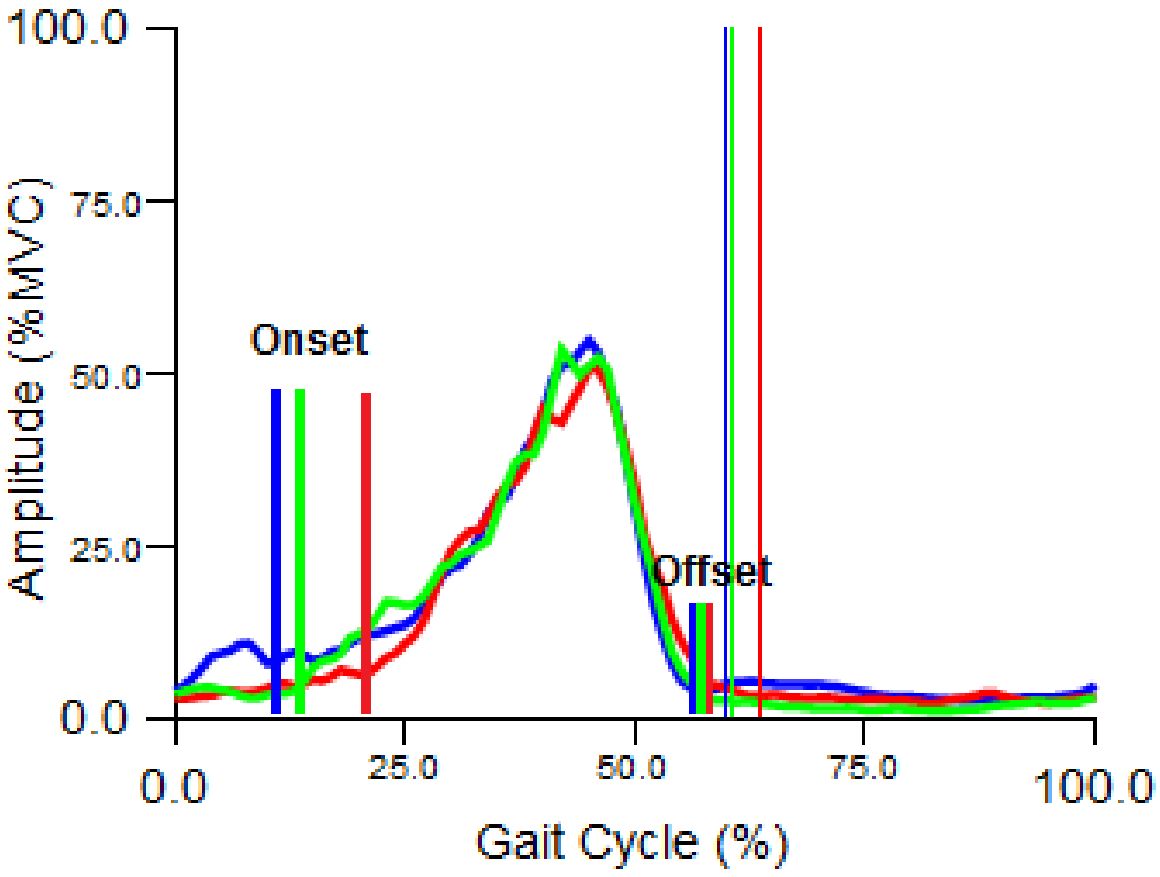


Figure 12. Ensemble-averaged EMG profile of the right *Gastrocnemius Medialis*.

Legend: DM group is depicted in blue, DPN group in red and the Control group in green. Vertical axis represents mean of electromyographic activity normalized by the maximal voluntary contraction. Horizontal axis represents the duration of a gait cycle expressed as a percentage. The vertical line represents the moment of toe-off.

For the left lower limb GM muscle activity (figure 13), similar EMG profiles were observed between the DM, DPN and control group.

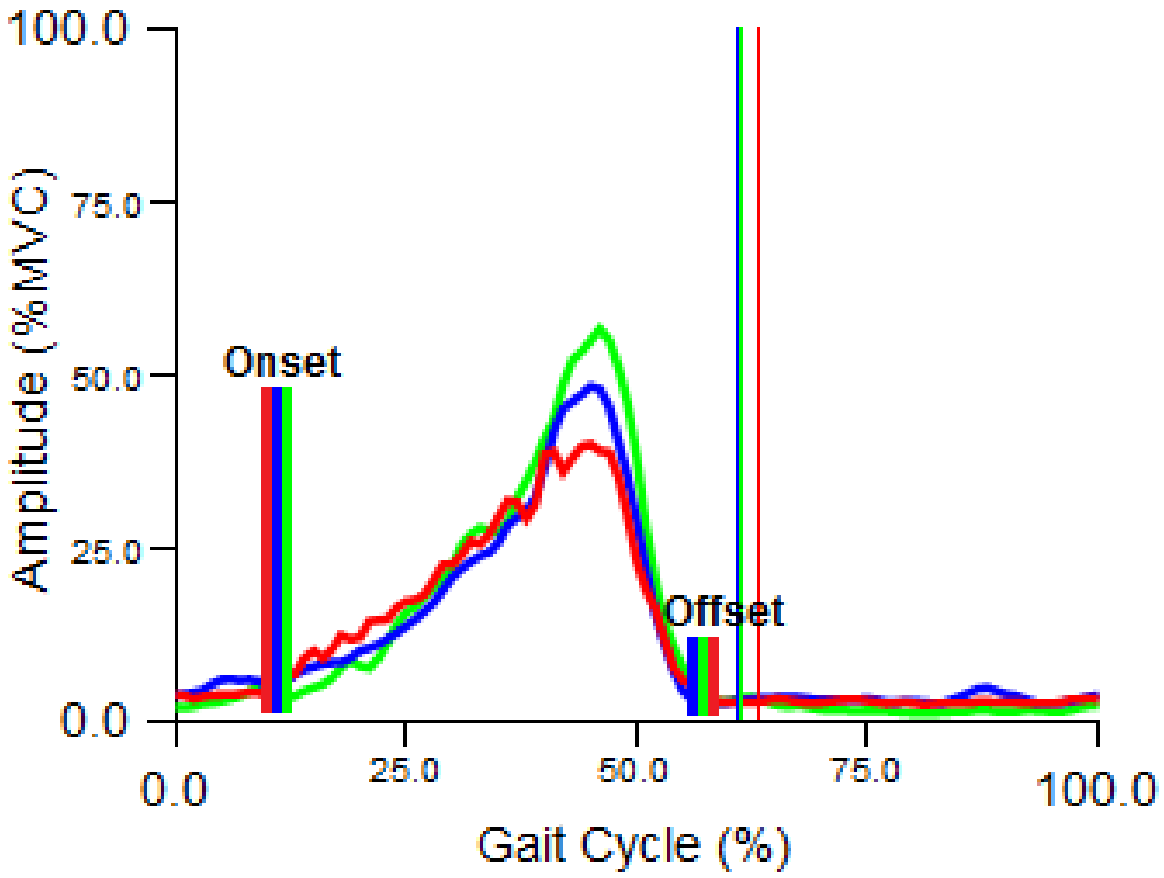


Figure 13. Ensemble-averaged EMG profile of the left *Gastrocnemius Medialis*.

Legend: DM group is depicted in blue, DPN group in red and the Control group in green. Vertical axis represents mean of electromyographic activity normalized by the maximal voluntary contraction. Horizontal axis represents the duration of a gait cycle expressed as a percentage. The vertical line represents the moment of toe-off.

Table 17 presents the results of EMG analysis of the TA muscle for both right and left leg of the DM, DPN and CG groups.

Similarly to what is observed to the GM muscle, the TA muscle of the right leg showed a significant difference between groups in the time to peak muscle activity when normalized to the activation time of the muscle (p=0.037).

The right TA muscle also showed significant differences in the activation time between groups (p=0.006). Although, to compare the duration of TA between groups its necessary to normalize this variable to the gait cycle time and no significant differences in right TA activity time (**norm1** %) were observed (p=0.537).

No significant differences were recorded for the remaining electromyographic parameters of the TA muscle between groups.

Table 17. Electromyographic parameters for the *Tibialis Anterior*.

Tibialis Anterior	**Group**									
	DM (n=7; 26%)			**DPN (n=5; 18%)**			**Control (n=15; 56%)**			
	Median	min	max	Median	min	max	Median	min	max	*p*£
Activation time L (s)	0.66	0.40	0.91	0.76	0.58	0.88	0.66	0.54	0.86	0.357
Activation time R (s)	0.63	0.51	0.66	0.75	0.68	0.83	0.63	0.50	0.75	***0.006***
	Mean	(sd)		Mean	(sd)		Mean	(sd)		*p*#
Activation time (**norm1**) L (%)	60.87	13.11		60.58	4.59		63.63	8.01		0.721
Activation time (**norm1**) R (%)	57.93	8.69		62.57	7.75		59.49	5.99		0.537
	Median	min	max	Median	min	max	Median	min	max	*p*£
Average Amplitude L (**norm2**) (%MVC)	28.87	16.23	107.60	25.83	17.05	31.66	29.37	16.24	118.26	0.569
Average Amplitude R (**norm2**) (%MVC)	29.89	16.21	63.65	25.06	15.24	41.51	36.01	18.35	46.22	0.392
	Mean	(sd)		Mean	(sd)		Mean	(sd)		*p*#
Time 2 Peak L (s)	0.41	(0.14)		0.35	(0.15)		0.36	(0.10)		0.588
Time 2 Peak R (s)	0.37	(0.08)		0.35	(0.11)		0.38	(0.09)		0.727
Time 2 Peak (**norm3**) L (%)	63.61	(19.66)		49.45	(19.46)		53.89	(12.31)		0.272
Time 2 Peak (**norm3**) R (%)	60.39	(9.80)		44.70	(11.02)		61.36	(12.83)		***0.037***

sd: Standard deviation; min: Minimum; Max: Maximum; *p*: Percentile; £ Kruskal-Wallis Test; # One-way ANOVA Test; R: Right; L: Left. Bold *p* values are significant with $p<0.05$. **norm1**- normalized to gait cycle time; **norm2**- normalized to the maximum voluntary contraction; **norm3**- normalized to the activation time of the muscle.

To identify between which groups a significance level was reached for the variable time to peak muscle activity (**norm3** %), the Bonferroni post-hoc test was applied (table 18).

Table 18. Bonferroni post hoc test result for *Tibialis Anterior* right time to peak normalized.

Multiple Comparisons				
Dependent Variable: *Tibialis Anterior* Time2Peak (**norm3**) R (%) Bonferroni Test				
(I) Group	(J) Group	Mean Difference (I-J)		*p*
DM	DPN	15.68		0.121
	Control	-0.97		1.000
DPN	DM	-15.68		0.121
	Control	-16.65*		***0.038***
Control	DM	0.97		1.000
	DPN	16.65*		***0.038***

p: Percentile; R: Right. Bold *p* values are significant with $p<0.05$.

From the analysis of table 18, it can be concluded that the right TA muscle demonstrated a significant difference in the time to peak muscle activity normalized to activation time only between the DPN and CG ($p=0.038$). Compared with the CG (61.36 ± 12.83 %), individuals with DPN (44.70 ± 11.02 %), reach the peak of activity of the right TA earlier.

There were no significant differences in TA time to peak muscle occurrence when compared individuals from the DM group with the DPN group and also with the control group.

In order to analyse the time of activation and cessation of the muscle along the gait cycle, the EMG profile for the TA muscle was obtained. Figure 14 and 15 presents the ensemble-averaged EMG profiles of the right and left lower extremity muscle TA, respectively, during walking in subjects with DM, DPN and control subjects.

For the right lower limb (figure 14), at the moment of heel-strike, similar EMG profiles in terms of activation and cessation were observed between the three groups.

Close to the event of toe-off, the DPN groups presents a premature activation of the right TA muscle.

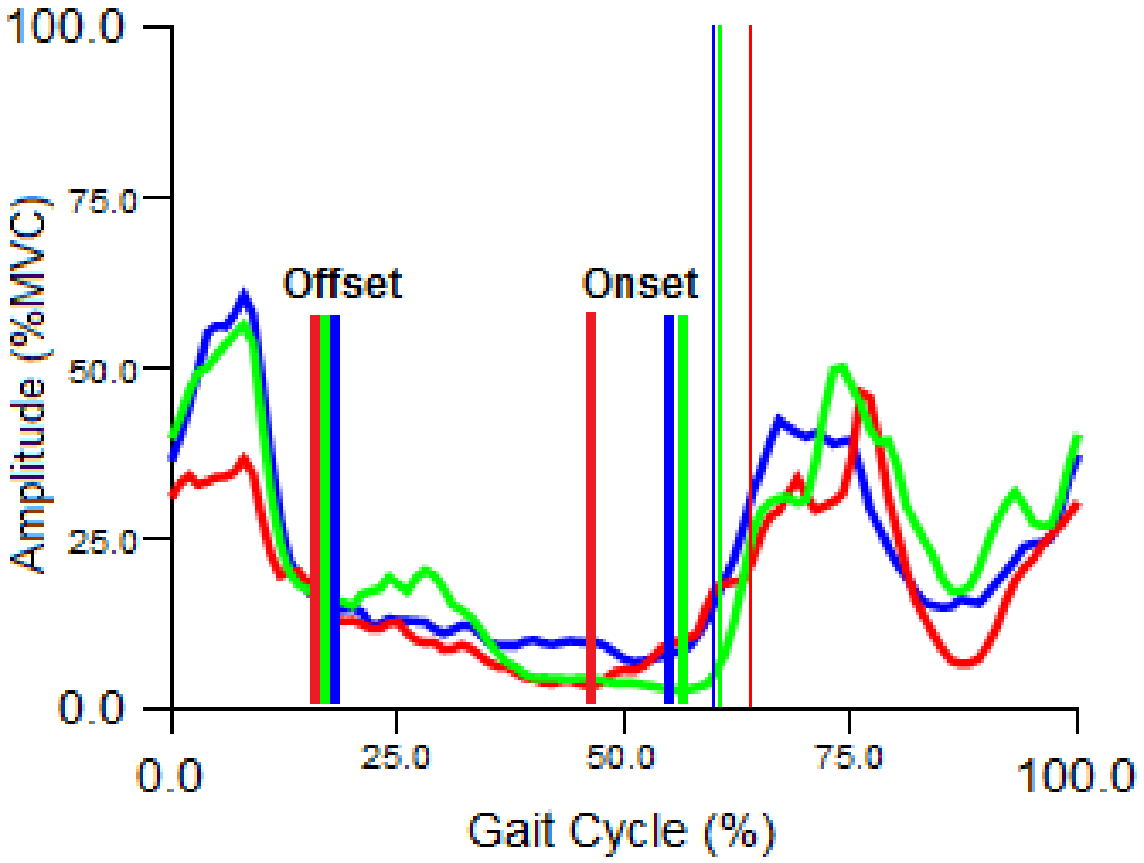


Figure 14. Ensemble-averaged EMG profile of the right *Tibialis Anterior*.

Legend: DM group is depicted in blue, DPN group in red and the Control group in green. Vertical axis represents mean of electromyographic activity normalized by the maximal voluntary contraction. Horizontal axis represents the duration of a gait cycle expressed as a percentage. The vertical line represents the moment of toe-off.

For the left lower limb TA muscle (figure 15), similar EMG profiles were observed between the three groups.

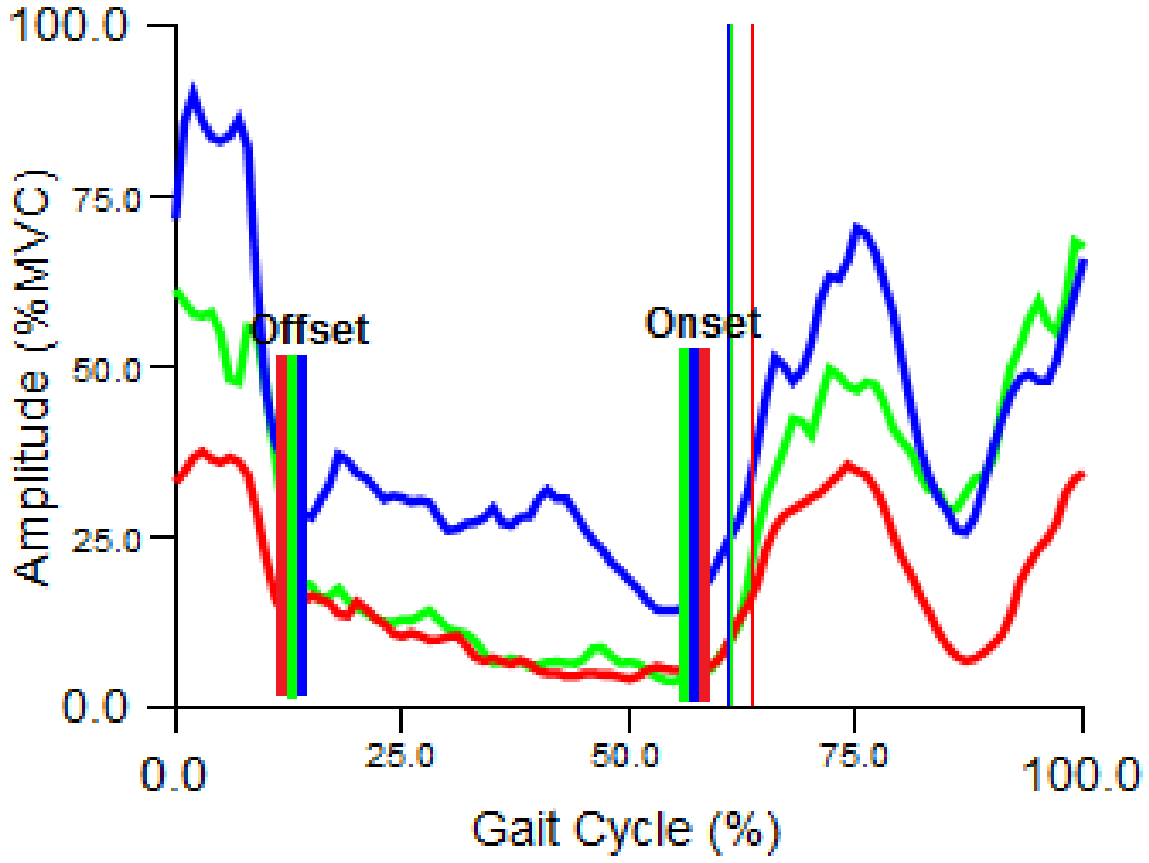


Figure 15. Ensemble-averaged EMG profile of the left *Tibialis Anterior*.

Legend: DM group is depicted in blue, DPN group in red and the Control group in green. Vertical axis represents mean of electromyographic activity normalized by the maximal voluntary contraction. Horizontal axis represents the duration of a gait cycle expressed as a percentage. The vertical line represents the moment of toe-off.

Chapter 6

Discussion

Discussion

Diabetic peripheral neuropathy is a critical complication of diabetes that accounts for significant morbidity and mortality. The consequences of DPN can be catastrophic for patients, as this leads to increased risk of falls, foot ulceration and limb amputation, significant healthcare costs, reduced quality of life and reduced mobility. Due to these several complications associated to DPN, the insight of the impact of this pathology on the biomechanical aspects of human locomotion is clinically important. Therefore, the use of 3D gait biomechanical analysis and EMG could be advantageous and crucial in early detection of health impairments related with diabetes.

The purpose of the present study was to characterize the gait and investigate EMG activity deviations during the entire gait cycle of people with diabetes in early stages of IWGDF classification risk. Comparative observations of dynamic and temporal gait data, lower extremity kinematics and GRF during gait were performed between diabetic individuals with and without peripheral neuropathy. The electric activity of GM and TA during gait was performed between diabetic individuals with and without peripheral neuropathy and a control group.

Gait was characterized in all three dimensions by means of a MoCap system and force platforms allowing to identify different movement patterns performed by the groups. The EMG activity was measured in synchrony with the MoCap system, in order to identify and describe muscle activation patterns during the entire gait cycle.

In this study, group's comparison showed statistical significant differences between most of the studies spatiotemporal variables. Consistently with other reports (128,130–132,135,136), in this study DPN participants presented a slower gait with reduced cadence, reduced stride and step length and higher stride width when compared to diabetic non-neuropathic individuals. The overall pattern of slow gait speed, shorter steps and lower cadence is quite consistent/acknowledged in people with diabetic peripheral neuropathy. This conservative gait pattern in neuropathy could be seen as a result of motor weakness (128,135) as well as underlying proprioceptive deficit (128,130,135) and cutaneous sensory loss (128,130). As peripheral neuropathy disturbs both efferent and afferent pathways (118), a combination of both explanations is possible.

Peripheral neuropathy-associated loss of proprioception and cutaneous sensory input at the feet contributes also to postural instability (119,121,124,130), and with subjects fearful of falling (121,123–125) the slow speed gait, smaller step length and higher stride width, can be seen as adopted strategies for a safer and stable gait. The fear of falling is inextricably linked to a higher risk of falls, therefore in the DPN group a cautious gait could be a demonstration of such fear. In general, no differences were found for spatiotemporal variables between the two lower limbs (right and left), probably due to the need of bilateral compensatory mechanisms of musculoskeletal nature in order to adapt gait to the sensitivity and proprioceptive alterations caused by the neuropathy.

MoCap approach of gait kinematics and complete lower limb analysis for DPN individuals is not easily found in the literature. The current study provided full assessment of pelvis and lower limbs to better

characterize the movement in all three planes (sagittal, frontal and transverse) during stance phase of walking. A range of motion differences has been found in kinematics of both groups (DM and DPN group), especially concerning the hip and knee.

At the moment of heel strike, the DPN group showed knee valgus and an increased hip adduction in the right lower limb, when compared to the diabetic non-neuropathic group. Although only the right hip presented a significant difference between diabetic groups, the left hip of the neuropathic group presented also a greater adduction. The increase knee valgus is an effect of the increased adduction of the hip, and could be seen as a strategy of gait stabilization by neuropathic participants. With a characteristic loss of proprioception, cutaneous sensory input and gait stability, this compensatory mechanism at the heel strike allows the neuropathic group a broad base of support as an attempt to a more balanced and safe gait initiation. Still on the right lower limb, the DPN group showed a significant decrease in rotation of the pelvis to the left, when compared to the DM group. This was expected since the neuropathic group with a reduced stride length, should present a smaller accentuation of the pelvic movement, that is, a reduced pelvic rotation to the left.

In midstance, when the swinging foot passes the standing foot, as was seen at the heel strike, the neuropathic participants continued to present a knee valgus and an increase hip adduction when compared to the DM group. Like in heel strike, this mechanism could be seen as a strategy of gait balance and stabilization.

The majority of significant differences found in midstance between both groups (DM and DPN group) occurred in the right lower limb, when the right foot was in contact with the floor. Although with no statistical significance, the same movement was found to the left lower limb (left foot in contact with the floor). Compared to the DM group, DPN subjects showed also a significantly reduced extension of the knee and hip on the right lower limb. This pattern of reduced overall extension could be recognized as a mean to approach the center of mass to the ground in order to have more balance, and it's associated with a movement inefficiency during gait phase's transition. Increased flexion may also be part of a compensatory movement, either to reduce the effective limb length in functional leg length discrepancy or as part of pattern of exaggerated hip, knee and arm movements to make up for a lack of plantar flexor power in push off.

In midstance was also observed an exaggerated pelvic elevation to the right for the DPN group, when the right foot was in contact with the floor. The neuromuscular damage implied by DPN generally starts with sensory nerve damage and advances to muscle weakness and atrophy (76,77,79,92,104), so the exaggeration of pelvic elevation observed in the neuropathic group may express a decrease in the hip stability muscular strength of the right abductor muscles.

In the final moment of the stance phase, the toe-off, a reduced plantar-flexion was observed in the DPN group when compared to the diabetic non-neuropathic group. This deficit can be associated with changes in neuromuscular recruitment of musculature surrounding the ankle, causing a diminished ability of these muscles to push-off, contributing to a reduced propulsion capacity during the last phase of the stance. In this phase the DPN group showed also a reduced extension of the hip, and we believe

that this pattern along with the reduced overall extension of the knee and hip at midstance can also be responsible for the reduced ankle mobility at toe-off. The ankle plays a significant role in many aspects of gait cycle, including forward propulsion, body weight support, and vertical acceleration of body mass center and maintenance of knee stability. Consequently, reduced plantar flexion in toe-off phase has the capability to disrupt the normal gait cycle, enhancing the potential for negative functional consequences, like balance disturbances and instability during gait.

At the pelvis during toe-off, the right lower limb of the DPN group continues to show an increase pelvic elevation to the right, as seen in midstance. This right elevation increase may be associated not only with the decrease in the hip stability muscular strength of the right abductor muscles, as seen in the midstance phase, but also as a need to compensate for the diminished ability of the plantar-flexor muscles to push-off. The left lower limb of the neuropathic group shows a reduced pelvic rotation to the left, what was expected since this group presented a reduced stride length, consequently, a reduced accentuation of the pelvic movement. The strategy of gait stabilization of the DPN group with a greater hip adduction is also seen in the end of stance-phase.

In sum, regarding kinematics, restriction of lower-limb joint mobility in DPN individuals during gait is well documented in the literature and has the potential to compromise balance and stability (131,132,135,137,138). A number of significant differences were found in kinematics between the DM and DPN groups throughout the stance phase. Still most of the significant differences were observed on the right lower limb when the right foot was in contact with the floor, the left lower limb showed the same movement towards what happened on the right.

The anterior-posterior, medio-lateral and vertical GRF for the right and left lower limb were also analysed between the diabetic non-neuropathic and diabetic peripheral neuropathic group.

Starting from the vertical GRF component, we observed a significant decrease of the second peak (at push-off/ toe-off) for both right and left lower limb of the neuropathic group when compared with the DM group. A similar reduction of this component at the toe-off phase has been also reported by Katoulis et al. (135), Uccioli et al. (139), Yavuzer et al. (116) and Raspovic et al. (140). However, these results contradict those obtained by Saura et al. (137) and Sawacha et al. (141), who observed a significant increase in the vertical GRF peak at push-off in patients with diabetic peripheral neuropathy. In both these late studies (137,141) the gait velocity of the diabetic neuropathic group wasn't reported, one of the most important factor influencing the ground reaction forces. Additionally, in the study by Sawacha et al. (141), the diabetic neuropathic group included subjects with cavus and flat foot, none of the subjects presented normal foot. These differences may generate discrepant results between studies.

In this study, the neuropathic group showed a significant decrease in the first and second anterior-posterior GRF peaks (braking and propelling force respectively/ heel strike and toe-off) for the right lower limb, when compared with the DM group. In the left lower limb, the anterior-posterior GRF component showed significant differences only in the second peak. These data are in agreement with those by Katoulis et al. (135), Uccioli et al. (139) and Savelberg et al. (142).

The DPN gait in this study is characterized with a minimal heel strike, expressed by a reduction of the anterior-posterior component of GRF in the heel area, and a minimal toe-off/push-off phase, as a result of a minimal involvement of the hallux in the final phase of the stance of the vertical and anterior-posterior GRF peak in this area.

It is well-established and accepted that joint angles and GRF components increase with walking speed (154). The body is subject to increasing deceleration and acceleration forces, so the force components must increase (154). Therefore, it is possible that the differences in the vertical and anterior-posterior components of GRF observed in the DPN group could be attributed to the decreased walking speed (135,154), as forces produced tend to have a shorter magnitude in this group. Lower values of vertical and anterior-posterior GRF can also be associated with proximal and distal muscular weakness of lower extremity, and reduced ankle and knee mobility (116,135,139,140,142). Decreased dorsiflexor strength aligned with reduced lower-extremity mobility in diabetic neuropathic patients, causes a diminished ability of the dorsiflexors muscles to brake and decelerate during the first phase of the stance. Additionally, a diminished ability of the plantar flexor muscles to push-off and generate propelling forces during the last phase of the stance, can be associated with reduced ankle mobility and plantar-flexor strength.

The medio-lateral component differs between subjects, with the type of footwear and surface also, being considered the most variable of the three GRF components (110). However, although expected to be variable, no significant differences were found in medio-lateral GRF component between DM and DPN group.

In brief, the peripheral neuropathy group shows a trend to produce less GRF in amplitude throughout the stance phase, except for medio-lateral forces, and occurs later during the gait cycle. The time delay of GRF can be associated to the propulsion inefficacy, relative slowness during the movement, and with a longer contact between foot and ground, so with a increased exposure time of the plantar surface to loads. This higher exposure could be a predisposing factor for foot ulceration.

In the illustrated results for gait kinematics and kinetics, a reference data is depicted in gray. Although the main purpose of this study was to characterize the gait of people with diabetes classified in different risk groups, when compared both diabetic groups with the reference data same differences were observed for the lower-limb joint mobility and GRF. In general, both diabetic groups presented the same pattern of joint motion as the reference data, with the exception of the hip joint in the transverse plane that shows an external rotation for the diabetic groups and an internal rotation for the reference. However, the DPN group exhibits greater differences when compared to the reference, not only in the pattern of joint motion, but also in terms of angular amplitude.

Regarding the data illustrated in the graphs of the anterior-posterior, medio-lateral and vertical GRF both diabetic groups showed a pronounced decrease in the force normalized to the body weight when compared to the reference data. Although with no statistical analysis between the diabetic groups and the reference, it's perceptible the greater deficit of the force for the DPN group than the DM group. Altered gait patterns of diabetic persons are largely related to peripheral neuropathy, as stated for

several studies (119-129,131-137,139-147), however identical abnormalities have been observed in diabetic patients without neuropathy (116,143). So, when compared the illustrated results for the DM and DPN group gait kinematics and kinetics with the reference data, the differences observed can be seen as a result of the harmful effect of neuropathy, and also as a compensatory strategy adopted to improve stability or to maintain balance in diabetic patients with and without neuropathy.

As for the electromyographical activity, there were no differences in the time of activation and mean amplitude of the GM and TA muscle. However, the right GM and TA presented significant differences for the time to peak normalized to the activation time, with an earlier peak of activity for the DPN group when compared to the CG.

Regarding the EMG profiles, during midstance a delayed activation of the right GM muscle in subjects with DPN was observed when compared to the DM and control group. Although the right GM muscle of the DPN activates later in the gait cycle, when activated this muscle reaches the peak significantly sooner than the control group, which can be seen as a way to compensate for the delay of activation. Considering that, the delayed activation with an earlier peak of activity may express abnormalities in the sensitivity inputs and mechanical loads received by the foot, and a compensatory mechanism to reach the maximum of activity earlier due to changes in input information from the lower limbs of the DPN group.

The GM is responsible for the forefoot contact with the surface, shock absorption at heel strike and impulse at the forefoot. Despite the compensatory mechanism, the delayed activity of the GM in the neuropathic group may be responsible for a propulsion inefficiency in gait motion, which is confirmed by the lower GRF amplitude and the greater amount of time in stance phase.

The TA is responsible for the impact reduction over the forefoot during the foot flat phase in gait, and during the swing phase provides active dorsiflexion for adequate ground clearance. In this study, regarding the EMG profile, near the event of toe-off a premature activation of the right TA muscle was observed for the neuropathic group, when compared to the DM and control group. This activity pattern may be related to the propulsion inefficacy, and the need to perform a higher co-contraction period with the GM toward a greater rigidity and stability of gait. These co-contraction of agonist and antagonist muscles may be related to an adaptive walking strategy that compensates for the diminished sensory information from the ankle and foot.

The TA and GM contribute during gait, TA to ankle dorsiflexion and GM as plantar flexor, therefore dysfunction on the activity pattern, together with loss of cutaneous sensibility and proprioception and reduced dynamic motion of the lower extremities, will impair gait and posture and reduce functional capacity, contributing to increased fall risk and impaired balance in DPN individuals (138,144–147).

Despite the fact that differences in the duration of the stance phase could contribute to differences in the cessation of activity of the muscles, and although the neuropathic participants spend a longer

amount of time in the stance phase, no differences were found regarding the EMG profiles between the DPN, DM and control group.

A similar delayed activation of the GM has been also reported by Gomes et al. (138); however some studies observed the exact opposite, a premature activation of the GM. Regarding the TA muscle, our results contradict those reported in other studies, that observed a delayed activation (145) or no significant changes during gait (138). These inconsistencies with the literature may be related to methodological and functional aspects, and given the scarcity of information it's not possible to conclude whether the differences are due to the nature of the disease or the own measurement form. Furthermore, the analysis of muscle activity alterations during gait in diabetic neuropathic patients is still insufficient, and remains controversial between studies.

The differences in spatiotemporal, kinematics and kinetic parameters between the DPN and DM group may be explained by several factors induced by neuropathy, including loss of sensory perception, decreased muscle strength, decreased ankle mobility and slow walking speed.

Regarding the results of electromyographic parameters, significant differences were only observed between the DPN and CG. With no significant differences between the DM and DPN group, as reported in the literature (116,143,148,), it can be assumed that diabetes may impact gait mechanisms before onset of peripheral neuropathy. In sum, diabetic subjects with and without neuropathy could present similar deviations of gait pattern and muscle activity, in order to improve stability and maintain balance rather than being a direct result of peripheral neuropathy.

There is sufficient evidence that DPN patients show gait abnormalities, however, once the changes of lower limb joint mobility, GRF and muscle activity and its consequences have become clear, it will be possible to intervene with appropriate prevention and rehabilitation programs. Nonetheless, the findings of the present study are important for a better understanding of the biomechanical changes that affect the gait function of diabetic subjects with and without neuropathy, showing definitively the fundamental key role of 3D-gait analysis in medical research and rehabilitation.

Several limitations should be considered when interpreting findings from this study. The small sample of subjects and the reduced set of muscles studied can be considered possible limitations. The small number of diabetic participants in the study is a reflection of the difficulties recruiting people with a medical condition that is associated with multi-professional care, heavy appointments with full days of review.

Another limitation was the age difference between DPN and DM participants. Although the effect of diabetic peripheral neuropathy on gait characteristics is widely recognized to be higher than that of ageing, a better matched between ages would have contributed to a higher statistical power.

Chapter 7

Conclusions

Conclusions

This study highlights the biomechanics differences in terms of spatiotemporal, kinematic, kinetic and electromyographic variables, in gait of people with diabetes differently classified in risk groups. Slower gait speed, shorter steps, lower cadence, restriction of lower-limb joint mobility, less GRF amplitude and altered EMG patterns are some features related to the presence of diabetes peripheral neuropathy. These differences portray the harmful consequences of the disease, as also reflect the compensatory strategies adopted from the DPN population to improve the efficiency and stability of locomotion.

The analysis and knowledge of biomechanical gait characteristics reveals the structure and function of the lower limbs, posture and movement control, underlining functional gait alterations. For diabetic population, 3D movement analysis will provide accurate and reliable knowledge of gait characteristics at a given time, and also over time, an essential factor for prevention and diagnosis of future impairments, future risks of ulcer development, development of innovative therapeutic options and goal oriented rehabilitation programs.

In terms of therapeutic aspects, besides better glycemic control, health professionals that implement treatments and rehabilitation programs focused on maintaining or even improving muscle strength, balance and coordination of lower-limbs, may be advantageous for the diabetic population, contributing to the improvement of gait pattern, optimizations of balance and stability, long-term health related quality of life, and prevention of complications.

In conclusion, insight of the biomechanics differences in gait of people with diabetes differently classified in risk groups is, without a doubt, clinically important for the identification of early signs that allow discriminating different stages of diabetes and its complications, and to prevent and evaluate the injurious effects.

Chapter 8

Future Work

Future Work

Further development of this project would be of interest, with more and age-matched participants, to increase the accuracy of the identification of biomechanics parameters and to better understand and underpinned the effects of DPN in the walking pattern.

For future research there are several interesting and possible paths to take. Future studies addressing the effect of DPN on mobility should focus more on performing different daily life tasks, to determine the impact of DPN on the actual mobility capability in diabetic patients.

In line with the outcomes of this study, more insight into the muscular changes in diabetes needs to be obtained. It may be useful to focus on muscle activity during more demanding tasks such as walking faster, or walking on irregular surface to optimize the output responses to different input information. Tests of the force-velocity relationship, maximal contractile velocity and isotonic muscle power generation might help understand DPN-related impacts on muscle function.

Physical exercise is essential in the prevention and treatment of diabetes. So another promising investigation may be the evaluation of combine exercises to improve muscle strength, balance and coordination during gait and especially during more difficult locomotor tasks to prevent injuries and impairments in the future, and to optimize motor rehabilitation processes.

Further studies exploring inverse dynamics and net joint moments are also recommended.

Appendices

Table 19. Description of the direction of movement for gait analysis.

Joint	Axis	Movement	Coordinate Sign
Pelvis	x	Retroversion	+
		Anteversion	-
	y	Right Elevation	+
		Left Elevation	-
	z	Left Rotation	+
		Right Rotation	-
Hip	x	Flexion	+
		Extension	-
	y	Adduction	+
		Abduction	-
	z	Internal Rotation	+
		External Rotation	-
Knee	x	Flexion	+
		Extension	-
	y	Varus	+
		Valgus	-
	z	Internal Rotation	+
		External Rotation	-
Ankle	x	Dorsiflexion	+
		Plantarflexion	-
	y	Inversion	+
		Eversion	-
	z	Pronation	+
		Supination	-

Table 20. Check-list of LABIOMEP Normal Gait marker setup for thorax, head and upper limbs markers.

Marker Name	Location	Static (21)	Dynamic (21)
RALH	Approximately over the temple and preferably aligned with the lateral commissure of the eye	X	X
LALH		X	X
RPLH	Over the Occipital bone and at the same level as RALH and LALH on the frontal and sagittal plane	X	X
LPLH		X	X
RAC	Acromial edge of the scapula	X	X
LAC		X	X
C7	7TH Cervical Vertebrae	X	X
IJ	Jugular Insertion/Notch of the Sternum	X	X
PX	Xiphoid Process of the Sternum	X	X
RLELB	Lateral Epicondyle of the Humerus	X	X
LLELB		X	X
RMELB	Medial Epicondyle of the Humerus	X	X
LMELB		X	X
RRAD	Radio-Styloid Process	X	X
LRAD		X	X
RULN	Ulna-Styloid Process	X	X
LULN		X	X
RLH	Lateral portion of the 5th metatarsal head	X	X
LLH		X	X
RMH	Medial portion of the 5th metatarsal head	X	X
LMH		X	X

Table 21. Check-list of LABIOMEP Normal Gait marker setup for lower limb markers.

Marker Name	Location	Static (26)	Dynamic (16)
RASIS	Anterior Superior Iliac Spine	X	X
LASIS		X	X
RPSIS	Posterior Superior Iliac Spine	X	X
LPSIS		X	X
RTROC	Trochanter	X	
LTROC		X	
RFLE	Lateral Epicondyle of the Femur	X	
LFLE		X	
RFME	Medial Epicondyle of the Femur	X	
LFME		X	
RFAX	Proximal tip of the head of the Fibula	X	
LFAX		X	
RTTC	Most anterior portion of the Tibia Tuberosity	X	
LTTC		X	
RLA	Lateral prominence of the lateral Malleolus	X	X
LLA		X	X
RMA	Medial prominence of the lateral Malleolus	X	X
LMA		X	X
RCA	Distal end of the posterior aspect of the Calcaneus. Should be vertically aligned with FM2.	X	X
LCA		X	X
RFM1	Lateral aspect of the 1st metatarsal head	X	X
LFM1		X	X
RFM2	Dorsal aspect of the 2nd metatarsal head. Calcaneus marker should be vertically aligned.	X	X
LFM2		X	X
RFM5	Lateral aspect of the 5th metatarsal head	X	X
LFM5		X	X

Table 22. Check-list of LABIOMEP Normal Gait marker setup for multisegmented foot (Oxford Model).

Marker Name	Location	Static (24)	Dynamic (24)
RLA	Lateral prominence of the lateral Malleolus	X	X
LLA		X	X
RMA	Medial prominence of the lateral Malleolus	X	X
LMA		X	X
RPCA	Proximal end of the posterior aspect of Calcaneus (superior heel)	X	X
LPCA		X	X
RCPG	Medial end of the posterior aspect of Calcaneus (medial heel)	X	X
LPCG		X	X
RHEE	Distal end of the posterior aspect of Calcaneus (inferior heel)	X	X
LHEE		X	X
RLCA	Lateral aspect of the Calcaneus at the same height and distance as STL	X	X
LLCA		X	X
RSTL	Medial aspect of the Calcaneus at the same height and distance as LCA	X	X
LSTL		X	X
RP1M	Dorsal aspect of the base of the 1st metatarsal	X	X
LP1M		X	X
RP5M	Lateral aspect of the base of the 5th metatarsal	X	X
LP5M		X	X
RD1M	Lateral aspect of the head of the 1st metatarsal	X	X
LD1M		X	X
RD5M	Lateral aspect of the head of the 5th metatarsal	X	X
LD5M		X	X
RTOE	Lateral aspect of the head of the 2nd metatarsal	X	X
RHLX	Lateral aspect of the base of the Hallux	X	X

Table 23. SENIAM recommendations for sensor location in *Gastrocnemius Medialis* (152).

Muscle	
Name	*Gastrocnemius*
Subdivision	*Medialis*
Muscle Anatomy	
Origin	Proximal and posterior part of medial condyle and adjacent part of the femur, capsule of the knee joint.
Insertion	Middle part of posterior surface of calcaneus.
Function	Flexion of the ankle joint and assist in flexion of the knee joint.
Recommended sensor placement procedure	
Starting posture	Lying on the belly with the face down, the knee extended and the foot projecting over the end of the table.
Electrode size	Maximum size in the direction of the muscle fibres: 10 mm.
Electrode distance	20 mm.
Electrode placement	
- location	Electrodes need to be placed on the most prominent bulge of the muscle.
- orientation	In the direction of the leg (see picture).
- fixation on the skin	(Double sided) tape / rings or elastic band.
- reference electrode	On / around the ankle or the proc. spin. of C7.
Clinical test	Plantar flexion of the foot with emphasis on pulling the heel upward more than pushing the forefoot downward. For maximum pressure in this position it is necessary to apply pressure against the forefoot as well as against the calcaneus.
Remarks	The SENIAM guidelines include a separate sensor placement procedure for the lateral gastrocnemius.

Figure 16. SENIAM recommendation for sensor position in *Gastrocnemius Medialis* (*Adapted from:* (152)).

Table 24. SENIAM recommendations for sensor location in *Tibialis Anterior* (152)).

Muscle	
Name	*Tibialis anterior*
Subdivision	
Muscle Anatomy	
Origin	Lateral condyle and proximal 1/2 of lateral surface of tibia, interosseus membrane, deep fascia and lateral intermuscular septum.
Insertion	Medial and plantar surface of medial cuneiform bone, base of first metatarsal bone.
Function	Dorsiflexion of the ankle joint and assistance in inversion of the foot.
Recommended sensor placement procedure	
Starting posture	Supine or sitting.
Electrode size	Maximum size in the direction of the muscle fibers: 10 mm.
Electrode distance	20 mm.
Electrode placement	
- location	The electrodes need to be placed at 1/3 on the line between the tip of the fibula and the tip of the medial malleolus.
- orientation	In the direction of the line between the tip of the fibula and the tip of the medial malleolus.
- fixation on the skin	(Double sided) tape / rings or elastic band.
- reference electrode	On / around the ankle or the proc. spin. of C7.
Clinical test	Support the leg just above the ankle joint with the ankle joint in dorsiflexion and the foot in inversion without extension of the great toe. Apply pressure against the medial side, dorsal surface of the foot in the direction of plantar flexion of the ankle joint and eversion of the foot.
Remarks	

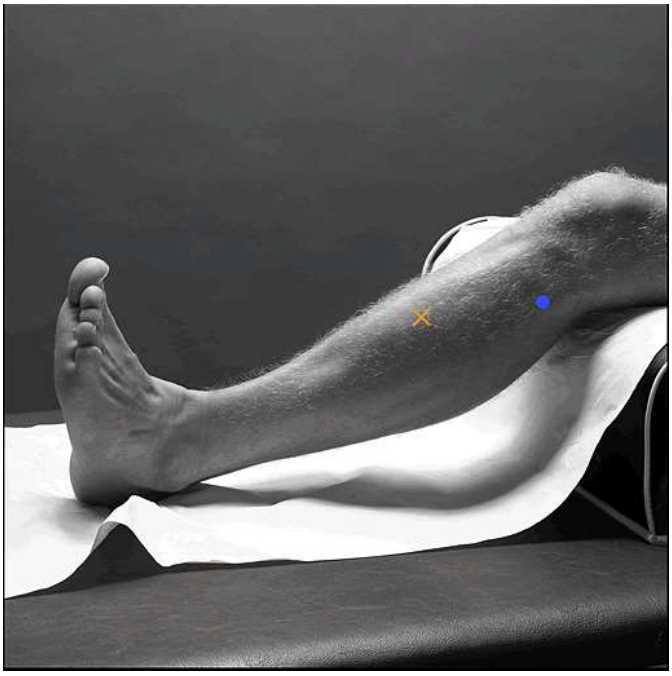


Figure 17. SENIAM recommendation for sensor position in *Tibialis Anterior* (*Adapted from:* (152).

Figure 18. MVC position for *Gastrocnemius Medialis*.

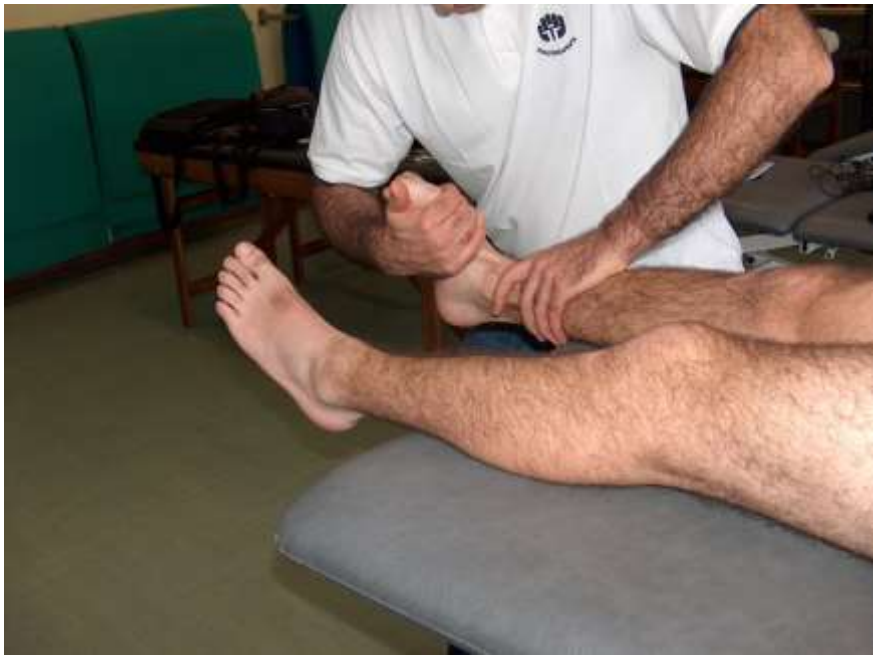
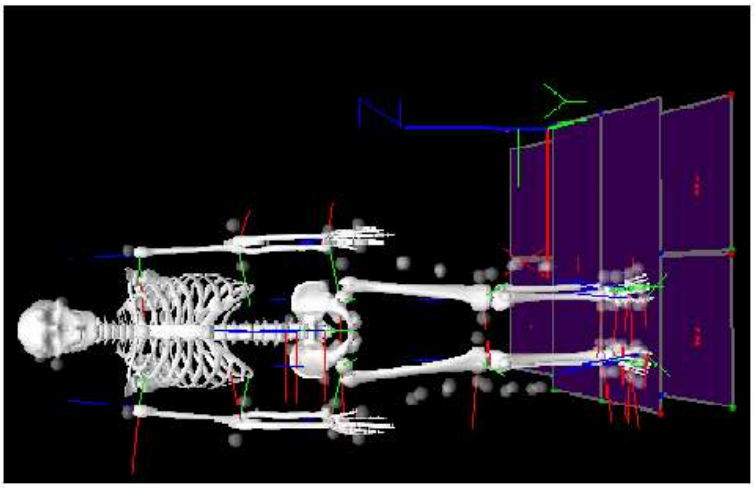
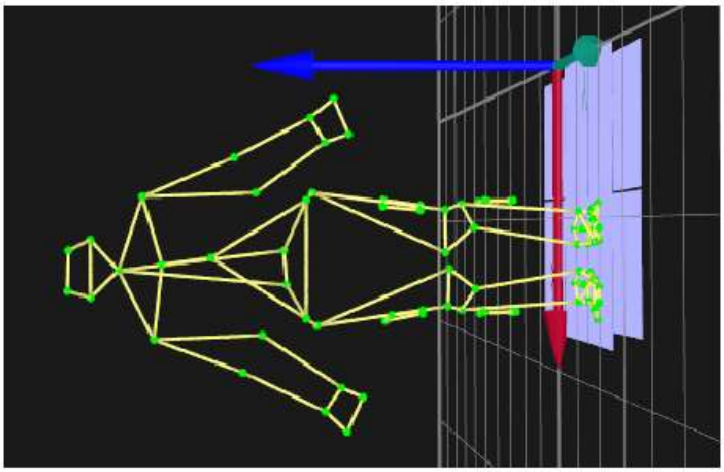


Figure 19. MVC position for *Tibialis Anterior*.





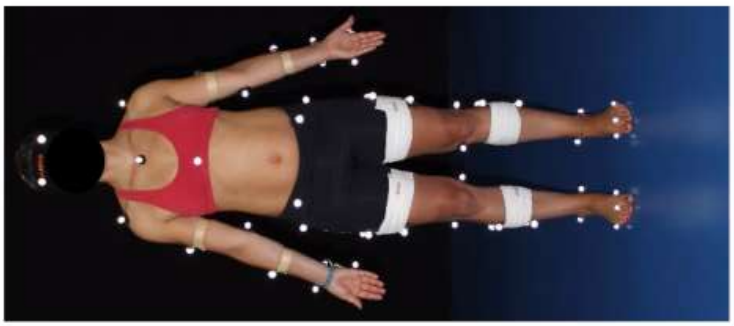


Figure 20. Data processing (LABIOMEP Normal Gait marker setup- Qualisys Track Manager- C-Motion's Visual3D).

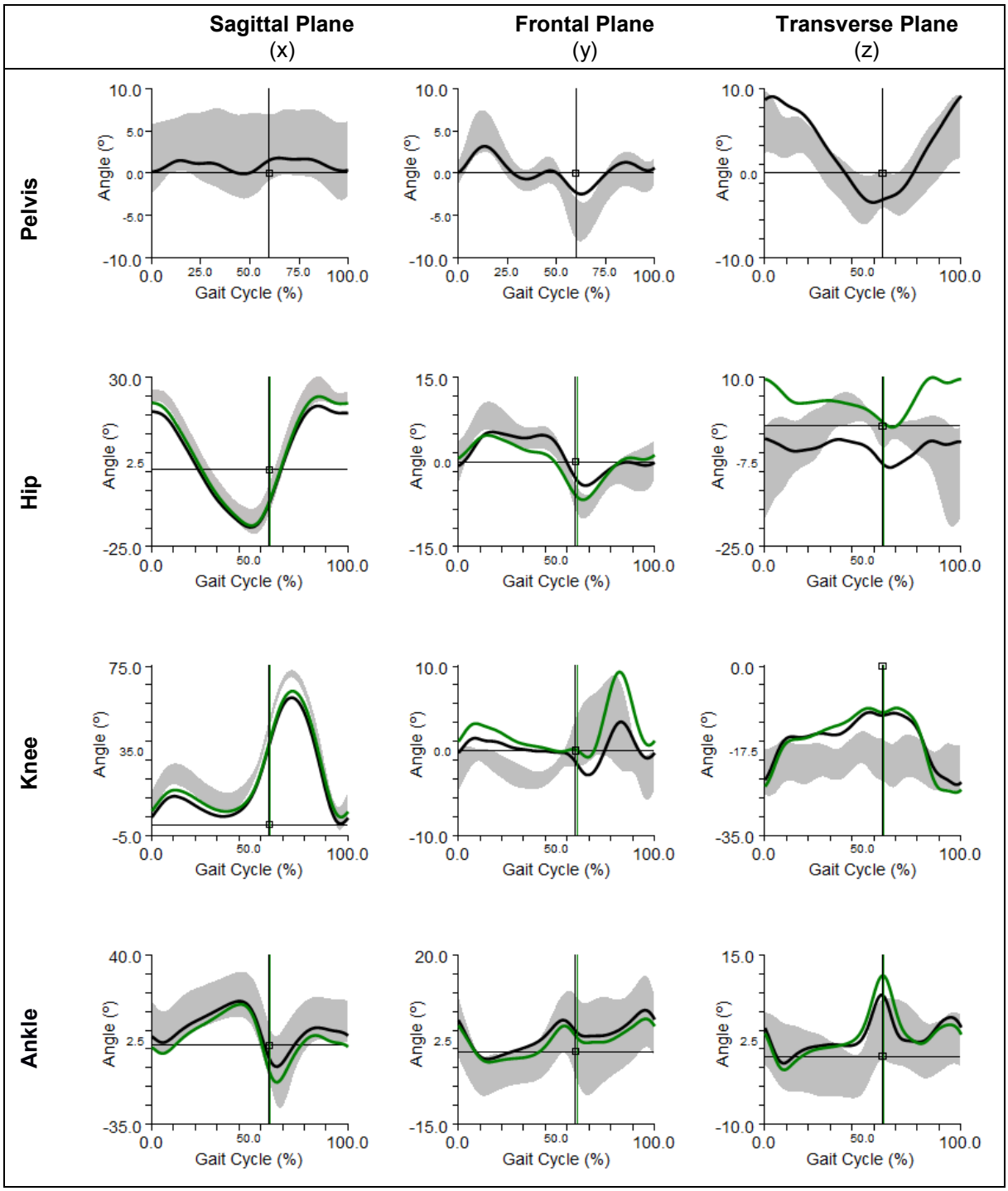


Figure 21. Gait analysis joint kinematic results of the DM group in sagittal, frontal and transverse plane.

Legend: The right side is depicted in black and the left side in green. Reference data is depicted in gray. Vertical axis represents angulation in degrees. Horizontal axis represents the duration of a gait cycle expressed as a percentage. The vertical line represents the moment of toe-off.

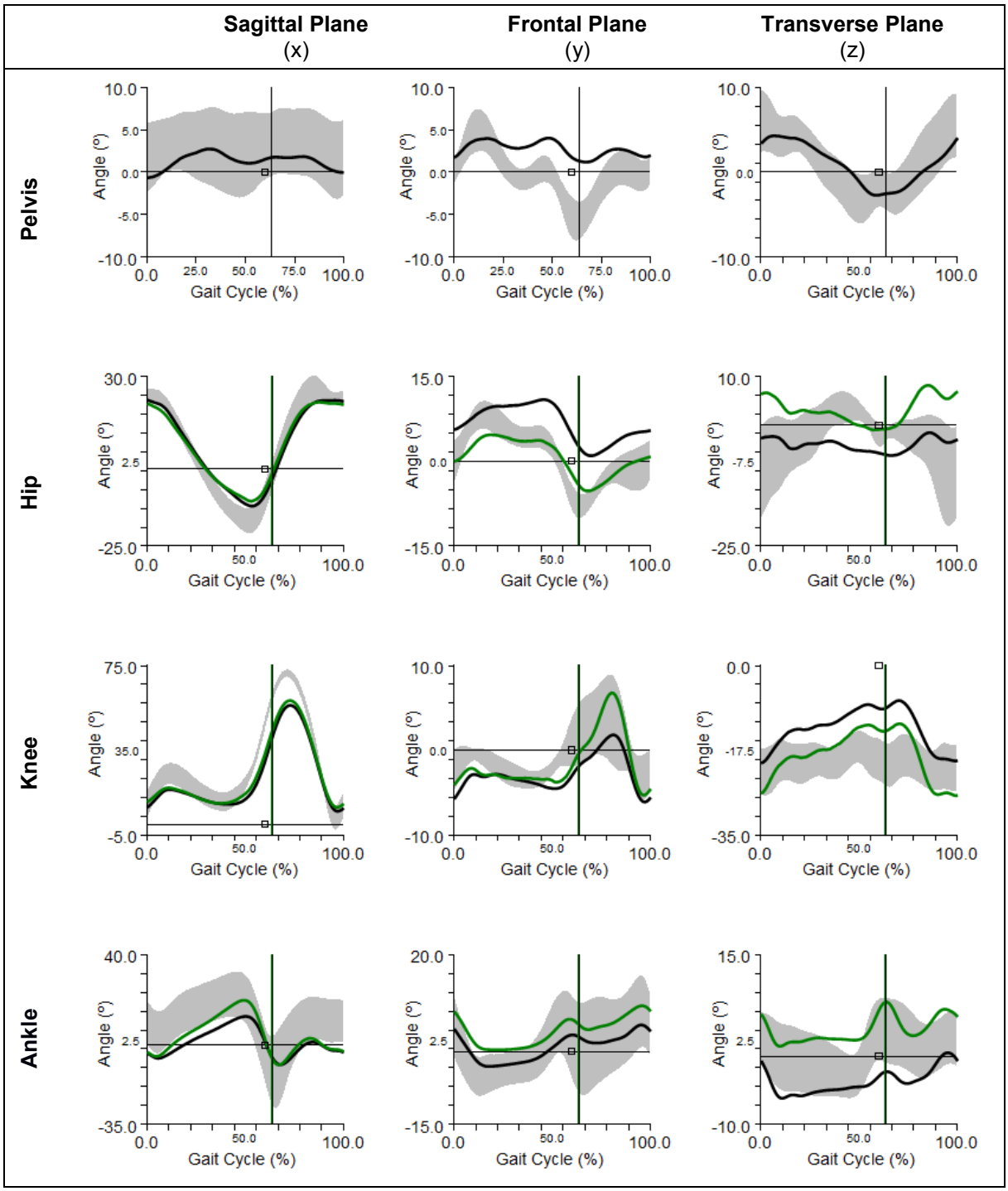


Figure 22. Gait analysis joint kinematic results of the DPN group in sagittal, frontal and transverse plane.

Legend: The right side is depicted in black and the left side in green. Reference data is depicted in gray. Vertical axis represents angulation in degrees. Horizontal axis represents the duration of a gait cycle expressed as a percentage. The vertical line represents the moment of toe-off.

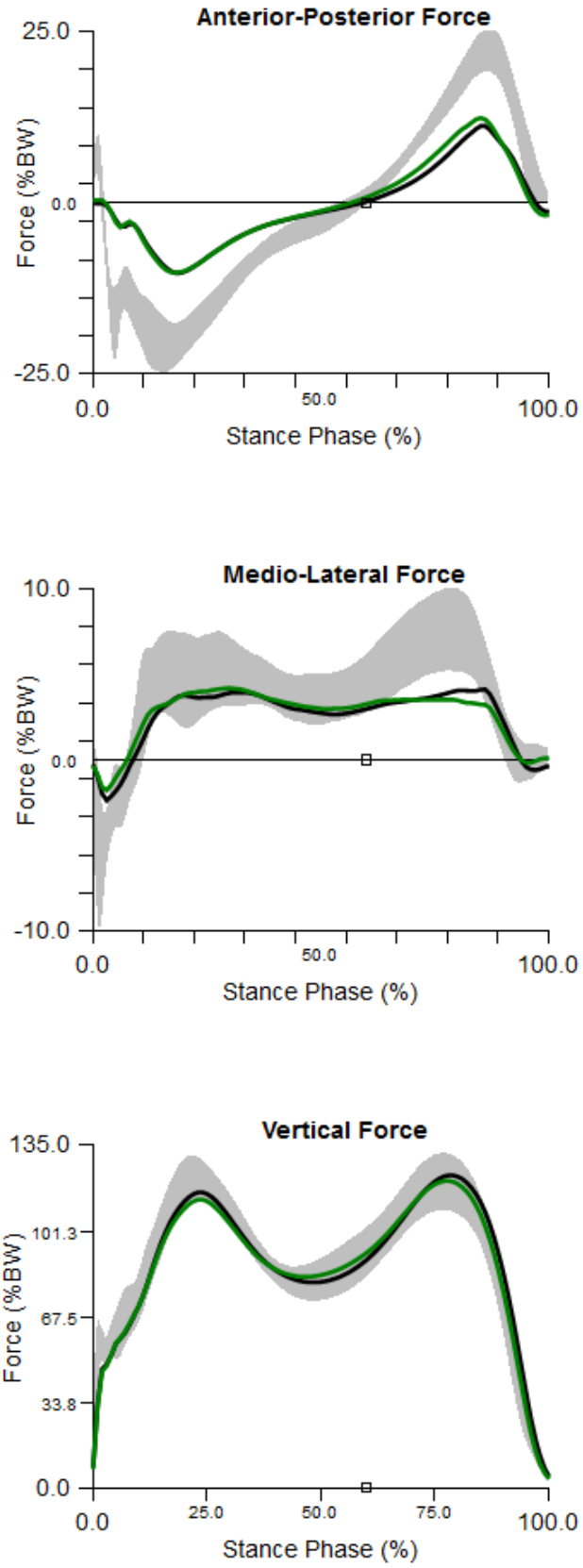


Figure 23. Averaged temporal evolution of the anterior-posterior, medio-lateral and vertical GRF of the DM group over the stance phase.

Legend: The right side is depicted in black and the left side in green. Reference data is depicted in gray. Vertical axis represents the ground reaction force normalized to body weight and expressed as a percentage. Horizontal axis represents the duration of stance phase, expressed as a percentage.

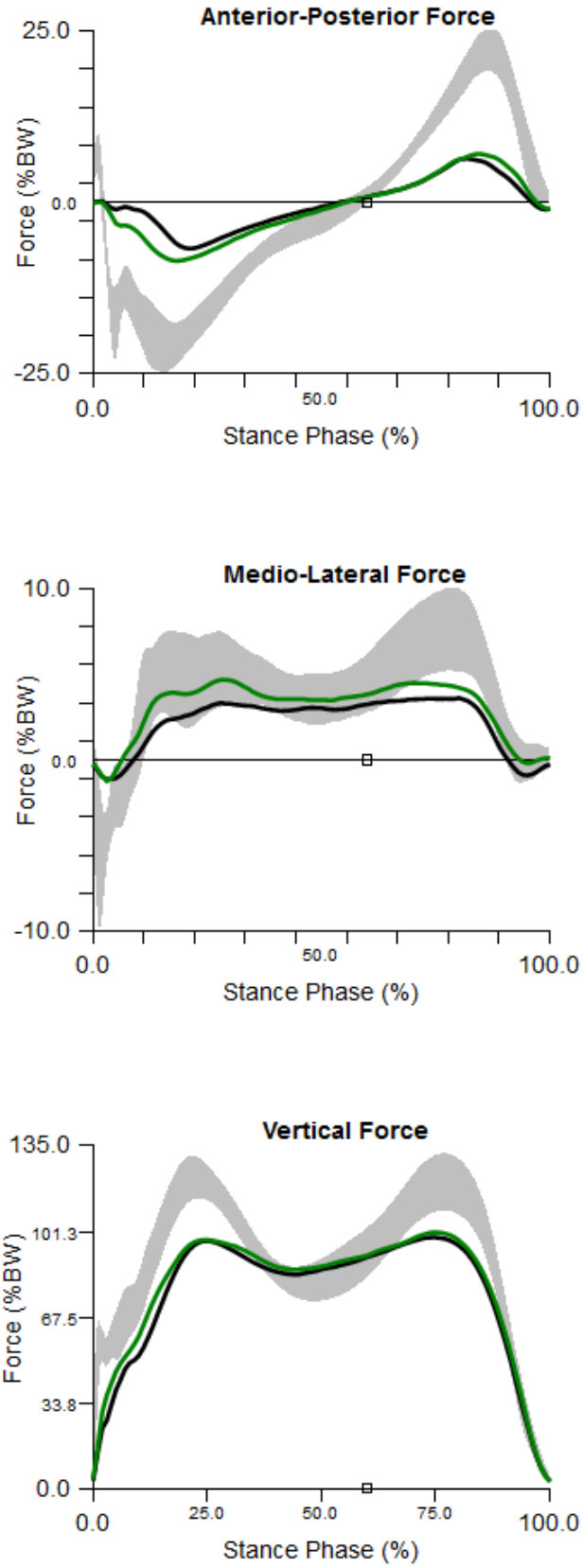


Figure 24. Averaged temporal evolution of the anterior-posterior, medio-lateral and vertical GRF of the DPN group over the stance phase.

Legend: The right side is depicted in black and the left side in green. Reference data is depicted in gray. Vertical axis represents the ground reaction force normalized to body weight and expressed as a percentage. Horizontal axis represents the duration of stance phase, expressed as a percentage.

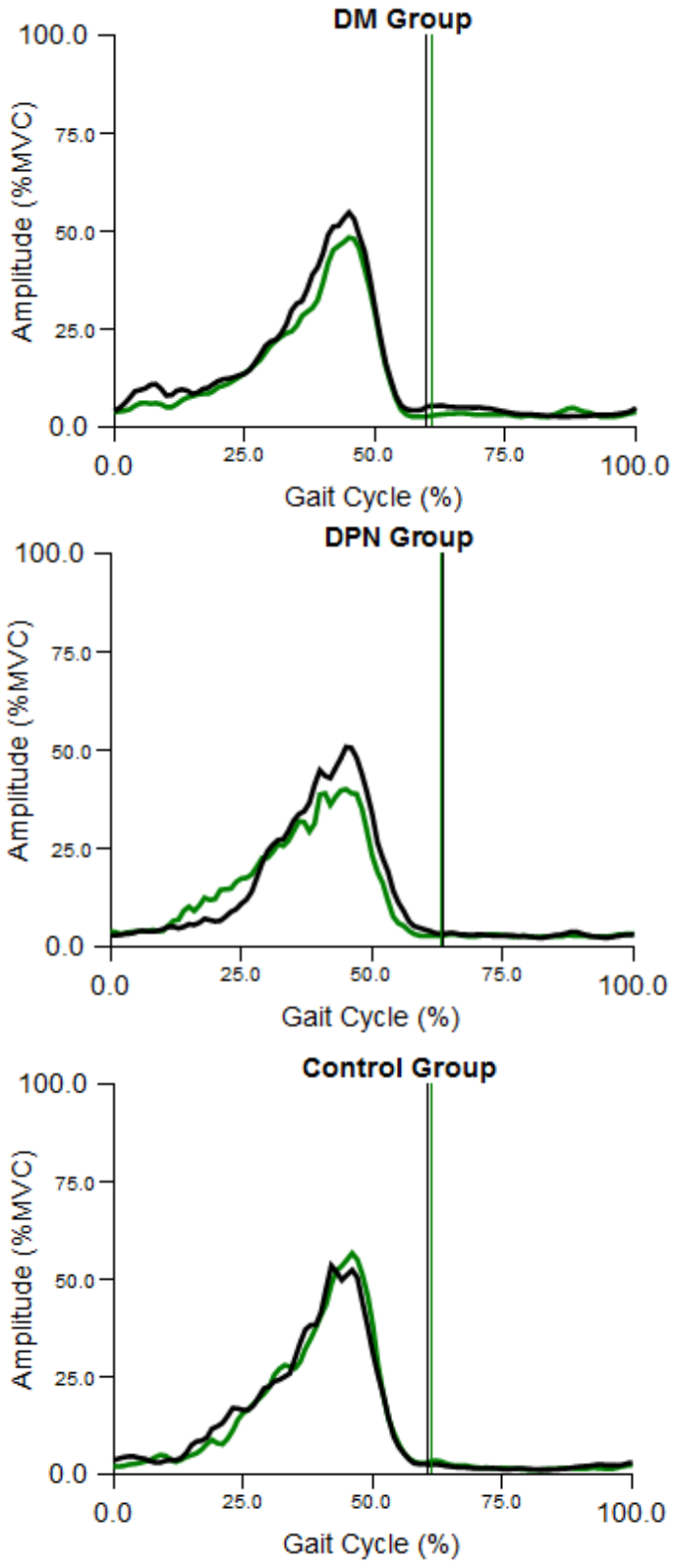


Figure 25. Ensemble-averaged EMG profile of the *Gastrocnemius Medialis* for the DM, DPN and control group.

Legend: The muscle from the right leg is depicted in black and the muscle from the left leg in green. Vertical axis represents mean of electromyographic activity normalized by the maximal voluntary contraction. Horizontal axis represents the duration of a gait cycle expressed as a percentage. The vertical line represents the moment of toe-off.

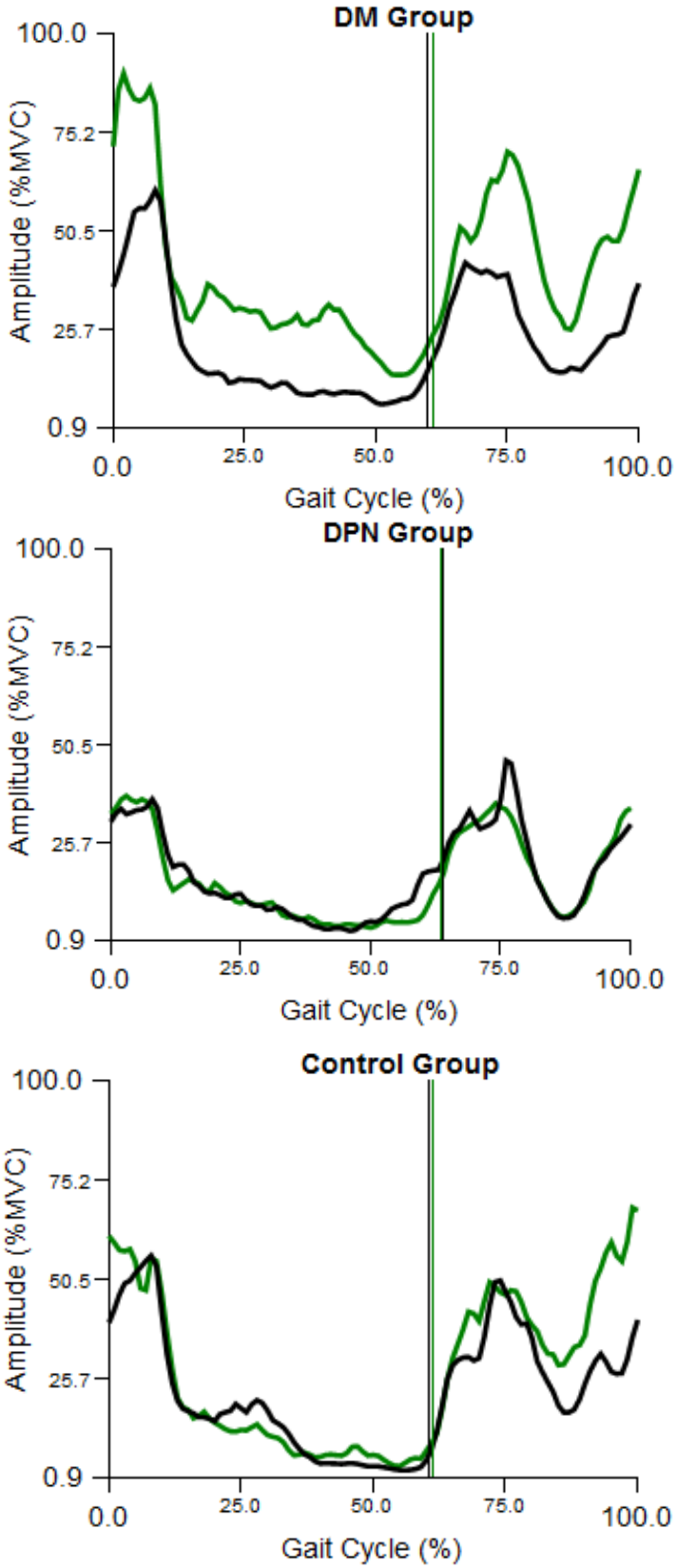


Figure 26. Ensemble-averaged EMG profile of the *Tibialis Anterior* for the DM, DPN and control group.

Legend: The muscle from the right leg is depicted in black and the muscle from the left leg in green. Vertical axis represents mean of electromyographic activity normalized by the maximal voluntary contraction. Horizontal axis represents the duration of a gait cycle expressed as a percentage. The vertical line represents the moment of toe-off.

Bibliography

1. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol. 2003; 17(1): 24–38.

2. Diabetes DOF. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2012; 35(SUPPL. 1).

3. Joshi SR, Parikh RM, Das AK. Insulin History , Biochemistry , Physiology and Pharmacology Biosynthesis of Insulin. Pharmacology. 2007; 55(JULY): 19–25.

4. Brown PM, Tompkins C V, Juul S, Sonksen PH. Mechanism of action of insulin in diabetic patients: a dose-related effect on glucose production and utilisation. New Zeal J Med Arthritis Rheum Sobrinho-Simoes, M, J Lab Clin Med Ann Rheum Dis J J, Ann Rheum Dis Am J Med. 1977; 13(39): 117–242.

5. Sonksen P, Sonksen J. Insulin: understanding its action in health and disease. BJA Br J Anaesth. 2000; 85(1): 69–79.

6. DeFronzo, R. A. . The triumvirate:beta-cell, muscle, liver. A Collusion Responsible for NIDDM. *Diabetes*. 1988; *37*(6): 667–687.

7. Kloppel G, Lohr M, Habich K, Oberholzer M, Heitz PU. Islet Pathology and the Pathogenesis of Type 1 and Type 2 Diabetes mellitus Revisited. Surv Synth Path Res. 1985; 4(2): 110–25.

8. Matveyenko A V., Butler PC. Relationship between β-cell mass and diabetes onset. Diabetes, Obes Metab. 2008; 10(SUPPL. 4): 23–31.

9. Campbell-Thompson M, Fu A, Kaddis JS, Wasserfall C, Schatz DA, Pugliese A, et al. Insulitis and β-cell mass in the natural history of type 1 diabetes. Diabetes. 2016; 65(3): 719–31.

10. Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA. β-cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: A new analysis. J Clin Endocrinol Metab. 2005; 90(1): 493–500.

11. Care D, Suppl SS. 2. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes—2018*. Diabetes Care. 2018; 41(Supplement 1): S13–27.

12. World Health Organization. Global Report on Diabetes. Isbn. 2016; 978: 88.

13. Turcotte LP, Fisher JS. Diabetes Special Issue. Phys Ther. 2008; 88(11): 1279–96.

14. Skyler JS, Bakris GL, Bonifacio E, Darsow T, Eckel RH, Groop L, et al. Differentiation of diabetes by pathophysiology, natural history, and prognosis. Diabetes. 2017; 66(2): 241–55.

15. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress—A concise review. Saudi Pharm J. 2016; 24(5): 547–53.

16. You W-P, Henneberg M. Type 1 diabetes prevalence increasing globally and regionally: the role of natural selection and life expectancy at birth. BMJ Open Diabetes Res Care. 2016; 4(e000161): 8.

17. Gallen I. Type 1 Diabetes: Clinical Management of the Athlete. Clin Chem. 2012; 367(9911): 194.

18. Alam U, Asghar O, Azmi S, Malik RA. General aspects of diabetes mellitus. 1st ed. Vol. 126, Diabetes and the Nervous System. Elsevier B.V. 2014; 211-222.

19. Group, D. C. and C. T. R.. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *The New England Journal of Medicine*. 1993; *329*(14): 977–986.

20. DeFronzo RA, Ferrannini E, Zimmet P, Alberti G. International Textbook of Diabetes Mellitus. Wiley. 2015.

21. Zimmet P, Alberti KGMM, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001 ;414: 782–7.

22. State A, Biology E. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. 2013; 4(4): 46–57.

23. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet. 2017; 389(10085): 2239–51.

24. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: From pathophysiology to prevention and management. Lancet. 2011; 378(9786): 169–81.

25. Hemminki K, Li X, Sundquist K, Sundquist J. Familial Risks for Type 2 Diabetes. Diabetes Care. 2010; 33(2).

26. Ríos MS, Fuentes JAG. Type 2. Diabetes Mellitus. Elsevier; 2009.

27. Harris MI, Klein R, Welborn TA, Knuiman MW. Onset of NIDDM occurs at least 4-7 yr before clinical diagnosis. Diabetes Care. 1992; 15(7): 815–9.

28. Colagiuri S. Diabesity: Therapeutic options. Diabetes, Obes Metab. 2010; 12(6): 463–73.

29. X P-S, G B, FL B. Reduction in Weight and Cardiovascular Disease Risk Factors in Individuals With One-year results of the Look AHEAD trial. Diabetes Care. 2007; 30(6): 1374–83.

30. International Diabetes Federation. IDF Diabetes Atlas Eighth Edition 2017. International Diabetes Federation. 2017; 150.

31. Buchanan T a, Xiang AH. Science in medicine Gestational diabetes mellitus. Diabetes. 2005; 115(3): 485–91.

32. Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. Diabet Med. 2004; 21: 103–13.

33. Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care. 2002; 25(10): 1862–8.

34. Chu SY, Callaghan WM, Kim SY, Schmid CH, Lau J, England LJ, et al. Maternal obesity and risk of gestational diabetes mellitus. Diabetes Care. 2007; 30(8): 2070–6.

35. Torloni MR, Betrán AP, Horta BL, Nakamura MU, Atallah AN, Moron AF, et al. Prepregnancy BMI and the risk of gestational diabetes: A systematic review of the literature with meta-analysis: Diagnostic in Obesity and Complications. Obes Rev. 2009; 10(2): 194–203.

36. Sanabria-Martínez G, García-Hermoso A, Poyatos-Leõn R, Álvarez-Bueno C, Sánchez-Lõpez M, Martínez-Vizcaíno V. Effectiveness of physical activity interventions on preventing gestational diabetes mellitus and excessive maternal weight gain: A meta-analysis. BJOG An Int J Obstet Gynaecol. 2015; 122(9): 1167–74.

37. Spaight C, Gross J, Horsch A, Puder JJ. Gestational diabetes mellitus. Endocr Dev. 2016; 31: 163–78.

38. Seino Y, Nanjo K, Tajim N, Kadowaki T, Kashiwagi A, Araki E, et al. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. J Diabetes Investig. 2010; 1(5): 212–28.

39. Slingerland AS. Monogenic diabetes in children and young adults: Challenges for researcher, clinician and patient. Rev Endocr Metab Disord. 2006; 7(3): 171–85.

40. Steck AK, Winter WE. Review on monogenic diabetes. Curr Opin Endocrinol Diabetes Obes. 2011; 18(4): 252–8.

41. Punthakee Z, Goldenberg R, Katz P. 2018 Clinical Practice Guidelines Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome Diabetes Canada Clinical Practice Guidelines Expert Committee. Can J Diabetes. 2018; 42: S10–5.

42. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L. The many faces of diabetes: A disease with increasing heterogeneity. Lancet. 2014; 383(9922): 1084–94.

43. Pandit MK, Burke J, Gustafson AB, Minocha A, Peiris AN. Drug-induced disorders of glucose tolerance. Ann Intern Med. 1993; 118(7): 529–39.

44. Leslie RD. Predicting adult-onset autoimmune diabetes clarity from complexity. Diabetes. 2010; 59(2): 330–1.

45. Nathan DM, Balkau B, Bonora E, Borch-Johnsen K, Buse JB, Colagiuri S, et al. International expert committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care. 2009; 32(7): 1327–34.

46. Genuth S, Alberti KGMM, Bennett P, Buse J, DeFronzo R, Kahn R, et al. Follow-up Report on the Diagnosis of Diabetes Mellitus. Diabetes Care. 2003; 26(11): 3160–7.

47. Unwin N, Shaw J, Zimmet P, Alberti KGMM. Impaired glucose tolerance and impaired fasting glycaemia: The current status on definition and intervention. Diabet Med. 2002; 19(9): 708–23.

48. WHO. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia. Who2. 2006; 50.

49. Diabetes DOF. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010; 33(SUPPL. 1).

50. Report A, Consultation WHO. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. Diabetes Res Clin Pract. 2011; 93(3): 299–309.

51. Ada. Tests of Glycemia in Diabetes FOR ROUTINE OUTPATIENT. Diabetes Care. 2003; 26(1): S106–8.

52. Inzucchi SE. Diagnosis of Diabetes. N Engl J Med. 2012; 367(6): 542–50. 53.

53. Sacks DB. A1C versus glucose testing: A comparison. Diabetes Care. 2011; 34(2): 518–23.

54. Rebekah Gospin, James P.Leu, and Joel Zonszein, Porestsky L. chapter 8, Diagnostic Cirteria and Classification of Diabetes. Principles of diabetes mellitus (3rd ed.). 2017; 123-158.

55. Ackermann RT, Cheng YJ, Williamson DF, Gregg EW. Identifying adults at high risk for diabetes and cardiovascular disease using hemoglobin A1c: National Health and Nutrition Examination Survey 2005-2006. Am J Prev Med. 2011; 40(1): 11–7.

56. Nam H, Cho WK, Kim JH, Rhie Y, Chung S, Lee K, et al. HbA1c Cutoff for Prediabetes and Diabetes Based on Oral Glucose Tolerance Test in Obese Children and Adolescents. 2018; 33(12): 1–11.

57. Dubowitz N, Xue W, Long Q, Ownby JG, Olson DE, Barb D, et al. Aging is associated with increased HbA $_{1c}$ levels, independently of glucose levels and insulin resistance, and also with decreased HbA $_{1c}$ diagnostic specificity. Diabet Med. 2014; 31(8): 927–35.

58. Lee JM, Wu EL, Tarini B, Herman WH, Yoon E. Diagnosis of diabetes using hemoglobin A1c: Should recommendations in adults be extrapolated to adolescents? J Pediatr. 2011; 158(6): 947–952.e3.

59. Yang L, Shen X, Yan S, Xu F, Wu P. The effectiveness of age on HbA1c as a criterion for the diagnosis of diabetes in Chinese different age subjects. Clin Endocrinol (Oxf). 2015; 82(2): 205–12.

60. Lipska KJ, Inzucchi SE, Van Ness PH, Gill TM, Kanaya A, Strotmeyer ES, et al. Elevated HbA1c and fasting plasma glucose in predicting diabetes incidence among older adults: Are two better than one? Diabetes Care. 2013; 36(12): 3923–9.

61. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006; 3(11): 2011–30.

62. Risk NCD, Collaboration F. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet (London, England). 2016; 387(10027): 1513–30.

63. Diabetologia SP. Relatório Anual do Observatório Nacional da Diabetes. 2016.

64. Seuring T, Archangelidi O, Suhrcke M. The Economic Costs of Type 2 Diabetes: A Global Systematic Review. Pharmacoeconomics. 2015; 33(8): 811–31.

65. Gilbert MP. Screening and Treatment by the Primary Care Provider of Common Diabetes Complications. Med Clin North Am. 2015; 99(1): 201–19.

66. Boulton AJM, Vileikyte L, Ragnarson-tennvall G, Apelqvist J. Review The global burden of diabetic foot disease. 2005; 1719–24.

67. Orasanu G, Plutzky J. The Pathologic Continuum of Diabetic Vascular Disease. J Am Coll Cardiol. 2009; 53(5 SUPPL.).

68. Forbes JM, Cooper ME. Mechanisms of Diabetic Complications. Physiol Rev. 2013; 93(1): 137–88.

69. Niemet A. Diabetic Complications. Chemist+Druggist. 2013; 18–20.

70. Cade WT. Diabetes-Related Microvascular and Macrovascular Diseases in the Physical Therapy Setting. Phys Ther. 2008; 88(11): 1322–35.

71. Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? Indian J Endocrinol Metab. 2016; 20(4): 546.

72. Papatheodorou K, Papanas N, Banach M, Papazoglou D, Edmonds M. Complications of Diabetes 2016. J Diabetes Res. 2016.

73. Naslafkih A, Sestier F. Diabetes mellitus related morbidity, risk of hospitalization and disability. J Insur Med. 2003; 35(2): 102–13.

74. Vinik AI. Diabetic Neuropathies. Contemp Endocrinol Controv Treat Diabetes Clin Res Asp. 2000; 109–34.

75. Duby JJ, Campbell RK, Setter SM, White JR, Rasmussen KA. Diabetic neuropathy: An intensive review. Am J Heal Pharm. 2004; 61(2): 160–76.

76. Pop-Busui R, Boulton AJM, Feldman EL, Bril V, Freeman R, Malik RA, et al. Diabetic neuropathy: A position statement by the American diabetes association. Diabetes Care. 2017; 40(1): 136–54.

77. Bansal V, Kalita J, Misra UK. Diabetic neuropathy. Postgrad Med J. 2006; 82(964): 95–100.

78. Boulton AJ, Gries FA, Jervell JA. Guidelines for the diagnosis and outpatient management of diabetic peripheral neuropathy. Diabet Med. 1998; 15(6): 508–14.

79. Tesfaye S, Selvarajah D. Advances in the epidemiology, pathogenesis and management of diabetic peripheral neuropathy. Diabetes Metab Res Rev. 2012; 28(S1): 8–14.

80. Singh R, Kishore L, Kaur N. Diabetic peripheral neuropathy: Current perspective and future directions. Pharmacol Res. 2014; 80: 21–35.

81. Tesfaye S, Stevens LK, Stephenson JM, Fuller JH, Plater M, Ionescu-Tirgoviste C, et al. Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: The EURODIAB IDDM Complications Study. Diabetologia. 1996; 39(11): 1377–84.

82. Hanssen KF. Blood glucose control and microvascular and macrovascular complications in diabetes. Diabetes. 1997; 46 Suppl 2(9): S101-3.

83. Risk Factors for Severity of Diabetic Polyneuropathy. Diabetes Care. 1999; 22(9): 1479–86.

84. Boulton AJM, Malik RA, Arezzo JC, Sosenko JM. Diabetic somatic neuropathies. Diabetes Care. 2004; 27(6): 1458–86.

85. Edwards JL, Vincent AM, Cheng HT, Feldman EL. Diabetic neuropathy: Mechanisms to management. Pharmacol Ther. 2008; 120(1): 1–34.

86. Said G. Diabetic neuropathy - A review. Nat Clin Pract Neurol. 2007; 3(6): 331–40.

87. Tesfaye S, Boulton AJM, Dyck PJ, Freeman R, Horowitz M, Kempler P, et al. Diabetic neuropathies: Update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care. 2010; 33(10): 2285–93.

88. Quattrini C, Tesfaye S. Understanding the impact of painful diabetic neuropathy. Diabetes Metab Res Rev. 2003; 19(SUPPL. 1): 2–8.

89. Davies M, Brophy S, Williams R, Taylor A. The prevalence, severity, and impact of painful diabetic peripheral neuropathy in type 2 diabetes. Diabetes Care. 2006; 29(7): 1518–22.

90. H. VD. The diabetic foot. Rev Med Liege. 2005; 60(5–6): 516–25.

91. Boulton AJM. Management of Diabetic Peripheral Neuropathy. Clin Diabetes. 2005;23(1):9–15.

92. Boulton AJM, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, et al. Diabetic neuropathies: A statement by the American Diabetes Association. Diabetes Care. 2005; 28(4): 956–62.

93. Al-Geffari M. Comparison of different screening tests for diagnosis of diabetic peripheral neuropathy in Primary Health Care setting. Int J Health Sci (Qassim). 2012; 6(2): 127–34.

94. Herman WH, Pop-Busui R, Braffett BH, Martin CL, Cleary PA, Albers JW, et al. Use of the Michigan Neuropathy Screening Instrument as a measure of distal symmetrical peripheral neuropathy in Type 1 diabetes: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications. Diabet Med. 2012; 29(7): 937–44.

95. Moghtaderi A, Bakhshipour A, Rashidi H. Validation of Michigan neuropathy screening instrument for diabetic peripheral neuropathy. Clin Neurol Neurosurg. 2006; 108(5): 477–81.

96. Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA. A Practical Two-Step Quantitative Clinical and Electrophysiological Assessment for the Diagnosis and Staging of Dianetic Neuropathy. Diabetes Care. 1994; 17(11): 1281–9.

97. Velazquez FR, Bartholomew D. Reproducibility of Michigan Neuropathy Screening Instrument (MNSI). Diabetes Care. 1996; 19(8): 904–5.

98. Said G. Diabetic neuropathy. Handb Clin Neurol. 2013; 115: 579–89.

99. Martin CL, Albers JW, Pop-Busui R. Neuropathy and related findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. Diabetes Care. 2014; 37(1): 31–8.

100. Otto-Buczkowska E, Jainta N. Pharmacological Treatment in Diabetes Mellitus Type 1-Insulin and What Else? Int J Endocrinol Metab. 2018; 16(1): 7.

101. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group TWT for the DC and CT of DI and CR. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. Jama. 2002; 287(19): 2563–9.

102. Turner R. Intensive blood glucose control reduced type 2 diabetes mellitus- related end points: Commentary. Evid Based Med. 1999; 4(1): 10–1.

103. Azad N, Emanuele N V., Abraira C, Henderson WG, Colwell J, Levin SR, et al. The effects of intensive glycemic control on neuropathy in the VA cooperative study on type II diabetes mellitus. J Diabetes Complications. 1999; 13(5–6): 307–13.

104. Tesfaye S. Advances in the management of diabetic peripheral neuropathy. Curr Opin Support Palliat Care. 2009; 3(2): 136–43.

105. Obrosova IG. Diabetic Painful and Insensate Neuropathy: Pathogenesis and Potential Treatments. Neurotherapeutics. 2009; 6(4): 638–47.

106. Whittle MW. Clinical gait analysis: A review. Hum Mov Sci. 1996; 15(3): 369–87.

107. Kuo AD, Donelan JM. Dynamic Principles of Gait and Their Clinical Implications. Phys Ther. 2010; 90(2): 157–74.

108. Vaughan CL, Davis BL, O'Connor JC. Dynamics of Human Gait. 2nd ed. Human Kinetics Publishers. 1992; 137.

109. Winter DA. Biomechanics and Motor Control of Human Movement. 2nd ed. John Wiley & Sons, Inc. 1990; 370.

110. Richards J. Biomechanics in Clinic and Research. Churchill Livingstone/Elsevier. 2008; 207.

111. Nigg BM, Herzog W. Biomechanics of the Musculo-skeletal System. John Wiley & Sons, Inc. 1994; 672.

112. Fonseca F, Completo A. Fundamentos de Biomecânica: Músculo-esquelética e Ortopédica. PUBLINDUSTRIA. 2011; 446.

113. Solnik S, Rider P, Steinweg K, Devita P, Hortobágyi T. Teager-Kaiser energy operator signal conditioning improves EMG onset detection. Eur J Appl Physiol. 2010; 110(3): 489–98.

114. Li X, Zhou P, Aruin AS. Teager-kaiser energy operation of surface EMG improves muscle activity onset detection. Ann Biomed Eng. 2007; 35(9): 1532–8.

115. Konrad P. The ABC of EMG. A Pratical Introduction to Kinesiological Electromyography. Noraxon U.S.A., Inc. 2006; 61.

116. Yavuzer G, Yetkin I, Toruner FB, Koca N, Bolukbas N. Gait deviations of patients with diabetes mellitus: looking beyond peripheral neuropathy. Eur J Phys Rehabil Med. 2006; 42(2): 127–33.

117. Abbott C a., Vileikyte L, Williamson S, Carrington AI, Bolton AJM. Multicenter Study of the Incidence of and Predictive Risk Factors for Diabetic Neuropathic Foot Ulceration. Diabet Care. 1998; 21(7): 1071–5.

118. Cavanagh P., Derrb J., Ulbrecht J., Maserc R., Orchardd T. Problems with Gait and Posture in Neuropathic Patients with Insulin Dependent Diabetes Mellitus. Diabet Med. 1992; 9(5): 469–74.

119. Simoneau GG, Ulbrech JS, Derr JA, Becker MB, Cavanagh PR. Postural Instability in Sensory Neuropathy. Diabetes Care. 1994; 17(12): 1411–21.

120. Corriveau H, Prince F, Hébert R, Raîche M, Tessier D, Maheux P, et al. Evaluation of postural stability in elderly with diabetic neuropathy. Diabetes Care. 2000; 23(8): 1187–91.

121. De Souza Fortaleza AC, Chagas EF, Minonroze D, Ferreira A, Mantovani AM, Federighi E, et al. Postural control and functional balance in individuals with diabetic peripheral neuropathy. Brazilian J Kinanthropometry Hum Perform. 2013; 15(3): 305–14.

122. Palma FH, Antigual DU, Martínez SF, Monrroy MA, Gajardo RE. Static balance in patients presenting diabetes mellitus type 2 with and without diabetic polyneuropathy. Arq Bras Endocrinol Metabol. 2013; 57(9): 722–6.

123. Fahmy IM, Ramzy GM, Salem NA, Ahmed GM, Mohammed AA. Balance disturbance in patients with diabetic sensory polyneuropathy. Egypt J Neurol Psychiatry Neurosurg. 2014; 51(1): 21–9.

124. Brown SJ, Handsaker JC, Bowling FL, Boulton AJM, Reeves ND. Diabetic peripheral neuropathy compromises balance during daily activities. Diabetes Care. 2015; 38(6): 1116–22.

125. Courtemanche R, Teasdale N, Boucher P, Fleury M, Lajoie Y, Bard C. Gait problems in diabetic neuropathic patients. Arch Phys Med Rehabil. 1996; 77(9): 849–55.

126. Menz HB, Lord SR, St George R, Fitzpatrick RC. Walking Stability and Sensorimotor Function in Older People with Diabetic Peripheral Neuropathy. Arch Phys Med Rehabil. 2004; 85(2): 245–52.

127. Richardson J, Thies S, DeMott T, Ashton-Miller J. A comparison of gait characteristics between older women with and without peripheral neuropathy in standard and challenging environments. 2004; 52(9): 1532–7.

128. Tareef AA. The effect of a cognitive or motor task on gait parameters of diabetic patients, with and without neuropathy. Can Fam Physician. 2011; 57(7): 771–6.

129. Martinelli AR, Mantovani AM, Nozabieli AJL, Ferreira DMA, Barela JA, Camargo MR de, et al. Muscle strength and ankle mobility for the gait parameters in diabetic neuropathies. Foot. 2013; 23(1): 17–21.

130. Lalli P, Chan A, Garven A, Midha N, Chan C, Brady S, et al. Increased gait variability in diabetes mellitus patients with neuropathic pain. J Diabetes Complications. 2013; 27(3): 248–54.

131. Mueller MJ, Minor SD, Sahrmann SA, Schaaf JA, Strube MJ. Differences in the Gait Characteristics of Patients With Diabetes and Peripheral Neuropathy Compared With Age-Matched Controls. Phys Ther. 1994; 74(4): 299–308.

132. Sawacha Z, Gabriella G, Cristoferi G, Guiotto A, Avogaro A, Cobelli C. Diabetic gait and posture abnormalities: A biomechanical investigation through three dimensional gait analysis. Clin Biomech. 2009; 24(9): 722–8.

133. Wuehr M, Schniepp R, Schlick C, Huth S, Pradhan C, Dieterich M, et al. Sensory loss and walking speed related factors for gait alterations in patients with peripheral neuropathy. Gait Posture. 2014; 39(3): 852–8.

134. Sacco ICN, Amadio AC. A study of biomechanical parameters in gait analysis and sensitive cronaxie of diabetic neuropathic patients. Clin Biomech. 2000; 15(3): 196–202.

135. Katoulis EC, Ebdon-Parry M, Lanshammar H, Vileikyte L, Kulkarni J, Boulton AJM. Gait abnormalities in diabetic neuropathy. Diabetes Care. 1997; 20(12): 1904–7.

136. Chiles NS, Phillips CL, Volpato S, Bandinelli S, Ferrucci L, Guralnik JM, et al. Diabetes, Peripheral Neuropathy, and Lower Extremity Function. Jorunal Diabetes Its Complicat. 2014; 28(1): 91–5.

137. Saura V, Godoy dos Santos AL, Ortiz RT, Parisi MC, Fernandes TD, Nery M. Predictive Factors of Gait in Neuropathic and Non-Neurophatic Diabetic Patients. Acta Ortop Bras. 2010; 18(3): 148–51.

138. Gomes AA, Onodera AN, Otuzi MEI, Pripas D, Mezzarane RA, Sacco ICN. Electromyography and kinematic changes of gait cycle at different cadences in diabetic neuropathic individuals. Muscle and Nerve. 2011; 44(2): 258–68.

139. Uccioli L, Caselli A, Giacomozzi C, Macellari V, Giurato L, Lardieri L, et al. Pattern of abnormal tangential forces in the diabetic neuropathic foot. Clin Biomech. 2001; 16(5): 446–54.

140. Raspovic A. Gait characteristics of people with diabetes-related peripheral neuropathy, with and without a history of ulceration. Gait Posture. 2013; 38(4): 723–8.

141. Sawacha Z, Guarneri G, Cristoferi G, Guiotto A, Avogaro A, Cobelli C. Integrated kinematics-kinetics-plantar pressure data analysis: A useful tool for characterizing diabetic foot biomechanics. Gait Posture. 2012; 36(1): 20–6.

142. Savelberg HHCM, Schaper NC, Willems PJB, De Lange TLH, Meijer K. Redistribution of joint moments is associated with changed plantar pressure in diabetic polyneuropathy. BMC Musculoskelet Disord. 2009; 10: 1–10.

143. Petrofsky J, Lee S, Bweir S. Gait characteristics in people with type 2 diabetes mellitus. Eur J Appl Physiol. 2005; 93(5–6): 640–7.

144. Akashi PMH, Sacco ICN, Watari R, Hennig E. The effect of diabetic neuropathy and previous foot ulceration in EMG and ground reaction forces during gait. Clin Biomech. 2008; 23(5): 584–92.

145. Abboud RJ, Rowley DI, Newton RW. Lower limb muscle dysfunction may contribute to foot ulceration in diabetic patients. Clin Biomech (Bristol, Avon). 2000; 15(1): 37–45.

146. Kwon O-Y, Minor SD, Maluf KS, Mueller MJ. Comparison of muscle activity during walking in subjects with and without diabetic neuropathy. Gait Posture. 2003; 18(1): 105–13.

147. Savelberg HHCM, Ilgin D, Angin S, Willems PJB, Schaper NC, Meijer K. Prolonged activity of knee extensors and dorsal flexors is associated with adaptations in gait in diabetes and diabetic polyneuropathy. Clin Biomech. 2010; 25(5): 468–75.

148. Sawacha Z, Spolaor F, Guarneri G, Contessa P, Carraro E, Venturin A, et al. Abnormal muscle activation during gait in diabetes patients with and without neuropathy. Gait Posture. 2012; 35(1): 101–5.

149. Barbosa M, Saavedra A, Severo M, Maier C, Carvalho D. Validation and Reliability of the Portuguese Version of the Michigan Neuropathy Screening Instrument. Pain Pract. 2017; 17(4): 514–21.

150. Cappozzo A, Catani F, Croce U Della, Leardini A. Position and orientation in space of bones during movement: anatomical frame definition and determination. Clin Biomech. 1995; 10(4): 171–8.

151. Wilken JM, Rodriguez KM, Brawner M, Darter BJ. Reliability and minimal detectible change values for gait kinematics and kinetics in healthy adults. Gait Posture. 2012; 35(2): 301–7.

152. Hermens HJ, Freriks B, Merletti R, Stegeman D, Blok J, Rau G, et al. European Recommendations for Surface ElectroMyoGraphy. Roessingh Research and Development, Enschede, the Netherlands. 1999.

153. VEVES A. Book_DPN. Diabetic Neuropathy:CLINICAL MANAGEMENT. 2007; 31-43.

154. Andriacchi TP, Ogle JA, Galante JO. Walking speed as a basis for normal and abnormal gait measurements. J Biomech. 1977; 10(4): 261–8.