APPLICATION OF SURFACE EMG
IN DIABETIC DISEASE

Direttore della Scuola: Ch.mo Prof Gaetano Thiene

Supervisore: Ch.mo Prof Carlo Reggiani

Dottorando: Fabiola Spolaor
# Contents

Summary: English ................................................................. 4  
Summary: Italian ............................................................... 5

1 Introduction ............................................................... 6  
1.1 Background ............................................................. 6  
1.2 Aim ........................................................................ 7  
1.3 Thesis Outlines .......................................................... 7

2. Diabetes Mellitus And Its Complications  
2.1 Diabetes Mellitus ......................................................... 9-11  
2.2 Diabetes Complications ............................................... 11-12  
2.2.1 Peripheral Neuropathy ............................................. 12-13  
2.2.2 Peripheral arterial disease ....................................... 13-14

3. Comparison Of Muscle Activity During Walking In Subjects With And Without Diabetes  
3.1 Background ............................................................. 15-16  
3.2 The Gait Cycle ............................................................ 16-18  
3.3 Gait Analysis And Surface Emg .................................... 18-20  
3.3.1 Gait Analysis And Surface Emg in patient with diabetes ........................................ 20-21  
3.4 Material and Methods  
3.4.1 Subjects ............................................................... 21-22  
3.4.2 The Protocol .......................................................... 22-23  
3.4.3 Clinical Evaluation .................................................. 23-25  
3.4.4 Gait Analysis .......................................................... 25  
3.4.5 Sterophotogrammetric System ................................... 25-27  
3.4.6 Force Plates .......................................................... 27-29  
3.4.7 Plantar Pressure System .......................................... 29-30  
3.4.8 Emg Analysis ........................................................ 30-31  
3.4.9 Dynamic Acquisition .............................................. 32  
3.4.10 Fullbody Protocol .................................................. 32  
3.5 Statistical analysis ...................................................... 33  
3.6 Results ..................................................................... 33-35  
3.7 Discussion ................................................................. 36-38  
Bibliography

4. Comparison Of Muscle Activity During Step Ascent And Descent In Subjects With And Without Diabetes  
4.1 Background ............................................................. 45-47  
4.2 Aim ....................................................................... 47  
4.3 Material and Methods  
4.3.1 Subjects ............................................................... 48  
4.3.2 Step Ascent and Descent Analysis ......................... 49-50  
4.3.3 Posture ................................................................. 50-51  
4.4 Statistical Analysis ..................................................... 51  
4.5 Results ................................................................... 51-54  
4.6 Discussion ................................................................. 54-56  
Bibliography ................................................................. 57-59
5. Evaluation Of Muscle Fatigue During Treadmill Walking In Patients With Type 2 Diabetes And Peripheral Vasculopathy

5.1 Background
5.1.1 Muscle Fatigue .......................................................... 60-63
5.1.2 Muscle Fatigue, PAD and Intermittent Claudication ............................. 63
5.2 Aim ............................................................................. 64

5.3 Material and Methods
5.3.1 Subjects ................................................................. 64-65
5.3.2 Clinical Evaluation (see paragraph 2.4.3 chapter 1) ................................. 65
5.3.3 Treadmill Protocol Exercise ............................................ 66
5.3.4 Muscle Fatigue Assessment ............................................ 67-68
5.4 Statistical analysis ...................................................... 68-69
5.5 Results ....................................................................... 70-71
5.6 Discussion ................................................................... 71

Bibliography ................................................................. 72-74
Conclusion ................................................................. 75
The World Health Organization warns that, in 2000, as many as 33 million Europeans suffered from diabetes, approximately 15% will likely develop foot ulcers, and approximately 15% to 20% of these patients will face lower-extremity amputation. In 2004, an estimated 3.4 million people died from consequences of high blood sugar. Diabetic neuropathy is the most common chronic complication associated with diabetes mellitus, affecting 20–50% of diabetic patients 10 years after their diagnosis. Peripheral neuropathy and peripheral arterial disease are the most common and invalidating diabetes’s complications, involved in the pathogenesis of diabetic foot. They account for the leading cause of non-traumatic lower limb amputations. It results from two factors. The first one is a reduced blow of blood in the inferior limbs, caused from the presence of obliterating peripheral arteriopathy disease. The second is the progressive laceration of nervous fibers (neuropathy) that cause a reduction of the sensitivity (also to the pain) and of the ability of movement, and that helps the appearance of lesions. Together with diabetes falls in older adults are a big public health concern and have provided much of the motivation for research into age-related changes in human gait. Tripping during walking is the predominant cause of falls not only in the elderly but also in the neuropathic subjects. Trips can occur during walking on a level ground, but also during crossing visible obstacle, stair ascending and descending. The social and economic weight of the diabetic foot and the tragic consequences that brings with it can be reduced through a prompt diagnosis and treatment from the very beginning. The aim of this thesis, was to evaluate differences in gait parameters, in performing stair ascending and descending task and evaluation of muscle fatigue during treadmill protocol in diabetes subjects with and without complications, in order to provide a further tool for early diagnosis which allows clinicians to change, if is necessary, or only to control, as soon as possible, the follow-up of patients according to their specific characteristics.
L'Organizzazione Mondiale della Sanità avverte che, nel 2000, ben 33 milioni di europei hanno sofferto di diabete, circa il 15% probabilmente svilupperà ulcere del piede, e circa il 15-20% di questi pazienti si troveranno ad affrontare l'amputazione degli arti inferiori. Nel 2004 5.2 milioni di persone sono morte a causa degli elevati livelli di zucchero nel sangue. La neuropatia periferica è la complicanza diabetica cronica più frequente e colpisce dal 20 al 50% dei pazienti diabetici a distanza di 10 anni dalla diagnosi. Neuropatia e vasculopatia periferica sono le complesanze del diabete più comuni e invalidanti, e le maggiori responsabili della patogenesi del piede diabetico. Insieme rappresentano la principale causa di amputazioni non traumatiche degli arti inferiori. La vasculopatia periferica causa un ridotto apporto di sangue agli arti inferiori, mentre la neuropatia periferica si manifesta attraverso la lacerazione progressiva delle fibre nervose che causa una riduzione della sensibilità (anche al dolore) e della capacità di movimento, che provoca di conseguenza la comparsa di lesioni. Insieme al diabete, le cadute nella popolazione anziana sono una grande preoccupazione per la sanità pubblica e sono state la spinta motivazionale per la maggior parte delle ricerche svolte nell’ambito delle alterazioni del cammino nell’uomo. Inciampare durante il cammino è la causa predominante delle cadute, non solo negli anziani, ma anche nei soggetti neuropatici e può accadere non solo durante il cammino su un terreno pianeggiante, ma anche su terreni sconnessi o durante la salita e la discesa di una scala. Il peso sociale ed economico del piede diabetico, assieme alla drammatiche conseguenze che porta con sé possono essere ridotti attraverso una diagnosi tempestiva e un trattamento immediato preferibilmente antecedente alla diagnosi clinica. L’obiettivo primario di questa tesi, è stato quello di valutare la presenza di alterazioni nelle attivazioni muscolari in soggetti diabetici con e senza complicanze durante l’esecuzione di diversi task motori con il fine ultimo di valutare se questo tipo di acquisizioni fossero in grado di fornire ai clinici un ulteriore strumento per la diagnosi precoce che consenta loro di modificare, se necessario, o semplicemente di valutare l’efficacia del follow-up dei pazienti in base alle loro caratteristiche specifiche.
1. Introduction

1.1 Background
Diabetes mellitus is a chronic disease widely used in the population and continuously increasing. The disease long term complications are multiple and invalidating, among these the diabetic foot, drifted from the contemporary presence of peripheral neuropathy and peripheral arterial disease, that altering the biomechanics of the foot, can carry to callosity formation and ulcerations. Diabetic neuropathy is the most common chronic complication associated with diabetes mellitus, affecting 20–50% of diabetic patients 10 years after their diagnosis and leads to a progressive loss of somatosensory sensitivity, proprioception and distal muscle function especially in the lower limbs, which may cause an alteration of the motor control during gait and during static posture [1]. Peripheral neuropathy and peripheral arterial disease are the most common and invalidating diabetes’s complications, involved in the pathogenesis of diabetic foot. They account for the leading cause of non-traumatic lower limb amputations. It results from two factors. The first one is a reduced blow of blood in the inferior limbs, caused from the presence of obliterating peripheral arteriopathy disease (PAD). PAD is a chronic obstructive disease of the arteries of the lower limb caused by atherosclerosis. The decrease in blood flow can result in symptoms of pain in the lower limb on exercise, known as intermittent claudication. Exercise induced pain is experienced in the calves, thigh or buttocks restricting activities of daily living and thus reducing quality of life. The second is the progressive laceration of nervous fibers (neuropathy) that cause a reduction of the sensitivity (also to the pain) and of the ability of movement, and that helps the appearance of lesions.

Together with diabetes falls in older adults are a major public health concern and have provided much of the motivation for research into age-related changes in human gait. Tripping during walking is the predominant cause of falls not only in the elderly but also in the neuropathic subjects. Trips can occur during walking on a level ground, but also during crossing visible obstacle, stair ascending and descending. The social and economic weight of the diabetic foot can be reduced through a prompt diagnosis and treatment from the very beginning. The World Health Organization warns that, in 2000, as many as 33 million Europeans suffered from diabetes, approximately 15% are likely to develop foot ulcers, and approximately 15% to 20% of those patients will face lower-extremity amputation. Diabetic foot problems are related to, peripheral neuropathy, foot trauma, foot deformity, increased foot pressures, and callus [2]. Mortality and morbidity related to ulceration is still high and healed ulcers often recur [3]. Distal symmetric sensorimotor polyneuropathy, is primarily confined to the axons of small and large-fiber sensory afferents. The
result is a "stocking feet" pattern of sensory loss that begins in the toes and progresses proximally
[4]. Peripheral neuropathy patients exhibit decreased stability while standing [5-7] as well as during
dynamic conditions [8]. Several authors [9,10] found gait pattern deviations in diabetic patients
with peripheral neuropathy (DPN) and without peripheral neuropathy (NoDPN). For instance
important deviations were revealed in hip, knee, ankle joints and trunk moment patterns over the
entire stance phase of gait in both DPN and NoDPN subjects [10]. These findings established the
need for investigating the role of muscles activation in diabetic gait abnormalities even when
neuropathy is not present. A few authors [11,12] reported abnormalities in the electromyographic
(SEMG) pattern of vastus lateralis and tibialis anterior, soleus, medial and lateral gastrocnemius
and, medial hamstrings of DPN subjects. However to our knowledge, the SEMG patterns of
NoDPN have not been investigated yet. The aim of this thesis, was to evaluate differences in gait
parameters, in performing stair ascending and descending task and evaluation of muscle fatigue
during treadmill protocol in diabetes subjects with and without complications, in order to provide a
further tool for early diagnosis which allows clinicians to change as soon as possible the follow-up
of patients according to their specific characteristics.

1.2 Aim
The aim of this thesis is to investigate the role of muscles activation in diabetic subjects gait in
presence of neuropathy, vasculopathy or none of the two. The present project was carried on in
collaboration with the Bioengineering of Movement Lab at the Department of Information
Engineering of Padova, the Department of Clinical Medicine and Metabolic Disease of the
University of Padova and the Department of Electronics of the Polytechnic of Torino.

1.3 Thesis Outlines
The Thesis is articulated as follows.
Chapter 2 depicts diabetes pathology, its characteristics and its history;
Chapter 3 presents the results about analysis of dynamic surface electromyography (SEMG) during
gait on fifty subjects (mean ± SD age 58.9±8.7, mean ± SD BMI 25.9±5.9): 10 were healthy
(control subjects (C)), 20 were NoDPN, 20 were DPN. Results of this study has been published in
Sawacha and Spolaor et al 2012 [13].
In Chapter 4 the results about dynamic surface electromyography (SEMG) during rising up and
down from a stair were presented. 43 patients were enrolled in the study: 17 NoDPN, 15 DPN and
11  C (mean ± SD age 58.5±10.2, mean ± SD BMI 25.7±2.8).
In Chapter 5 the results about dynamic surface electromyography (SEMG) during treadmill exercise in diabetic patient with and without PAD were reported. 39 subjects were recruited from the patients attending the outpatient clinic of the Department of Metabolic Disease at the University of Padova (Italy) as well as from university personnel: 10 C (mean age 58,0±12,3; mean BMI 23,1±4,8), 13 diabetic patients without PAD (NoPAD) (mean age 57±14,3; mean BMI 25,4±6,4), and 16 diabetic patient with PAD (DPAD) (mean age 64,3 ±7 mean BMI 26,3±2,7).
2. Diabetes Mellitus

Clinical features similar to diabetes mellitus were described 3000 years ago by the ancient Egyptians. The term "diabetes" was first coined by Araetus of Cappodocia (81-133AD). Later, the word mellitus (honey sweet) was added by Thomas Willis (Britain) in 1675 after rediscovering the sweetness of urine and blood of patients (first noticed by the ancient Indians). It was only in 1776 that Dobson (Britain) firstly confirmed the presence of excess sugar in urine and blood as a cause of their sweetness. In modern time, the history of diabetes coincided with the emergence of experimental medicine. An important milestone in the history of diabetes is the establishment of the role of the liver in glycogenesis, and the concept that diabetes is due to excess glucose production Claude Bernard (France) in 1857. The role of the pancreas in pathogenesis of diabetes was discovered by Mering and Minkowski (Austria) 1889. Later, this discovery constituted the basis of insulin isolation and clinical use by Banting and Best (Canada) in 1921. Trials to prepare an orally administrated hypoglycemic agent ended successfully by first marketing of tolbutamide and carbutamide in 1955 [14]

Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycaemia). Type 1 diabetes (previously known as insulin-dependent or childhood-onset diabetes) is characterized by a lack of insulin production. Type 2 diabetes (formerly called non-insulin-dependent or adult-onset diabetes) is caused by the body’s ineffective use of insulin. It often results from excess body weight and physical inactivity. Gestational diabetes is hyperglycaemia that is first recognized during pregnancy. The World Health Organization warns that, in 2000, as many as 33 million Europeans suffered from diabetes, approximately 15% are likely to develop foot ulcers, and approximately 15% to 20% of those patients will face lower-extremity amputation.

Always WHO warns that at moment 346 million people worldwide have diabetes. In 2004, an estimated 3.4 million people died from consequences of high blood sugar. WHO projects that diabetes deaths will double between 2005 and 2030 (Figure 1) [15]
Diabetes increases the risk of heart disease and stroke, 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke). Combined with reduced blood flow, neuropathy in the feet increases the chance of foot ulcers and eventual limb amputation. Diabetic retinopathy is an important cause of blindness, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. After 15 years of diabetes, approximately 2% of people become blind, and about 10% develop severe visual impairment. Diabetes is among the leading causes of kidney failure. 10-20% of people with diabetes die of kidney failure. Diabetic neuropathy is damage to the nerves as a result of diabetes, and affects up to 50% of people with diabetes. More than 80% of diabetes deaths occur in low- and middle-income countries (Figure 2)
Although many different problems can occur as a result of diabetic neuropathy, common symptoms are tingling, pain, numbness, or weakness in the feet and hands. The overall risk of dying among people with diabetes is at least double the risk of their peers without diabetes [15].

### 2.2 Diabetes Complications

Diabetes is characterized by elevated morbidity and mortality. Of the 52.8 million deaths occurred globally in 2010, 1.3 million were due to diabetes, twice as many as in 1990 [16]. One person with diabetes has a lifespan shortened of about 6 years [17], showing a risk of death of 3.03 (95% CI 2.59-3.55) for the decades 40-59 years, compared to the non-diabetic counterpart. Because of this elevated morbidity and mortality, diabetes represents an economic burden, with serious implications for the public health systems. Accordingly, the need of effective tools aimed to the prevention of its chronic complications is urgent and cannot be deferred [18]. Among the chronic complications of diabetes, diabetic foot problems are very common and can lead to much morbidity and some mortality: foot disease represents the leading cause of non-traumatic lower-limb amputation in the developed world [19]. Of all amputations in diabetic patients, 85% are preceded by a foot ulcer.

**Figure 2** Distribution of diabetic disease in Developed and Developing Countries
which can subsequently deteriorate to a severe infection or gangrene [19,20]. Three main pathologies, which can occur singly or in combination, contribute to foot disease in individuals with diabetes, i.e. diabetic peripheral neuropathy, peripheral arterial disease and infections; the most important factors related to the development of foot ulcers are peripheral neuropathy, minor foot trauma, foot deformity and decreased tissue perfusion [21-23]. Diabetic neuropathy is the most common cause of peripheral neuropathy in the world, and affects more than half of the patients with diabetes [22-24]. Diabetic neuropathy is a major cause of disability and health care expense. Peripheral vascular disease and neuropathy are frequently present in the same patient [25]. The clinical consequences of peripheral neuropathy, and possibly vasculopathy, are ulceration, Charcot foot [26] and foot deformity, painful diabetic neuropathy, gangrene and amputation. For these reasons the management of diabetic foot problems is a complex clinical challenge, that needs the involvement of multidisciplinary teams, including several competences, from the initial assessment to the management of a very complex disease. Recurrence of ulcers is common [27], so good foot health education, adequate footwear and regular podiatry (if necessary) must be an integral part of the patient's review process. In this scenario, the role of prevention is mandatory, because the early identification of diabetic patients at risk for foot disease should significantly reduce the burden of this complication.

2.2.1 Peripheral Neuropathy

Diabetic neuropathies are a family of nerve disorders caused by diabetes. People with diabetes can, over time, develop nerve damage throughout the body. Some people with nerve damage have no symptoms. Others may have symptoms such as pain, tingling, or numbness-loss of feeling-in the hands, arms, feet, and legs. Nerve problems can occur in every organ system, including the digestive tract, heart, and sex organs.

About 60 to 70 percent of people with diabetes have some form of neuropathy. People with diabetes can develop nerve problems at any time, but risk rises with age and longer duration of diabetes. The highest rates of neuropathy are among people who have had diabetes for at least 25 years. Diabetic neuropathies also appear to be more common in people who have problems controlling their blood glucose, also called blood sugar, as well as those with high levels of blood fat and blood pressure and those who are overweight.

As a result, many wounds go unnoticed and progressively worsen as the affected area is continuously subjected to repetitive pressure and shear forces from ambulation and weight bearing.
Neuropathy is manifested in the motor, autonomic, and sensory components of the nervous system [21]. Damage to the innervations of the intrinsic foot muscles leads to an imbalance between flexion and extension of the affected foot leading to ischemia, which will promote nerve cell injury and death. Hyperglycemia and oxidative stress also contribute to the abnormal glycation of nerve cell proteins and the inappropriate activation of protein kinase C, resulting in further nerve dysfunction and ischemia.

### 2.2.2 Peripheral arterial disease

PAD is a contributing factor to the development of foot ulcers in up to 50% of cases [27,28]. It commonly affects the tibial and peroneal arteries of the calf. Endothelial cell dysfunction and smooth cell abnormalities develop in peripheral arteries as a consequence of the persistent hyperglycemic state [23]. There is a resultant decrease in endothelium-derived vasodilators leading to constriction. Further, the hyperglycemia in diabetes is associated with an increase in thromboxane A2, a vasoconstrictor and platelet aggregation agonist, which leads to an increased risk for plasma hypercoagulability. There is also the potential for alterations in the vascular extracellular matrix leading to stenosis of the arterial lumen [28]. Cumulatively, this leads to occlusive arterial disease that results in ischemia in the lower extremity and an increased risk of ulceration in diabetic patients.

Potential risk factors for PAD include elevated levels of C-reactive protein (CRP), fibrinogen, homocysteine, apolipoprotein B, lipoprotein(a), and plasma viscosity. In people with diabetes, the risk of PAD is increased by age, duration of diabetes, and presence of peripheral neuropathy. The true prevalence of PAD in people with diabetes has been difficult to determine, as most patients are asymptomatic, many do not report their symptoms, screening modalities have not been uniformly agreed upon, and pain perception may be blunted by the presence of peripheral neuropathy. For these reasons, a patient with diabetes and PAD may be more likely to present with an ischemic ulcer or gangrene than a patient without diabetes.

Peripheral artery disease symptoms include:

- painful cramping in hip, thigh or calf muscles after activity, such as walking or climbing stairs (intermittent claudication)
- leg numbness or weakness
- coldness in lower leg or foot, especially when compared with the other side
- sores on toes, feet or legs that won't heal
- change in the color of legs
- hair loss or slower hair growth on feet and legs
- slower growth of toe nails
- shiny skin on legs
- No pulse or a weak pulse in legs or feet

Intermittent claudication is the primary symptom of PAD, the condition causing reduced flow of blood and oxygen to tissues. Claudication comes from the Latin word "to limp." Claudication is crampy leg pain that occurs during exercise, especially walking. The pain is due to insufficient blood flow in the legs (caused by blocked arteries). Intermittent means the pain comes and goes. Intermittent claudication is the most prominent symptom of PAD. The arterial obstruction or narrowing causes a reduction in blood flow during exercise or at rest. Clinical symptoms are caused by the consequent ischemia. The most common symptom of peripheral arterial disease is a pain upon exertion – intermittent claudication. The pain usually occurs in the calf and is described as a cramp or tightness or severe fatigue. The pain is usually bilateral. The cause of pain is not only reduced oxygen delivery, but also an increase in the production of toxic metabolites and cellular free radicals. These free radicals accumulate and react with the lipid constituents of the cell membrane. Patients with PAD and diabetes thus may present later with more severe disease and have a greater risk of amputation. Moreover, the presence of PAD is a marker of excess cardiovascular risk. It is important to diagnose PAD in patients with diabetes to elicit symptoms, prevent disability and limb loss, and identify a patient at high risk of MI, stroke, and death. Diagnosis of PAD is composed by ABI index and assessment of the posterior tibial and pedal pulses of both leg.
3. Comparison Of Muscle Activity During Walking In Subjects With And Without Diabetes

3.1 Background

Neuropathy in diabetic patients is manifested in the motor, autonomic, and sensory components of the nervous system [21]. Damage to the innervations of the intrinsic foot muscles leads to an imbalance between flexion and extension of the affected foot, leading to ischemia, which will promote nerve cell injury and death. Hyperglycemia and oxidative stress also contribute to the abnormal glycation of nerve cell proteins and the inappropriate activation of protein kinase C, resulting in further nerve dysfunction and ischemia. As a result, many wounds go unnoticed and progressively worsen as the affected area is continuously subjected to repetitive pressure and shear forces from ambulation and weight bearing. Peripheral arterial disease (PAD) is a contributing factor to the development of foot ulcers in up to 50% of cases [27,28]. It commonly affects the tibial and peroneal arteries of the calf. Endothelial cell dysfunction and smooth cell abnormalities develop in peripheral arteries as a consequence of the persistent hyperglycemic state [23]. There is a resultant decrease in endothelium-derived vasodilators leading to constriction. Further, the hyperglycemia in diabetes is associated with an increase in thromboxane A2, a vasoconstrictor and platelet aggregation agonist, which leads to an increased risk for plasma hypercoagulability. There is also the potential for alterations in the vascular extracellular matrix leading to stenosis of the arterial lumen [28]. Cumulatively, this leads to occlusive arterial disease that results in ischemia in the lower extremity and an increased risk of ulceration in diabetic patients. With such a scenario, treatment optimization is critical for improving the prognosis and quality of life, and for minimizing the economic impact. Diabetic peripheral neuropathy either reduces or even abolishes the protective sensation; it also induces changes in foot structure and function [29]. These conditions predispose to high foot plantar pressure, an important predictive risk factor for the development of diabetic foot ulceration. A number of authors found that increased tangential stress is also an important determinant of tissue breakdown in diabetic neuropathic subjects [13]. However their exact role in the aetiology of diabetic foot has not been understood yet. Some authors demonstrated that also diabetic subjects’ gait is characterized by an altered kinematics [30-32] which has been recognized also to affect plantar pressure [33]. Plantar pressure and kinematics measurement are widely employed to study foot function, the mechanical pathogenesis of foot disease and as a diagnostic and outcome measurement tool for many treatment interventions [29]. These findings established the need for investigating the role of muscles activation in diabetic gait abnormalities with and without neuropathy.
The aim of this chapter is to investigate whether muscle activity patterns change along with the evolution of the disease and what consequences altered muscle activity has on the kinetics of diabetes gait function in regard to ground reaction forces. Previous studies have not distinguished between the degrees of neuropathy in their experimental groups; therefore, it has not been possible to identify differences in gait patterns between the early and advanced stages of the disease [12]. This established the need for investigating the role of muscles activation in diabetic gait abnormalities even when neuropathy is not present.

3.2 The Gait Cycle

We define walk as "a series of rhythmic movements of the lower limbs, upper trunk and pelvis, causing a forward displacement of the center of gravity, produce, through a series of translations and of rotations of the bony segments and joints involved, moving the body forward". The walking can also be defined simply as the ability to move the center of pressure (CPS: projection on the ground of the center of gravity) from one foot to ' alternatively and more dynamically, to maintain the dynamic equilibrium. The conditions for the neuro mechanical bipedal locomotion in an upright position are:

- support for anti-gravity of the body, where the upright posture depends on the righting reflex and antigravity reflexes that allow the passage from supine to sitting upright. This is due to the integration of pulses vestibular, proprioceptive, tactile and visual, in the spinal cord, stem, basal ganglia;
- execution of steps, which is a grassroots movement, present at birth, integrated in the spinal, midbrain, diencephalon;
- maintaining balance;
- a means of propulsion.

The normal gait is mostly with head erect, upright, arms hanging loosely at his sides and harmonious, rhythmically moving forward and together with the opposite leg. The feet are slightly apart and the steps of moderate length with internal malleoli almost touch when it surpasses the other foot. The medial portions of the heel provide a straight line when they touch the ground at every step. As the leg moves forward, there is a coordinated reduction of the hip and knee, a dorsi flexion of the foot and a barely perceptible elevation of the hip that allows the foot to touch the ground. At each step the chest moves slightly forward and the side opposite to that of the lower limb that progresses. The heel rests on the ground first. The way you walk differs from one individual to another, between men and women (cadence, heaviness and lightness in the step). Is called a cycle or walk "gait cycle" the period between two successive
supports of the same foot to the ground. The gait cycle is divided into two main phases (Figure 3)

- stance or support - STANCE PHASE -: during which the foot remains in contact with the ground. This phase occupies approximately 60% of the gait cycle and decreases more gradually you increase the speed of walking (in the race is reduced to about 37%)

- cessation or swing or flying - SWING PHASE -: during which the limb is lifted and brought forward to the support to prepare for the next. This phase is also called the transfer phase.

The stance phase can be divided into four different phases:

- Contact the heel (heel strike = initial contact + loading response) is a very brief phase in which the heel of the foot is thrown forward into contact with the ground;

- Full support (mid stance) is the longest phase that begins with the detachment of the contralateral foot and ends when the foot is fully supported on the ground (heel, metatarsal and toes resting on the ground);

- Posting heel (heel off): this phase ends when the contralateral limb touches the ground while we see the detachment from the soil of the heel of lead foot;

- Posting finger (toe off): is a phase that ends with the toe of the ground, after which body weight is transferred forward.

Swing phase is divided, however, in three further stages:

- Initial phase of acceleration: the leg of interest moves forward through the work of hip flexor after the detachment of the toes;

- Midswing or intermediate stage: the limb examined moves from a posterior position to a position anterior to the body. Simultaneously flexes the ankle through the work of the tibialis anterior;

- Final phase of deceleration in this phase continues, and ends the preceding movement, the knee and ankle reach their maximum extension at the same time preparing the limb to ground contact (heel and support the resumption of the cycle path [34].
Therefore the present study aimed at evaluating the role of altered muscle activity in gait alterations during different phases of diabetic subjects with and without neuropathy. The electrical activity of 6 muscles was collected bilaterally on the lower limb during gait: gluteus medius (GM), rectus femoris (RF), tibialis anterior (TA), peroneous longus (PL), gastrocnemius lateralis (GL), and extensor digitorum communis (EDC). Electromyographic activity was represented through linear envelopes. Time and space parameters were also evaluated by means of 2 Bertec force plates and a 6 cameras motion capture system (BTS, 60-120 Hz).

3.3 Gait Analysis And Surface Emg
Aristotle (384–322 BCE) can be attributed with the earliest recorded comments regarding the manner in which humans walk. It was not until the renaissance that further progress was made through the experiments and theorising of Giovanni Borelli (1608–1679). Although several scientists wrote about walking through the enlightenment period it was the brothers Willhelm (1804–1891) and Eduard (1806–1871) Weber, working in Leipzig who made the next major contribution based on very simple measurements. Both Jules Etienne Marey (1830–1904), working in France, and Eadweard Muybridge (1830–1904), working in America, made significant advances in measurement technology. These were developed further by Otto Fischer (1861–1917) in collaboration with Willhelm Braune (1831–1892). The major developments in the early twentieth century were in the development of force plates and the understanding of kinetics. The team headed by Verne Inman (1905–1980) and Howard Eberhart (1906–1993) made major advances in America shortly after the Second War. David Sutherland (1923–2006) and Jacquelin Perry [34] pioneered
clinical applications in America and Jurg Baumann (1926–2000) in Europe. It was not until the advent of modern computers that clinical gait analysis became widely available [35].

The origins of the science of gait analysis began in Europe in the 17th century and continued through the early 20th century. The discoveries of such notables as Borelli [36], Galvani [37], Newton [38], Descartes [39], Marey [40-42], Carlet [43], the Weber brothers [44,45], Scherb [46-48], Duchenne [49], Muybridge [50], and Braune and Fischer [51-55] provided a solid scientific foundation for our current understanding of human walking. Braun and Fischer employed the principles of Newtonian classical mechanics, the coordinate geometry of Descartes, and Borelli’s mathematical concepts for estimating muscle action, to create an elegant representation of the gait of their military subjects carrying backpacks. Although the principles of investigation employed by Braun and Fischer are recognized as valid today, their methods of study were far too labor intensive to permit any practical application for subjects in a clinical setting. Vern Inman, and colleagues moved the science of gait analysis dramatically forward by adding kinesiological electromyography (KEMG), 3-D force, and energy measurements in the study of walking in normal subjects and amputees (1944–1947) [56,57]. The remarkable contributions of this inspired team, led by Inman, are contained in a report to the National Research Council [58] and are printed in a limited number of publications [59]. Nonetheless, their methods of study were still too labor intensive, invasive, and computationally demanding to permit their application in a clinical setting. Now in its second edition [60], this book contains a distillation of much of the original work of the team, as well as many new contributions by contemporary researchers. The search for improved methods of gait data acquisition began in the decade of the 50’s. Former orthopedic residents of Inman, and other investigators inspired by his research, embarked on time-consuming studies, utilizing equipment that had to be conceived, created, constructed and tested. We can see movements although we are unable to measure them by visual observation alone. Muscles are the engines that produce active movements. It follows that an understanding of the forces causing or contributing to movements must include KEMG. This reality was uppermost in the minds of those who struggled to begin clinical gait analysis. They persevered and utilized KEMG to gain insights into normal and pathological gait. For those who have recently started clinical gait analysis using commercial electromyography (EMG) hardware and software, it may be difficult to imagine the immense difficulties confronting those who first began to employ KEMG. The electromyographs available at that time had been developed primarily to search for abnormal action potentials, such as, fibrillation and fasciculation, and to test for delays in nerve conduction velocity. The disciplines of neurology, neurosurgery, orthopedics and physiatry often concern themselves with diagnostic electromyography while most gait laboratories usually employ only dynamic (KEMG). KEMG can be defined as a
technique to determine the relationship of the muscle activation signal to joint movement and to the
gait cycle. In the early years of KSEMG most of the commercially available electromyographs had
too few channels to monitor multiple muscles. As subjects were usually examined sitting or lying
down, little attention had been given to eliminate, let alone reduce, extraneous noise from cables, or
60-cycle or 50-cycle interference. Although Dr Inman had developed the use of indwelling wire
electrodes, they were large (0.006-in. diameter), relatively inflexible, and painful during walking.
Two insertions were required to record SEMG from one muscle. The primary reference has thus far
eluded our best efforts, but Johanson credits Inman with the discovery that bending the tip of the
electrode after it has been passed through a needle causes the electrode to remain in the muscle after
the needle is withdrawn [61]. Even more daunting was the fact that no methods had been developed
to record and synchronize SEMG with the events of the gait cycle. Telemetry had not made its
appearance; it took the challenge of sending humans into space before practical, reliable telemetry
was developed. Initially, computers were not available. The early investigators were forced to use
available tools, which were usually custom-made, by engineering colleagues. The earliest clinical
studies consisted of dynamic electromyograms coupled with gait movies.

3.3.1 Gait Analysis And Surface Emg in patient with diabetes

A few authors [1, 10, 12] reported abnormalities in the electromyographic (SEMG) pattern of vastus
lateralis and tibialis anterior, soleus, medial and lateral gastrocnemius and, medial hamstrings of
DPN subjects. To our knowledge, for the first time the SEMG patterns of NoDPN have been
investigated. In previous literature [1, 10, 12] diabetic subjects with previous ulcerations or without
diabetic complications, with the exception of PN, were considered.

Changes in some gait parameters that appear to be specific in diabetes have been identified in the
literature: shorter stride length, reduced walking speed, and altered lower limb and trunk mobility.
During gait cycle most of the major muscle groups are active at or around both heel strike and toe-
off (i.e., at the beginning and end of the stance and swing phases of the cycle). These are the periods
of deceleration and acceleration of the legs, when body weight is transferred from one foot to the
other [56]. In normal subjects it is expected that just after heel strike, for example RF SEMG
increases [12]. Some authors found marked reduction in hip mobility in a group of DPN and
NoDPN subjects [10]. Kwon et al [11] reported an early activation of GL and TA, while Sacco and
Amadio [1] registered a delay of onset instead. During midstance and midswing, most muscles
(with the exception of MG and triceps surae during stance, and TA during swing) are relatively
quiescent [12]. However, it is during these two periods (midstance and midswing) that the greatest
observable movement takes place. During midstance, MG acts as a hip abductor to stabilize the
pelvis as the contralateral leg swings through, while the GL and the soleus prevent excessive
dorsiflexion of the ankle and then prepares to drive the body forward [12]. Rigidity of the ankle is a
characteristic widely recognized in diabetic subjects in different studies [2,10,62-65].
During midswing, the TA (as well as EDC) provides active dorsiflexion and thus prevents the toes
from dragging on the ground. One of the principal actions of these muscles is to accelerate and
decelerate the angular joint motion [66]. Previous literature reported that DPN patients are not able
to develop any compensatory strategy for the lack of stability because of their sensorimotor deficit
[5-8,67]. In his study Greenman [68] found that small muscle atrophy is present in diabetes before
clinical peripheral neuropathy can be detected using standard clinical techniques. The RF muscle is
characterized as a bi-articular muscle that acts at the hip and the knee [69] while VL crosses the
knee joint only and acts as a knee extensor [69]. Previous studies have suggested that the
hamstrings play a major role in running, especially at higher speeds [70]. Kyrolainen et al. [71]
found that the BF had the largest increase in SEMG activity among the muscles examined in their
study, as running speed increased. GL is characterized as a bi-articular muscle that acts at the knee
and the ankle, while soleus only acts at the ankle [60]. Since previous studies using sophisticated
experimental techniques have shown either subclinical nerve function abnormalities or muscle
tissue properties [68] in most diabetic patients, it seems reasonable to suspect that such changes
were also present in the NoDPN patients.

3.4 Material and Methods

3.4.1 Subjects
Fifty subjects participated in the study (mean age 58.9±8.7, mean BMI 25.9±5.9): 10 were healthy,
20 were NoDPN and 20 were DPN. Diabetic subjects were recruited among the patients attending
the outpatient Clinic of the Department of Metabolic Disease of the University of Padova (Italy)
(Table 1)
Subjects belonging to the C group were recruited among hospital personnel. All subjects gave
written informed consent. The protocol was approved by the local Ethics Committee (of the
University Polyclinic). Inclusion criteria included: either type 1 or type 2 diabetic subjects who
were able to walk, no history of ulcers or neurological disorders (apart from PN), and no history of
orthopedic problems, lower limb surgery, or cardiovascular disease.
Table 1. Demographic and clinical parameters (mean ± standard deviation). Results of One Way Anova (P<0.05) and Z-Test (P<0.05) performed among the three populations: diabetic neuropathic subjects (DPN), diabetic non neuropathic subjects (NoDPN), control subjects (C).

<table>
<thead>
<tr>
<th>Group</th>
<th>DPN</th>
<th>NoDPN</th>
<th>C</th>
<th>p</th>
<th>p1</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>61.2(±7.7)</td>
<td>56.53(±13.3)</td>
<td>61.2(±5.07)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172(±8.02)</td>
<td>172(±7.8)</td>
<td>167.5(±12.6)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>78.7(±13.5)</td>
<td>78.3(±10.2)</td>
<td>69.5(±17.3)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>BMI</td>
<td>26.8(±3.4)</td>
<td>26.4(±2.5)</td>
<td>24.4(±2.8)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>100%</td>
<td>0</td>
<td>/</td>
<td>0</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Autonomic Neuroapthy</td>
<td>42.09%</td>
<td>0</td>
<td>/</td>
<td>1.00E-004</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>42.10%</td>
<td>0</td>
<td>/</td>
<td>0.01</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Peripheral Vasculopathy</td>
<td>14.30%</td>
<td>0</td>
<td>/</td>
<td>0.02</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>TSA</td>
<td>33.30%</td>
<td>21.05%</td>
<td>/</td>
<td>N.S.</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Coronary vasculopathy</td>
<td>19.05%</td>
<td>9.52%</td>
<td>/</td>
<td>N.S.</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>HbA1c</td>
<td>8.3 (±1.3)</td>
<td>8.6 (±5.6)</td>
<td>/</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Years of disease</td>
<td>13 (±6.5)</td>
<td>23.3 (±13.7)</td>
<td>/</td>
<td>0.0005</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

3.4.2 The Protocol

For each subjects the feet were checked for skin lesions, bone deformities, ulcerations, signs of infection and previous amputations. Height (m) and weight were recorded and the body mass index (BMI, kg/m2) was calculated. The neurological evaluation included the assessment of symptoms and signs compatible with peripheral nerve dysfunction. The peripheral nerve conduction test to confirm DSP was obtained in all subjects. The Michigan Neuropathy Screening Instrument questionnaire which evaluates motor and sensory symptoms (subjects were classified as pathologic if 3 positive scores out of 15 were found) was completed. The physical examination consisted of: (1) patellar and ankle reflexes, with the patient in the sitting position; (2) assessment of muscle strength by ability to walk on heels, bilateral dorsi/plantar flexion of the feet, flexion/extension of legs, abduction/adduction of both forearms and fingers, all against resistance; (3) sensory testing carried out on the index finger, and on the hallux (pin-prick with a disposable 25 mm/7 mm needle), touch (10 g Semmens Weinstein monofilament, pathologic if no response on 3 out of 10 sites) and vibration perception threshold (VPT, 128 MHz tuning fork and Biothesiometer, pathologic if
>25V); (4) pain sensitivity; (5) electroneurophysiological study; (6) Index of Winsor (ankle-to-brachial index).

The cardiovascular autonomic tests were also performed: if 2 or more tests were abnormal, the patient was considered to have autonomic neuropathy. HbA1c values in the preceding ten years were collected. Each patient had at least an ophthalmologic examination, a urinary albumin-to-creatinine ratio, a carotid artery Doppler examination, Index of Winsor, and an electrocardiogram in the preceding three months.

### 3.4.3 Clinical Evaluation

**Diagnosis of peripheral neuropathy:**

- Questionnaire MNSI (Michigan Neuropathy Screening Instrument) [72]
- Assessment of patellar and Achilles tendon reflexes left and right limb
- Assessment of vibration sensitivity (VPT Vibration Perception Threshold): the determination of the vibration perception threshold is routinely used as one of the quantitative sensory tests to determine the level of neuropathy in patients with diabetic neuropathy [73]. An increased VPT has been reported as one of the first signs in peripheral nerve disorders such as polyneuropathy and nerve entrapment [74]. It was used biothesiometry consists of a piston diameter of 1.3 cm, which transmits a vibratory stimulus at 120 Hz with an intensity that varies from 0 to 50 V. During clinical exam the piston is placed on the patient's skin at the ankle and hallux, the perceived value of vibration represents the VPT. Values above 25 are not received are considered high risk disease and ulcer index (Figure 4 and 5)
- Semmes Weinstein Monofilament Test: used for the evaluation of tactile protective, is a predictor of risk of ulceration. The instrument consists of a series of monofilaments of a nylon fiber of 10 grams of weight applied to the skin folds pressure exerting an effect. The test is performed on one or more different points of the foot, in our case 8 (one on the back foot and 7 on the plan) after the removal of any calluses that would alter the test results. The absence of perception in two points of eight is indicator of pathology.
Diagnosis of autonomic neuropathy:
Operated through the execution of cardiovascular tests, using a system of collecting and compiling data driven (Cardionomic), which evaluates the function through the sympathetic and parasympathetic Deep Breath Test or deep breathing test and the Lying to standing and standing to lying tests that detect changes in heart rate during forced respiration and change of posture, it also performs the test of orthostatic hypotension, instead, evaluates the pressure variations in the transition from supine to ortostatic position: decreases in systolic blood pressure below 10 mm Hg is considered normal, decreases between 11 and 29 mmHg is borderline, and greater reductions of 30 mmHg are pathological. If at least two of the three tests are positive we have a diagnosis of autonomic neuropathy

Diagnosis of peripheral vascular disease:
- Assessment of the posterior tibial and pedal pulses of both leg.
- Ankle/Brachial Index: the ankle-arm pressure index (also known as the Ankle / Brachial Index, ABI) compares the systolic blood pressure of the ankle to that of the arm (brachial). (Figure 6). These pressure measurements are useful in the assessment, follow-up and treatment of patients with peripheral vascular disease (PVD). ABI's provide an objective baseline to follow the progression of the disease process and evaluate the effectiveness of the treatment plan. The ankle / brachial index (ABI) is calculated by dividing the ankle pressure by the higher of the two brachial pressures. [75,76].
3.4.4 Gait Analysis

Subjects were asked to walk barefoot at their preferred walking speed on the 8 m gait laboratory. A minimum of three walking trials per subject were collected. Gait analysis was performed at the Bioengineering of Movement Lab at the Department of Information Engineering of Padova, with a BTS motion capture system (6 cameras, 60-120 Hz) synchronized with 2 Bertec force plates (FP4060-10). The electrical activity of 6 muscles for each lower limb were collected by means of a portable SEMG system (POCKETEMG, 16 channels, BTS Padova) together with the ground reaction forces and the kinematic data. Dynamic surface electromyography (SEMG) during gait was assessed on 20 NoDPN, 20 DPN and 10 control (C) subjects. SEMG of lower limb muscles were collected. Surface EMG signals of the following muscles were recorded at 1000 Hz: MG, RF, TA, PL, GL, and EDC. Sensors were positioned according to Blumenstein [58] after appropriately cleaning and preparing the skin. Sensors were 3 cm of diameter and positioned 1 cm apart. SEMG of rectus femoris (RF) gluteus medius (MG), tibialis anterior (TA), gastrocnemius lateralis (GL), peroneus longus (PL), and extensor digitorum communis (EDC) were collected. The right and left muscle activation patterns were analyzed and the envelope of the signal computed (the peak (POP) and the position of the peak both in milliseconds (POPs) and with respect to the stair ascending and descending cycle (POP%)).

3.4.5 Sterophotogrammetric System

In this study it has been used a sterophotogrammetric system Smart (BTS,Padova Figure 7), consisting of 6 optoelectronic cameras.
The details of the system were reported in the following figure 8.

![Smart System](image)

**Figure 7** Smart System

The analysis of a motor task requires the reconstruction of the instantaneous position and orientation (pose) of systems of axes that are, in principle, embedded in the bones under analysis (bone embedded technical frames), relative to a laboratory frame. To this purpose stereophotogrammetric systems are most commonly used. These allow for the reconstruction of the
3-D position of markers attached to the surface of body segments in each sampled instant of time. Modern clinical gait analysis traces its origins back to the early 1980s with the opening of the laboratory developed by the United Technologies Corporation at Newington, Connecticut and those provided with equipment by Oxford Dynamics (later to become Oxford Metrics) in Boston, Glasgow and Dundee. Retro-reflective markers were placed on the skin in relation to bony landmarks. These were illuminated stroboscopically and detected by modified video cameras. If two or more cameras detect a marker and the position and orientation of these cameras are known then it is possible to detect the three-dimensional position of that marker [78-91].

3.4.6 Force Plates

In this study two plantar pressure-dynamometric platforms were obtained by fixing each pressure platform (Imago S.n.c, Piacenza) onto two commercial force platforms (Bertec Corp., Worthington, OH, USA Figure 9), in order to allow the resultant GRF to be transmitted unaltered from one platform to the other. The use of a plantar pressure-dynamometric integrated system, obtained by superimposing a pressure platform over a force platform, allowed the estimation of the three components of GRF expressed by specific selected foot subareas as previously done by Giacomozzi [93]. Force Plate BERTEC are six component load transducers which measure the three orthogonal components of the resultant force acting on the platform, and the three components of the generated moment in the same orthogonal co-ordinate system. The point of application of the force and the couple acting on the platform can be readily calculated from the measured force and moment components. Each platform includes a pre-amplifier mounted inside the force plate. The pre-amplifier improves the signal-to-noise ratio and permits the use of long connector cables.

Figure 9: Bertec force plate and their Reference System
In this work two Bertec 4060H force plates have been used by mounting them hidden into the middle of the gait laboratory floor (at the Bioengineering of Movement Laboratory, Department of Information Engineering, University of Padova, Padova). Placement and orientation of the force platforms (FP) has been chosen such that ground reaction forces during gait can be acquired from each foot individually. This configuration of the FPs was found suitable for healthy young and older subjects. Linoleum tile sample floors matching the rest of the walkway flooring material are attached to the FPs. The details of the force plate can be found in the following two Tables.

![Diagram of force plate connection](image)

**Table 2** Bertec force plates connection

The force plate can measure the following variables:

- the 3 components Fx, Fy, and Fz of a force F acting on the platform
- the 3 components Mx, My, and Mz of the resulting moment vector M related to the origin of the coordinate system
- the 2 coordinates ax and ay of the force application point on the force plate surface
- the free moment M'z about an axis normal to the platform surface.
### Table 3 Bertec technical specification (Bertec Manual)

<table>
<thead>
<tr>
<th>Force Plate</th>
<th>4060H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td></td>
</tr>
<tr>
<td>Dimensions</td>
<td></td>
</tr>
<tr>
<td>Relative size</td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td>400 mm (15.75 in)</td>
</tr>
<tr>
<td>Length</td>
<td>600 mm (23.62 in)</td>
</tr>
<tr>
<td>Height</td>
<td>100 mm (3.94 in)</td>
</tr>
<tr>
<td>Force range</td>
<td></td>
</tr>
<tr>
<td>$F_z$ (vertical force)</td>
<td>10 kN (2,250 lb)</td>
</tr>
<tr>
<td>$F_x$, $F_y$ (shear forces)</td>
<td>5 (1,125 lb)</td>
</tr>
<tr>
<td>Overload capacity</td>
<td>% 50</td>
</tr>
<tr>
<td>Sensitivity</td>
<td></td>
</tr>
<tr>
<td>$F_z$</td>
<td>0.5 mVNm</td>
</tr>
<tr>
<td>$F_x$, $F_y$</td>
<td>0.85 mVNm</td>
</tr>
<tr>
<td>$M_x$</td>
<td>1.6 mVNm</td>
</tr>
<tr>
<td>$M_y$</td>
<td>2.25 mVNm</td>
</tr>
<tr>
<td>$M_z$</td>
<td>2.75 mVNm</td>
</tr>
<tr>
<td>Linearity</td>
<td>% FSO** ( \pm 0.2 )</td>
</tr>
<tr>
<td>Hysteresis</td>
<td>% FSO** ( \pm 0.2 )</td>
</tr>
<tr>
<td>Cross-talk (typical)</td>
<td>% ( \pm 0.1 )</td>
</tr>
<tr>
<td>Natural frequency</td>
<td>Hz 1000</td>
</tr>
<tr>
<td>$F_z$</td>
<td>Hz 600</td>
</tr>
<tr>
<td>$F_x$, $F_y$</td>
<td></td>
</tr>
<tr>
<td>Operating temperature</td>
<td>°C 0-50</td>
</tr>
<tr>
<td>Mass</td>
<td>kg (lb) 25 (60)</td>
</tr>
</tbody>
</table>

#### 3.4.7 Plantar Pressure System

The pressure platform is made of a matrix of resistive sensors covered by a flexible plastic surface (40x40 cm), 1.5 mm height, deprived of the protective rigid frame generally used in the commercial product, in order to allow the mechanical coupling with the underneath force plate. The full number of strain-gauge sensors placed on the flexible frame is 2304 which allows a spatial resolution of 1.44 sensors/cm. (Figure 10 Left). The electronic equipment, A/D conversion and PC interface are placed on a rigid data acquisition system located 3 cm aside to the platform and connected with it.
The software WINPOD allows the acquisition of the plantar pressure data, controls the sampling rate frequency (60 Hz, 90 Hz, 120 Hz and 150 Hz) and enables posture, static and dynamic type of acquisition (Figure 10 Right)

The technical specification of this device can be found in the following Figure 12

<table>
<thead>
<tr>
<th>Technical Specification</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimension (height/length/height)</td>
<td>530 x 600 x 45mm</td>
</tr>
<tr>
<td>Sensors Thickness</td>
<td>4mm</td>
</tr>
<tr>
<td>Sensors Area</td>
<td>380 x 382mm</td>
</tr>
<tr>
<td>Weight</td>
<td>6.8kg</td>
</tr>
<tr>
<td>Electronic Characteristics</td>
<td></td>
</tr>
<tr>
<td>Sensors</td>
<td>Resistive Sensor calibrated under IEEE specification</td>
</tr>
<tr>
<td>Dimension</td>
<td>8mm x 8mm</td>
</tr>
<tr>
<td>Sensor Thickness</td>
<td>0.15mm</td>
</tr>
<tr>
<td>N’ of sensors on the platform</td>
<td>2304 x 40 x 48</td>
</tr>
<tr>
<td>Vacuum Resistance</td>
<td>&gt; 1MΩ</td>
</tr>
<tr>
<td>Sensor Precision</td>
<td>±5%</td>
</tr>
<tr>
<td>“Rise time”</td>
<td>1 to 2ms</td>
</tr>
<tr>
<td>Temperature</td>
<td>da -40 a +85°C</td>
</tr>
<tr>
<td>Life time of a sensor</td>
<td>&gt; 1 Milton activations</td>
</tr>
<tr>
<td>Minimum measurable pressure</td>
<td>4 N/cm²</td>
</tr>
<tr>
<td>Maximum measurable pressure</td>
<td>100 N/cm²</td>
</tr>
<tr>
<td>PC connection</td>
<td>USB</td>
</tr>
<tr>
<td>Power</td>
<td>External Transducer 12VDC</td>
</tr>
<tr>
<td>Acquisition Frequency (sampling rate)</td>
<td>≤ 150 images/sec</td>
</tr>
<tr>
<td>Conversion analogic/digital A/D</td>
<td>8 bit, 255 values</td>
</tr>
</tbody>
</table>

Figure 12 Winpod technical specification

3.4.8 Emg Analysis

The electrical activity of 6 muscles for each lower limb were collected by means of a portable SEMG system: Pocket Emg with 16 channels BTS Padova. (Figure 13) together with the ground reaction forces and the kinematic data. Surface EMG signals of the following muscles were recorded at 1000 Hz: sensors were positioned according to Blumenstein [77] after appropriately cleaning and preparing the skin. Sensors were 3 cm of diameter and positioned 1 cm apart. SEMG of rectus femoris (RF) gluteus medius (MG), tibialis anterior (TA), gastrocnemius lateralis (GL), peroneus longus (PL), and extensor digitorum communis (EDC) were collected. The recorded
signals were band pass filtered between 10 and 450 Hz with a 5th order Butterworth filter and full wave rectified. The envelope was computed by low-pass filtering the signals with a 4th order butterwort filter and a cut off frequency of 5 Hz [11]. The right and left muscle activation patterns were analyzed and the envelope of the signal computed (the peak and the position of the heel marker trace together with the ground reaction force curve were used. Hence phases of normal activation for each signal in the gait cycle were defined (see Table 1) according to the normal SEMG patterns proposed by Perry [34] (Figure 14). The same analysis was performed for each signal in the gait cycle considering both phase of normal and non normal activation (Matlab R2008b). The time of peak muscle activity occurrence was evaluated as a function of the gait cycle in order to assess the effects of any deviations in diabetic muscle phasic activity on gait.

![Figure 13 Pocket Emg 16 channels on left and Free Emg 8 channels on right (Bts Bioengineering Padova)](image)

**Figure 13** Pocket Emg 16 channels on left and Free Emg 8 channels on right (Bts Bioengineering Padova)

![Figure 14 Example of normal muscular activation of Peroneus Longus (grey band). During MidStance (10-50% of gait cycle) this muscle is active](image)

**Figure 14** Example of normal muscular activation of Peroneus Longus (grey band). During MidStance (10-50% of gait cycle) this muscle is active
3.4.9 Dynamic Acquisition

Gait analysis is the systematic measurement, description and assessment of quantities that characterize human locomotion: in a few words, it is the evaluation of a subject's walking pattern. The core of most contemporary gait analysis is the measurement of joint kinematics and kinetics. Other measurements regularly made are electromyography (SEMG), oxygen consumption and foot pressures. A systematic physical examination of the patient is usually conducted as part of a gait analysis. Therefore in order to obtain all the necessary parameters at least three walking patterns per subject should be collected by means of motion analysis system, and force plates. In this protocol the gait analysis section consisted in 3 walking trials with 3 right and 3 left foot contacts on both the force and pressure plates acquired simultaneously by means of motion analysis system, force and plantar pressure plates.

3.4.10 Fullbody Protocol

The following anatomical landmarks (ALs) were tracked in space by applying a 10-mm-diameter spherical marker (see above figure) to: the vertebra L5, the vertebra C7 and the most anterior borders of the acromions (right and left), the two most anterior and the two most posterior margins of the iliac spines (ASIS, PSIS), the most lateral prominence of the great trochanter (GT), of the lateral and medial epicondyle (LE, ME), the proximal tip of the head of the fibula (HF), the most anterior border of the tibial tuberosity (TT), the lateral prominence of the lateral malleolus (LM), lower ridge of the calcaneus posterior surface (CA), and the dorsal margins of the first (FM) and fifth (VM) metatarsal heads (Figure 15). The centre of the femoral head (FH) were assumed to coincide with the centre of the acetabulum, which is reconstructed by a functional method according to Cappozzo et al. 1995 [76]. Each ALs were calibrated by means of a static acquisition without the aid of a pointer. The position of the ALs were assumed to coincide with the center of the marker applied onto it. Each ALs position in the dynamic trials were compared throughout an algorithm with the corresponding position in the static trial by means of comparing the mutual distance of ALs belonging to the same body segment. In case of disagreement the trial could be excluded from the analysis according to the entity of the difference registered between the two measures. The 4 markers of the two clusters applied on the thigh (right and left) were used to correct the measurement of the position of GT, LE and ME during the dynamic acquisition [95].
3.5 Statistical analysis
Confidence interval of observed proportion was determined with the Z-Test (the staRt Package) of R in order to compare the clinical characteristics of study subjects. The level of significance was set to $p \leq 0.05$. Age, duration of the disease, HbA1c, BMI, SEMG variables were compared between groups by using the T-Test, after evidence of normality (Kolmogorov-Smirnov Test). We considered the differences as statistically significant for $p \leq 0.05$. Envelopes peaks and the timing of envelope peaks were compared by means of One Way ANOVA, with SPSS (v13) statistical software after evidence of normality (Levene's Test for Equality of Variances), or Kruskal Wallis Test.

3.6 Results
The results of Space Time parameters and SEMG analysis were reported respectively in Table 2 and 3 in terms of POP of muscle activity. DPN subjects showed significantly longer stance and stride time (see Table 2), together with earlier activation of RF at initial contact ($p < 0.0007$) and reduced POP during pre swing phase of gait (see Table 3). In contrast with DPN subjects, NoDPN showed normal temporal and space parameters and altered muscle activation on RF, MG and GL ($p < 0.04$).
**Table 2.** Temporal and space parameters (mean ± standard deviation). Results of One Way Anova (p<0.05) performed among the three populations: diabetic neuropathic subjects (DPN), diabetic non neuropathic subjects (NoDPN), control subjects (C).

P= statistical significance between DPN and NoDPN; p¹= statistical significance between DPN and C; p²= statistical significance between NoDPN and C; N.S.= not significant.

<table>
<thead>
<tr>
<th>Phase</th>
<th>DPN</th>
<th>NoDPN</th>
<th>C</th>
<th>p</th>
<th>p¹</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.7 (±0.108)*</td>
<td>0.64 (±0.072)*</td>
<td>0.63 (±0.06)*</td>
<td>0.0000044</td>
<td>0.0000</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>Swing</strong></td>
<td>0.44 (±0.047)</td>
<td>0.43 (±0.043)</td>
<td>0.43 (±0.04)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>% Stance</td>
<td>61.07 (±3.14)*</td>
<td>59.7 (±2.20)*</td>
<td>59.4 (±2.32)*</td>
<td>0.0001769</td>
<td>0.0002</td>
<td>N.S.</td>
</tr>
<tr>
<td>% Swing</td>
<td>38.9 (±3.14)*</td>
<td>40.3 (±2.20)*</td>
<td>40.5 (±2.32)*</td>
<td>0.0001769</td>
<td>0.0002</td>
<td>N.S.</td>
</tr>
<tr>
<td>Stride Time</td>
<td>1.14 (±0.137)*</td>
<td>1.07 (±0.104)*</td>
<td>1.07 (±0.085)*</td>
<td>0.0000483</td>
<td>0.0003</td>
<td>N.S.</td>
</tr>
<tr>
<td>Stride Length</td>
<td>1.24 (±0.19)</td>
<td>1.33 (±0.2)</td>
<td>1.207 (±0.11)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>% Stance</td>
<td>61.07 (±3.14)*</td>
<td>59.7 (±2.20)*</td>
<td>59.4 (±2.32)*</td>
<td>0.0001769</td>
<td>0.0002</td>
<td>N.S.</td>
</tr>
<tr>
<td>% Swing</td>
<td>38.9 (±3.14)*</td>
<td>40.3 (±2.20)*</td>
<td>40.5 (±2.32)*</td>
<td>0.0001769</td>
<td>0.0002</td>
<td>N.S.</td>
</tr>
<tr>
<td>Stride Time</td>
<td>1.14 (±0.137)*</td>
<td>1.07 (±0.104)*</td>
<td>1.07 (±0.085)*</td>
<td>0.0000483</td>
<td>0.0003</td>
<td>N.S.</td>
</tr>
<tr>
<td>Stride Length</td>
<td>1.24 (±0.19)</td>
<td>1.33 (±0.2)</td>
<td>1.207 (±0.11)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Gait Velocity</td>
<td>1.11 (±0.21)</td>
<td>1.23 (±0.21)</td>
<td>1.12 (±0.184)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phase</th>
<th>DPN</th>
<th>NoDPN</th>
<th>C</th>
<th>p</th>
<th>p¹</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rectus Femoris</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC 0-2% LR 0-10%</td>
<td>5.46 (±1.26)</td>
<td>6.82 (±1.24)</td>
<td>11.8 (±1.29)</td>
<td>N.S.</td>
<td>0.0007</td>
<td>0.0062</td>
</tr>
<tr>
<td>Psw 50-60% ISw 60-73%</td>
<td>59.6 (±1.81)</td>
<td>58.8 (±1.50)</td>
<td>60.6 (±1.62)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Tsw 85-100%</td>
<td>91.93 (±2.34)</td>
<td>95.89 (±1.43)</td>
<td>89.96 (±1.81)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>0.0230</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phase</th>
<th>DPN</th>
<th>NoDPN</th>
<th>C</th>
<th>p</th>
<th>p¹</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tibialis Anterior</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC 0-2% LR 0-10%</td>
<td>11.71 (±1.13)</td>
<td>6.96 (±1.10)</td>
<td>9.27 (±1.63)</td>
<td>0.0032</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>PSw 50-60% ISw 60-73% MSw 70-85% Tsw 85-100%</td>
<td>75.04 (±1.57)</td>
<td>72.75 (±1.45)</td>
<td>73.6 (±2.26)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Muscle</td>
<td>IC 0-2% LR 0-10%</td>
<td>MSt 10-30% Tst 30-50%</td>
<td>Tsw 85-100%</td>
<td>Gastrocnemius Lateralis</td>
<td>MSt 10-30% Tst 30-50%</td>
<td>Peroneus Longus</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------</td>
<td>-----------------------</td>
<td>-------------</td>
<td>-------------------------</td>
<td>-----------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Gluteus Medius</td>
<td>7.55 (±2.68)</td>
<td>11.7 (±1.87)</td>
<td>13.2 (±1.89)</td>
<td>N.S. N.S. N.S.</td>
<td>94.0 (±2.87)</td>
<td>96.1 (±1.57)</td>
</tr>
<tr>
<td>Gastrocnemius lateralis</td>
<td>38.1 (±1.66)</td>
<td>35.9 (±1.38)</td>
<td>41.60 (±2.29)</td>
<td>N.S. N.S. 0.0410</td>
<td>41.81 (±0.09)</td>
<td>37.2 (±0.11)</td>
</tr>
<tr>
<td>Peroneus Longus</td>
<td>41.81 (±0.09)</td>
<td>37.2 (±0.11)</td>
<td>33.4 (±0.211)</td>
<td>N.S. N.S. N.S.</td>
<td>41.81 (±0.09)</td>
<td>37.2 (±0.11)</td>
</tr>
<tr>
<td>Extensorum Digitorum</td>
<td>9.91 (±1.63)</td>
<td>7.72 (±2.22)</td>
<td>7.68 (±4.63)</td>
<td>N.S. N.S. N.S.</td>
<td>9.91 (±1.63)</td>
<td>7.72 (±2.22)</td>
</tr>
<tr>
<td></td>
<td>69.3 (±2.56)</td>
<td>78.0 (±3.45)</td>
<td>67.2 (±7.25)</td>
<td>0.0470 N.S. N.S.</td>
<td>69.3 (±2.56)</td>
<td>78.0 (±3.45)</td>
</tr>
</tbody>
</table>

**Table 3.** Temporal pattern of muscle activation during gait (% of gait cycle). Mean value (±SD) of the time of the activation peak of the right and left Rectus Femoris, Tibialis Anterior, Gluteus Medius, Gastrocnemius Lateralis, Peroneus Longus and Extensorum Digitorum Communis muscles. Results of One Way Anova (P<0.05) performed among the three populations: diabetic neuropathic subjects (DPN), diabetic non neuropathic subjects (NoDPN), control subjects (C).

p= statistical significance between DPN and NoDPN; p1= statistical significance between DPN and C; p2= statistical significance between NoDPN and C; N.S.= not significant.

I.C. = Initial Contact (0-2% of gait cycle (g.c.)); L.A.= Loading Response (0-10% of g.c.); M.St.= Midstance (10-30% of g.c.); T.St = Terminalstance (30-50% of g.c.); P.Sw.= Preswing (50-60% of g.c.); I.Sw.= Initialswing (60-73% of g.c.); M.Sw.= Midswing (70-85% of g.c.); T.Sw.= Terminalswing (85-100% of g.c.)
3.7 Discussion

The key finding of the present study can be considered the presence of statistically significant alterations in NoDPN subjects’ SEMG activity. To our knowledge this has not been previously documented in the literature. Most of the major muscle groups are active at or around both heel strike and toe-off (i.e., at the beginning and end of the stance and swing phases of the cycle). These are the periods of deceleration and acceleration of the legs, when body weight is transferred from one foot to the other [56]. SEMG activity differed significantly from C. Indeed at initial contact or loading response an early POP was revealed both in DPN (p = 0.0007) and NoDPN’s (p = 0.0063) RF. Meanwhile, it is expected that just after heel strike, the RF SEMG increases in normal subjects [34]. This because the RF is either a hip flexor or a knee extensor, and in this specific phase the knee is extended and the hip is flexed. In order to stabilize the joints and to permit this action rectus femoris has to work in eccentric conditions. It should be further considered that in this phase the contralateral foot is about to leave the ground. The early POP of RF may be interpreted as an attempt to anticipate the heel strike or to decelerate the forward motion at the hip (flexion) and the knee (extension). This finding is in agreement with the reduction in hip mobility previously reported in a group of DPN and NoDPN subjects [10]. Indeed the primary role of the RF in normal gait is to stabilize the hip and knee at heel strike. Future research may include assessment of lower limb joint motion in order to establish the role of joint rigidity in muscle activity deviations in diabetic subjects. However, similar studies in the literature, presented contradictory results, and did not report any deviation of activity on this specific muscle during this phase of the gait cycle. Kwon et al. [11] reported an early activation of GL and TA, while Sacco and Amadio [1] registered a delay of onset instead. During midstance and midswing, most muscles (with the exception of MG and triceps surae during stance, and TA during swing) are relatively quiescent [34]. However, it is during these two periods (midstance and midswing) that the greatest observable movement takes place. During midstance, MG acts as a hip abductor to stabilize the pelvis as the contralateral leg swings through, while the GL and the soleus prevent excessive dorsiflexion of the ankle and then prepares to drive the body forward [34]. In our study an early POP was found only in NoDPN’s GL (p = 0.0365). This would suggest an attempt to cope with the rigidity of the ankle, which is a characteristic widely recognized in diabetic subjects [2,10,62-65]. In this contest it would be interesting to evaluate the displacement of the centre of pressure together with SEGM. This would test the hypothesis that a change in the walking strategy of NoDPN subjects did occur and would be in agreement with previous publications [63-66]. The absence of such a mechanism in DPN subjects could be due to their inability to develop compensatory mechanisms because of the altered proprioception typically present in PN. During midswing, the
TA (as well as EDC) provides active dorsiflexion and thus prevents the toes from dragging on the ground. One of the principal actions of these muscles is to accelerate and decelerate the angular joint motion [56]. However, in this specific phase no significant differences were registered in our study group with respect to TA and EDC. Our study revealed a delay in POP in both RF (p=0.0024) and GL (p=0.0420) of NoDPN instead. These alterations, if considered together with the early POP registered at RF during the loading response, may explain how the NoDPN subjects walked without significantly altering the time and space gait parameters. They stabilized their lower limb joints during the loading response and the single support by recruiting these muscles. The DPN time and space parameters were significantly different from the C in our study, thus suggesting an inability to counterbalance the consequences of tissue glycation. This is in agreement with previous literature which reported that DPN patients are not able to develop any compensatory strategy for the lack of stability because of their sensorimotor deficit [5-8,67]. It should be noted that in the present study NoDPN subjects were characterized by a significantly higher disease duration than DPN. This indicates the important role of the prolonged chronic exposure to hyperglycaemia in diabetics’ SEMG activity alterations. Furthermore these subjects were not characterized by lower HbA1c, even though they did not show larger presence of diabetes complications. It could be speculated that these conditions may have also played a role in the muscle activity alterations revealed in this study. This agrees with Greenman et al. [68] who found that small muscle atrophy is present in diabetes before clinical peripheral neuropathy can be detected using standard clinical techniques. Since our study included a large proportion of DPN subjects affected by diabetes complications other than PN and no previous history of ulcers, the differences between our results and those of previous studies are justified. In previous literature [10–12] diabetic subjects with previous ulcerations or without diabetic complications, with the exception of PN, were considered. Limitations of the present study include the small sample of subjects and the reduced set of muscles studied. In contrast with previous literature we did not consider the vastus medialis, soleus and biceps femoris. The choice of reducing the number of muscles was based on the consideration that in normal gait monitoring the activity of RF and GL could lead to exhaustive information about hip, and knee motion allowing experimental set up reduction. This by considering that RF and GL are bi articular muscles. The RF muscle is characterized as a bi-articular muscle that acts at the hip and the knee [69] while VL crosses the knee joint only and acts as a knee extensor [70]. Previous studies have suggested that the hamstrings play a major role in running, especially at higher speeds [71]. Kyrolainen et al. [71] found that the BF had the largest increase in SEMG activity among the muscles examined in their study, as running speed increased. GL is characterized as a biarticular muscle that acts at the knee
and the ankle, while soleus only acts at the ankle [70]. Based on these considerations we did not consider vastus medialis, soleus and biceps femoris. Finally our study revealed the presence of significant muscle activity deviations in NoDPN subjects. Since previous studies using sophisticated experimental techniques have shown either subclinical nerve function abnormalities or muscle tissue properties [68] in most diabetic patients, it seems reasonable to suspect that such changes were also present in the NoDPN patients who participated in this study. Despite this, we believe that the results of our study indicate that changes in foot muscles occur before changes in nerve function can be detected. This lead to the conclusion that this technique might be adopted as a screening method for early detection of patients at risk for diabetic foot before clinical peripheral neuropathy can be detected using standard clinical techniques. This would allow the development of prevention program directed to NoDPN patients. This supports the need of planning prevention programs and rehabilitation activities directed at reducing the consequences of diabetes and not only of diabetic neuropathy. Finally we can conclude that this technique has proven to be a useful diagnostic tool in identifying early-stage diabetic foot problems thus allowing inclusion of these patients in prevention programs.


15. WHO 2011


41. Marey EJ. Development de la methode graphique par l’emploi de la Photographie, 1885.

42. Marey EJ. Le Mouvement, 1894.


87. Baker R, Rodda J: All you ever wanted to know about the conventional gait model but were afraid to ask. Melbourne, Women and Children's Health; 2003.
88. Davis RB, Ounpuu S, Tyburski D, Gage JR: A gait analysis data collection and reduction technique. Human Movement Science 1991, 10:575-
4. Comparison Of Muscle Activity During Step Ascent And Descent In Subjects With And Without Diabetes

4.1 Background

Stair climbing is a common activity of daily life. Kinematic and kinetic studies have shown that, in comparison to level walking, larger ranges of knee flexion angle and knee flexion moment are required during stair climbing. Andriacchi et al. (1980) [1] found the maximum external knee flexion moment during stair ascent to be three times greater than level walking and maximum hip flexion moments during stair descent to be a maximum of 1.5 times greater than level walking. Jevsevar et al. (1993) [2] found an average of 98.6 (st.dev 6.5) of knee flexion was required to ascend stairs, 90.3 (st.dev 4.9) of knee flexion to descend stairs and 64.6 (st.dev 6.7) of knee flexion to walk on level ground. Analysis of the biomechanical requirements involved in stair climbing can add to our understanding of the diverse demands of this common activity in human locomotion.

In comparison to level walking, only a small number of studies have investigated normal human stair ascent and descent [3-7] Researchers have also used stair climbing to describe changes in a patient’s functional performance following knee arthroplasty [1], anterior cruciate ligament deficiency [8,9], transtibial amputations [10] and patellofemoral pain [11,12]. Understanding the biomechanics and pathomechanics of the lower limb during stair climbing is important for therapists attempting to integrate scientific findings into clinical examination and management of patients with lower extremity dysfunction.

Andriacchi et al. (1980) investigating hip, knee, ankle joint angles and moments in ten young healthy male subjects during stair climbing found maximum external knee flexion moments during stair descent to be 2.7 times greater than during ascent. They used the ground reaction method for the calculation of joint moments. This method involves calculation of joint moment by calculating the product of the ground reaction force vector and the perpendicular distance from the joint center to that vector. Wells (1981) [13] found that the ground reaction method is a good predictor of net joint moments for slow gait, but increasing the velocity of gait results in increased errors, especially at the hip. Therefore for healthy populations, the linked segment method is preferable to calculate joint moments; the linked segment method takes into consideration the mass-acceleration products of the foot, leg and thigh, that the ground reaction method neglects [12]. McFayden and Winter 1988 [14] used the linked segment method for the calculation of joint moments during stair climbing. However, the small sample size (n = 3) in their study limits the power and usefulness of the results. Kowalk et al. [15] reported external abduction–adduction moments at the knee joint in young adults (n = 10) ranging in age from 22 to 40 years, while [3] reported only external hip, knee
moments (n = 35, mean age = 24.6). Further studies that include larger numbers of subjects and more developed analysis of joint moments are required before definitive conclusions can be made.

Livingston, L.A et al. [5] investigated stair climbing kinematics of the hip, knee, and ankle joints on stairs of differing dimensions. Fifteen young healthy women were divided into short, medium, and tall subject groups with five subjects in each group ranging in age from 19 to 26 years. Subject height appeared to influence knee motion during stair climbing. Short subjects used greater maximum knee flexion angles than taller subjects during stair ascent and descent. Riener et al [7] investigating how stair inclination affects the kinematic and kinetic patterns of stair climbing (n = 10, mean age = 28.8 years) found joint ranges and maximum flexion angles to increase with increasing inclination of the staircase.

In clinical gait analysis, the determination of the timing of muscle activation (“on-off”) is of paramount importance [16,17]. The evaluation of the “on-off” pattern of one or more muscles, particularly when examined together with kinematics (joint angles) and kinetics (joint moments and powers), provides an insight into the performance of muscles and their role in accomplishing a motor task [18-20].

Therefore providing this information also with respect to stair ascending and descending should be considered highly important. In 1967 J. Joseph and R. Watson, wrote the first work about the actions of muscles involved during walking up and down stairs [21]. The results of their study show that: raising the body on to the stair above is brought about by the contraction of the soleus, quadriceps femoris, hamstrings and gluteus maximus; the gluteus medius at the same time prevents the body falling on to the unsupported side; tibialis anterior dorsiflexes the foot during the swing phase and helps the limb to clear the stair on which the supporting limb is placed; hamstrings flex the leg at the knee in the early part of the swing phase and control the terminal part of extension at the knee at the end of this phase; both erectors spinae contract twice in each step and control the forward bending of the body at the vertebral column. Walking down stairs revealed that the body is lowered on to the stair below by the controlled lengthening of the soleus and quadriceps femoris; the gluteus medius at the same time prevents the body from falling on to the unsupported side; tibialis anterior inverts the foot at the beginning of the supporting phase as the toe is placed on the stair below and dorsiflexes the foot in the middle of the swinging phase; hamstrings control the extension of the leg at the knee during the middle of the swing phase; both erectors spinae contract twice in each step and prevent forward bending of the trunk at the vertebral column.

Going up and down stairs is a common activity of daily living. From a mechanical viewpoint, it is quite different from level walking. The differences are reflected by changes in the ranges of motion of the different joints during gait, and changes in the phasic muscle activities and in the maximum
joint forces and moments. Observations of phasic muscle activity have indicated that there are major differences in the activities of the muscles during stair-climbing as opposed to level walking. These differences in activity are mainly in the muscles responsible for vertical movement of the body [10-13]. In climbing up stairs, the differences are reflected by changes in the contractions of the soleus, quadriceps femoris, hamstrings, and gluteus maximus during the support phase; going down stairs, the differences are reflected by changes in the contractions of the soleus and quadriceps femoris muscles [21,22].

The duration of the activity of the flexor muscles of the knee has been observed to be small compared with the activity of the extensor muscles of the knee, both ascending and descending. Furthermore, the knee extensor muscles are required to generate larger forces during stair-climbing than during level walking. Morrison and Paul confirmed this observation using data derived by means of electromyography, a force-plate, and high-speed moving pictures of three subjects ascending and descending stairs. [23,24].

Recently Benedetti et al 2010 [25] provided a reference data set, referred to an adult population (mean age 27 years), of the muscular activation timing during common activities of daily living - such as level walking and stair ambulation - for clinical and research use. To this purpose, raw surface EMG signals from trunk and lower limb muscles were recorded and processed in a sample population of healthy young volunteers. Eight muscles were considered for the right side of each subject: ipsilateral and contralateral erector spinae at lumbar site, gluteus medius, rectus femoris, medial hamstrings, lateral hamstrings (biceps femoris, long head), gastrocnemius (medial head), and tibialis anterior. Results of their study were in agreement with previous literature on healthy subjects [9-15]. In this contest it would be interesting to evaluate the differences in performing a stair ascending and descending task in diabetes subjects with and without neuropathy.

4.2 Aim

The aim of the study was to evaluate differences in performing a stair ascending and descending task in diabetes subjects with and without neuropathy. In this contest temporal parameters and lower limb muscles SEMG were evaluated together with the displacement of the centre of pressure during quiet standing both with eyes open and closed. This allowed to test the hypothesis that a change in the stair ascending and descending strategy of NoDPN and DPN subjects could be related to the altered proprioception which is a typical consequence of diabetes neuropathy.
4.3 Material and Methods

4.3.1 Subjects

Forty two subjects participated in the study (mean age 58.6±10.4; mean BMI 25.6±3): 10 were healthy, 17 were NoDPN and 15 were DPN. Diabetic subjects were recruited among the patients attending the outpatient Clinic of the Department of Metabolic Disease of the University of Padova (Italy). Subjects belonging to the C group were recruited among hospital personnel. All subjects gave written informed consent. The protocol was approved by the local Ethics Committee (of the University Polyclinic). Inclusion criteria included: either type 1 or type 2 diabetic subjects who were able to walk, no history of ulcers or neurological disorders (apart from PN), and no history of orthopedic problems, lower limb surgery, or cardiovascular disease (see Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>DPN</th>
<th>NoDPN</th>
<th>C</th>
<th>p</th>
<th>p1</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>60(±8)</td>
<td>56.7(±14)</td>
<td>60.2(±6)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171(±0.06)</td>
<td>170(±0.08)</td>
<td>166(±0.10)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>76(±12.8)</td>
<td>78.2(±10)</td>
<td>67(±11)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI</td>
<td>26(±3.2)</td>
<td>26.5(±2.5)</td>
<td>24(±2)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>0.02</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>100%</td>
<td>0</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Autonomic Neuroapthy</td>
<td>40%</td>
<td>0</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Microalambinuria</td>
<td>40%</td>
<td>0</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Peripheral Vasculopathy</td>
<td>27%</td>
<td>0</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>TSA</td>
<td>27%</td>
<td>23,50%</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Coronary vasculopathy</td>
<td>40%</td>
<td>12%</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>HbA1c</td>
<td>8.04(±1.2)</td>
<td>8.6(±6)</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Years of disease</td>
<td>24.2(±13)</td>
<td>12(±6)</td>
<td>/</td>
<td>0.001</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

Table 1. Demographic and clinical parameters (mean ± standard deviation). Results of One Way Anova (p<0.05) and Z-Test (p<0.05) performed among the three populations: diabetic neuropathic subjects (DPN), diabetic non neuropathic subjects (NoDPN), control subjects (C).

p = statistical significance between DPN and NoDPN; p1 = statistical significance between DPN and C; p2 = statistical significance between NoDPN and C; N.S. = not significant.

Motion analysis and EMG acquisition protocol together with clinical examination descriptions can be found in paragraph 3.4.2 and 3.4.3 previous chapter.
4.3.2 Step Ascent and Descent Analysis

Patients were asked to rise up and down from a stair 3 times (2 steps, total height of 32 cm, each step of 16 cm (see figures from Figure 2 to Figure 5) at the Bioengineering of Movement Lab at the Department of Information Engineering of Padova.

**Figure 2** Low step up
(ascending 1 step, 16 cm)

**Figure 3** High step up
(ascending 2 steps, 32 cm)

**Figure 4** Low step down
(descending 1 step, 16 cm)

**Figure 5** High step down
(descending 2 steps, 32 cm)
Step analysis was performed with a BTS motion capture system (6 cameras, 60-120 Hz) synchronized with 1 Bertec force plate (FP4060-10). The electrical activity of 6 muscles for each lower limb were collected by means of a portable EMG system (POCKETEMG, 16 channels, BTS Padova) together with the ground reaction forces and the kinematic data. Dynamic surface electromyography (SEMG) during stair ascent and descent was assessed on the following muscles (recorded at 1000 Hz): rectus femoris (RF), gluteus medius (MG), tibialis anterior (TA), gastrocnemius lateralis (GL), peroneus longus (PL), and extensor digitorum communis (EDC). Sensors were positioned according to Blumenstein [26] after appropriately cleaning and preparing the skin. Sensors were 3 cm of diameter and positioned 1 cm apart. SEMG of MG, RF, TA, PL, GL, and EDC were collected. The SEMG recorded signals were band pass filtered between 10 and 450 Hz with a 5th order Butterworth filter and full wave rectified. The envelope was computed by low-pass filtering the signals with a 4th order butterworth filter and a cut off frequency of 5 Hz [27]. The right and left muscle activation patterns were analyzed and the envelope of the signal computed (the peak (POP) and the position of the peak both in milliseconds (POPs) and with respect to the stair ascending and descending cycle (POP%)). Stair ascending and descending cycle were defined by means of the kinematics data together with the ground reaction forces. The traces of the right and left heel markers were used. The formers were also employed in estimating the duration of the stair ascending and descending phases.

4.3.3 Posture
The human balance control system relies on feedback from the somatosensory, vestibular, and visual systems. Diminished somatosensation is associated with increased postural instability during quiet standing with eyes closed, which is clinically referred to as sensory ataxia and is assessed via the Romberg test [28-30]. Somatosensory loss is a characteristic of patients with diabetic neuropathy and is also observed in approximately 50% of patients with stroke. In patients with diabetic neuropathy, somatosensory deficits are associated with increased sensory thresholds of mechanoreceptors and changes in the characteristics of the afferent fibers. Postural sway during quiet standing is typically increased in patients with diabetic neuropathy compared with healthy control subjects, and some work suggests that the loss of sensitivity in the soles of the feet in patients with diabetic neuropathy leads to postural instability [28-30]. Posturography is one of the biomechanical techniques dealing with the study of the neuromuscular control system and trying to provide quantitative information on the strategies underlying balance mechanisms. Posturographic analysis has the advantage that only a relatively simple experimental set up is required, which does not noticeably interfere, either physically or psychologically, with patients comfort. For this reason,
it is also suitable for subjects with impairments. A typical experimental set-up for posturographic studies consists of a force plate and a signal conditioning and acquisition chain to store transduced mechanical signals on a computer, which in turn is generally used for the extraction of suitable parameters from the Centre of Pressure (COP) trajectories [28-30]. By this technique it is possible to calculate the displacement of the COP (Centre of Pressure, i.e. the application point of the foot-to-ground reaction force) during experiments with a variety of different set-ups. Several measures were proposed in literature for describing the COP motion. They can be either summary statistic scores directly computed from COP time-series [28-30]. In this study the posturographic analysis has been carried on by collecting the data of each subject while performing a Roemberg test on a strain gage force plate. It was then chosen to analyze all the parameters (temporal and stochastic) that could be derived from the COP pattern, in order to identify the set of variables most suitable to describe the diabetic foot pathology: ellipsis 95%, sway area, path, path x, path z, mean velocity, mean velocity x, mean velocity z (where x and Z are the medio-lateral and anterior-posterior direction) [30].

4.4 Statistical Analysis

One-way ANOVA using SPSS (SPSS, version 13.0) and Pearson product moment correlation coefficients between pairs of quantitative variables were calculated across all subjects. A significance level of p< 0.05 was adopted for all above mentioned statistical analysis, using a Bonferroni correction when appropriate. Correlation analysis was performed between temporal parameters of stair ascending and descending and posturographic parameters of Romberg test in order to evaluate possible relationship between an altered performance of the former task with alterations in the postural control.

4.5 Results

The time parameters were reported in Table 2 in terms of mean and standard deviation (ms). The results of SEMG analysis were reported in Table 3 in terms of POP of muscle activity, position of the peak. DPN subjects are faster than NoDPN and C group, meanwhile NoDPN group is faster than C group. The subjects of control group are the slowest. Concerning with muscles DPN group show an iper and delay activation of all muscles during rising up and down all type of step (Table 2 and 3). When considering correlation analysis this revealed interesting results in the association between temporal parameters of stair ascending and descending and posturographic parameters of Romberg test. A negative correlation was found between stair descending duration and each posturographic parameter in eyes open condition (R>0.5 p<0.009).
<table>
<thead>
<tr>
<th>Step</th>
<th>Groups</th>
<th>Mean (ms)</th>
<th>± St Dev</th>
<th>p</th>
<th>p*</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High Step</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up</td>
<td>DPN</td>
<td>.656</td>
<td>.016</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NoDPN</td>
<td>.605</td>
<td>.014</td>
<td>0.0197</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>.668</td>
<td>.020</td>
<td></td>
<td></td>
<td>0.0121</td>
</tr>
<tr>
<td><strong>High Step</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Down</td>
<td>DPN</td>
<td>.579</td>
<td>.017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NoDPN</td>
<td>.595</td>
<td>.015</td>
<td>0.0129</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>.646</td>
<td>.021</td>
<td></td>
<td></td>
<td>0.0434</td>
</tr>
<tr>
<td><strong>Low Step</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up</td>
<td>DPN</td>
<td>.586</td>
<td>.015</td>
<td>0.0168</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NoDPN</td>
<td>.537</td>
<td>.014</td>
<td>0.0309</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>.637</td>
<td>.018</td>
<td>2.555E-05</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Low Step</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Down</td>
<td>DPN</td>
<td>.551</td>
<td>.014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NoDPN</td>
<td>.532</td>
<td>.014</td>
<td>0.0169</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>.607</td>
<td>.018</td>
<td></td>
<td></td>
<td>0.0011</td>
</tr>
</tbody>
</table>

**Table 2.** Temporal parameters (mean ± standard deviation). Results of One Way Anova (p<0.05) performed among the three populations: diabetic neuropathic subjects (DPN), diabetic non neuropathic subjects (NoDPN), control subjects (C).

p= statistical significance between DPN and NoDPN; p*= statistical significance between DPN and C; p**= statistical significance between NoDPN and C
<table>
<thead>
<tr>
<th>Step</th>
<th>Muscles</th>
<th>p &lt;0.05</th>
<th>PPES</th>
<th>PPE%</th>
<th>PPM</th>
<th>Groups</th>
<th>Mean in PPES ± St Dev in PPES</th>
<th>Mean in PPE% ± St Dev in PPE%</th>
<th>Mean in PPm ± St Dev in PPm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High Step Up</strong></td>
<td>RF</td>
<td>p</td>
<td>0.00</td>
<td>DPN</td>
<td>2,97</td>
<td>0.20</td>
<td>24.03 ± 1.46</td>
<td>153.85 ± 29.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>2,90</td>
<td>0.13</td>
<td>25.82 ± 0.97</td>
<td>33.03 ± 19.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>2.75</td>
<td>0.17</td>
<td>24.79 ± 1.23</td>
<td>38.82 ± 24.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GM</td>
<td>p</td>
<td>0.00</td>
<td>DPN</td>
<td>3.23</td>
<td>0.24</td>
<td>23.82 ± 1.80</td>
<td>251.45 ± 39.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>2,75</td>
<td>0.13</td>
<td>24.61 ± 1.02</td>
<td>34.50 ± 22.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>2.76</td>
<td>0.17</td>
<td>25.58 ± 1.27</td>
<td>22.91 ± 28.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TA</td>
<td>p</td>
<td></td>
<td>DPN</td>
<td>3.09</td>
<td>0.14</td>
<td>22.64 ± 1.03</td>
<td>251.18 ± 20.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>2.81</td>
<td>0.12</td>
<td>23.88 ± 0.86</td>
<td>219.43 ± 16.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>2.44</td>
<td>0.15</td>
<td>23.47 ± 1.12</td>
<td>232.84 ± 21.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GL</td>
<td>p</td>
<td></td>
<td>DPN</td>
<td>2.99</td>
<td>0.18</td>
<td>22.72 ± 1.37</td>
<td>57.85 ± 11.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>2.70</td>
<td>0.14</td>
<td>23.65 ± 1.06</td>
<td>55.49 ± 8.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>2.33</td>
<td>0.18</td>
<td>21.33 ± 1.37</td>
<td>68.14 ± 11.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ECD</td>
<td>p</td>
<td>0.01</td>
<td>DPN</td>
<td>3.07</td>
<td>0.16</td>
<td>23.02 ± 0.93</td>
<td>204.03 ± 16.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>2.76</td>
<td>0.14</td>
<td>23.68 ± 0.89</td>
<td>159.50 ± 15.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>2.50</td>
<td>0.19</td>
<td>22.66 ± 1.24</td>
<td>119.28 ± 22.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>p</td>
<td></td>
<td>DPN</td>
<td>3.01</td>
<td>0.19</td>
<td>23.37 ± 1.12</td>
<td>69.309 ± 8.238</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>2.77</td>
<td>0.13</td>
<td>24.20 ± 0.895</td>
<td>67.356 ± 6.547</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>2.652</td>
<td>0.176</td>
<td>24.519 ± 1.176</td>
<td>59.996 ± 8.604</td>
<td></td>
</tr>
<tr>
<td><strong>High Step Down</strong></td>
<td>RF</td>
<td>p</td>
<td>0.00</td>
<td>DPN</td>
<td>10.16</td>
<td>0.48</td>
<td>80.61 ± 1.57</td>
<td>140.33 ± 24.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>9.17</td>
<td>0.33</td>
<td>81.60 ± 1.09</td>
<td>44.99 ± 16.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>8.96</td>
<td>0.43</td>
<td>80.43 ± 1.40</td>
<td>31.63 ± 21.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GM</td>
<td>p</td>
<td>0.00</td>
<td>DPN</td>
<td>11.30</td>
<td>0.51</td>
<td>84.16 ± 2.83</td>
<td>242.39 ± 40.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>9.08</td>
<td>0.30</td>
<td>80.58 ± 1.66</td>
<td>35.86 ± 23.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>8.70</td>
<td>0.35</td>
<td>80.27 ± 1.96</td>
<td>32.65 ± 27.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TA</td>
<td>p</td>
<td>0.01</td>
<td>DPN</td>
<td>10.85</td>
<td>0.46</td>
<td>80.00 ± 1.32</td>
<td>215.80 ± 15.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>9.43</td>
<td>0.36</td>
<td>78.58 ± 1.04</td>
<td>109.07 ± 12.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>8.20</td>
<td>0.48</td>
<td>77.71 ± 1.38</td>
<td>101.12 ± 16.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GL</td>
<td>p</td>
<td>0.01</td>
<td>DPN</td>
<td>10.58</td>
<td>0.59</td>
<td>80.70 ± 1.54</td>
<td>204.80 ± 20.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>9.68</td>
<td>0.43</td>
<td>81.67 ± 1.12</td>
<td>137.73 ± 14.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>9.03</td>
<td>0.59</td>
<td>81.39 ± 1.54</td>
<td>63.17 ± 20.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ECD</td>
<td>p</td>
<td>0.00</td>
<td>DPN</td>
<td>3.29</td>
<td>0.14</td>
<td>23.02 ± 0.93</td>
<td>204.03 ± 16.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>2.76</td>
<td>0.14</td>
<td>23.68 ± 0.89</td>
<td>159.50 ± 15.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>2.50</td>
<td>0.19</td>
<td>22.66 ± 1.24</td>
<td>119.28 ± 22.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PL</td>
<td>p</td>
<td>,010</td>
<td>DPN</td>
<td>11.066</td>
<td>0.508</td>
<td>81.375 ± 1.392</td>
<td>153.509 ± 26.472</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>,003</td>
<td>NoDPN</td>
<td>9.407</td>
<td>0.423</td>
<td>79.427 ± 1.160</td>
<td>94.153 ± 22.060</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>8.694</td>
<td>0.568</td>
<td>81.095 ± 1.557</td>
<td>81.406 ± 29.597</td>
<td></td>
</tr>
<tr>
<td><strong>Low Step Up</strong></td>
<td>RF</td>
<td>p</td>
<td>0.00</td>
<td>DPN</td>
<td>2.31</td>
<td>0.22</td>
<td>17.34 ± 1.65</td>
<td>150.46 ± 27.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.01</td>
<td>NoDPN</td>
<td>1.94</td>
<td>0.15</td>
<td>17.40 ± 1.10</td>
<td>32.68 ± 18.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>N.S</td>
<td>1.72</td>
<td>0.17</td>
<td>15.41 ± 1.30</td>
<td>28.16 ± 21.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GM</td>
<td>p</td>
<td>0.02</td>
<td>DPN</td>
<td>2.58</td>
<td>0.21</td>
<td>18.72 ± 1.54</td>
<td>265.38 ± 45.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p*</td>
<td>0.00</td>
<td>NoDPN</td>
<td>1.95</td>
<td>0.15</td>
<td>17.98 ± 1.11</td>
<td>29.73 ± 32.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p**</td>
<td></td>
<td>C</td>
<td>1.70</td>
<td>0.18</td>
<td>14.82 ± 1.34</td>
<td>27.87 ± 39.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DPN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NoDPN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>p</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>p</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>p</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Temporal pattern of muscle activation during step rise up and down (% of total step). Mean value (±SD) of the time of the activation peak of the right and left RF, TA, GM, GL, PL and EDC muscles. Results of One Way Anova (p<0.05) performed among the three populations: diabetic neuropathic subjects (DPN), diabetic non neuropathic subjects (NoDPN), control subjects (C). p= statistical significance between DPN and NoDPN; p*= statistical significance between DPN and C; p** statistical significance between NoDPN and C.

4.6 Discussion
The key finding of the present study can be considered the presence of statistically significant alterations both in NoDPN and DPN subjects’ SEMG activity and stair climbing ascending and descending phases duration. To our knowledge this has not been previously documented in the
literature. In term of stair ascending and descending phases duration NoDPN and DPN subjects displayed a statistically significant shorter period. This result is in disagreement with what was previously demonstrated with respect to gait analysis temporal parameters, where diabetic neuropathic subjects showed a longer stride time due to a longer stance phase [27]. However this could be connected to the shorter swing phase that characterizes the gait of DPN and NoDPN subjects as reported in [31], if we consider that either stair ascending and descending or swing phase of gait require a dynamic postural control in a monopodalic position.

When considering that most of the major muscle groups are active at or around both heel strike and toe-off (i.e., at the beginning and end of the stance and swing phases of the stair ascending and descending cycle). These are the periods of deceleration and acceleration of the legs, when body weight is transferred from one foot to the other. These are the phases where most differences were reported on the SEMG activity of DPN and NODPN subjects with respect to C during gait. However some similarities were found with diabetic subjects SEMG activity during stair ascending and descending. When rising up from a stair an example of the starting position is with the left foot placed on top of the step and the right on the verge of pushing.

In this phase DPN subjects displayed a delay in the activity of RF oppositely to what was previously reported during gait where the same group of subjects showed an earlier activation of the same muscle with respect to C [27]. However this result could be interpreted as a possible reason for the lack of stability previously reported with respect to the alterations found in DPN temporal parameters.

After the initial phase each limb performs a complete cycle of movements out of phase, so that while the left limb supports the weight of the body, the right is ending its movement. The right foot pushes a plantar flexion of the ankle due to the action of the calf muscles which produce a passive extension of the toes that stretch the flexors muscles. Then flexors muscles immediately flexing the toes and produce the final push of the right foot. At this instant the whole body is tilted forward; however, both the knee and the hip are in extended position while the trunk is flexed, so as to move the sliding body weight. It is in this phase that both DPN and NoDPN displayed a delay in the SEMG activity of both TA and EDC. If we considered that simultaneously the quadriceps femoris of the left showed a delayed activation in DPN while it was suppose to work on his maximum speed/intensity to extend the left knee and lift the body up to the next step.

Now begins the phase of oscillation of the right leg, which involves the extension of the toes with the action of the long extensor muscle of the toes and the hallux. The dorsiflexion of the ankle is also involved due to the tibialis anterior and to the extensor of the hallux, the flexion of the knee to the action of the hamstrings, the flexion of the hip given by psoas major and by iliac and finally the
anterior rotation of the pelvis on the same side for the action of the gluteus medius and small limb that bears weight. This movement continues until the foot goes over the left leg and rests on the next step. It is in this phase that we registered a delayed activation of PL in DPN together with TA and ECD in both DPN and NoDPN. A similar behaviour was also reported during gait with respect to TA during the loading response [27]. With respect to this specific muscle a delayed activity was shown in DPN subjects when compared to C, even though less delayed than NoDPN. This results is in contrast with what observed during gait where an earlier activation was registered in DPN subjects.

In this phase, the foot is rested on the step thanks to the eccentric contraction of the hip flexors, and the weight is transferred on it, thus beginning the next stance phase. At this point the intrinsic muscles of the right foot are contracted to stabilize the plantar arches, while the long flexor lead fingers towards the bearing surfaces. The ankle, which is the in a slightly dorsally flexed position due to the inclination forward of the tibia, reaches a neutral position thanks to the soleus and, in part, thanks to the extension of the knee. The movements of both the ankle and the knee contribute to the force that stretches the hip, which helped turn the gluteus maximus and the hamstrings produces a backward tilt of the pelvis. The muscles of the trunk, use this stable base to extend the trunk in a standing position. The swing phase of the limb is increased by the anterior rotation of the pelvis at the level of the hip joint from the side of support determined by the action of the gluteus medius and small of the leg that support the weight. When the hip and the knee is fully extended, the other foot is placed on the step and following the ankle plantar-flexed limb support, is to push the body forward on the left leg to complete the cycle. Making step required different transition from mono to bipodalic support, it is for this reason that, in this task, a correctly and effectively postural control proves to be very important. Indeed correlation analysis between posturographic measurements and temporal duration showed a significant negative correlation which outlined the lack of postural control for those subjects that registered a lower phase duration (namely the DPN and NoDPN subjects). Thus confirming the hypothesis that DPN and NoDPN performed the stair ascending and descending task in a shorter time than C due to an inability to maintain a postural control for a time comparable to C.
25. Maria Grazia Benedetti, Valentina Agostini, Marco Knaflitz and Paolo Bonato (2012). Muscle Activation Patterns During Level Walking and Stair Ambulation, Applications of EMG in Clinical and Sports Medicine, Catriona Steele (Ed.), InTech,
5. Evaluation Of Muscle Fatigue During Treadmill Walking In Patients With Type 2 Diabetes And Peripheral Vasculopathy

5.1 Background

5.1.1 Muscle Fatigue

Over a half century ago, Muscio [1] argued that the then current interpretation of the word "fatigue" was too general in meaning for scientific use and should be abandoned. This timely advice induced professions to subdivide the concept of fatigue into subsets. This approach was exemplified by Bills [2] who suggested that fatigue be divided into three major categories. The first was subjective fatigue, characterized by a decline of alertness, mental concentration, motivation, and other psychological factors. The second was objective fatigue, characterized by a decline in work output. The third was physiological fatigue, characterized by changes in physiological processes. These categories have been further subdivided into areas with identifiable origins and symptoms [3]. One type of physiological fatigue is induced by sustained muscular contractions. It is associated with such external manifestations as the inability to maintain a desired force output, muscular tremor and localized pain. The effects of this fatigue are localized to the muscle or group of synergistic muscles performing the contraction. This category of fatigue has been termed localized muscular fatigue by Chaffin [4]. Although this term originally had its roots in the field of Ergonomics, it was subsequently popularized by a research group at Chalmers Institute of Technology and Sahlgren Hospital in Sweden. However, according to Merton [5] and various other investigators, even this category of fatigue may have its source peripherally (in the muscle tissue or neuromuscular junction) or centrally (in the brain and spinal cord).

In the study of localized muscular fatigue, analysis of the median frequency of the power density spectrum of the myoelectric (ME) signal, detected on the surface of the skin over a muscle, has been extensively employed. Since the historic work of Piper [6] in 1912, the frequency components of the surface ME signal have been known to decrease when a contraction is sustained. Cobb and Forbes [7] noted this shift in frequencies toward the low end with fatigue, and also observed a consistent increase in amplitude of the ME signal recorded with surface electrodes. Many other investigators have also noted an increase in ME signal amplitude [8-14]. The frequency shift (towards the lower frequencies) has also been observed often and in a variety of muscles throughout the human body [15-20].
These two phenomena, which are pictorially represented in Figure 2, are in fact related. Lindstrom et al [21] and De Luca [22] explained the interrelationship by noting that during a sustained contraction the low-frequency components of the ME signal increase and, hence, more ME signal energy will be transmitted through the low-pass filtering effect of the body tissue. Therefore, the magnitude of the two related phenomena is dependent on many factors, such as force level of contraction, time into the contraction, the type of electrode used to obtain the ME signal, the thickness of the subcutaneous tissue, and the particular muscle investigated. A minor digression is necessary at this point. It is commonly observed that the spectral shift is most dramatic near the beginning of a sustained contraction, whereas the amplitude of the ME signal shows a more pronounced increase near the end of a sustained contraction. Such divergent behavior of these two measurements would seem to indicate that they might have separate origins, were it not for the fact that the firing rates of the motor units decrease, even during constant-force contractions. This decrease in the firing rate is more pronounced near the beginning of the contraction. The decreasing firing rates will decrease the amplitude of the ME signal and thus offset the increase induced by the frequency shift. In a sustained contraction, muscle fatigue can be considered proportional to the decline in the mean frequency of the SEMG power spectral density. For more than 10 years muscle fatigue has been studied mainly during isometric and constant force contractions, because under these conditions, the SEMG can be considered as a realization of a colored and wide-sense stationary random process [23].

Figure 3 (Top) Myoelectric signal amplitude and force during an attempted constant-force contraction in the first dorsal interosseous muscle. (Bottom) Power density spectra of the myoelectric signal at the beginning and at the end of the constant-force segment of the contraction.
However, in dynamic exercises, the SEMG must be model as a non stationary random process. Hence, a proper quantification of muscle fatigue requires spectral analysis techniques capable of dealing with signal non-stationarities. The most used techniques for the spectral analysis of SEMG recorded during dynamic exercises are the time–frequency transforms belonging to Cohen’s class [24]. In the past, the analysis of the electrical manifestations of muscle fatigue was developed mainly for exercises during which the interested joint is kept in isometric conditions and force may be considered as constant, or, at most, slowly varying. In this case, the accumulation of chemical by products within the muscle induces a slow and progressive modification of the interstitial fluid pH, which causes a progressive decrement of the propagation velocity of depolarization along the muscle fibers. The principal effect of the progressive reduction of muscle fiber conduction velocity is a scaling of the power density function of the signal towards the lower frequencies. The surface myoelectric signal detected during isometric constant force contractions may be considered as a stochastic process with gaussian distribution of amplitudes. It is generally accepted [25] that it is wide sense stationary over time intervals ranging from 0.5 s up to 2 s, depending on the contractile force exerted and on the properties of the investigated muscle. In this contraction paradigm, the electrical manifestations of muscle fatigue are quantified by first estimating the power spectral density function of the signal within subsequent epochs during which it may be considered as wide sense stationary, and then by computing either the mean or the median frequency of each spectral estimate. The time series of the considered spectral variables usually show a decreasing trend over time. This phenomenon may be quantified in different ways and is the most typical electrical manifestation of muscle fatigue. Only in the last decade the study of localized muscle fatigue has been utilized in clinics, although mainly in pilot studies. Recently, different authors reported applications of surface myoelectric signal analysis that contributed to an important advancement of knowledge in physiology [26,27], in the clinical assessment of muscle dystrophy [28,29], and in rehabilitation [30-32]. Surface electromyography provides a particularly interesting application. It is well known that the Power Spectral Density function (PSD) of the surface myoelectric signal (MES) undergoes progressive compression and change of shape during sustained contractions. This phenomenon is reflecting a number of physiological changes taking place at the muscle fiber level that are associated with variations of pH [33], mean and distribution of muscle fiber conduction velocity [33-36] and motor unit action potential shape, and spatial width [37]. Although the analysis of the myoelectric signal detected under stationary conditions has not yet been thoroughly explored in clinics, it is evident that isometric contractions are not usual in most daily activities. In sports medicine, ergonomics, and rehabilitation, it would be preferable to study muscle fatigue while the subject is performing a functional task, in order to evaluate muscle endurance during an exercise
very similar to the activity in which the subject is usually involved or in which he experiences discomfort. When the muscle contracts in dynamic conditions, the myoelectric signal generated by the muscle may no longer be considered as a stationary process. This observation is crucial, since it follows that the spectral estimation techniques adopted when working with stationary processes must be substituted by techniques suitable to analyzing non stationary processes.

More specifically, we can classify the non stationarities that affect the myoelectric signal recorded during dynamic contractions as slow and fast. The slow non stationarities are generally related to the accumulation of chemical byproducts within the muscle tissue, and hence are those reflecting the effects of localized muscle fatigue. Fast ones are due to numerous phenomena, some related to the control strategy of the central nervous system system and others associated with the biomechanics of the movement. When the purpose is to evaluate the progression of the electrical manifestations of muscle fatigue during dynamic contractions, fast non stationarities appear as confounding factors. Kaflitz and Bonato in 1999 proposed a successful analysis of the myoelectric signal requires: first, suitable spectral estimation techniques, then proper instantaneous spectral parameters to track the progression of muscle fatigue, and finally processing methods and contraction modalities that allow for discriminating the slow variations due to fatigue from the confounding factors that cause fast non stationarities. In this work we focused our attention on surface emg’s analysis during dynamic contraction, in particular during walking protocol exercise on treadmill.

5.1.2 Muscle Fatigue, PAD and intermittent claudicatio
The diagnosis is made with a determination of the ABI. Previous study [38] have highlighted that claudicating patients have altered kinematic gait patterns that can be fully characterized utilizing advanced biomechanical analysis. The most important alteration were greater ankle plantar flexion in early stance and ankle range of motion during stance, time to maximum ankle plantar flexion was shorter and time to maximum ankle dorsi flexion was longer in PAD patients. The link between IC PAD and surface emg is represented by the fact that arterial obstruction causes lower blood circulation than normal and this causes in turn pain in the muscle interested. In this study we focalized our attention on surface emg’s analysis during fatigable treadmill exercise, to understand if it was possible to highlight the presence of muscle fatigue as result of arterial obstruction for provide a further support to clinician in early PAD diagnosis.
5.2 Aim
The aim of this study was, firstly, to evaluate the effects of RT on muscle fatigue assessed by means of surface electromyography (SEMG) [39], in dynamic conditions, in a group of type 2 diabetic patients with peripheral arterial disease (DPAD) and without (NoPAD). In addition a methodology for quantifying the localized muscle fatigue of lower limbs in patients suffering from type 2 diabetes with and without micro and macroangiopathy complications was developed. This methodology is based on the analysis of surface electromyography (SEMG) signals recorded during treadmill walking and allows studying electrical manifestation of muscle fatigue in dynamic conditions [40]. The present project was carried on in collaboration with the Department of Information Engineering of Padova, the Department of Clinical Medicine and Metabolic Disease of the University of Padova and the Electronics of the Polytechnic of Torino.

5.3 Material and Methods
5.3.1 Subjects
Thirty nine subjects were recruited from the patients attending the outpatient clinic of the Department of Metabolic Disease at the University of Padova (Italy) as well as from university personnel: 10 control subjects (C) (mean age 58,0±12,3; mean BMI 23,1±4,8), 13 diabetic patients without PAD (NoDPAD) (mean age 57±14,3; mean BMI 25,4±6,4), and 16 diabetic patient with PAD (DPAD) (mean age 64,3 ±7 mean BMI 26,3±2,7). Demographic data were reported in Table 1. Subjects belonging to the C group were recruited among hospital personnel. All subjects gave written informed consent. Sixteen subjects had PAD, stage I of Leriche-Fontaine classification. Diagnosis of PAD included a lower limb arterial Doppler ultrasound examination, ankle-to-brachial systolic pressure ratio (Index of Winsor), examination of tibialis posterior and foot peripheral pulses. On every subjects a history of intermittent claudication was also assessed.
## Table 1. Demographic and clinical parameters (mean ± standard deviation).

<table>
<thead>
<tr>
<th>Group</th>
<th>DPAD</th>
<th>NoDPAD</th>
<th>C</th>
<th>p</th>
<th>p1</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>64.3(±7)</td>
<td>57(±14.3)</td>
<td>58.0(±12.3)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179(±0.09)</td>
<td>160(±0.4)</td>
<td>160(±0.3)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>77(±13)</td>
<td>70(±17)</td>
<td>67(±18)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI</td>
<td>26.3(±2.7)</td>
<td>25.4(±6.4)</td>
<td>23.1(±4.8)</td>
<td>N.S.</td>
<td>N.S.</td>
<td>0.02</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>37.50%</td>
<td>23%</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Autonomic Neuroapthy</td>
<td>25%</td>
<td>15.40%</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Peripheral Vasculopathy</td>
<td>100%</td>
<td>0</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

During SEMG analysis patients were instrumented bilaterally with foot-switches, knee goniometers and surface SEMG probes over Tibialis Anterior, Gastrocnemius Lateralis, Vastus Lateralis, Biceps Femoris and Lateral Hamstrings. Patients were then asked to walk on a treadmill. After a warm-up of 2.5 minutes at 2 km/h patients walked for 35 minutes at 4 km/h, with an inclination of 2%. Then a period of 2.5 minutes of cool-down followed, again at 2 km/h. The signal acquisition started after 2-3 minutes from the beginning of the treadmill walk at 4 km/h, in order to give the patient time to acquire a fluid and natural gait at this velocity. Foot-switch, knee flexo-extension angle and SEMG signals were recorded synchronously for 30 minutes by means of the system STEP32 (DemItalia, Italy). SEMG signals were acquired with a sampling frequency of 2kHz and high-pass filtered at 20 Hz (FIR filter, 100 taps) to attenuate motion artefacts and low-pass filtered at 350 Hz to reduce high-frequency noise (anticausal IIR filter, 14th order).

For each SEMG signal we estimated the mean frequency of the Power Spectral Density function on each gait cycle. Then, in order to reduce the estimation variability, we averaged the mean frequency over 30 consecutive gait cycles.

### 5.3.2 Clinical Evaluation (see paragraph 2.4.3 chapter 1)

Furthermore, to classify patient, clinical evaluation includes transthoracic echo color Doppler which allow us to evaluate systolic function (FE% normal if > 50-55%) and the presence of hypo-and akinetic areas and valvulopathy. Three groups show normal parameters.
5.3.3 Treadmill Protocol Exercise

Patients were asked to walk on a treadmill at 4 km/h (inclination 2%) for 30 minutes (Figure 1). During walking the informations of foot-switch (Figure 2), knee flexo-extension angle (Figure 3), and SEMG signals (Figure 4) from Tibialis Anterior (TA), Gastrocnemius Lateralis, (GL) Vastus Lateralis (VL), Biceps Femoris (BF) and Lateral Hamstrings (LH) were recorded bilaterally (R=right, L=Left) with the system STEP32 (DemItalia, Italy).

Figure 1 Patient during exercise session with all instrument activated

Figure 2 Foot-switch Figure 3 Electrogoniometer Figure 4 Emg’s probes
5.3.4 Muscle Fatigue Assessment

Evaluation of local muscle fatigue by means of SEMG signal processing requires complex analysis especially in dynamic tasks, therefore this specific analysis was performed by Agostini et al (Polytechnic of Torino). In treadmill walking, it is necessary to perform pre-processing steps for ensuring the quality and reliability of the results obtained. A first issue is the estimation of the Signal-to-Noise ratio (SNR) of SEMG signals. A second issue is the evaluation of the variations in the muscle activation timing due to learning or adaptation, that maybe a confounding effect with respect to the fatigue phenomena. For each SEMG signal the mean frequency of the Power Spectral Density function on each gait cycle was estimated by Agostini et al as reported in [41]. Then, in order to reduce the estimation variability, the mean frequency was averaged over 30 consecutive gait cycles. Evaluation of local muscle fatigue by means of SEMG signal processing was performed as follows:

a pre-processing steps for ensuring the quality and reliability of the results obtained through the estimation of the Signal-to-Noise ratio (SNR) of SEMG signals (Figure 5)

Figure 5 Identification of gait cycle [41]
5.4 Statistical analysis

Confidence interval of observed proportion was determined with the Z-Test (the staRt Package) of R in order to compare the clinical characteristics of study subjects. The level of significance was set to $p \leq 0.05$. Clinical variables were compared between groups by using the T-Test, after evidence of normality (Kolmogorov-Smirnov Test). We considered the differences as statistically significant for $p \leq 0.05$. K-means and Hierarchical cluster analysis were also performed by mean of Orange Canvas software considering the data obtained from SEMG analysis together with clinical examination parameters (see Table n. 2). Clustering techniques are typically based on a minimization/maximization of a global objective function. The clustering problem, then, becomes an optimization problem and, as a result, a number of techniques for optimizing a global objective function have been developed in the past [42]. One approach to optimize a global objective function is to rely on algorithms, which find solutions that are often good, but not optimal. This is the case of K-means clustering algorithm which tries to minimize the sum of the squared distances (error) between objects and their cluster centers. Another approach is to forget about global objective functions as through Hierarchical Clustering (HC) procedures that proceed by making local decisions at each step of the clustering process. Whenever the function k-means was applied, it was performed using the standard Euclidean distance to form the clusters. K-means uses an iterative algorithm that minimizes the sum of distances from each object to its cluster centroid over all clusters and moves objects between clusters until the sum cannot be decreased further. The number of cluster is increased at each solution until an empty cluster is created and the solution that generates clusters that are better separated than previous solutions is chosen. In this data analysis, the Silhouette score was applied [43] as optimum cluster criterion to manage different numbers of clusters: maximizing the index allows choosing the most appropriate number of clusters to use in data processing and statistics. HC is an agglomerative clustering technique based on a distance measure between clusters and on a linkage method. In the adopted implementation, the user can set
the linkage method (single, complete, average, centroid or ward), the distance measure (correlation, cross correlation or Euclidean distance), the number of clusters and the number of nodes to be shown in the tree-based dendrogram. In order to explore how the subjects were distributed in the proposed cluster, after each clustering technique was performed, descriptive statistics was used. Statistical differences of all variables between the obtained clusters were, then, investigated using one-way ANOVA using SPSS (SPSS, version 13.0). Pearson product moment correlation coefficients between pairs of quantitative variables were calculated across all subjects. A significance level of P < 0.05 was adopted for all above mentioned statistical analysis, using a Bonferroni correction when appropriate.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NoDPAD group</th>
<th>DPAD group</th>
<th>C group</th>
<th>NoDPAD group</th>
<th>DPAD group</th>
<th>C group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± St. Dev.</td>
<td>Mean ± St. Dev.</td>
<td>Mean ± St. Dev.</td>
<td>Parameters</td>
<td>Mean ± St. Dev.</td>
<td>Mean ± St. Dev.</td>
<td>Mean ± St. Dev.</td>
</tr>
<tr>
<td>BPM Pre</td>
<td>72.15 ± 11.09</td>
<td>72.07 ± 15.82</td>
<td>66.5 ± 6.95</td>
<td>BPM Post</td>
<td>81.85 ± 12.25</td>
<td>80.87 ± 15.32</td>
</tr>
<tr>
<td>Aorta Pre</td>
<td>30.38 ± 2.9</td>
<td>33.04 ± 2.21</td>
<td>32.46 ± 3.39</td>
<td>Aorta Post</td>
<td>31.31 ± 3.3</td>
<td>33.47 ± 2.33</td>
</tr>
<tr>
<td>D Asn Pre</td>
<td>38 ± 2.98</td>
<td>38.17 ± 2.63</td>
<td>36.01 ± 2.93</td>
<td>D Asn Post</td>
<td>39.42 ± 2.71</td>
<td>39.15 ± 3.52</td>
</tr>
<tr>
<td>VOL Asn/SA Pre</td>
<td>24.67 ± 6.55</td>
<td>23.93 ± 5.42</td>
<td>23.76 ± 6.24</td>
<td>VOL Asn/SA Post</td>
<td>26.06 ± 10.69</td>
<td>28.31 ± 8.52</td>
</tr>
<tr>
<td>DT Pre</td>
<td>28.35 ± 3.3</td>
<td>28.19 ± 3.52</td>
<td>27.7 ± 2.31</td>
<td>DT Post</td>
<td>27.27 ± 5.37</td>
<td>31.14 ± 7.43</td>
</tr>
<tr>
<td>DTD Pre</td>
<td>48.12 ± 3.66</td>
<td>48.75 ± 4.49</td>
<td>49.74 ± 2.65</td>
<td>DTD Post</td>
<td>48.58 ± 3.07</td>
<td>48.49 ± 5.77</td>
</tr>
<tr>
<td>FA% Pre</td>
<td>41.27 ± 3.5</td>
<td>42.14 ± 3.06</td>
<td>44.31 ± 3</td>
<td>FA% Post</td>
<td>43.88 ± 4.98</td>
<td>41 ± 3.33</td>
</tr>
<tr>
<td>SIV Pre</td>
<td>9.38 ± 1.03</td>
<td>10.94 ± 1.31</td>
<td>9.68 ± 1.34</td>
<td>SIV Post</td>
<td>10.42 ± 1.42</td>
<td>11.49 ± 1.55</td>
</tr>
<tr>
<td>VTS Pre</td>
<td>27.35 ± 6.63</td>
<td>30.22 ± 9.25</td>
<td>28.32 ± 6.5</td>
<td>VTS Post</td>
<td>30.84 ± 7.58</td>
<td>34.71 ± 11.77</td>
</tr>
<tr>
<td>VTD Pre</td>
<td>74.19 ± 16.19</td>
<td>80.57 ± 22.98</td>
<td>73.78 ± 14.78</td>
<td>VTD Post</td>
<td>90.08 ± 21.57</td>
<td>82.27 ± 29.02</td>
</tr>
<tr>
<td>FE% Pre</td>
<td>62.93 ± 4.32</td>
<td>62.61 ± 4.28</td>
<td>61.35 ± 2.63</td>
<td>FE% Post</td>
<td>65.59 ± 4.62</td>
<td>61.52 ± 4.39</td>
</tr>
<tr>
<td>TEI Pre</td>
<td>0.34 ± 0.08</td>
<td>0.37 ± 0.08</td>
<td>0.37 ± 0.05</td>
<td>TEI Post</td>
<td>0.36 ± 0.06</td>
<td>0.42 ± 0.11</td>
</tr>
<tr>
<td>TRIV Pre</td>
<td>65.77 ± 15.12</td>
<td>68.67 ± 21.59</td>
<td>68 ± 13.17</td>
<td>TRIV Post</td>
<td>74.23 ± 16.44</td>
<td>83.27 ± 26.02</td>
</tr>
<tr>
<td>TCIV Pre</td>
<td>53.85 ± 24.25</td>
<td>59.33 ± 18.21</td>
<td>47 ± 18.59</td>
<td>TCIV Post</td>
<td>55.38 ± 19.09</td>
<td>66.33 ± 21.42</td>
</tr>
<tr>
<td>E Pre</td>
<td>66.88 ± 9.57</td>
<td>66.87 ± 10.99</td>
<td>64.07 ± 5.37</td>
<td>E Post</td>
<td>71.23 ± 14.64</td>
<td>73.07 ± 13.45</td>
</tr>
<tr>
<td>A Pre</td>
<td>82.23 ± 9.48</td>
<td>86.33 ± 13.22</td>
<td>64.77 ± 9.06</td>
<td>A Post</td>
<td>95.23 ± 19.76</td>
<td>96.2 ± 16.26</td>
</tr>
<tr>
<td>F/A Pre</td>
<td>0.81 ± 0.12</td>
<td>0.78 ± 0.14</td>
<td>1.01 ± 0.2</td>
<td>F/A Post</td>
<td>0.76 ± 0.12</td>
<td>0.77 ± 0.18</td>
</tr>
<tr>
<td>T Dec Pre</td>
<td>259.23 ± 39.31</td>
<td>267.67 ± 43.83</td>
<td>243.5 ± 28.09</td>
<td>T Dec Post</td>
<td>268.85 ± 46.42</td>
<td>252 ± 54.21</td>
</tr>
<tr>
<td>T</td>
<td>302.31 ± 31.07</td>
<td>304.67 ± 37.44</td>
<td>309 ± 21.45</td>
<td>T</td>
<td>308.46 ± 29.4</td>
<td>302.33 ± 37.65</td>
</tr>
<tr>
<td>AT Pre</td>
<td>61.92 ± 12.84</td>
<td>60.59 ± 7.58</td>
<td>67 ± 4.22</td>
<td>AT Post</td>
<td>66.92 ± 11.82</td>
<td>60.67 ± 9.8</td>
</tr>
<tr>
<td>VEL PICCO Pre</td>
<td>139.62 ± 21.37</td>
<td>137.47 ± 21.65</td>
<td>133.7 ± 27.7</td>
<td>VEL PICCO Post</td>
<td>140.77 ± 22.49</td>
<td>145.4 ± 26.19</td>
</tr>
<tr>
<td>IM Pre</td>
<td>0.46 ± 0.66</td>
<td>0.36 ± 0.6</td>
<td>0.6 ± 0.52</td>
<td>IM Post</td>
<td>0.62 ± 0.65</td>
<td>0.6 ± 0.63</td>
</tr>
<tr>
<td>IT Pre</td>
<td>0.23 ± 0.44</td>
<td>0.21 ± 0.58</td>
<td>0.1 ± 0.32</td>
<td>IT Post</td>
<td>0.23 ± 0.44</td>
<td>0.2 ± 0.56</td>
</tr>
<tr>
<td>T Ao Pre</td>
<td>0.31 ± 0.48</td>
<td>0.07 ± 0.27</td>
<td>0.2 ± 0.42</td>
<td>T Ao Post</td>
<td>0.31 ± 0.48</td>
<td>0 ± 0.4</td>
</tr>
<tr>
<td>Tai</td>
<td>0.09 ± 0.21</td>
<td>0.24 ± 0.75</td>
<td>0.02 ± 0.08</td>
<td>Microglobulina</td>
<td>2.28 ± 0.5</td>
<td>2.4 ± 0.64</td>
</tr>
<tr>
<td>Gil</td>
<td>-0.08 ± 0.15</td>
<td>-0.28 ± 0.39</td>
<td>-0.07 ± 0.1</td>
<td>Cstatina _Pr</td>
<td>337.52 ± 118.03</td>
<td>333.58 ± 160.39</td>
</tr>
<tr>
<td>Rlf</td>
<td>-0.17 ± 0.29</td>
<td>-0.08 ± 0.68</td>
<td>-0.3 ± 0.34</td>
<td>PCR Post</td>
<td>47.9 ± 86.48</td>
<td>31.36 ± 29.31</td>
</tr>
<tr>
<td>Lhl</td>
<td>-0.12 ± 0.21</td>
<td>-0.09 ± 0.16</td>
<td>-0.16 ± 0.24</td>
<td>Microglobulina</td>
<td>2.34 ± 0.47</td>
<td>2.87 ± 0.65</td>
</tr>
<tr>
<td>Vll</td>
<td>-0.33 ± 0.43</td>
<td>-0.33 ± 1.25</td>
<td>-0.2 ± 0.27</td>
<td>Cstatina _Post</td>
<td>354.14 ± 130.37</td>
<td>375 ± 129.03</td>
</tr>
<tr>
<td>Tar</td>
<td>-0.03 ± 0.12</td>
<td>0.15 ± 0.39</td>
<td>0.02 ± 0.1</td>
<td>PCR Pre</td>
<td>51.24 ± 96.74</td>
<td>31.67 ± 31.29</td>
</tr>
<tr>
<td>Gir</td>
<td>-0.11 ± 0.16</td>
<td>-0.05 ± 0.17</td>
<td>-0.11 ± 0.15</td>
<td>Lhr</td>
<td>-0.12 ± 0.26</td>
<td>-0.24 ± 0.37</td>
</tr>
<tr>
<td>Rfr</td>
<td>-0.17 ± 0.28</td>
<td>-0.2 ± 0.41</td>
<td>-0.08 ± 0.49</td>
<td>Vlr</td>
<td>-0.21 ± 0.56</td>
<td>-0.05 ± 0.58</td>
</tr>
</tbody>
</table>

Table n. 2 Mean and Standard Deviation for each clinical variable take in account. For muscles, the parameter take in account is the Decrease of mean frequency.
5.5. Results

Signs of muscular fatigue were observed with respect to the following muscles of PAD subjects: TA, VL, RF, GL, LH (Figure 7). One way-Anova analysis performed among the three populations of subjects displayed significant differences for the following parameters: SIV PP and EA pre exercise and SIV PP FE% and Microglobulina post exercise.

Cluster analysis by means of K-means (Euclidean distance) generated two different clusters one containing only C and one containing both DPAD and NoPAD (Figure 8). Thus showing the capability of both clinical and SEMG parameters to distinguish pathological subjects from controls.

Figure 7 In this figure the muscles with blu circle presents muscle fatigue, while others not.

Agostini et al. [41]
5.6 Discussion

The present methodology allowed to highlight signs of muscular fatigue on DPAD subjects thus showing that also a simple task as treadmill walking can be used. In particular the following muscle showed signs of muscular fatigue on TA, VL, RF, GL, LH as you can see in Figure 7. If we considered that generally muscular fatigue has been tested by means of demanding protocols which employed cycle ergometers, the advantage of such a methodology is that even much more complicated subjects would be able to perform this type of task. However it should be noticed that very little significant differences were found among the three populations of subjects. Even though signs of muscular fatigue were highlighted also in some of the subjects with a low degree of PAD, this could be attributed to the fact that only a small sample of subject could be considered affected by severe PAD. This lead to the conclusion that the present methodology can be very useful in highlighting presence of muscular fatigue on a specific subject even though the overall statistical analysis by mean of either One way Anova or Pearson correlation didn’t allowed to register a large number of statistically significant differences. It should be mentioned that cluster analysis based on a combination of clinical and SEMG parameters allowed to distinguish C from the diabetes subjects. However the latter failed in distinguishing DPAD from NoPAD, thus showing that differences in the parameters were much more related to diabetes per se than to PAD. Future development should include recruiting a larger number of subjects characterized by severe PAD.
Bibliography

Conclusion

Diabetes mellitus is a chronic disease widely used in the population and continuously increasing. The disease long term complications are multiple and invalidating, among these the diabetic foot, drifted from the contemporary presence of peripheral neuropathy and peripheral arterial disease, that altering the biomechanics of the foot, can carry to callosity formation and ulcerations. Together with diabetes falls in older adults are a major public health concern and have provided much of the motivation for research into age-related changes in human gait. The World Health Organization warns that, in 2000, as many as 33 million Europeans suffered from diabetes, approximately 15% will likely develop foot ulcers, and approximately 15% to 20% of these patients will face lower-extremity amputation. The social and economic weight of the diabetic foot and the and the tragic consequences that brings with it can be reduced through a prompt diagnosis and treatment from the very beginning. The aim of this thesis was to investigate the role of muscles activation in diabetic subjects gait in presence of neuropathy, vasculopathy or none of the two. The present project was carried on in collaboration with the Bioengineering of Movement Lab at the Department of Information Engineering of Padova, the Department of Clinical Medicine and Metabolic Disease of the University of Padova and the Electronics of the Polytechnic of Torino.

With regard to alterations relative to the gait DPN subjects showed significantly longer stance and stride time, together with earlier activation of RF at initial contact (p< 0.0007) and reduced POP during pre swing phase of gait. In contrast with DPN subjects, NoDPN showed normal temporal and space parameters and altered muscle activation on RF, MG and GL (p<0.04). During step DPN subjects are faster than NoDPN and C group, meanwhile NoDPN group is faster than C group. The subjects of control group are the slowest. Concerning with muscles DPN group show an iper and delay activation of all muscles during rising up and down all type of step. When considering correlation analysis this revealed interesting results in the association between temporal parameters of stair ascending and descending and posturographic parameters of Romberg test. A negative correlation was found between stair descending duration and each posturographic parameter in eyes open condition (R>0.5 p<0.009). Finally in treadmill protocol our data lead to the conclusion that the proposed methodology can be very useful in highlighting presence of muscular fatigue on a specific subject even though the overall statistical analysis by mean of either One way Anova or Pearson correlation didn’t allowed to register a large number of statistically significant differences. We can conclude, therefore, that the surface electromyography is an effective tool in the early detection of diabetic complications, in order to identify the biomechanical alterations of the diabetic population when there is still no clinical evidence of complications.