Diabetic peripheral neuropathy: Primary pathologies and the role of physical activity as a treatment intervention. A preliminary study

Candidate: Alejandro Meana-Esteban

Supervisors: Dr Robert van Deursen
Professor Patricia Price

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School of Healthcare Studies
Cardiff University
Cardiff, Wales, UK

AUTHOR'S DECLARATION

The work submitted has not been accepted in substance for any other degree or award, and is not being submitted concurrently in candidature for any degree or other award.

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ABSTRACT

Diabetic peripheral neuropathy (DN) is the most common complication in diabetes mellitus affecting up to 50% of this population. Foot ulceration in DN is a major health problem, often leading to lower-limb amputations and increased mortality rates. A combination of gait and microcirculatory alterations increases the risk of foot ulcerations in DN subjects. DN is also linked to an increased risk of cardiovascular diseases and poor quality of life (QOL). Physical activity (PA) plays an important role in the prevention and treatment of diabetes mellitus. Thus, PA has been associated with positive changes in glucose control, obesity and blood pressure. However, almost all the studies investigating PA interventions in subjects with diabetes mellitus have been carried out in individuals without neuropathic complications, whereas the effect of PA programmes in DN subjects is still unknown. In addition to that, the vast majority of studies have investigated the association between PA and health problems commonly linked to type 2 diabetes whereas the relationship between PA and additional problems associated with DN (i.e. risk of foot ulceration, sensory neuropathy or QOL) have received minimal attention. Therefore, the principal aim of this study was twofold: 1) to quantify differences between DN and healthy individuals in the primary pathologies that may co-exist in DN, with special attention to gait and microcirculation due to their association with foot problems; and 2) to evaluate the overall effect of a PA intervention, based on strengthening and foot mobility exercises, in modifying the primary pathologies linked to DN. Prior to the main study, a number of reliability studies were carried out to determine the reliability of some the methods used in the main part of the study.

Preliminary studies

Three reliability studies were carried in the present investigation. One study investigated the reliability (within- between-day) of near infrared spectroscopy to quantify muscular blood flow and oxygen consumption in the lower limb using a venous occlusion method (microcirculation). The other two studies investigated the reliability (within-day) of two different approaches to calculate the time differences between electromyography data and mechanical output (force) (electromechanical delay) during different conditions. Substantial reliability (ICC>0.6) or higher was found in all the three studies. Electromechanical delay values for the distal leg muscles were significantly higher in DN subjects compared to healthy individuals.

Main study

The main study was composed of two parts. Part 1 (cross-sectional study) investigated group differences between subjects with DN (N=53) and healthy individuals (N=25) whereas part 2 (intervention study) investigated group differences over time between two groups of subjects with DN; one participating in a 16 week PA programme (N=21) and the other as controls (N=20). Both studies followed the same experimental protocol and investigated the same domains (general health, gait, microcirculation and QOL).

Cross-sectional study

This study confirmed that DN is a complex condition that affects all the domains measured in the present investigation. Thus, the DN group showed significant differences ($p<0.05$) in: 1) traditional cardiovascular risk factors (blood pressure); 2) gait (spatial-temporal characteristics, forefoot foot pressures and muscular activity patterns); 3) microcirculation (blood flow and oxygen consumption in response to exercise stress); and 4) QOL compared to the healthy group. Interestingly, the present investigation showed that EMG alterations in DN may be associated with changes in plantar foot pressures and consequently with higher risk of foot complications. Furthermore, results from the present study showed for the first time impairments in exercise-induced microcirculatory responses in subjects with DN compared to healthy individuals. These alterations in the microcirculation were observed both in the muscular vasodilatory capacity as well as in the ability of the muscle to consume oxygen.

Intervention study

This study demonstrated for the first time that 16 weeks of a PA programme based on strengthening and foot mobility exercises can influence a number of aspects of health that are altered in DN subjects. The most remarkable finding was that the exercise programme improved sensory neuropathy ($p=0.027$) whilst 16 weeks of strength training did not produce significant changes in strength levels ($p>0.115$ at least) in the DN subjects. In addition to this results from the present investigation showed that a well controlled strengthening training program does have beneficial effects ($p<0.05$) on the microcirculation, obesity, blood pressure as well as on mental health QOL. On the other hand, the exercise program did not seem to have a substantial effect on any aspect of gait and HbA_{1c}. Importantly, no adverse effects related to the intervention were reported in any of the volunteers who participated in the physical activity program.

Conclusions

The present study demonstrated that DN is a condition that affects different aspects of health, of which some are modifiable by a well controlled PA programme (i.e. QOL, blood pressure and weight loss). Furthermore, the intervention seemed to trigger positive adaptations in the microcirculation, in particular in the ability of the muscle to recover from a stress condition (exercise stress). However, the most striking finding was that the intervention improved sensory neuropathy in individuals with DN. Surprisingly, changes in sensory neuropathy did not coincide with changes in muscular strength. This suggests that the lack of muscular adaptation to a PA programme may be caused by intrinsic changes in the muscle and not necessarily to lack of efferent muscle stimulation. In summary, results from the present investigation highlighted 1) the importance of PA as a therapeutic tool in subjects with DN to modify outcome measures associated with type 2 diabetes as well as sensory neuropathy; 2) the need to investigate the effect of PA on DN subjects to challenge the assumption that similar adaptations may occur in DN compared to individuals with type 2 diabetes and no neuropathic complications.

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GLOSSARY

ANOVA: analysis of variance
ANCOVA: analysis of covariance
ANS: autonomic nervous system
ATP: adenoside triphosphate
BicFem: biceps femoris
BF: blood flow
BMI: body mass index
BP: blood pressure
CA: contact area
CON: control group
COP: centre of pressure
CT: contact time
CV: coefficient of variation
DF: dorsi-flexion
DN: diabetes peripheral neuropathy
ECG: electrocardiogram
EMD: electromechanical delay
EMG: electromyography
EXE: exercise group
GC: gait cycle
GRF: ground reaction force
HAMS: hamstrings
HbA_{1c}: glycosylated haemoglobin
HbO: oxygenated haemoglobin
HbdO: deoxygenated haemoglobin
HDL: high density lipoprotein
HEALTH: healthy group
HR: heart rate
ICC: intraclass correlation
KE: knee extension
KF: knee flexion
LDL: low density lipoprotein
LGast: lateral gastrocnemious
MGast: medial gastrocnemious
MVC: maximal voluntary contraction
$\boldsymbol{mVO_2}$: muscular oxygen consumption
NO: nitric oxide
NIRS: near infrared spectroscopy
$\boldsymbol{O_2}$: oxygen
PA: physical activity
PF: plantar-flexion
PP: peak plantar pressure
PTI: pressure time integral
QOL: quality of life
QUADS: quadriceps
ROM: range of motion
ROP: roll over process
TA: tibialis anterior
TC: total cholesterol
tHb: total haemoglobin content
TS: triceps surae
VL: vastus lateralis
VO: venous occlusion
$\boldsymbol{VO_{2max}}$: maximal oxygen consumption
VPT: vibration perception threshold

CHAPTER 1

1 Introduction

Diabetes mellitus has been defined as the disease of the 21st century (Zimmet et al., 2003). In fact the overall burden of diabetes mellitus is immense with a current worldwide estimation between 150 million (Goruns et al., 2004) and 170 million (Kahn et al., 2006). In addition to that, it is estimated that there is a further one third to one half of this number of undiagnosed cases (International Working Group on the Diabetic foot, 1999). Although there are several distinct forms of diabetes, for classification purposes they have been divided into two main categories. Type 1, which is characterized by a marked reduction in the amount of insulin produced by the beta cells of the pancreas, and Type 2, in which peripheral tissue insulin resistance is a common feature (Krentz, 2000). Obesity is a very common cause of insulin resistance and a major risk factor for the development of Type 2 diabetes. In fact, about 80% of people with type 2 diabetes are overweight (Beck-Nielsen & Hother-Nielsen, 2004). Obesity in general and increased fatty acid concentrations in particular result in lipid accumulation in muscle and liver, which diminishes the availability of the protein glucose transporter type 4 (GLUT4) (insulin-responsive glucose transporter) and compromises the glucose transport into the muscle (Savage et al., 2007). However, diabetes is a complex metabolic disorder and other mechanisms in which obesity may lead to type 2 diabetes have been proposed (Kahn et al., 2006; Kelley & Goodpaster, 2001). In addition to obesity, physical inactivity has also been proposed as a key factor in the development of type 2 diabetes. Due to the rising rates of obesity and physical inactivity in our society, prevalence of type 2 diabetes is expected to double in the next 20 years (Cheng, 2005). Type 2 diabetes accounts for between 85 and 95 per cent of all people with diabetes (International Working Group on the Diabetic foot, 1999).

Persistently elevated levels of glucose are thought to trigger long term complications of diabetes, which typically affect the eyes (retinopathy), kidneys (nephropathy) and nerves (neuropathy) (Clark &Anthony, 1995; Nathan, 1993). Diabetic neuropathy is the

most common complication among diabetic patients affecting up to 50% of this population (Greene et al., 1997; Reiber et al., 1999). It is clear that high glucose leads to peripheral nerve injury over time, which can affect somatic sensory and motor nerves, as well as autonomic nerves. In fact the prevalence of diabetic neuropathy ranges from 7% within 1 year of diagnosis to 50% for those with diabetes for >25 years (Pirart, 1978). The mechanism by which hyperglycaemia leads to peripheral nerve injury has been a matter for debate over the years. Thus, there have been two main view points. On the one hand, some investigators have suggested that vascular problems may lead to peripheral nerve damage. Since nerve function depends on adequate blood flow and blood flow is known to be diminished in patients with diabetes, the vascular hypothesis was proposed as a possible mechanism for diabetic neuropathy (Dyck et al., 1986; England et al., 1995; Johnson et al., 1986). In line with this idea, therapeutic interventions to improve vasodilatation have been shown to increase nerve perfusion in diabetic rats (Maxfield et al., 1993) and diabetic subjects (Reja et al., 1993), which have led some investigations to conclude that vascular abnormalities are the cause of neuropathies (Cameron & Cotter, 1994). On the other hand, some investigators have associated neuropathy with metabolic mechanisms related to hyperglycaemia, among which the polyol pathway has been stated as being the most important. While, most body cells require the action of insulin for glucose to gain entry into the cell, the cells of the retina, kidney and nervous tissue (which are the parts of the body commonly associated with diabetic complications) are insulin-independent (Tortora & Derrickson, 2006). Therefore, there is a free interchange of glucose from inside to outside the cell, regardless of the action of insulin, in the eye, kidney and neurons. The cells will use glucose for energy as normal, and any glucose not used for energy will enter the polyol pathway and be converted into sorbitol (Dyck & Thomas, 1999). Activation of the polyol pathway is dependent on the enzyme aldose reductase (AR) and there is little doubt that this metabolic cascade contributes to the development of neuropathy (Yagihashi et al., 2011). However, the detailed mechanism of how the polyol pathway is involved in neuropathy remains elusive. In addition to the polyol pathway, other hyperglycaemia-dependent metabolic pathways such as advanced glycation end-products or increased oxidative stress have been also proposed as possible mechanisms of the disease (Cameron & Cotter, 1994; Figueroa-Romero et al., 2008). Although neuropathy can occur in every organ including the heart (cardiovascular autonomic neuropathy), peripheral neuropathy affecting peripheral nerves in a distal-proximal

manner is the most common manifestations of the disease (Vinik et al., 2000). For this reason type 2 diabetic patients with peripheral neuropathy (DN) were selected for the present investigation.

Foot ulceration in patients with DN is a major health problem, often leading to lower-limb amputations and increased mortality rates (Leung, 2007; Ramsey et al., 1999). Among persons diagnosed as having diabetes mellitus, the prevalence of foot ulcers is 4% to 10% (Garrow et al., 2005; Singh et al., 2005; Ramsey et al., 1999), the annual population-based incidence is 1 % to 4% (Lavery et al., 2003a), and the lifetime incidence may be as high as 25% (Singh et al., 2005). Unsurprisingly in the presence of neuropathy the annual incidence of foot ulceration has demonstrated an approximately 10 fold increase (McGill et al., 2005). Moreover, Gordois et al. (2003) estimated that the annual cost of DN and its complications in the United States was between $5.6 and $13.7 billion, which accounts for up to 27% of the direct medical cost of diabetes. These figures highlight the enormous impact foot complications have in DN subjects and the total economic burden of the condition.

Commonly, ulceration in patients with peripheral neuropathy is triggered by a cascade of events. A minor trauma, which in the presence on neuropathy (sensory loss) is unattended, is believed to be the starting point. Thereafter, a lack of adequate blood supply at this critical phase contributes to the risk of significant infection and hence further tissue breakdown and risk of ulceration (Dinh & Veves, 2005). Changes in gait characteristics are considered a common mechanism by which tissue damage may occur in DN subjects (Cavanagh et al., 1996; Veves et al., 1991); while impairments in the microcirculation are thought to play a key role in the wound healing process (Hile & Veves, 2003; Tooke & Brash, 1995).

Most of the investigations analyzing gait characteristics in neuropathic patients have been interested in kinetic data, which is believed to predict the risk of foot ulceration in this population (Boulton et al., 1983; Guldemond et al., 2006). Thus, foot pressures in the form of peak plantar pressures (PP) and pressure time integrals (PTI) are considered substitute measures to determine risk of foot ulceration in DN subjects (Frykberg et al., 1998; Stokes et al., 1975; Veves et al., 1992). It is well established that DN subjects suffer from excessive foot pressures during walking (Payne et al., 2002; Uccioli et al.,

2001), especially under the metatarsals heads (Mueller et al., 2005; Salsich et al., 2005), which not surprisingly is the foot area with the highest rate of foot complications (Boulton, 1994; Mueller et al., 2005). However, kinematic data by itself provide limited information about gait characteristics. The study of muscular activity has played a very important role over the decades in the understanding of motor control and human movement (Perry & Burnfield, 2010; Winter, 2009). However, the study of muscular activity in DN subjects is very limited and only recently a few investigations have attempted to assess whether neuropathy affects muscular activity patterns (Abboud et al., 2000; Kwon et al., 2003). Although the results from these investigations are controversial, early evidence indicates alterations in muscular activity patterns between healthy and DN subjects. More interestingly some of these changes have been linked to alterations in kinetic parameters (Abboud et al., 2000; Kwon et al., 2003). For instance, Kwon et al. (2003) found an early activation of the triceps surae in the DN group compared to healthy controls, which may likely result in an early contact of the forefoot with the ground and consequently higher PTI values under this foot area. These early findings highlight the importance of investigating muscular activity patterns alongside kinetic data to develop a more comprehensive understanding of walking characteristics in individuals with DN.

Microcirculation has been observed to be impaired in diabetic patients under conditions of stress (Kingwell et al., 2003; Mohler et al., 2006), which limits the ability of the foot of patients with DN to respond to injury and infection in the usual manner (Schramm et al., 2006). Vasodilatory abnormalities in DN subjects have been related to the dysfunction of the endothelial cells (endothelial dependent vasodilatation) (Pitei et al., 1997; Arora et al., 1998) and vascular cells of the arterioles (endothelial independent vasodilatation) (Pitei et al., 1997; Veves et al., 1998) as well as to impairments of the nerve-axon reflex (Vinik et al., 2001). In addition to that, mounting evidence suggests that microcirculatory abnormalities are also responsible for the reduced exercise capacity observed in diabetic patients (Regensteiner et al., 1998). Several investigations have reported diminished exercise-induced vasodilatory responses in subjects with diabetes compared to healthy controls (Kingwell et al., 2003; Pichler et al., 2004). It has been therefore concluded that impairments in the microcirculation were responsible for the reduced exercise capacity observed in diabetic patients (Scheuermann-Freestone et al., 2003). However, it is still unclear whether the muscle oxidative capacity in these

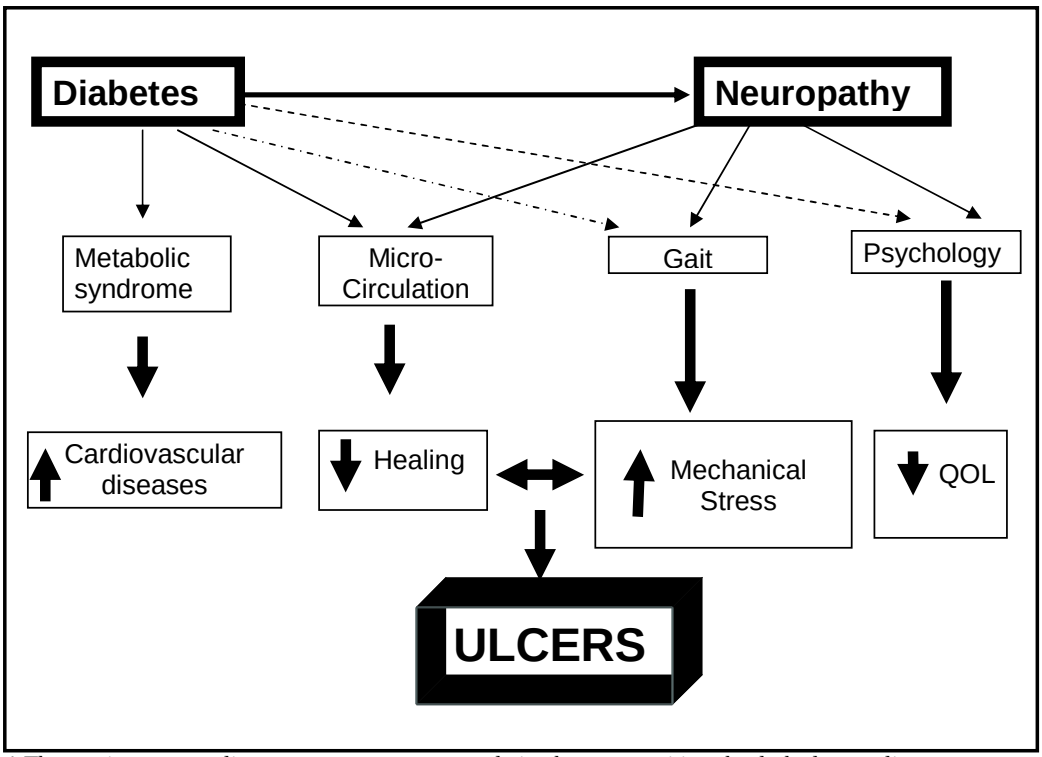
patients may be also affected, which could also be a limiting factor for the reduced exercise capacity in this population (Baldi et al., 2003). It is noteworthy that all the evidence presented above was acquired on type 2 diabetic patients without neuropathic complications, while the magnitude of the exercise-induced microcirculatory impairments in patients with DN is still unknown.

In addition to gait and microcirculatory alterations, which can predispose foot ulcers formation in DN subjects, this population carries further health related problems. On the one hand, it is well known that type 2 diabetes increases the risk of cardiovascular diseases. In fact cardiovascular diseases are listed as the cause of death in approximately 65% of persons with diabetes (Grundy et al., 1999). On the other hand, increasing evidence suggests that diabetes has an impact on everyday living and consequently a diminished health related quality of life (QOL) (Price & Harding, 2000). Furthermore, it appears that there is a correlation between diabetes and its complications and QOL, with subjects with more severe complications showing the poorest QOL (Lloyd et al., 2001). Figure 1-1shows an overview of the health problems related to patients with type 2 diabetes and peripheral neuropathy.

Overall, the evidence presented above demonstrates that DN is a very complex condition that affects multiple aspects of health. Thus, pathologies associated with DN include: 1) metabolic abnormalities secondary to diabetes mellitus; 2) alterations in traditional cardiovascular risk factors; 3) gait alterations which result in increased mechanical stress on plantar surface; 4) microcirculation impairments under stress conditions and; 5) poor QOL.

A fundamental goal of rehabilitative exercise programs is promotion of health, which should be viewed as a multi-factorial construct that includes several components (ACSM, 2000). Diabetes in general and diabetic neuropathy in particular is a very complex condition, which results in metabolic, biomechanical, physiological as well as psycho-social impairments. Therefore it is imperative that researchers investigate interventions that may influence these primary pathologies linked to DN patients.

Figure 1-1. Overview of the health problems linked to DN

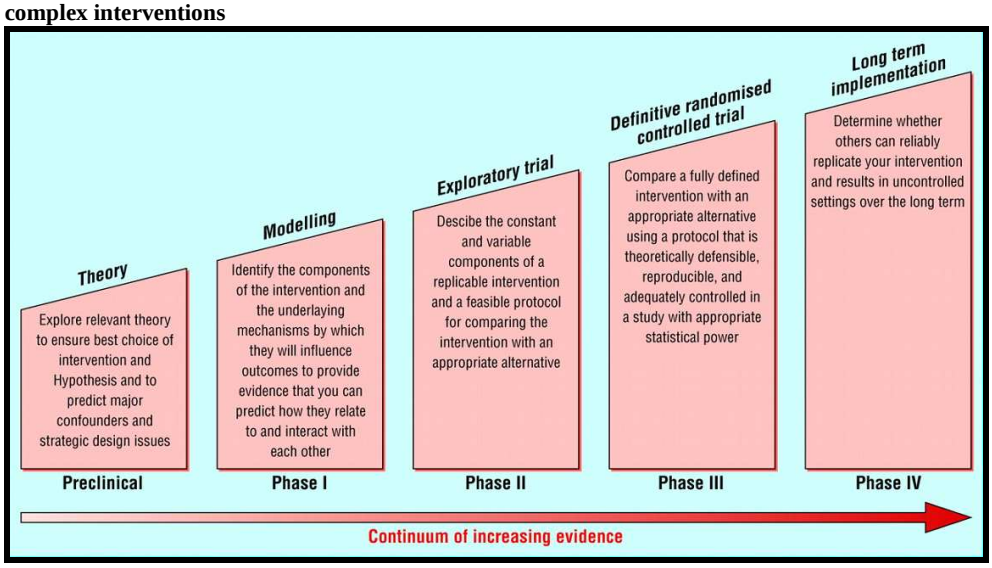


* The continous arrow lines represent a strong correlation between entities; the dashed arrow lines represent a weaker correlation between entities (i.e. although diabetes may lead to some changes in gait, neuropathy is the main factor explaining gait changes in DN subjects).

The Medical Research Council (MRC) framework for design and evaluation of complex interventions provides a methodology for designing and evaluating complex interventions (Campbell et al., 2000; Craig et al., 2008). Five distinct phases have been identified in this framework to ensure the intervention is fully defined, developed and evaluated before long term implementation is appropriate (Figure 1-2 represents these phases). The preclinical phase deals with exploring the relevant theory to ensure best choice of intervention. This phase is followed by the modelling phase, which aims to identify the components of the intervention and the underlying mechanisms by which the intervention may possibly influence health in the population under investigation. In the exploratory phase the information gathered in the previous phase is used to develop the optimum intervention and study design. An important characteristic of this framework is that progression from one phase to another may not be linear and in many cases phases may need to be repeated. For example, at the exploratory phase different versions of the intervention may need to be tested or the intervention may have to be

adapted to achieve optimal effectiveness. In exercise based interventions various trials using different treatment components (i.e. type of exercise, duration, intensity, etc.) may need to be tested in this phase of the framework to ensure the definitive trial achieves optimal effectiveness. This highlights the importance of the early phases of the intervention to successfully develop the definitive randomized controlled trial (Craig et al., 2008).

Figure 1-2. Framework proposed by Campbell et al. (2000) for the development and evaluation of complex interventions



For decades, exercise has been considered a cornerstone of a healthy lifestyle both in healthy and in type 2 diabetic patients. Physical activity (PA) is well documented to reduce glucose levels in patients with type 2 diabetes (Thomas et al., 2006). There is also mounting evidence that PA is associated with significantly lower cardiovascular risk and overall mortality in type 2 diabetes (Wei et al., 2000). It is generally believed that this protective effect of PA against cardiovascular diseases may be partly achieved by positive changes in the metabolic syndrome (ACSM, 2000, McArdle et al., 2010). In agreement with this idea, interventions on subjects with diabetes using different types of PA programmes (aerobic, resistance exercises and a combination of both) have shown beneficial changes in blood pressure (Castaneda et al., 2002; Loimaala et al., 2003; Yeater et al., 1990) and obesity (Cauza et al., 2005; Ibañez et al., 2005). It is therefore becoming clear that PA should be considered a therapeutic tool in patients with diabetes

and for this reason regular PA is considered an important component of diabetes treatment.

However, all the evidence shown above has been gathered in individuals with diabetes without neuropathic complications whereas there is no information about the effect of PA programs on glucose control and cardiovascular risk factors in DN subjects. Furthermore, findings from subjects with type 2 diabetes cannot automatically be generalized to other diabetic groups such as DN. In addition to this, it is generally believed that the amount of weight-bearing activity among individuals with DN is likely to influence the amount of mechanical trauma accumulated by plantar tissues (Cavanagh et al., 1996). This suggests that at least an adaptation of the exercises used is going to be necessary to avoid foot complications in this population (Kanade et al., 2006). It is therefore crucial to evaluate the effect of PA programmes on health related outcome measures in subjects with DN.

Since maximising health with its multiple components is the treatment goal of rehabilitation programs in a clinical setting, it is important to understand the whole range of effects an intervention may trigger on the recipients (Craig et al., 2008). The vast majority of studies assessing the effect of PA in diabetic patients have investigated the association between physical activity and health problems commonly linked to type 2 diabetes (i.e. glycaemic control and cardiovascular risk factors), whereas the relationship between PA and the health problems associated with peripheral neuropathy (i.e. sensory neuropathy, motor neuropathy, the risk of foot ulcerations or poor QOL) have received minimal attention. For instance, as explained previously, gait abnormalities together with microcirculatory impairments play an important role in the development of foot ulcers in DN patients. Therefore, it is essential to evaluate the effect of PA, not only on glucose levels and traditional cardiovascular risk factors but also on outcome measures relevant to neuropathy (sensory and motor neuropathy) or foot ulcer formation in this population.

If this is considered within the context of the complex intervention research framework proposed by Campbell et al. (2000), studies in the exploratory phase of the framework are required at this stage to evaluate the effect of an exercise-based rehabilitation program on the multiple pathologies linked to DN. Therefore, the majority of the work

presented in this thesis could fit within the definition of an exploratory study. Prior to reaching the exploratory trial phase, it was necessary to review the theoretical basis for the intervention (preclinical phase) and to understand the possible mechanism by which the intervention may influence the outcome measures (modelling phase) (see Figure 1-2)

In the present investigation the preclinical phase was fulfilled by reviewing relevant theory and evidence to develop a comprehensive understanding of the different pathologies associated with DN patients. This work is presented in part 1 of the literature review. This information was supplemented by a cross-sectional study (part 1 of the main study), which aimed to 1) broaden the understanding of the multiple pathologies associated with DN and 2) compare the findings in this study to previously published investigations.

In the present study the modelling phase was to some extent addressed by reviewing empirical evidence on the potential influence of the different components of physical activity programmes (type of exercise, duration, intensity, etc.) on the multiple health problems associated with DN. Since, there is no published data on the effect of a physical activity program on DN subjects, evidence gathered in this phase was on patients with type 2 diabetes with no neuropathic complications. This work is presented in part 2 of the literature review. Since foot ulceration is a major problem in patients with DN, the components of the PA program were selected to optimize its influence on gait characteristics and microcirculation in DN subjects. Thereafter, the information gathered in the preclinical phase and in the modelling phase was used to develop an intervention and study design. Thus, the second part of the main study of the present thesis designed and evaluated an exploratory trial to determine the effect of a physical activity program on 1) general health (glucose control, sensory neuropathy, cardiovascular risk factors; 2) gait; 3) microcirculation and 4) QOL in DN subjects. Table 1-1shows an overview of the present study and how it relates to Campbell´s framework.

Table 1-1. Adaptation of the framework proposed by Capmbell et al. (2000) for the development and evaluation of complex interventions

Phases	Action
Theory	Review available information in the health problems associated with DN (Part 1 literature review). Cross-sectional study to broaden understanding about pathologies linked to DN diabetes and compare results with literature. (Part 1 main study)
Modelling	Identify the components of the intervention and underlying mechanism they may influence outcomes. To some extent this was covered in Part 2 of the literature review
Exploratory trial	Design and evaluation of a physical activity intervention to influence health in DN subjects
Definitive randomised controlled trial	More exploratory trials are required before designing a definitive randomised controlled trial
Long term implementation	Substantial evidence needs to be gathered before this phase can be achieved.

CHAPTER 2

2 Literature review

The objective of this literature review chapter is twofold. The first objective is to build up a comprehensive understanding of the different health problems associated with DN. Part 1 of the literature review provides a critical review of the pathologies associated with type 2 diabetes and DN. Figure 1-1 shows an overview of the health problems associated with "each condition". Since this topic is very broad, the main focus of this doctoral thesis is on pathologies linked to DN with particular attention to gait and microcirculation alterations due to their association with foot ulcers. The second objective of this chapter is to review the current literature on the effect of PA interventions on the different pathologies associated with DN. This review is presented in part 2 of this literature review chapter.

2.1 Literature search

The structure of the literature search was split into three stages. The first stage of the process was to identify relevant articles concerning the primary pathologies associated with DN, namely: cardiovascular risk, gait, microcirculation and QOL. The second stage was to identify available literature on the effect of PA interventions in these identified pathologies. For both stages of the literature search process a computer search of the medical literature was carried out using the databases AMED, MEDILINE and EMBASE. Keywords related to the different pathologies associated with DN were combined for the search strategy. A list of the keywords used is included in Appendix 1. The comprehensive search was limited to publications between 1996 and 2011, due to the large number of articles retrieved, and to articles in the English language. Duplicates were removed prior to screening the resultant publications for relevance. Animal studies were also excluded. A total of 104 and 49 articles from stage 1 and stage 2 of the search, respectively, were considered relevant and consequently included in the review. A search history is included in Appendix 1.

The third stage of the search process was to review the references cited in the 153 retained publications to broaden the search strategy, regardless of years of publication. Animal studies were considered during this stage of the search process when they were relevant to the topic and no studies with humans were available. During this stage 62 further publications were identified, and a total of 215 references retained for critical review.

2.2 PART 1: Health problems in patients with type 2 diabetes and peripheral neuropathy

DN is a very complex condition that carries pathologies associated with type 2 diabetes and pathologies linked to peripheral neuropathy. Part 1 of this chapter will be composed of two sections that review each "condition" separately. The following section provides a review of the health problems associated with type 2 diabetes.

2.2.1 Health problems linked to type 2 diabetes

It is well established that there is an association between type 2 diabetes and increased risk of cardiovascular diseases and mortality (Grundy et al., 1999; Luscher et al., 2003). In fact, a large body of epidemiological and pathological data documents that diabetes is an independent risk factor for cardiovascular diseases (McCallum & Fisher, 2006; Wilson, 1998). Individuals with type 2 diabetes often suffer from other medical conditions such as hypertension and dyslipidemia, whose common denominator is thought to be insulin resistance (Gray et al., 1998). The common clustering of these risk factors in a single individual is known as metabolic syndrome, and it is partly responsible for the 4-fold increased risk of cardiovascular diseases in type 2 diabetes compared to healthy individuals (McCallum & Fisher, 2006; Stamler et al., 1993). The association between hypertension and dyslipidemia and type 2 diabetes is briefly described below.

Patients with type 2 diabetes are likely to have dyslipidemia, which is characterized by elevated low density lipoprotein (LDL), very low density lipoprotein and triglycerides as well as reduced high density lipoprotein (HDL). One of the major mechanisms behind the dyslipidemia of insulin resistant states is the increased flux of free fatty acids from adipose tissue to the liver. Free fatty acids promote increased triglyceride synthesis in the liver, which can lead to the secretion of very-low-density lipoprotein (Rader, 2007). An elevated concentration of serum LDL and very low density lipoprotein is a major risk factor for vascular diseases. In fact, some elevation of LDL cholesterol appears to be necessary for the initiation and progression of atherosclerosis (Grundy et al., 1999). Thereby, several elevations in LDL lipoproteins can develop atherosclerosis and premature vascular diseases in the complete absence of other risk factors (Grundy et

al., 1990). Clinical manifestations of atherosclerosis in diabetes include coronary artery disease, cerebrovascular disease and peripheral arterial disease (Luscher et al., 2003). Thus, diabetes is associated with a 4-fold increase in the risk of developing coronary artery disease (Luscher et al., 2003) and peripheral arterial disease (Abbott et al., 1990), and with a more than 10-fold increase in the risk of stroke in diabetic patients younger than 44 years of age (Rohr et al., 1996). Alongside hyperglycaemia insulin resistance is another mechanism that is generally believed to play an important role in atherosclerosis formation in diabetic patients. Wollesen et al. (2002) carried out a multivariate study with 136 individuals to determine the association between insulin resistance and atherosclerosis. They found that insulin resistance independently predicts atherosclerosis in type 2 diabetic patients, but not in type 1 diabetes. However, the mechanisms underlying insulin resistance and atherosclerosis are still not fully understood (Semenkovich, 2006). More detailed information about the pathophysiology of atherosclerosis in diabetes can be found in a well presented review paper written by Creager & Lusher (2003).

High blood pressure is another cardiovascular risk factor commonly associated with diabetes, and largely independent of age and obesity (DeFronzo, 1992). Thus, some studies have reported a prevalence of hypertension as high as 60% in persons with type 2 diabetes (Albright et al., 2000). The Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (1997) concluded that diabetes markedly increases cardiovascular disease risk at any stage of hypertension. Thus, some reports have estimated a two fold increase in the number of cardiovascular events when hypertension and diabetes coexists (Grossman et al., 2000; Grundy et al., 1999). Obesity (Narkiewicz, 2006), impairments in endothelial function (Tooke & Brash, 1995) and insulin resistance (Grundy et al., 1999) are some pathologies linked to type 2 diabetes that are known to result in hypertension. Based on population studies, risk estimates indicate that at least two-thirds of prevalence of hypertension can be directly attributed to obesity (Krause et al., 1998). The precise mechanism linking obesity to hypertension is not fully understood. However, it is thought that obesity may lead to hypertension by activating the rennin-angiotensin-aldosterone system (Engeli & Sharma, 2000) which is a hormone system that regulates blood pressure and water balance. It appears that abnormalities in the endothelial function may also contribute to hypertension in patients with diabetes (Pitei et al.,

1997). Diabetes mellitus is known to diminish nitric oxide (NO) availability (Tooke, 2000), which is the most important vasodilator substance in the microvasculature (Tortora & Derrickson, 2006). Impairments in the endothelial function in subjects with diabetes will be discussed in more detail in Section 2.2.2.2.1. In addition to that, it seems that there is a particular association between insulin resistance and hypertension; however the mechanisms are still not fully understood (Grundy et al., 1999).

Overall it is well established that diabetes is a major risk factor for cardiovascular morbidity and mortality, partly due to the association between diabetes and traditional risk factors such as insulin resistance, hypertension and dyslipidemias (McCallum & Fisher, 2006; Stamler et al., 1993). Treatments to minimize cardiovascular risk include correction of the metabolic disturbances and modification of related atherosclerotic risk factors, such as hypertension, obesity and dyslipidemia (Grundy et al., 1999). Gaede et al. (2003) carried out a follow up interventional study (7.8 years) on 80 patients with type 2 diabetes to assess the effect of a targeted, intensified, multifactorial pharmacological intervention on modifiable risk factors for cardiovascular disease. Pharmacological treatment targeted hyperglycaemia, hypertension, dyslipidemia, and microalbuminuria. This study demonstrated that a long term intervention which targeted multiple risk factors in patients with type 2 diabetes and microalbuminuria reduced the risk of cardiovascular events by about 50%. This highlights the importance of finding interventions that may influence these cardiovascular risk factors in patients with type 2 diabetes. Part 2 of this literature review chapter will review the role of exercise programmes in modifying the metabolic syndrome in patients with type 2 diabetes.

Alongside the increased risk of cardiovascular diseases, there is little doubt that type 2 diabetes also affects microcirculatory function and QOL. However, those problems are worsened by neuropathy, and for this reason these issues will be discussed in the next section that covers health problems associated with neuropathy.

2.2.2 Health problems linked to peripheral neuropathy

Foot ulceration in diabetic patients with peripheral neuropathy is a major health problem, often leading to lower-limb amputations and an increased mortality rate (Leung, 2007; Ramsey et al., 1999). Peripheral neuropathy is well documented as an independent risk factor for foot ulceration and amputation (Boulton et al., 1997; Veves et al., 1992). Potter et al. (1998) investigated the incidence of peripheral neuropathy in the contralateral limb of 38 diabetic persons with unilateral amputations. Evidence of neuropathy in the contralateral limb was found in 97% of diabetic patients at the time of the amputation. McGill et al. (2005) carried out a follow up study on 477 patients with diabetes mellitus of which 250 were diagnosed with DN and 222 were not. They found that during the follow up period, 34 new ulcers occurred in the neuropathy group and three ulcers in the control group, resulting in an annual incidence of 6.3% and 0.5%, respectively. These figures highlight the impact foot complications have in DN subjects.

Commonly, ulceration in patients with peripheral neuropathy is triggered by a cascade of events. A minor trauma, which in the presence of neuropathy (sensory loss) is unattended, is believed to be the starting point. Changes in gait characteristics are considered a common mechanism by which tissue damage may occur in diabetic patients with peripheral neuropathy. This population shows changes in gait characteristics compared to non-neuropathic counterparts (Courtemanche et al., 1996; Kwon et al., 2003), which result in higher stress on the plantar surface and, consequently, higher risk of tissue damage. Thereafter, a lack of adequate blood supply at this critical phase contributes to the risk of significant infection and hence, further tissue breakdown and risk of amputation. Microcirculatory impairments are thought to play a key role in the diminished blood flow responses under stressful conditions (Tooke & Brash, 1995; Veves et al., 1998), which prevent the diabetic neuropathic foot to respond to injury and infection in the usual manner. To sum up, it seems clear that a combination of gait biomechanics and microcirculatory changes are responsible for the increased risk of foot ulcerations observed in subjects with peripheral neuropathy.

In addition to gait and microcirculatory impairments, diabetes in general and neuropathic complications in particular have also been associated with diminished

QOL. Furthermore, it seems that there is a positive correlation between diabetes and its complications and QOL, with subjects with more severe complications showing the poorest QOL (Price & Harding, 2000).

This section provides a critical review of the primary pathologies linked to DN with particular attention to changes in gait characteristics and microcirculation function, due to their association with foot ulcers. Thus, the pathologies associated with DN will be review in the following order: Gait biomechanics, microcirculation and QOL.

2.2.2.1 Gait biomechanics

Gait analysis has been used to objectively quantify changes in the biomechanics of walking. Given the fact that normal walking is the end product of a healthy neuro-musculo-skeletal system, the analysis of walking strategy in DN may lead to a better understanding of the dysfunctions produced by this condition.

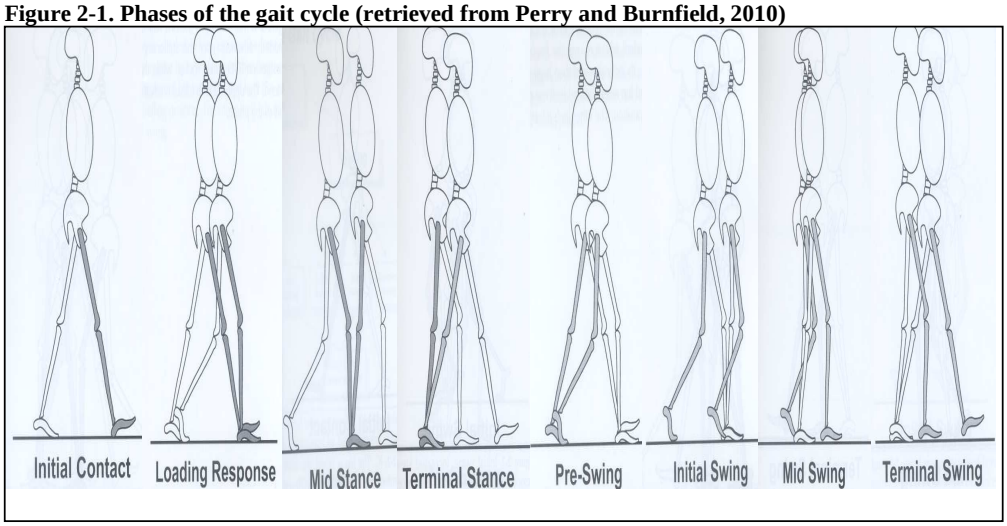
Most of the investigations analyzing gait characteristics in neuropathic patients have been interested in kinetic data, which is believed to predict the risk of foot ulceration in this population (Boulton et al., 1983; Guldemond et al., 2006). However, kinetic data alone provides limited information about gait characteristics. Therefore, gait should be investigated alongside other perspectives (i.e. kinematics and muscular activity) to develop a more comprehensive understanding of walking patterns in patients with diabetic neuropathy. This section will then investigate the differences between healthy and DN walking characteristics from different angles (kinematics, kinetics and muscular function). In addition to this, attention will also be paid to the factors that may be responsible for those differences.

2.2.2.1.1 Gait characteristics: Healthy vs. Neuropathy

Walking is a very complex movement that requires the coordination of the whole body. However, this chapter will only investigate lower limb gait biomechanics since it is the part of the body in which most of the walking-related complications occur.

According to Perry & Burnfield (2010), the gait cycle (GC) consists of two basic components: the stance phase, during which the foot is in contact with the floor and the swing phase when the foot is in the air for limb advancement. The stance phase can be divided into three parts. The first is the contact phase (initial contact and loading response). It starts with the initial contact, which is normally made with the heel, and continues until the other foot is lifted for swing. The second is the midstance phase. It begins as the other foot is lifted and continues until the heel raises the ground. The third phase, the propulsion phase, can be further subdivided into two components: terminal stance and pre-swing. Terminal stance starts with heel rise and ends when the other foot strikes the ground. Pre-swing begins with opposite heel contact and terminates with support-side toe-off (Figure 2-1 provides a graphic representation of the GC).

Figure 2-1. Phases of the gait cycle (retrieved from Perry and Burnfield, 2010)



Each part of the stance phase is characterized by a rocker action of the foot and ankle (Perry & Burnfield, 2010). During the contact phase, the heel ("heel rocker") serves as an axis to allow both smooth plantarflexion and full contact of the foot with the ground. Action by the dorsi-flexor muscles is required to reduce the rate of the foot drop and direct the tibia forward. The second rocker (midstance phase) is the ankle rocker. The pivotal arc of the ankle rocker advances the tibia over the stationary foot. During this phase, plantar-flexor muscles play an important role to make the tibia a stable base for knee extension and allow tibia advancement. The third rocker is referred to as the

forefoot rocker. During terminal stance, the first metatarsal-phalangeal joint allows progression of the limb over the forefoot and accelerates the heel lift. Both gastrocnemius and soleus muscles act vigorously to both decelerate the rate of tibial advancement and allow the foot to push off (Perry & Burnfield, 2010; van Schie et al., 2005).

As briefly described above, the normal GC in the sagittal plane starts at the heel (contact phase) and terminates at the toes (propulsion phase). In contrast, people with DN are characterized by a significant reduction of the motion along the longitudinal axis. There is general consensus that subjects with DN tend to approach the floor with the most anterior part of the heel and perform their push off phase at the metatarsals level (Giacomozzi et al., 2002; Uccioli et al., 2001). The resulting gait, similar to a flat-footed gait, is characterized by a minimum heel strike and a minimum push-off phase. Giacomozzi et al. (2002) carried out a very interesting study in which the evolution of the centre of pressure (COP) during gait was measured in 21 healthy and 19 DN patients. The COP records the point of application of the ground reaction force on a force platform during the stance phase, and it is plotted as a sequence of points on the ground plane. This study supports the idea that there is a reduction of the COP progression along the longitudinal axis in DN individuals when compared to the healthy. COP progression was calculated as the maximum COP excursions (cm) along the longitudinal axis of the foot. In line with these results, some investigations have reported reduced contact areas at the heel and toes in DN patients compared to healthy counterparts (Veves et al., 1991). These results further support the idea that during gait DN subjects show a shift in their loading pattern from the heel and toes towards the midfoot and forefoot, respectively.

Apart from the motion in the longitudinal axis, the transverse plane mobility also plays a pivotal role in the normal GC (Perry & Burnfield, 2010). Excursions along the medio-lateral axis of the foot mainly depend on the inversion-eversion movements performed by the subtalar joint. In normal gait eversion begins as part of the loading response immediately after the heel contacts the floor. Peak eversion is reached by early midstance. Subtalar motion then slowly reverses toward inversion throughout terminal stance. Peak inversion is attained at the onset of the passive propulsion phase (Perry & Burnfield, 2010). Neuropathic gait is characterized by a significant reduction of the

motion along the medio-lateral axis and a concurrent shift of the loading pattern from the lateral toward the medial part of the foot (Giacomozzi et al., 2002; Gutierrez et al., 2001).

In addition to the gait alterations reported above, there is general agreement in the literature that neuropathic patients show different spatial-temporal pattern characteristics compared with age-matched control subjects. Hence, DN patients use a more conservative walking strategy than healthy subjects, which is characterized by shorter steps, less time spent in the single support phase and slower walking speed (Courtemanche et al., 1996; Kwon, et al., 2003).

Up to date, numerous studies have assessed gait characteristics in diabetic subjects with neuropathic complications and it is well established that individuals with diabetic neuropathy adopt a more cautious walking strategy than non-neuropathic patients. This altered gait is characterized by shorter and slower steps as well as by a significant reduction of the motion along the longitudinal and medio-lateral axis. The section below discusses the factors that are thought to be responsible for the modified walking strategy observed in DN subjects.

2.2.2.1.1.1 Factors for altered walking strategy in neuropathic patients.

Diabetic neuropathy is a very complex condition and there are several factors that may be responsible for the differences in walking characteristics described. Although neuropathy is considered a key factor in the explanation of gait changes in patients with DN, recent studies have reported gait alterations in diabetic patients without neuropathy (Petrofsky et al., 2005a; Yavuver, et al., 2006). This suggests that neuropathy may not be the only reason for gait deviations in this population. In this section, neuropathic and non-neuropathic factors that may influence walking patterns in DN patients will be reviewed separately.

Neuropathic factors

The loss of protective sensation is generally considered the single most important factor in order to explain the gait changes observed with neuropathy (Frykberg et al., 1998). It

has been hypothesized that sensory feedback plays a role in adjusting step-to-step limb trajectories to maintain balance during locomotion (Gandevia & Burke, 1992). The loss of this feedback information resulting from DN may then lead to loss of stability in these patients during gait. Consequently, this may increase the need for producing a more cautious gait in this population. This idea is supported by previous investigations that have assessed stability in DN subjects by measuring the distance travelled by the COP during different conditions. It is assumed that stability is negatively related to the distance travelled by the COP during a fixed period of time. Therefore, subjects with poorer stability are expected to show higher distances and vice versa. Numerous studies have reported a high correlation between the severity of DN and COP distance in this population (Simoneau et al., 1994; Simmons et al., 1997b), which demonstrates that sensory neuropathy may produce instability in this population. Furthermore, the level of neuropathy has been also associated with step times (negative correlation), gait velocity (negative correlation) and step length (negative correlation) during gait (Yavuzer, et al., 2006). This suggests that feeling of instability secondary to sensory neuropathy may lead to a more cautious gait in DN subjects characterized by slower gait velocity, shorter steps and longer contact times.

Eils and colleagues (2002) carried out an interesting study to investigate the role of cutaneous sensory information during walking. In this study walking characteristics were assessed in 40 healthy subjects (25.3± 3.3 years of age) with no history of sensory disorders during normal conditions (control condition) and after reducing foot sensation via foot ice immersion (ice condition). The study found that a short-term reduction in the afferent information of the sensors located in the foot plantar surface substantially influenced walking characteristics by shifting the load from the heel and toes towards the midfoot and forefoot, respectively. Data was collected on a pressure distribution platform (EMED ST4 platform, Novel) with a good resolution (4 sensors$\cdot cm^{-2}$), which enables accurate determination of different foot areas. Interestingly, these changes in walking characteristics show similarities with the gait characteristics described above for DN patients. Loss of sensation produces a feeling of instability, which leads to a change in the walking behaviour so that the body mass centre is positioned more directly above the foot during the whole GC (Katoulis, et al., 1997). In disagreement with Eil's study, Hohne et al. (2009) found that reduced plantar cutaneous sensation did not cause any changes in plantar loading distribution while walking. It should be noted

that Hohne et al. (2009) applied intradermal anaesthesia to reduce plantar cutaneous sensation contrary to Eil's study, in which ice immersion was used. Intradermal anaesthesia is thought to target only the end-organs of the plantar cutaneous mechanoreceptors while the foot and ankle proprioception as well as intrinsic foot muscles are unaffected (Meyer et al. 2004). On the other hand, ice immersion is likely to affect the intrinsic foot muscles and joint receptors. This methodological difference could explain the differences on the results. These conflicting results show that it is still not well understood how the effect of insensitive skin, secondary to peripheral sensory neuropathy, may influence changes in walking characteristics. However, it is certainly likely that postural instability in subjects with DN may be the result of a loss of peripheral sensory receptor function in the lower legs and cannot be attributed exclusively to loss of plantar cutaneous sensation (van Deursen et al., 1999).

In support of this idea, several studies have reported that an impairment of the sensory or afferent function of the foot diminishes the ability of the foot to perceive movement and furthermore may affect the normal foot progression along the GC (Uccioli et al., 2001; Yavuzer et al., 2006). Van den Bosch et al. (1995) reported that the inversion/eversion proprioceptive thresholds in subjects with DN were about five times greater than in age matched subjects without peripheral neuropathy. It should be noted that a small sample size (7 volunteers in each group) was used in this study. In line with these results, Son et al. (2009) carried out a similar study on 11 DN subjects and 11 matched control and found that inversion/eversion proprioception thresholds were greater in the DN group compared to the healthy group. However, the group differences reported by Son et al. (2009) were significantly smaller (2 fold differences) than the ones reported by van den Bosch (1995) (5 fold differences). Methodological differences are likely to explain the diversity of the magnitude of the differences between both studies. Son et al. (2009) set up angle rotation constantly at 5 degrees$\cdot s^{-1}$ while van den Bosch et al. (1995) reported maximum values of 18 and 23 degrees$\cdot s^{-1}$ in inversion and eversion, respectively (no information was published about the mean rotation velocity). Higher rotation velocity is likely to explain the higher group differences reported by van den Bosch et al. (1995) when compared to the results shown by Son et al. (2009). A reduced ability to perceive movement due to afferent impairment increases the excursion of the centre of mass over the foot before the movement is perceived by the patient, which, in turn, increases the risk of falls in this population (Yavuzer et al., 2006). In support of this idea, many investigations have reported an increased risk of

falls in patients with DN (Dingwell & Cavanagh, 2001; Richardson & Hurvitz, 1995). This may partly explain why individuals with DN show a significant reduction of the motion along the longitudinal and medio-lateral axis. Overall, it appears that loss of stability secondary to DN is an important contributor factor for the gait characteristics described above.

Afferent impairments in isolation would appear to be enough to explain the changes in walking strategy observed in neuropathic patients. However, while some peripheral nerves have a purely sensory or afferent function, most are mixed and carry both sensory and motor fibres (Gutierrez et al., 2001). An efferent/motor deficit, which is identified by muscle weakness and is well established in DN subjects (Andersen et al., 1997; Andersen et al., 1998; van Schie et al., 2004), could further explain the previously described neuropathic walking strategy.

At the ankle joint, at least three major muscles contribute substantially to joint moments during gait. The tibialis anterior muscle (TA) contributes to ankle dorsi-flexion, while the soleus and gastrocnemius muscles, which form the triceps surae (TS), work as plantar-flexors (Perry & Burnfield, 2010). On the one hand, TA plays a very important role during the early contact phase of the GC to enable normal heel strike. Weakness of the TA may result in a reduced dorsi-flexion angle during heel strike, which provokes the foot to contact the floor with the most anterior part of the heel (Giacomozzi et al., 2002). This may partly explain why during gait DN subjects show a shift in their loading pattern from the heel to the midfoot. On the other hand, TS is responsible for driving the body forward by generating plantar-flexor moment during to the late stance phase (Perry & Burnfield, 2010). Weakness of the TS may require compensation of proximal hip musculature to push the legs forward. In line with this idea, DN have been shown to rely more on the hip flexor muscles and less on the plantar-flexor muscles during the push off phase of the GC, compared to healthy individuals (Giacomozzi et al., 2002; Mueller et al., 1994). This compensatory mechanism characterized by a shift from the distal (ankle muscles) to the proximal musculature (hip muscles) is known as hip strategy. Hip strategy is also well known to be highly effective to stabilize body posture during weight bearing (Horak & Nashner, 1986) by reducing the displacement of the centre of mass along the sagittal and frontal planes (Runge et al., 1999). This suggests that the shift from an ankle to a hip strategy is also responsible for the limited

excursions of the centre of pressure of the foot observed in DN subjects. Thus, hip strategy in DN individuals may be a mechanism not only to compensate for the plantar-flexor weakness but also to improve stability during weight bearing conditions.

Both sensory neuropathy and motor neuropathy are well known to be responsible for the modifications in gait characteristics observed in DN subjects. On the one hand, a general loss of peripheral sensory receptor function in the lower legs appears to be responsible for the feeling of postural instability observed in DN individuals. Furthermore, feeling of instability has been associated with a more cautious gait walking strategy. On the other hand, weakness of the distal musculature appears to result in compensation of proximal hip musculature, especially during the push off to drive the body forward. Furthermore hip strategy is believed to reduce the displacement of the centre of mass along the longitudinal and medio-lateral axis.

Non-neuropathic factors: Hyperglycaemia

Besides well known dramatic alterations in all components of the peripheral nerves, diabetes mellitus has a spectrum of hyperglycaemic complications including the mechanical characteristics of bones and soft tissues, which may further alter gait characteristics in this population (Duffin et al., 2002; Gefen et al., 2003). Abnormally high sugar levels promote glycosylation of proteins and the consequent accumulation of advanced glycosylation end-products in most human tissues (Rahman et al., 2006; Yavuzer et al., 2006). It is well established that glycosylation plays an important role in the limited range of motion (ROM) of the foot and ankle joints in patients with (Frykberg et al., 1998; Simmons et al., 1997a; Yavuzer et al., 2006; Zimny et al., 2004) and without diabetic neuropathy (D´Ambrogi et al., 2003; Duffin et al., 2002). Normal joint mobility in the articulations of the foot is necessary to allow for normal foot function. Articular mobility of tibiotalar and metatarsophalangeal joints in the sagittal plane permits normal initial contact and push-off during the gait cycle, respectively (Perry & Burnfield, 2010; van Schie et al., 2005). In addition, an adequate range of motion in the subtalar joint is necessary to allow the excursion of the foot along the medio-lateral axis (Perry & Burnfield, 2010; van Schie et al., 2005). Therefore, reduction of ankle ROM, secondary to hyperglycaemia, may partly explain

the reduction of the COP progression along the anterior-posterior and the medio-lateral axis in diabetic individuals (with and without neuropathic complications) when compared to healthy counterparts (Giacomozzi et al., 2002; Gutierrez et al., 2001).

The plantar aponeurosis (plantar fascia) is rich in collagen (Gefen, 2003) and can therefore be susceptible to non-enzymatic glycation of the collagenous component (Duffin et al., 2002). In agreement with this suggestion, some studies have observed a significantly higher plantar aponeurosis thickness in non-neuropathic diabetic subjects when compared with non-diabetic individuals (D´Ambrogi et al., 2003; D´Ambrogi et al., 2005; Giacomozzi et al., 2005). This is further supported by Duffin et al. (2002), who found that plantar aponeurosis was significantly thickened in 216 young diabetic individuals (15.6 years of age) as compared with a non-diabetic age-matched control group. This study confirms that structural changes appear soon after the onset of diabetes regardless of whether neuropathic complications are present or not. It is believed that the thickening of the plantar fascia may provoke the development of a rigid foot, fixed in a cavus configuration, with a high longitudinal arch for the entire stance period (D´Ambrogi et al., 2005). Thus, the foot is less adaptable to the floor during the foot-floor interaction. As a result, most of the plantar surface makes early contact with the ground, and propulsion is poorly effective. Therefore, it is likely that structural changes in the plantar fascia may be partly responsible for the reduced foot motion along the longitudinal axis observed in diabetic patients with and without neuropathy (D´Ambrogi et al., 2003; Giacomozzi et al., 2005).

Overall, neuropathy, both sensory and motor neuropathy, is considered the main factor in the explanation of gait changes in DN subjects. However, recent studies show that gait changes in patients with diabetes are present even in the absence of neuropathic complications. Glycosylation changes secondary to hyperglycaemia have been linked to reduced joint mobility and/or structural changes in diabetic patients. Mounting evidence suggests that these changes may also contribute to gait alterations in diabetic subjects even without peripheral neuropathy. Nevertheless, differences in walking characteristics by themselves cannot explain the high rate of ulcers observed in diabetic neuropathic patients. Next, other variables that may better predict ulceration among those patients will be discussed.

2.2.2.1.2 Gait analysis: Kinetic perspective

During weight-bearing activities such as standing and walking, the plantar surface of the foot is exposed to ground reaction forces (GRFs), leading to tissue deformation (van Deursen, 2004). The relationship between force and deformation is expressed as the stress-strain relationship. Stress, commonly known as pressure, is equal to normalized force (force per unit to which the force is applied) (Hall, 1995; Nigg & Herzog, 1999). When standing the magnitude of the GRF is equal to body weight, with each foot experiencing about 50% of body weight distributed over the plantar surface area, which is predominantly a vertically directed force. During walking, the stresses applied to the feet (pressures) are much higher than when standing for a number of reasons. First, weight is supported by only one foot for a substantial amount of time. Second, the stance phase of gait is characterized by a rollover of the foot. As a result, the plantar surface area changes in size and location while the GRFs progress from the heel to hallux. Third, the GRFs vary in magnitude, with a peak during heel landing and a second peak during push-off with the forefoot (van Deursen, 2004). It is noteworthy that in order to represent GRFs as vectors, they must be considered to act at a point that is known as COP (COP has been mentioned earlier to describe gait characteristics in DN subjects). Besides the double-hump pattern of the vertical force, there are also GRFs in the horizontal direction (shear forces) causing stress parallel to the foot skin. However, the magnitude of the shear forces is much smaller than the vertical GRF (van Deursen, 2004).

Beside neuropathy, which is well documented as being related to skin breakdown due to the lack of protective sensation, foot plantar pressure (PP) is known to be an important contributing factor to plantar tissue damage in people with diabetes and peripheral neuropathy (Frykberg et al., 1998; Van Schie., 2005). Thus, PP, which accounts for the highest stress generated under the foot plantar surface during stance phase of gait, is well known to be higher in DN subjects and has been associated for decades with foot ulcerations in this population (Guldemond et al., 2006; Stokes et al., 1975; Uccioli et al., 2001). However, there is an ongoing debate whether other kinetic variables may also predict foot complications in this population. Thus, PTI, calculated as the area under the peak pressure curve, has been postulated to be a better predictor for foot ulceration compared to PP since it represents both, the magnitude and duration of plantar loading

through stance phase (Mueller & Maluf, 2002). Thus, recent investigations have found higher PTI values in DN subjects compared to healthy or diabetic individuals with no neuropathic complications (D'Ambrogi et al., 2005; Sacco et al. 2009). The next two sections will investigate these two parameters (PP and PTI) in individuals with peripheral neuropathy as well as the factors responsible for those alterations.

2.2.2.1.2.1 Peak plantar pressures

Peak plantar pressures have been extensively investigated in the literature as a substitute measure to determine risk of foot ulceration (Frykberg et al., 1998; Veves et al., 1992). Stokes et al. (1975) was the first to notice that foot ulcers in neuropathic patients tended to develop at the sites of the highest load (i.e. metatarsal heads and toes). Since then, many other authors have described "high plantar pressures" in the diabetic neuropathic foot and their relationship with ulcer development (Guldemond et al., 2006; Payne et al., 2002; Uccioli et al., 2001). Veves et al. (1992) carried out a follow up study on 86 diabetic patients in which 17% developed ulcers over a mean period of 30 months. Of these 17%, 93% had neuropathy at baseline, and all had abnormally high foot pressures measured by pedobarography. Similar findings were reported by Lavery et al. (2003b) who carried out a follow up study over 24 months on 1666 individuals and found that PP was significantly higher in patients who developed ulcers during the follow up period than in patients who did not develop ulcers. However, none of these prospective studies provided a measure of association between foot pressures and ulceration risk. Frykberg and colleagues (1998) reported in a study with 251 subjects, that diabetic patients with foot pressures >588.6 kPa as measured with the F-scan mat system, were twice more likely to develop ulcers than those without high pressures, even after adjustment for age, sex, diabetes duration and race. This investigation attempted to determine a threshold point, above which the neuropathic foot is at increased risk. However, results should be taken with caution. This cross-sectional study assessed the relationship between high foot pressures and diabetic foot ulceration retrospectively, in a group of diabetic patients with a range of foot complications (diabetes, neuropathy, previous ulcer and active ulcer). Kanade et al. (2006) showed differences in PP values among neuropathic patients with different foot complications. She reported significantly higher peak pressure values in a group of 23 neuropathic patients with current foot ulcer

compared to a group of 23 neuropathic patients with no history of foot ulcer. This finding suggests that the measure of association between foot pressure and ulceration risk used by Frykberg could have been overestimated since the presence of ulcer may have been related to higher foot pressures. In addition to that, it should be noted that different plantar pressure measurement devices are not always directly comparable, and therefore, a universal threshold set for ulceration cannot be established. Pressure measurement devices consist of a number of force sensor elements and the variation in the size of these elements has major consequences for the calculation of pressure. Thus, a focal area of pressure under the foot will appear to have a lower value on a device with a larger element size (Nigg & Herzog, 1999; van Schie, 2005).

At this time, it is well established that subjects with peripheral neuropathy suffer from excessive foot pressures during walking (Payne et al., 2002; Uccioli et al., 2001), especially at the metatarsals (Mueller et al., 2005; Salsich et al., 2005) and that excessive pressures on the surface on the insensitive skin lead to tissue damage and ulcers (Lavery et al., 2003b). Current investigations are focused on the understanding of the different factors that modify peak foot pressure distribution in subjects with diabetes neuropathy. The factors that are widely accepted to potentially disrupt foot loading during gait will be discussed next.

Limited ROM in the ankle and first metatarsophalangeal joints has important implications in the mechanical loading of the foot and has been found to increase peak plantar pressures (Fernando et al., 1991; Frykberg et al., 1998; Zimny et al., 2004) as well as the risk of ulceration (Fernando, et al., 1991) in diabetic patients. Limited dorsi-flexion of the ankle has been associated with abnormal foot pressure distributions during gait in different ways; 1) it may lead to an earlier heel rise in the gait cycle and an earlier loading of the forefoot (van Deursen, 2004), and; 2) it may increase the pressure under the forefoot, particularly when the tibia rolls over the foot during the late stance phase of gait (Mueller et al., 1989; Salsich et al., 2005). Some investigations have also associated reduced mobility at the subtalar joint with increased foot pressures during the first phase of the stance phase (from heel strike to midstance phase) (Fernando, et al., 1991). It is believed that the subtalar joint plays an important role preventing excessive strain at the ankle as the limb is loaded (Perry & Burnfield, 2010).

The elevated plantar foot pressure in neuropathic patients has also been associated with structural changes secondary to this condition such as toe deformities and plantar fat-pad displacement (Mueller et al., 2005; Rahman et al., 2006). Atrophy of the ankle muscles as a result of motor neuropathy, has been suggested to produce an imbalance between flexors and extensor of the toes. This may lead to the development of hammer toes, claw toes and prominent metatarsal heads, making the metatarsals more likely to undergo higher pressure in neuropathic subjects (Mueller et al., 2005; Rahman et al., 2006; Robertson et al., 2002; van Schie et al., 2004). Since the toes no longer contribute to the support area the same amount of force will be transmitted to a smaller area, which obviously increases foot pressures on the metatarsal region. In clawing and hammering of the toes, the plantar fat pads under the metatarsal heads are believed to migrate distally as a result of hypertension of the metatarsal-phalangeal joint, exposing the now prominent and unprotected metatarsal heads to elevated levels of mechanical pressure during gait (Bus et al., 2002; Bus et al., 2004). Beside the structural changes associated to muscle atrophy, the thickening of the plantar fascia (discussed above) may provoke the development of a rigid foot, fixed in a cavus configuration, with a high longitudinal arch for the entire stance period (D´Ambrogi et al., 2003; D´Ambrogi et al., 2005; Giacomozzi et al., 2005). This foot configuration has been associated to higher foot pressures at the heel during the heel strike (Morag & Cavanagh, 1999) as well as higher stress on the forefoot underneath the metatarsal heads (Cavanagh et al., 1997; D´Ambrogi et al., 2003). Nevertheless, the most devastating structural changes in patients with neuropathic complications occur when suffering from Charcot foot (Mabilleau & Edmonds., 2010). Charcot foot is a condition that causes significant disruption of the bony architecture of the foot. Although the pathogenesis of this condition is not fully understood, it is generally believed that minor trauma may trigger a cascade of events, including inflammation, which may result in progressive osteolysis (resorption of bone matrix by osteoclasts) (Mabilleau & Edmonds., 2010). Due to excessive resorption of bone matrix the bones of the foot become soft and weak, which can lead to multiple fractures, joint disruptions and joint dislocation. It often results in foot deformities and causes abnormal pressure distribution on the plantar surface, which predispose the foot to ulcer formation. Armstrong & Lawrence (1998) proved that neuropathic patients with Charcot foot (N=21) had significantly higher foot pressures (1000± 80.5 kPa) than subjects with neuropathy and no Charcot foot (N=21) (650± 256 kPa).

In addition to the factors described above, it has been pointed out that reduction in plantar sensory input may be related to increased impact of the foot on the ground. As the foot has an impaired ability to sense the ground when landing, this may increase pressures under the foot (Payne et al., 2002). This interpretation has been supported by some studies that addressed a positive relationship between levels of neuropathy and plantar pressure (Boulton et al., 1987; Frykberg et al., 1998; Lavery et al., 2003b). Payne et al. (2002) carried out an interesting study on 50 subjects with diabetes mellitus to determine, by the use of regression analysis, the factors that may be associated with high peak plantar pressures during walking in different regions in the diabetic foot. A number of factors, identified as potentially increasing foot pressures, were included in the multivariate analysis. Thus, body weight, limited joint mobility, plantar fat pad thickness, muscle strength, motor and sensory neuropathy, foot structure, and foot deformity, were investigated. They found that sensory neuropathy was the single most important factor to explain plantar foot pressures in different regions of the foot (heel, forefoot and hallux). They also reported that the range of motion of the first metatarsophalangeal joint was related to hallux pressures while body weight was associated to pressures under the heel. Unexpectedly, structural changes in the foot and limited joint mobility were not significantly correlated to plantar pressures under the metatarsal heads. This finding contradicts the widely published belief that structural changes are important factors in the high foot pressures observed in subjects with DN (Fernando et al., 1991; Frykberg et al., 1998; Salsich et al., 2005). This result could be due to the high correlation that would be expected to exist between limited joint mobility and structural changes and neuropathy. It is likely that both structural changes (including limited ROM) and neuropathy are both secondary to a longer duration of diabetes, rather than each one having a causal relationship with the other (Gefen et al., 2003).

It should be also noted that, for Payne's study, an in-shoe pressure measuring system was employed, contrary to investigations that have determined foot pressures during bare foot walking over a pressure platform (Boulton et al., 1987; Fernando et al., 1991; Uccioli et al., 2001). Footwear has been shown to significantly reduce plantar pressure in both diabetic and control groups with a greater reduction in the diabetic group (Sarnow et at., 1994). Healthy subjects with intact protective sensation are likely to adopt a safer walking style to avoid excessive foot pressures during barefoot tasks. On

the other hand diabetic patients with impaired sensation are not aware of excessive pressure and gait is not readjusted. In support of this idea, as mentioned earlier, the level of neuropathy has been positively correlated to foot pressure (Frykberg et al., 1998; Lavery et al., 2003b). This could have affected the range of variables that explained the variability of plantar pressures in the study carried out by Payne et al. (2002).

Overall, it is well established that peak plantar pressures are associated with foot ulcers in patients with DN. Multiple factors account for the higher foot pressures observed in DN subjects including loss of sensation, limited joint mobility, structural changes and foot deformities. Neuropathy appears to be the single most important factor to explain plantar foot pressures in different regions of the foot in this population. However, structural changes secondary to hyperglycaemia are also considered to play an important role in the high peak pressures observed in DN subjects.

2.2.2.1.2.2 Pressure-time Integral

PTI is a relatively recently used parameter, which has not been widely investigated in DN subjects. However, all the investigations up to date point in the same direction demonstrating differences in regional PTI values between neuropathic and healthy individuals (D'Ambrogi et al., 2005; Rao et al., 2010; Sacco et al., 2009).

D´Ambrogi et al. (2005) investigated the PTI of the vertical GRFs under the heel, metatarsal heads and hallux, both during the landing (0-27% of stance) and propulsion (66-100% of stance) phases of the GC on 21 healthy and 19 DN patients. They found that neuropathic patients compared to healthy controls had; 1) lower heel integrals and higher metatarsal integrals during landing, which is typical of a flat landing; and 2) higher heel and metatarsal integrals and lower hallux integrals during propulsion, which again is typical of a flat walking (hip strategy). As a result neuropathic patients experienced an earlier and more prolonged loading of the metatarsal heads. Similar results were obtained by Sacco et al. (2009), who found higher PTI values during the push off at the heel and forefoot areas in the DN group compared to the control group. It should be noted that Sacco et al. (2009) failed to find group differences in PTI values both at the heel and forefoot during the landing phase, which disagrees with the results

presented by D'Ambrogi et al. (2005) and described above. The fact that the stance phase in both studies was calculated differently may explain those differences. D'Ambrogi et al. (2005) defined landing as the time period from 0 to 27% of the stance phase, whereas Sacco et al. (2009) calculated this period as the time period from heel strike (0% of the stance phase) to the instant of the minimum vertical force between the two vertical peaks (approx 50% of the stance phase). It is likely that differences in PTI values during the landing phase between DN and healthy individuals only occur during the first phase of the stance phase, which could explain why Sacco et al. (2009) who accounted for a longer period of time did not find any group differences. In addition to this, Rao et al. (2010) investigated PTI values at the heel and forefoot during the whole GC on 15 DN and 15 control subjects and found higher PTI values in the DN group both at the heel and forefoot when compared to the control group.

These results prove that not only the magnitude of the stress (pressure) is altered in DN subjects but also the amount of time stress is applied to the different foot regions. In addition to this, the forefoot seems the foot region with higher PTI differences between DN and healthy individuals, which coincides with the foot region in which more ulcers occur in this population (Boulton, 1994; Mueller et al., 2005). This suggests that PTI may be associated with foot complications in this population. Next, possible factors that may account of the higher PTI observed in DN subjects will be postulated.

Foot mobility during walking has been identified as an important contributor to plantar loads, especially in individuals with diabetic neuropathy (Fernando et al., 1991, Zimny et al., 2004). Thus, a few recent investigations have studied the relationship between reduced mobility of the foot-ankle complex and increased sustained plantar loads. Turner et al. (2007), in agreement with previous studies (Frykberg et al., 1998; Simmons et al., 1997a), found reduced ROM (subtalar joint and 1st metatarsalsophalangeal joint) in a group of 78 diabetic patients (with a range of diabetic complications) compared to healthy subjects. However those differences were only significant during passive movements while ROM values during gait, which was measured using a 3D electromagnetic tracking system, did not differ between groups. In addition to that, they found no statistical correlation between any of the gait-derived ROM variables and PTI over the whole foot. A more recent study carried out by Rao et al. (2010) went a step further and investigated the relationship between segmental foot

mobility (first metatarsal relative to the calcaneus, lateral forefoot relative to the calcaneus and calcaneus relative to the tibia), measured with a 3D infrared system, and regional PTI (heel and forefoot) (studied presented earlier). They found that frontal plane excursion of the calcaneus was negatively associated with PTI values at the forefoot and heel and that first metatarsal sagittal plane excursion during gait was negatively associated with PTI values under the forefoot. These findings support the theory that subtalar joint mobility plays a key role in mediating plantar load distribution (Perry & Burnfield, 2010), particularly in individuals with diabetes. The relationship between first metatarsal sagittal plane excursion and PTI values under the forefoot could be explained by the association between increased plantar fascia thickness and reduced mobility at the 1st metatarsalphalangeal joint (D'Ambrogi et al., 2002). Thickening of the plantar fascia may provoke the development of a rigid foot, fixed in a cavus configuration, with a high longitudinal arch for the stance period (D'Ambrogi et al., 2002; Giacomozzi et al., 2005), which may explain prolonged forefoot contact time and consequently increase PTI forefoot values in DN patients. Overall, this study highlights the importance of segmental foot mobility in individuals with DN. However, more investigations in this direction are needed to understand better those associations.

Turner et al. (2007) also compared PTI values between diabetic patients with and without neuropathy and they found higher PTI values in the neuropathic group (N=28) compared to the non-neuropathic group (N=25). This result suggests that neuropathy may also influence sustained plantar loads during gait. Sensory neuropathy is known to cause postural instability in DN subjects (van Deursen & Simoneau, 1999), which leads to walking alterations to increase stability during the gait cycle. Gait in DN individuals is characterized by a longer period of time during the stance phase especially at the heel and the metatarsals (Courtemanche et al., 1996; Giacomozzi et al., 2002; Sacco & Amadio, 2000; Zimny et al., 2004). Higher contact times (longer duration of the stress) at those sites may increase PTI values at the heel and forefoot, which agrees with the data presented above (D'Ambrogi et al., 2005; Sacco et al., 2009; Rao et al., 2010). However, it should not be neglected that another well known characteristic of the way DN subjects walk is that they tend to demonstrate an increase in flat foot contact time (larger contact area) (Courtemanche et al., 1996), which should decrease the magnitude of the stress (pressures) on the plantar surface. D'Ambrogi et al. (2005) and Rao et al. (2010) found that PTI at the heel during the push off phase was increased in DN

subjects compared to healthy individuals. Heel involvement during the push off can theoretically be associated with less stress on the forefoot during this phase of the gait cycle. However, PTI at the forefoot during the push off continued to be higher in the DN group compared to the control group. This highlights the role of factors intrinsic to the foot, such as segmental foot mobility and/or foot deformities, in contributing to PTI values in patients with DN.

Beside sensory neuropathy, motor neuropathy has also been proposed to influence PTI during gait. Muscle weakness is well known to play a crucial role in altering the foot rollover process in DN patients (Giacomozzi et al., 2005; van Schie, 2005), which, in turn, may modify load distribution over the plantar surface. Weakness of the TA has been proposed to reduce ankle flexion prior the heel contacts the ground, which may lead to premature contact of the forefoot to the ground during the first phase of stance. This earlier contact of the forefoot to the ground in the first phase of stance adds loads on the anterior areas of the foot that will be loaded at late stance phase during the propulsion phase. The consequence will be an accumulation of loads on the anterior regions of the foot (forefoot) during the whole stance phase and higher PTI values on this foot region.

It is well established that subjects with DN show alterations in the foot loading patterns during gait, and that increased peak pressures and PTI are undoubtedly associated with foot complications in this population. Although the exact mechanisms underlying these changes are not well understood, there is little doubt that loss of sensation, weakness of the distal musculature, limited ROM and structural changes secondary to hyperglycaemia are all contributing factors. Most of the studies assessing gait characteristics in diabetic patients have investigated kinetic or kinematic data, whereas the underlying functional factors that may be driving these changes are not well understood. Thus, the assessment of muscle activities in the lower limb may be useful for interpreting and clarifying those kinetic and kinematics changes as well as for gaining additional insights into the relative influence of biomechanical and neural factors on neuropathic patient's gait. The next section will attempt to summarize the current understanding about this issue.

2.2.2.1.3 Gait analysis: muscular activity perspective

Electromyography (EMG) is a widely used technique for recording the electrical signal associated with the contraction of a skeletal muscle (Winter, 2009). EMG can be used to measure the timing and relative intensity of muscular function during different conditions and therefore has played a crucial role in the understanding of human movement over the last decades (Perry & Burnfield, 2010; Winter, 2009). It should be noted that EMG signal refers to the electrical event produced by the muscle and not to the mechanical output (force). The time lag between muscle activation and muscle force production is known as electromechanical delay (EMD) (Cavanagh & Komi, 1979) and it should be taken into consideration when attempting to associate EMG signal and muscle function. Otherwise the prediction of segmental movement from EMG data could be mistimed and could lead to misinterpretation.

Although EMG data has provided valuable information about gait biomechanics over the years in healthy as well as in clinical populations (Perry & Burnfield, 2010), the study of EMG data during gait in DN patients is very limited. To the best of my knowledge only 4 investigations have studied this issue (Abboud et al., 2000; Akashi et al., 2008; Kwon et al., 2003; Sacco & Amadio, 2003) and the findings reported by the different studies are not in agreement. It should be noted that none of these investigations took into account EMD when processing EMG data, which as mentioned above could compromise the estimation of segment movements from EMG data. Next, results from previous investigations that have assessed muscular activity patterns in DN subjects will be presented. Special attention will be given to the association between EMG data and both spatial-temporal characteristics and foot loading patterns.

Gait changes in DN subjects, characterized by slower velocity and shorter steps, are thought to be a mechanism to improve motor control and reduce instability during gait in this population (Yavuzer et al., 2006). EMG alterations in DN subjects appear to support this hypothesis. It has been postulated that co-contraction is the result of poor motor control and that it may contribute to stiffen the joint and enhance stability during the stance phase of the GC (Benjuya et al., 2004; Manchester et al., 1989). Thus, Kwon et al. (2003) reported that the activity of the TA and TS was significantly prolonged in the subjects with DN compared to the healthy group, which suggested higher co-

contraction between the dorsi-flexor and plantar-flexor muscles. In addition to that, Petrofsky et al. (2005a) found similar results in a population of 25 subjects with diabetes mellitus without neuropathic complications compared to 25 healthy controls. Thus, Petrofsky et al. (2005a) reported that during the initial contact and toe off phases of the GC, the diabetic group showed muscle activity to be at least five fold higher in antagonist muscle groups compared to healthy controls. Therefore, early evidence suggests that co-contraction may be a strategy used by DN subjects (even diabetic subjects without neuropathy) to improve the feeling of instability associated with this condition. It is not surprising then that gait in this population is slower and the steps shorter, since agonist and antagonist muscles are firing at the same time.

In addition to this, the assessment of EMG patterns throughout the GC on different lower limb muscles may provide additional insights into the association between individual muscle function and kinetic and/or kinematic changes. At the ankle joint, at least two major muscle groups contribute substantially to joint moments during gait. The TS works as plantar-flexor, while the TA muscle contributes to ankle dorsi-flexion. Next, the activation patterns of those muscles in DN subjects will be examined.
The onset of TS activity normally occurs once the forefoot strikes the floor as it contracts to make the tibia a stable base for knee extension and to allow tibial advancement (Perry & Burnfield, 2010). From that moment in time TS activity rises consistently throughout the mid stance phase to peak at the middle of terminal stance phase (push off). The main function of the plantar-flexors is to drive the body forward by generating plantar-flexor moment during the late stance phase (Perry & Burnfield, 2010). Kwon et al. (2003) found an earlier activation of this muscle group in neuropathic patients. The premature activation of TS could be a mechanism to facilitate early forefoot contact with the ground and therefore provide the foot with a more stable base of support during walking in subjects with DN resulting in enhanced feeling of stability (Courtemanche et al., 1996). An early activation of the TS could be also the result of an anticipatory strategy of the DN muscles to efficiently produce force during the most demanding phase of the stance phase, the push off. Although an anticipatory mechanism has not been considered in the literature to explain differences in muscular activity patterns in DN subjects, it is a likely option.
Gutierrez et al. (2001) demonstrated a decrease in the ability to rapidly develop torque about the ankle in a group of 6 DN subjects compared to 6 diabetic individuals without

neuropathy. Force generation in this study was determined from the rate of change of the ground reaction force vector in the lateral direction (measured with a force platform) during 1) a rapid lateral loss of balance and 2) a quick voluntary inversion movement of the ankle. The results show that DN subjects were able to produce about half the rate of torque than the healthy group in both conditions. Although the reasons for this slower force production are not fully understood, evidence from animal studies suggests that the fast-twitch fibres (type 2) of the muscles are highly sensitive to the loss of strength due to atrophy (Bishop & Milton, 1997). Therefore, a generalized DN is likely to cause distal loss of fast twitch muscle fibres, which may result in slower force production by the muscles, especially the distal ones (Gutierrez et al., 2001). This limitation to generate force quickly might require DN subjects to activate the muscles earlier as a mechanism to overcome the slower rate of force production and ensure the peak force is produced at the right moment in time. In addition to that, some investigations have reported slower muscle fibre conduction velocities in DN subjects compared to individuals with non-neuropathic complications (Chisari et al., 2002; Meijer et al., 2008). Slower conduction velocities may result in longer delay from muscle activation to force production (EMD) and therefore it could be another factor that may contribute to the need in this population to develop an anticipatory mechanism. Few studies have reported differences in EMD due to gender (Winter & Brookes, 1991), type of muscle contraction (Cavanagh and Komi, 1979), movement velocity (Howatson et al., 2009), after ligament reconstruction (Kaneko et al., 2002), in response to a training programme (Grosset at al., 2009), and in cases of neuropathies (Granata et al., 2000). However, no investigation has assessed whether EMD is affected in patients with DN. Therefore, further studies investigating the rate of force production during gait as well as EMD in DN subjects are needed to determine whether an anticipatory muscular activation is a likely adaptive mechanism in this population.

Regardless of the exact mechanism by which DN subjects require an earlier activation of the TS muscle, it is clear that a premature activation of the TS may result in an earlier forefoot contact. This, in turn, may partly explain the abnormal forefoot PTI values observed in this population (Sacco et al., 2009; Turner et al., 2007). It should be noted that Abboud et al. (2000), Akashi et al. (2008) and Sacco & Amandi, (2003) failed to find differences in TS activation times between diabetic neuropathic and healthy subjects. However, activations times in these studies were defined as the time at which

peak activity was achieved, in contrast to Kwon et al. (2003) who defined activity onset as the moment in time when EMG activity raised 3 standard deviations above resting EMG. The results from these three studies (Abboud et al., 2000; Akashi et al., 2008; Sacco & Amandi, 2003) suggest that the moment in time at which peak TS activity is produced do not differ between healthy and DN subjects, however, no conclusion can be drawn in relation to whether TS activation is earlier in DN compared to healthy individuals.

Together with the TS, TA also plays a very important role during gait. TA is active at heel strike and is responsible for the impact reduction over the forefoot during the flat foot phase (Perry & Burnfield, 2010). EMG data shows a delayed activation of the TA muscle during the initial phase of the GC (Abboud et al., 2000; Sacco & Amadio, 2003). A later firing of the TA means that its normal modulating role in lowering the foot to the ground after heel strike through eccentric contraction is disturbed, and the result is that the foot reaches the flat stage quicker and in a less controlled manner. Although the exact reason why TA activation appears to be delayed in DN subjects is not clear, a few theories have been proposed. Following the lack of stability theory, an early contact with the ground by the forefoot could be a mechanism to enlarge the base of support quicker after the heel strike and therefore to increase stability during the initial phase of the GC (Courtemanche et al., 1996).

In addition to this, sensory neuropathy has been hypothesized to be responsible for those changes in activation patterns. The neuromuscular system generates responses according to the afferent sensory information caused by mechanical loads placed on the foot (Dingwell & Cavanagh, 2001). It has been suggested that due to the fact DN subjects have less sensitivity in the foot; the muscular and joint mechanisms responsible for the load attenuation are altered in these patients. As a consequence, a delayed muscular activation pattern during gait may be expected, especially in the initial contact, and mainly with respect to muscles related to the shock attenuation: dorsi-flexor muscles and knee extensors (Sacco & Amandi, 2003). In agreement with this theory, vastus lateralis (VL), that decelerates knee flexion during load reception, working as a shock absorber by transferring part of the impact to the thigh muscles mass (Perry & Burnfield, 2010), has also been reported to activate later in DN subjects compared to participants with no neuropathic complications (Abboud et al., 2000; Akashi et al.,

2008; Sacco & Amadio 2003). Sacco et al. (2010) carried out a study which supports this theory. In this study muscular activity patterns were compared between barefoot and shod gait in participants with diabetic neuropathy and healthy controls. They reported a delayed muscular activation pattern during shod gait in the healthy group when compared to the barefoot condition. These differences were only noticeable in the muscles responsible for the shock attenuation during the initial ground contact (TA and VL). Interestingly, the diabetic group kept the same pattern of VL activation during both conditions, whereas they had a delayed VL activation compared to the control group when walking barefoot. This may support the idea that a reduction in afferent information may alter muscular activation patterns. However, the fact that healthy individuals show different muscular activity patterns during in shoe and barefoot conditions does not necessarily prove that the lack of afferent information is responsible for those differences in activation patterns between healthy and DN subjects. Healthy individuals, having intact sensory information, can adapt to different conditions easier than DN subjects who perform tasks in a more automatic way. Therefore, since walking in shoes reduces the stress on the sole of the foot, the requirements for the TA and VL to work as shock absorbers are also reduced, which could have also altered the way these muscle were engaged during this part of the GC by the healthy group.

Although the exact mechanism behind this delayed activation of the TA is not clear, the result is that the foot reaches the foot flat stage in a less controlled manner, which may lead to increased pressure values and longer contact times of the metatarsal heads with the ground in this population compared to healthy individuals (Abboud et al., 2000). Moreover, Bevans (1992) justified the higher prevalence of ulcers in the forefoot of neuropathic patients by the delayed activation of the TA, which consequently may change the distribution of foot pressure beneath the metatarsal heads. However, it should be pointed out that some studies failed to find any delay in the activation of the TA at the beginning of the stance phase (Akashi et al., 2008). It is possible that the fact that these studies used different population groups may partly explain the difference in the results. For instance, Sacco & Amadio (2003) and Akashi et al. (2008) recruited DN subjects with no other previous foot complications whereas Kwon et al. (2003) studied DN subjects with history of ulcers and Abboud et al. (2000) included patients with diabetes mellitus who were not assessed for neuropathy. Another possible explanation for the discrepancies in the results is that muscular activation was not interpreted

consistently among all the studies. On the one hand, Sacco et al. (2003), Abboud et al. (2000) and Akashi et al. (2008) defined muscle activation as the time delay from heel strike to peak muscular activity. On the other hand, Kwon et al. (2003) defined activation onset as the EMG activity 3 standard deviations above resting EMG activity. Therefore, it is likely that methodological difference may explain the discrepancies in the results

Overall, studies investigating EMG patterns during gait in subjects with diabetic neuropathy are limited in number, and all of them have processed EMG data without considering EMD, which makes interpretation of the data difficult in relation to segment movement. However, it appears that DN subjects show different EMG activity patterns compared to healthy individuals and that those alterations in EMG patterns may lead to changes in kinetic and kinematic parameters. On the one hand, higher co-contraction, which has been associated with poor motor control, has been observed in DN subjects. This may partly explain some of the changes in the spatial-temporal characteristics (i.e. lower velocity) observed in this population. On the other hand, changes in the onset activation times of the TA and TS have been linked to changes in kinetic data. Thus, although the exact mechanisms underlying those changes are not understood, it appears that a delayed activation of the TA and an early activation of the TS may be partly responsible for the higher pressures on the forefoot (PP and PTI) shown by DN subjects. This early findings highlight the importance of investigating EMG data alongside kinetics and kinematics data to develop a more comprehensive understanding of walking patterns in individuals with DN. In addition to that, future studies using EMG must take into account EMD when relating EMG signal to muscle function. Furthermore, it appears very important to determine whether EMD values are different in DN subjects, which could have compromised the interpretation of the results presented above.

Evidence presented demonstrates that patients with DN present a variety of gait alterations compared to healthy individuals. Thus, DN subjects show changes in 1) gait parameters, in the form of spatial-temporal and floor-foot interaction characteristics (COP); 2) foot pressures, especially at the metatarsals region; and 3) muscle activation patterns. Although neuropathy, both sensory and motor neuropathy, has for decades been considered the main factor altering walking patterns and foot pressure in DN

subjects, there is growing evidence that changes in gait characteristics occur even before neuropathy. Thus, structural changes secondary to diabetes (hyperglycaemia) have been recently acknowledged to affect gait and foot loading patterns in this population. In addition to that, early evidence suggests that differences in EMG activity patterns during gait may also be partly responsible for the changes in gait characteristics observed in DN subjects. Moreover, changes in the onset activation times of the TA and TS have been linked to changes in kinetic data, which suggests that EMG data should be considered as a potential contributing factor for the higher foot pressures (PP and PTI) observed in DN subjects, especially in the forefoot.

Beside changes in gait biomechanics, vascular alterations in the microcirculation are also known to play an important role in the increased risk of foot ulcers observed in patients with DN. The next section will review alterations in the microcirculation in type 2 diabetic patients with and without neuropathy and their link to foot complication in this population.

2.2.2.2 Microcirculation

It has been postulated that abnormalities in foot microcirculation could play a significant role in the development of foot ulcers in diabetic neuropathic patients (Tooke et al., 2000). In fact, abnormalities in microcirculation are caused directly by diabetes and they are not secondary to impairments in macrocirculation as previously believed (Goldenberg et al., 1959; Tooke & Brash, 1995). In agreement with this theory, numerous investigations have reported impairments in microcirculation despite normal macrocirculation (Jorneskog et al., 1998; Kizu et al., 2003). Therefore, due to the primary role microvasculature plays in the development of foot ulcers in diabetic patients, this chapter will focus solely on the microcirculatory function.

This section will describe the main structural and functional abnormalities of microcirculation in the diabetic neuropathic foot and will assess the importance of these alterations in the pathogenesis of ulceration. The last section in this chapter will investigate the responses of the microcirculation to an exercise bout in diabetic patients.

Definition and regulation of microcirculation

The microcirculation is composed of a dense network of tiny vessels (diameter 5-10 um) known as capillaries. These capillaries are interposed between feeding arterioles and draining venules. The walls of these vessels consist of a single layer of endothelial cells; this is the site at which the vital functions of the circulatory system occur (i.e. transport and exchange of nutrients and hormones, clearance of waste products of metabolism, tissue defence and repair or maintenance of fluid homeostasis) (Tooke, 2004; Tortora & Derrickson, 2006).

Flow in the microvasculature is regulated by the relaxation and contraction of smooth muscle sphincters on the arterial side of the capillaries (Tuma et al., 2008). Pressure and flow in these vessels is controlled through alteration in vessel diameter. At a local level this is achieved by the balance of pre-post-capillary resistance under neurogenic control. An increase in pressure is believed to bring about a reflex pre-capillary vasoconstriction via the local sympathetic axon (venoarteriolar reflex) (Tortora & Derrickson, 2006; Tuma et al., 2008). The purpose of this mechanism is to protect the capillaries from excessive hydrostatic pressure on standing (Korzon-Burakowska & Edmonds, 2006). An impaired venoarteriolar reflex leads to hyperperfusion on dependency, increases venous pressure, and contributes to a reduction in skin capillary flow, increased fluid filtration, and oedema (Korzon-Burakowska & Edmonds, 2006).

In many tissues the simultaneous accommodation and control of multiple organic functions lead to the development of another category of microvessels, called shunts, which specialize in the regulation of blood flow between compartments. When blood pressure is reduced, flow in the shunts is reduced, while flow in the preferential channel (nutritional capillaries) is maintained due to its lower hydraulic resistance. However, when the nutritional capillaries have an increased resistance, much of this flow is probably diverted through the shunts, a path of lesser resistance, which “steals” the needed blood flow (Korzon-Burakowska & Edmonds, 2006; Tooke & Brash, 1995). It is generally believed that a common denominator of some of the major complications in diabetic patients is the pathological regulation of these shunts, leading to a maldistribution of blood flow between nutritional and non-nutritional microvessels (Watkins & Edmonds, 1983).

Structural Changes

While there are no occlusive lesions in the diabetic microcirculation, as generally believed in the early days (Goldenberg et al., 1959), structural changes do exist, most notably, thickening of the basement membrane (Raskin et al., 1983; Tooke, 2000). The capillary basement membrane is thicker in diabetic patients and these alterations are more pronounced in the legs, most likely due to the higher hydrostatic pressure in this part of the body (Ward, 1997). These changes decrease the elastic properties of the capillary vessel walls and therefore limit vasodilatation capacity (Tooke, 1995). The basement membrane thickening may also act as a barrier to the normal exchange of nutrients and cellular migration, decreasing the ability of the diabetic foot to fight infection (Schramm et al., 2006).

The first step in the development of capillary basement membrane thickening is increased hydrostatic pressure in the microcirculation. High hydrostatic pressure is thought to trigger an inflammatory response in the microvascular endothelium, which, over time may result in basement membrane thickening (Schramm et al., 2006). The association between elevated pressure and basement membrane thickening is supported by the fact that capillary basement is increased in the legs, where hydrostatic pressure is highest in the upright posture (Tooke, 2004). Metabolic control has also been linked to structural changes. Raskin et al. (1983) reported a correlation between the extent of basement membrane thickening and the level of glycaemic control, with poorly controlled diabetic patients showing more basement membrane thickening than well-controlled diabetic patients.

Functional changes

Despite these structural changes, it is currently understood that the most important changes in the microcirculation are functional. Over the last decade, abnormalities in microcirculation have been reported in neuropathic patients both at rest as well as when responding to vasodilatory stimulus.

Resting blood flow

Resting peripheral blood flow has been demonstrated not to be diminished in neuropathic patients when compared to non-diabetes individuals. Furthermore, foot blood flow has been shown to be either increased (Flynn et al., 1988; Tooke & Brash, 1995; Urbancic-Rovan et al., 2004) or unchanged (Schramm et al., 2006) in diabetic neuropathic patients when compared to healthy individuals. Although such findings suggest no reduction in blood flow to the foot, capillary ischemia can be present, which may compromise nutrient delivery to the tissues. Microcirculation of the lower limb is rich in arteriovenous shunts. The arteriovenous shunts are innervated by sympathetic nerves; therefore, the presence of diabetic neuropathy with sympathetic denervation may lead to the opening of these shunts with blood flow bypassing the skin capillaries, the so-called capillary steal syndrome (Korzon-Burakowska & Edmonds, 2006; Schramm et al., 2006). In support of this theory, studies with nail fold capillaroscopy have shown reduced capillary nutritional flow in neuropathic patients compared to healthy controls (Jorneskog et al., 1998; Nabuurs-Franssen et al., 2002). In addition several studies have reported an increased skin temperature in this population, which therefore increases metabolic demand (Flynn et al., 1988; Nabuurs-Franssen et al., 2002). This increase in metabolic demand will further amplify the potential problems originating from the reduction in nutritive capillary flow in diabetic neuropathic patients.

Vasodilatory responses

The most critical functional changes in diabetic patients with and without neuropathic complications occur in response to tissue injury. Maximal vasodilatation has been observed to be impaired in diabetic patients under conditions of stress (Kingwell et al., 2003; Petrofsky et al., 2005b). This defect is known to play a crucial role in the wound healing process (Hile & Veves, 2003). Commonly, ulceration is precipitated by relatively minor trauma, which, in the presence of sensory neuropathy is unattended. A lack of adequate blood supply at this critical phase contributes to the risk of significant infection and hence, further tissue breakdown.

Vasodilatory abnormalities in diabetic patients are mainly related to the dysfunction of the endothelial cells and vascular smooth cells of the arterioles, as well as to impairments of the nerve-axon reflex (Hile & Veves, 2003; Schramm et al., 2006). Functional alterations in the microcirculation are also reported in response to exercise stress, which are believed to the responsible for the reduced exercise capacity observed in patients with type 2 diabetes. These changes will be reviewed in more detail next.

2.2.2.2.1 Endothelial dysfunction

Vascular endothelium plays an important role in controlling the microvascular tone by synthesizing and releasing substances that modulate the vasomotor tone and regulate blood flow. NO is the most important vasodilator substance in endothelium-dependent vasodilatation. After its production and secretion from the endothelium, it diffuses to the adjacent smooth muscle cells (and stimulates the guanylate cyclase enzyme), which leads to smooth muscle relaxation and vasodilatation (Palmer et al., 1988; Tortora & Derrickson, 2006).

Endothelial dependent vasodilatation

The majority of studies agree that endothelium-dependent vasodilatation is impaired in diabetes, regardless of the presence or absence of neuropathy (Kingwell et al., 2003; Pitei et al., 1997). Furthermore, it has been demonstrated that changes in the endothelial function precede the development of diabetes and are present at the prediabetic stage (Tooke, 2000). It has been postulated that endothelial changes may be associated with reduced insulin sensitivity, which commonly precedes type 2 diabetes (Tooke, 2000). To further validate this theory, impaired microvascular vasodilatory capacity has been reported in individuals with impaired fasting glucose (Jaap et al., 1994). Furthermore, Jaap et al. (1997) found a correlation between vasodilatory impairments in microcirculation and the level of insulin sensitivity in pre-diabetic subjects.
It has been recently proposed that oxygen derived radicals, which in excess produce oxidative stress, inactivate endothelium-derived NO affecting the endothelium-dependent function (Schramm et al., 2006; Taddei et al., 1998). Evidence on the association between oxidative stress and vascular dysfunction come from experimental

studies using antioxidants administration. Vitamin C, an effective antioxidant, has been found to improve endothelial function in normal individuals with high insulin levels (Arcaro et al., 2002), hypertensive subjects (Taddei et al., 1998) as well as in diabetic patients (Ting et al., 1996). It should be pointed out that oxidative stress is aggravated by hyperglycaemia, hypertension and/ or hyperinsulinemia (Tooke et al., 2000). This may partly explain why changes in the endothelial function are present at the prediabetic stage.

However, it is important to consider that some studies failed to report microcirculatory impairments in the form of reduced endothelium-dependent vasodilatation in diabetic patients without neuropathy (Pfutzner et al., 2001; Veves et al., 1998). In both studies, a large number of the non-neuropathic subjects had type 1 diabetes of long duration and were therefore less likely to have microvascular complications. Oxidative stress, which results in changes in the endothelial function (as mentioned above), is known to be aggravated by hyperglycaemia, hypertension and/or hyperinsulinemia (Tooke et al., 2000). These metabolic conditions are commonly associated with type 2 diabetes and not necessarily with type 1 diabetes, which could partly explain why individuals with type 2 diabetes are more likely to suffer from endothelial dysfunction (Tooke, 2000).

Endothelial independent vasodilatation

Endothelium-independent vasodilatation reflects the function of the vascular smooth muscle cell. Smooth muscle vasodilatation is stimulated by direct donors of NO (such as sodium nitropusside, which is normally administrated transdermally using a iontophoresis technique), which act directly on the smooth muscle cell, independent of the endothelium (Dinh & Veves, 2004). There is substantial evidence to suggest that neuropathic patients show abnormalities in the endothelial independent vasodilatation (Pitei et al., 1997; Veves et al., 1998). However, conflicting results have been reported regarding whether abnormalities in smooth muscle vasodilatation are present before neuropathy develops. Although early studies demonstrated impairment of endothelium-independent vasodilatation in non-neuropathic patients (Furchgott & Zawadzki, 1980), subsequent work failed to find such abnormalities (Kingwell et al., 2003; Veves et al., 1998). It has therefore been suggested that dysfunction at the smooth muscle level might be associated specifically with neuropathy (Pitei et al., 1997). However, the exact

mechanism explaining this association between smooth muscle function and neuropathy is still unclear.

2.2.2.2.2 Neurogenic factors

The ability to increase blood flow depends on the existence of a normal neurogenic vascular response referred to as Lewis' triple flare response. In healthy subjects, skin trauma results in stimulation of the C nociceptive nerve fibres leading to antidromic stimulation (nerve conduction in the opposite direction, therefore along the axon away from axon terminal and towards the soma) of the adjacent C-fibres, which secrete vasodilators and cause increased local blood flow in injured tissues and thereby promote healing (Dinh & Veve, 2004; Vinik et al., 2001). This response is typically equal to one third of the maximal vasodilatory capacity (Schramm et al., 2006).

Neurogenic vascular responses have been shown to be impaired in the feet of neuropathic patients, which can predispose a significant reduction in blood flow under stressful conditions (Pfutzner et al., 2001; Veves et al., 1998). Thus, the diabetic neuropathic foot fails to respond to injury and infection in the usual manner, which may explain the lack of hyperaemia observed in the infected or injured diabetic foot (Dinh & Veves, 2004). It has been suggested that the impaired flare response observed in neuropathic patients may be related to both impaired C-nociceptive fibre function and impaired ability of the microvasculature to response to vasomodulators (i.e. NO) secreted by these fibres (Vinik et al., 2001).

Neurogenic vascular response impairments are usually associated with vasodilatory dysfunction in DN patients. However, vasodilatory responses to local warming, which are largely controlled by small C fibre nociceptors, have also been reported to be diminished in non-neuropathic individuals (Colberg et al., 2005). Nevertheless, more evidence is required to understand better the way diabetes may affect neurogenic vascular responses.

Overall, vasodilatory abnormalities in DN patients are mainly related to the dysfunction of the endothelial (endothelial dependent vasodilatation) and vascular smooth cells of

the arterioles (endothelial independent vasodilatation), as well as to impairments of the nerve-axon reflex (neurogenic responses). It appears that alterations in the endothelial cell function are associated with type 2 diabetes, whereas changes in the endothelial independent vasodilatation and in the nerve-axon reflex are related to neuropathy. In addition to this, functional alterations in the microcirculation have also been reported in response to exercise stress, which are believed to be responsible for the reduced exercise capacity observed in patients with type 2 diabetes. The next section will review microcirculatory responses to an exercise bout in subjects with type 2 diabetes.

2.2.2.2.3 Microcirculatory responses to an exercise bout

Patients with diabetes have shown a 20-30 % reduction in peak aerobic capacity (Ozdirenç et al., 2003; Regensteiner et al., 1998), which may limit their daily walking activities and functional capacity (Baldi et al., 2003; Ozdirenç et al., 2003), especially in sedentary elderly people with low fitness levels. Martin et al. (1995) reported that when exercising during 40 minutes on a bicycle ergometer at the same sub-maximal intensity (60% of maximal oxygen uptake) patients with type 2 diabetes (N=8) showed higher lactate values (determined in whole blood) compared to healthy individuals (N=7). This demonstrates that the aerobic capacity in this population is impaired. In line with this finding, Scheuermann-Freestone et al. (2003), who assessed blood acidity levels (pH levels) throughout a fatiguing exercise bout reported a faster decrease in pH (more acidity due to lactate acid accumulation) during exercise in the group of subjects with type 2 diabetes (N= 21) compared to the healthy group (N=15). They also demonstrated that all subjects (diabetic and healthy) fatigued at the same acidic pH. These findings prove that impairment in the aerobic capacity may be responsible for the reduced exercise capacity observed in diabetic patients.

Some investigations have demonstrated that reduced circulatory responses to exercise in diabetic patients are present even in the absence of peripheral arterial disease (Mohler et al., 2006; Petrofsky et al., 2005b). This suggests that vascular impairment may originate via changes in microcirculation. In line with this idea, Baldi et al. (2003) demonstrated that maximal oxygen consumption (VO_{2max}) in a group of 11 diabetic patients is mostly limited by abnormalities in the peripheral oxygen (O_2) extraction (microcirculation) and

not by cardiac output abnormalities. Cardiac output and arteriovenous differences in this study were estimated noninvasively during cycling by the carbon dioxide re-breathing equilibration method using the Fick equation. It should be noted that although microcirculatory abnormalities are believed to be a crucial contributor to the reduced exercise capacity observed in diabetic patients, other abnormalities, secondary to diabetes, such as autonomic neuropathy (Petrofsky et al., 2005b; Saltin et al., 1998) or peripheral arterial diseases (Adler et al., 1999, Boyko et al., 1997) may also contribute to this limitation. However, due to the primary role microvasculature plays both in reduced exercise capacity and in the development of ulcers in diabetic patients, this chapter will focus solely on the microcirculatory responses to exercise. It should be noted that all the evidence included in this section has been acquired from diabetic patients without neuropathy since no investigation has been carried out assessing microcirculatory responses to an exercise bout on DN subjects. Although similar alterations in the microcirculation may be expected from both populations, it is likely that DN patients have more diminished circulatory responses to exercise compared to non-neuropathic individuals. Therefore, the results presented might underestimate the microcirculatory responses to exercise in DN subjects. Moreover, this highlights the importance to investigate this issue in DN patients.

2.2.2.2.3.1 Blood flow during exercise

Investigations assessing acute circulatory responses to exercise in diabetic patients have reported that the increase in exercise-induced blood flow was reduced in diabetic patients compared to healthy individuals (Kingwell et al., 2003; Mohler et al., 2006; Petrofsky et al., 2005b; Pichler et al., 2004). These findings have shown consistency regardless of the method used and/or the vascular bed investigated (microvasculature and macrovasculature). Thus, diminished vascular responses to exercise have been reported when investigating total limb blood flow as measured by Plethysmography (Petrofsky et al., 2005b) and local muscle blood flow as measured by near infrared spectroscopy (NIRS) (Mohler et al., 2006; Pichler et al., 2004). It is noteworthy that plethysmography and NIRS are the most commonly used techniques to measure blood flow during exercise.

Volume plethysmography measures changes in whole limb volume. An arterial occlusion cuff is placed around the distal part of the limb (wrist and ankle for upper limb and lower limb measurements, respectively) and a venous cuff on the proximal part of the limb (upper arm or upper leg for upper limb and lower limb measurements respectively). During flow measurements the change in arm size due to arterial flow is transduced to an electrical output to determine total limb blood flow (Whitney, 1953).

Skeletal muscles form approximately 40% of the total body mass, thus representing an important percentage of cardiac output and total oxygen consumption even at rest. Under working conditions, muscle blood flow can increase to 80-85% of cardiac output and VO_2 can exceed the resting value by 50-fold (McArdle et al., 2000; Tortora & Derrickson, 2006). Therefore, NIRS is becoming a widely used research instrument to measure muscle haemodynamics during exercise bouts (van Beekvelt et al., 2001a; Boushel et al., 2000a). NIRS is based on the relative transparency of tissue to light in the near- infrared region, and on the oxygen- dependent absorption changes of haemoglobin and myoglobin. NIRS enables non-invasive continuous measurement of changes in the concentration of oxygenated haemoglobin (HbO) and deoxygenated haemoglobin (HbdO). The sum of HbO and HbdO concentrations reflects the total amount of haemoglobin (tHb), and changes in tHb can be interpreted as changes in blood volume in the tissue. In addition, this technique allows quantifications not only of blood flow but also oxygen consumption in the muscle (van Beekvelt et al., 2001a). Therefore, NIRS provides valuable information about overall muscular function, in the form of oxygen delivery and consumption.

Although circulatory responses to exercise are well known to be altered in diabetic patients, the exact mechanisms controlling exercise hyperaemia are still unclear. Early investigations found that NO blockade, which stops NO production, diminished blood flow at rest and during recovery, but it had no effect during exercise (Shoemaker et al., 1997). It was therefore concluded that other mechanisms instead of endothelium-related responses, were more likely to control blood flow during exercise. Thus, Saltin et al. (1998) in a comprehensive review of the literature suggested that an elevation in muscle sympathetic nerve activity, secondary to the muscle contraction, was likely to have an important functional role in controlling blood flow during exercises. In conflict with the findings shown above, Boushel et al. (2002) demonstrated that a combined

inhibition of NO and prostaglandins, which are considered the most important endothelium-derived vasodilator substances, reduced muscle blood flow during exercise in healthy individuals (up to 50%). Contrary to the study from Shoemaker et al. (1997), in which only NO production was blocked, Boushel et al. (2002) inhibited NO as well as prostaglandins production. This suggests that compensatory responses may result to ensure flow matches metabolic demand when only one vasodilator substance is inhibited. In line with this, Kingwell et al. (2003) reported the first study providing evidence that impaired endothelium-dependent vasodilatory responses in patients with type 2 diabetes limits blood flow during exercise. They measured leg blood flow responses in the right femoral venous blood flow by constant-rate infusion of cold saline (thermodilution). Thus, responses to 1) intrafemoral arterial infusions of acetylcholine (endothelium-dependent vasodilator); and 2) to a standardized 25-min cycling bout at 60% VO_{2max} were measured in 9 males with type 2 diabetes and 9 matched controls. They found that a reduced blood flow response to exercise was significantly correlated ($r=0.54$) with a reduced blood flow response to acetylcholine. It is worth mentioning that they also investigated endothelium independent vasodilatation by the injection of sodium nitroprusside and no relationship between smooth cell function and blood flow response to exercise was found. These results suggest that abnormalities in the endothelial function may be responsible for the reduced circulatory responses during exercise in individuals with type 2 diabetes.

Although more evidence supporting these initial findings is needed, abnormalities in endothelial function may be a potential mechanism responsible for the diminished circulatory responses to exercise observed in diabetic patients. However, it is likely that other abnormalities related to diabetes, such as impairments in the autonomic nervous system (ANS) (Petrofsky et al., 2005b; Saltin et al., 1998) or peripheral arterial diseases (Adler et al., 1999; Boyko et al., 1997) may also contribute to this limitation in subjects with type 2 diabetes.

2.2.2.2.3.2 Oxygen uptake during exercise

It is widely accepted that the reduced exercise capacity observed in diabetic patients reflects a reduced delivery rate of oxygen through a restricted circulation to the muscle

mitochondria. However, diabetic patients have increased oxidative stress as well as a spectrum of muscle metabolic abnormalities that may have implications in the mitochondrial function (Scheuermann-Freestone et al., 2003; Sivitz, 2010).

Submaximal oxygen consumption has been reported to be lower in diabetic patients compared to healthy individuals during identical submaximal workloads (Baldi et al., 2003; Regensteiner et al., 1998). In addition to that, Baldi and colleagues (2003) demonstrated that arteriovenous oxygen differences, which assess the muscular oxidative capacity, were also reduced in type 2 diabetic subjects compared to healthy controls when working at maximal (100% VO_{2max}) as well as submaximal intensities (70% VO_{2max}). They concluded that a reduction in the oxidative capacity of the skeletal muscle due to muscle metabolic abnormalities, may be responsible for less O_2 being consumed by working muscles during the exercise bouts. In line with these findings, Martin et al. (1995) found that when exercising at the same sub-maximal intensity (60% VO_{2max}), the total leg oxygen uptake was similar between groups while lactate production was higher in diabetic patients compared to healthy counterparts. This finding suggests impairments in the ability to use oxygen efficiently in the diabetic groups.

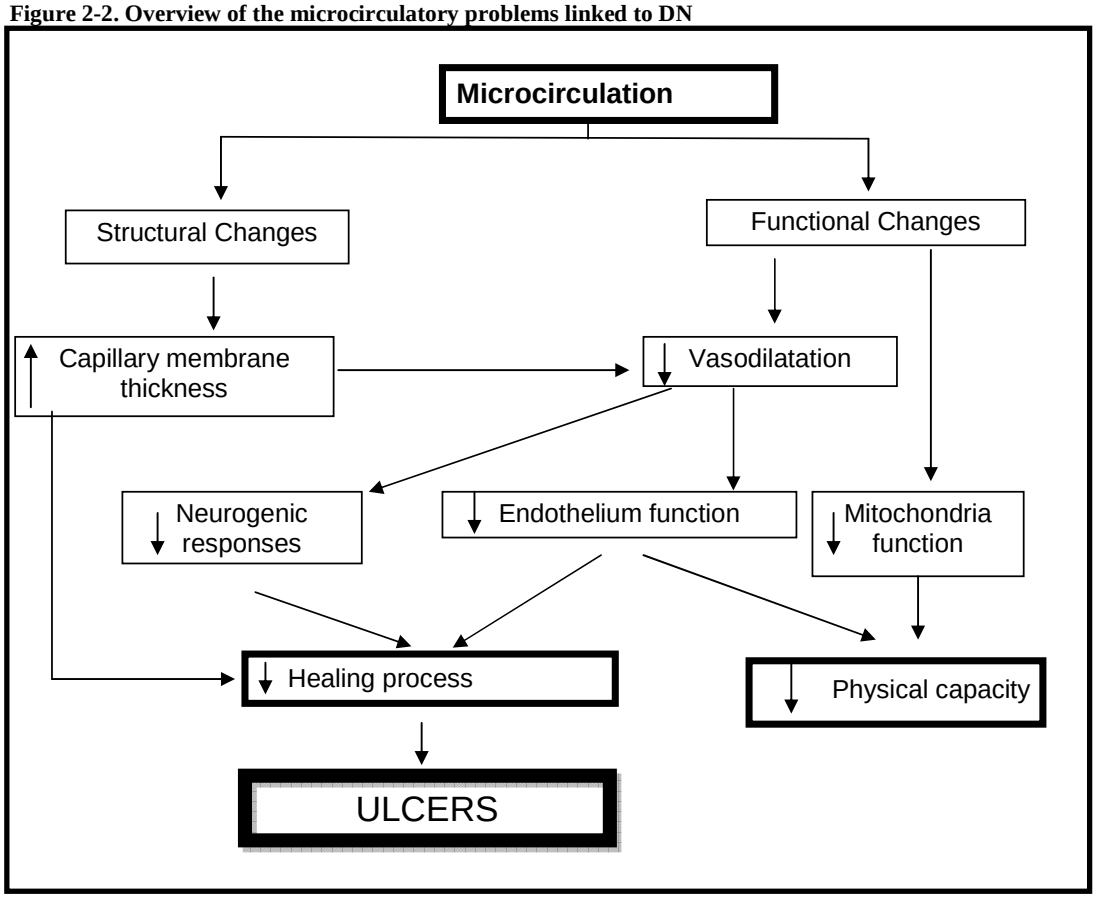
Contrary to the findings shown above, Bauer et al. (2007) reported that the diminished aerobic capacity in diabetic patients is due to abnormalities in the microvascular blood flow. They measured systemic oxygen uptake (breath to breath gas analyzer) and muscular oxygen uptake (NIRS) simultaneously during a submaximal workout (85% of the individual's estimated lactate threshold) and they found that oxygen uptake (breath to breath gas analyzer) was reduced after the onset of exercise while muscle deoxygenation was unchanged in diabetic patients compared to healthy controls. They therefore concluded that the limitation of oxygen uptake during submaximal exercise in type 2 diabetes may be related to impaired control or maldistribution of muscle blood flow. In agreement with this, Mohler et al. (2006) did not find differences in exercise-induced muscular deoxygenation, measured with NIRS, when comparing healthy and diabetic individuals.

There is some evidence that metabolic abnormalities such as increased type II to type I fibre ratio (Marin et al., 1994) and lower oxidative enzyme activity (Kelley et al., 2002)

may be present in the diabetic skeletal muscle. However, it is unclear whether oxygen uptake responses are affected in this population. To date, only a few studies have investigated muscular oxygen uptake during exercise in diabetic patients and more studies are necessary to better understand whether and to what extent muscular oxidative capacity is impaired in this population.

In summary, it looks clear that patients with type 2 diabetes show alterations in the microcirculatory responses to exercise stress. However, it is still unclear whether those abnormalities are solely related to exercise-induced vasodilatory responses or whether muscular oxidative capacity is also impaired in this population. It should be highlighted that all the studies presented in this section have been carried out on patients without neuropathy. The magnitude of microcirculatory impairments in response to an exercise bout is therefore unknown in DN subjects. Future investigations are warranted to investigate microcirculatory responses to exercise in this group of patients.

Figure 2-2. Overview of the microcirculatory problems linked to DN



Overall, it is well established what the association is between microcirculatory impairments and risk of foot ulceration in individuals with diabetes mellitus, especially in DN individuals (see Figure 2-2). Thus, diabetes carries a complex set of changes in the microcirculation, including structural and functional changes. Despite structural changes, such as the development of capillary basement membrane thickening, it is currently understood that the most important changes in the microcirculation are functional. Vasodilatation has been observed to be impaired in diabetic patients with and without neuropathic complications under conditions of stress (Kingwell et al., 2003; Petrofsky et al., 2005b) and this defect is known to play a crucial role in the wound healing process. Vasodilatory abnormalities in DN patients are mainly related to the dysfunction of the endothelium (endothelial dependent vasodilatation) and vascular smooth cells of the arterioles (endothelial independent vasodilatation), as well as to impairments of the nerve-axon reflex (neurogenic responses). On the one hand, alterations in the endothelial cells function are associated with metabolic impairments (reduced insulin sensitivity, hypertension and hyperinsulinemi), which commonly precede type 2 diabetes. On the other hand, alterations in the endothelial independent vasodilatation and in the nerve-axon reflex are thought to be related to neuropathy. This suggests that microcirculatory problems in type 2 diabetic patients are worsened by neuropathy, which explains, in conjunction with gait alterations, why this population is approximately 10 times more likely to develop foot ulcers compared to non-neuropathic subjects (McGill et al., 2005).

In addition to this, functional changes have also been observed in response to exercise stress. Patients with type 2 diabetes show a diminished exercise-induced vasodilatory capacity compared to healthy individuals, and preliminary evidence suggests that changes in the endothelial function may be partly responsible for these alterations. It is believed that these modifications are associated with the reduced exercise capacity observed in subjects with type 2 diabetes (Ozdirenç et al., 2003; Regensteiner et al., 1998). However, there is an ongoing debate about whether the diminished exercise capacity observed in diabetic patients is caused by solely a reduction in oxygen delivery or in combination with a reduction in the muscular oxidative capacity. It should be noted that all the evidence included in this section has been acquired from diabetic patients without neuropathy since no investigation has been carried out assessing

microcirculatory responses to an exercise bout on DN subjects. This highlights the importance to investigate this issue in DN patients.

Foot ulcerations are well established as a major health problem in individuals with diabetic neuropathy. Commonly, ulceration is precipitated by repetitive and/or excessive pressure on the surface of the insensitive skin during gait activities. A lack of adequate blood supply at this critical phase contributes to the risk of significant infection and hence, further tissue breakdown (van Schie, 2005; Schramm et al., 2006). Thus, the previous two sections have investigated gait and microvascular alterations in individuals with diabetes neuropathy and their association with foot problems.

Beside the foot complications associated with diabetes neuropathy, increasing evidence suggest that diabetes in general and DN in particular impact everyday living and consequently diminish health related QOL in this population (Price & Harding, 2000). Thus, QOL measurements are increasingly being used to assess the impact of a given health state on functioning in three key areas: physical functioning, social functioning and psychological well-being, which reflects the World Health Organization definition of health (WHO, 1959). The next section will discuss the impact of diabetes and DN on individual's quality of life.

2.2.2.3 Quality of life

Although glycaemic control, the development of foot complications and mortality represent critical outcomes in people with type 2 diabetes, health-related QOL outcomes are also important (ACSM, 2000). The value of optimizing QOL is increasingly recognised as it represents an important goal for health care. Also, importantly, associations between poor QOL and adverse outcomes in people with type 2 diabetes, including poor response to therapy, disease progression and even mortality have been well documented (Ali et al., 2010; Kleefstra et al., 2008). Landman et al. (2010) reported that lower physical and mental QOL were associated with a higher total mortality and cardiovascular mortality in patients with type 2 diabetes regardless of confounders such as age and sex. It has been therefore hypothesized that QOL instruments may become an increasingly important clinical tool to not only identify

those patients with a low QOL but also to identify those patients with an associated increase mortality risk (Kleefstra et al., 2008).

The adverse effects of diabetes on health-related quality of life are well established in the literature (Luscombe et al., 2000; Price & Harding, 2000). Furthermore, Lloyd et al. (2001) assessed the effect of diabetic complications (i.e. neuropathy, retinopathy, and nephropathy) on QOL in 1,233 patients with type 2 diabetes. It was found that even mild complications can have a profound effect on the patient's perceived QOL, as recorded using the SF-36 questionnaire. They also reported that biochemical markers of the disease (i.e. blood pressure or glycaemic control) did not appear to directly affect QOL. This disagrees with other studies that found a direct relationship between blood glucose and QOL in hyperglycaemic subjects (Wikblad et al., 1996). Currie et al. (2006) demonstrated that the severity of diabetic peripheral neuropathy symptoms was predictive of poor health-related utility and decreased quality of life. Moreover, they also related the effect of neuropathic symptoms such as burning or numbness (as measured with the Neuropathic Total Symptom Score) to different dimensions of health-related QOL (as measured with the SF-36 questionnaire). These findings suggest that there may be specific symptoms associated with diabetes as well as biochemical markers of the disease that may have a direct effect on QOL. Although these early findings represent a step forward in the understanding of the association between diabetes and reduced QOL, more studies are needed to understand the relationship between physiological and psychological markers**.**

Even though diabetic foot problems are the most common cause of hospitalization for patients with diabetes (Boulton, 1997), the QOL aspect of the diabetic foot has received little attention in the literature. Price & Harding (2000) published a comprehensive review of the literature in which they investigated the impact of foot complications secondary to diabetes on QOL. They concluded that there is a relationship between number of and severity of complications and QOL, and that the everyday experiences of patients with foot ulceration may be even poorer than for those with amputations. It has been hypothesized that limitations on physical functioning and mobility secondary to foot ulceration can reduce self-reported health-related quality of life in these subjects and may explain why QOL in patients with foot ulceration is worse than in mobile diabetic amputees (Price, 2004). A study carried out by Brod (1998) highlights the

impact of foot complications on everyday living for both the patients with ulceration and their carers. This study of 14 patients and 11 caregivers reported that reduction in social activities, increased family tensions, lost time from work, and a negative impact on general health were experienced by both groups.

In summary, QOL represents an important goal for health care professionals and it has been associated with adverse outcomes in people with type 2 diabetes, including poor response to therapy, disease progression and even mortality. It is well established that diabetes is associated with poor QOL. Furthermore, it appears that there is a positive correlation between diabetes and its complications and QOL, with subjects with more severe complications showing the poorest QOL. Interestingly, early evidence suggests that QOL could be associated with biochemical markers; however, more studies are needed to understand the relationship between physiological and psychological markers**.**

In conclusion, this part of the literature review has demonstrated that type 2 diabetes and peripheral neuropathy are associated with a complex set of complications including increased risk of cardiovascular problems (mainly due to metabolic abnormalities linked to type 2 diabetes), increased risk of foot problems, (partly due to gait alterations and partly due to microcirculatory problems) and reduced QOL. The next section will provide a critical review of the potential effect of PA programmes on the primary pathologies linked to DN.

2.3 PART 2: Physical activity, type 2 diabetes and peripheral neuropathy

For decades, exercise has been considered a cornerstone of a healthy lifestyle. It is well documented that PA reduces hyperglycaemia, insulin resistance, hypertension and dyslipidemias and provides a protective effect against cardiovascular diseases and death in healthy individuals (ACSM, 2000; McArdle et al., 2000). Moreover, it is becoming increasingly clear that the growing prevalence of type 2 diabetes is associated with decreasing levels of activity and an increasing prevalence of obesity. Yates et al. (2007) demonstrated in a systematic review, that included 4 studies, that the risk of diabetes was reduced by approximately 50% (range 42-63%) in individuals who were encouraged to reduce their body mass through changes in diet and PA. However, Yates and colleagues failed to demonstrate the effect of exercise independent of other factors on the risk of diabetes in individuals with impaired glucose tolerance and they concluded that weight loss may largely explain those results. In type 2 diabetes, the promotion of weight loss is one mechanism through which exercise may be beneficial since obesity, especially abdominal obesity, is associated with metabolic abnormalities in diabetes (Kahn et al., 2006; Kelley & Goodpaster, 2001). Thomas et al. (2006) carried out a systematic review with 14 studies to explore the independent effect of exercise in people with type 2 diabetes. They reported a clinically significant improvement in glycaemic control in the exercise groups compared to controls. Interestingly, they found that improvements in glycaemic control were achieved over a range of exercise intensities as well as types of exercises (aerobic exercises, strength training and combination of aerobic and strengthening exercises). It was also stated that the beneficial effect of exercise on glycosylated haemoglobin (HbA_{1c}) was independent of body mass. This finding is supported by Jeon et al. (2007) who, in his systematic review, found that the inverse association between PA and type 2 diabetes persisted after adjusting for body mass index (BMI). This proves PA as an independent factor to reduce type 2 diabetes prevalence even in the absence of weight loss.

In addition to this, there is mounting evidence that physical activity is associated with significantly lower cardiovascular risk and overall mortality in healthy individuals (ACSMS, 2000) as well as in type 2 diabetes (Wei et al., 2000). It is generally believed

that this protective effect of PA against cardiovascular diseases and mortality may be partly produced by positive changes in metabolic syndrome (blood pressure, dyslipidemia) (ACSMS, 2000). It is, therefore, becoming increasingly clear that physical activity may be a therapeutic tool in a variety of patients with, or at risk of diabetes. For this reason exercise should be considered an important component of diabetes treatment.

Current physical activity recommendations for diabetic patients include aerobic exercises as well as resistance exercises. A consensus statement from the American Diabetes Association (Sigal et al., 2006) recommends: at least 150 $min \cdot week^{-1}$ of moderate intensity aerobic physical activity; and/or at least 90 $min \cdot week^{-1}$ of vigorous aerobic exercise and 3 sessions a week of resistance training. The resistance training should target 3 sets of 8-10 repetitions at a weight that cannot be lifted more than these repetitions. However, the separate effect of each type of exercise in health-related outcome measures has not been widely investigated in diabetic patients. Recent review papers have evaluated the role of physical activity in potentially enhancing health in type 2 diabetes (American Diabetes Association, 2010; Thomas et al., 2006); however, those reviews included studies that used aerobic training, resistance training or/ and a combination of both; whereas the particular effect of each mode of exercise was neglected. Different types of exercise will trigger different physiological adaptations; therefore it is important to understand the potential benefits that each type of exercise may have on diabetic patients so better and more efficient rehabilitation programmes can be put into place.

The vast majority of studies have investigated the association between physical activity and health problems commonly linked to type 2 diabetes (i.e. glycaemic control and cardiovascular risk factors), whereas the relationship between PA and the health problems associated with peripheral neuropathy (i.e. the risk of foot ulcerations or poor QOL) have received minimal attention. For this reason, this review of the literature will attempt for the first time to gather evidence on the potential effect of PA interventions on the whole range of pathologies linked to DN. This section will therefore include two subsections. Subsection 1 will review the relationship between different types of exercises programmes (aerobic, anaerobic or combination of both) and pathologies linked to type 2 diabetes (glucose control, blood pressure and cholesterol levels).

Subsection 2 will review the relationship between different types of exercise programmes (aerobic, anaerobic or combination of both) and pathologies related to DN (gait, microcirculation and QOL).

It should be noted that almost all the studies investigating PA interventions in type 2 diabetic patients have been carried out in individuals with no neuropathic complications. In fact, after a comprehensive search only 1 study was found in which an exercise programme was carried out on subjects with DN (Allet et al., 2010). For this reason the majority of studies presented in this section were carried out with type 2 diabetic patients with no neuropathic complications.

2.3.1 Physical activity and type 2 diabetes

The traditional management of type 2 diabetes focuses on lifestyle interventions, lowering cardiovascular risk factors (metabolic syndrome), and maintaining blood glucose levels within the normal range. Therefore, evidence increasingly supports the beneficial effect of PA programmes on the management of glycaemic control and cardiovascular risk factor both in healthy individuals (Yates et al., 2007) as well as in type 2 diabetes patients (Thomas et al., 2006). However, evidence from PA programmes include aerobic training, strength training and a combination of both, while the separate effect of each mode of exercise have not been fully investigated (Sigal et al., 2006; Thomas et al., 2006). In the next section, evidence will be presented on the effect of different types of exercise on glycaemic control and traditional risk factors.

2.3.1.1 Glycaemic control

Thomas et al. (2006) carried out a meta-analysis in which 13 studies assessing the effect of an exercise programme on glycaemic control were included. They reported a clinically significant reduction in glycated haemoglobin of 0.62% when the exercise group (N=185) was compared to the non exercise group (N=176). An absolute decrease of 1% in HbA_{1c} levels has been associated with a 15% to 20 % decrease in major cardiovascular disease events (Selvin et al., 2004). Thus, the observed reduction of 0.62% HbA_{1c} levels might be expected to produce a 9.3% reduction in cardiovascular

disease risk. It should be pointed out that these risk reduction estimates are likely to be conservative because they are derived from medication studies and do not take into account other risk factors associated with exercising, such as improvements in cardio-respiratory fitness and strength, or reductions in fat mass and waist circumferences (Selvin et al., 2004). Unfortunately, this powerful review of the literature did not distinguish between different modes of exercising.

The positive effect of physical activity programmes to lower HbA_{1c} levels have been reported for aerobic training (Raz et al., 1997; Ronnema et al., 1986; Yeater et al., 1990), strength training (Castaneda et al., 2002; Dunstan et al., 2006) and combined aerobic and resistance training (Loimaala et al., 2003, Maiorana, et al., 2001). However, it is impossible to compare the results from different investigations due to methodological differences such as training intensity [ranging from low intensity (Yeater et al., 1990) to high intensity (Ronnemaa et al., 1986)]; duration of the intervention [ranging from 2 months (Yeater at al., 1990) to 14 months (Dunstan et al., 2006)]; or baseline HbA_{1c} levels [ranging from 7.8% (Dunstan et al., 2006) to 12.9% (Raz et al., 1997)].

Cauza et al. (2005) undertook the first investigation directly comparing the effect of aerobic and resistance training in glycaemic control after a 4 month exercise programme. They found that the strength training programme decreased HbA_{1c} levels significantly more than the aerobic training programme (-1.2% versus -0.3%). It should be pointed out that the exercise groups reported different, although not significant, baseline HbA_{1c} levels (8.3% the resistance group versus 7.7% the aerobic group), which could have partly compromised the findings. Thus, there is evidence that exercise-induced improvements in glycaemic control are greater among persons with higher baseline HbA_{1c} values (Sigal et al., 2007). In addition to this finding, there have been three recent powerful studies (N>250 in each study) that have assessed the effects of long-term (>22 weeks) aerobic training, resistance training, or both (combined aerobic and resistance training) on glycaemic control in subjects with type 2 diabetic (Church et al., 2010; Dunstan et al., 2008; Sigal et al., 2007). All three have agreed that a combination of aerobic and resistance training was the most efficient exercise modality to reduce HbA_{1c} levels. Furthermore, Dunstan et al. (2008) and Sigal et al. (2007) reported that the effect of aerobic training and resistance training on HbA_{1c} were

approximately equal (approximately a reduction of 0.40% when combining both studies), and those of the combined exercise training were twice those of aerobic or resistance exercises (approximately a reduction of 0.90% when combining both studies). On the other hand, Church et al. (2010) reported that only the combination of aerobic training and resistance training was associated with a reduction in HbA_{1c}. It should be pointed out that, whereas Dunstan et al. (2008) and Sigal et al. (2007) attempted to minimize hypoglycaemic medication changes, resulting in low changes in medications, Church et al. (2010) did not interfere with that, resulting in substantial changes in hypoglycaemic medications across groups. This may explain the lower effect size reported by Church et al. (2010) on all types of exercise. These findings suggest that a combination of aerobic and resistance training may be more effective for blood glucose management than either type of exercise alone, hence suggesting that each type of exercise may trigger different and complementary metabolic adaptations. Therefore, any increase in muscle mass that may result from resistance training could contribute to blood glucose uptake without altering the muscle's intrinsic capacity to respond to insulin. On the other hand, aerobic exercise may enhance its uptake via a greater insulin action, independent of changes in muscle mass or aerobic capacity (American Diabetes Association et al., 2010).

The evidence shown above suggests that physical activity can reduce blood glucose levels in patients with type 2 diabetes, and that a combination of aerobic and anaerobic exercises may trigger further reductions than either aerobic or anaerobic training alone. In addition to this, aerobic and anaerobic training programmes appear to trigger comparable improvements in HbA_{1c} levels.

2.3.1.2 Blood pressure

Although glucose control is essential for preventing microvascular diseases, intensive blood pressure control is needed to reduce cardiovascular events in diabetic patients with hypertension (Grossman et al., 2000). Luscher and colleagues (2003) carried out a review study and reported that lowering blood pressure reduced the risk of cardiovascular diseases in diabetic patients by approximately 40%. This figure highlights the importance of controlling blood pressure in this population.

Aerobic training is commonly recommended to lower blood pressure both in healthy and hypertensive individuals. Thus, a meta-analysis of 54 randomized trials carried out on healthy subjects found that aerobic exercise was associated with an overall reduction in mean blood pressure of 3.9 (systolic)/2.6 (diastolic) mmHg across all initial blood pressure levels (Whelton et al., 2002). Evidence on the role strength training may play to reduce blood pressure in healthy subjects is less clear. However, there is growing evidence to support the benefits of strengthening exercises on reducing blood pressure (Carter et al., 2003; Collier et al., 2008). It is believed that the positive effect of PA on endothelial function, due to an increased NO release, may be a strong exercise-related hypotensive mechanism (McArdle et al., 2000; Tortora & Derrickson, 2006). Additionally, central adaptations such as attenuation of the sympathetic pathway (responsible for increasing heart rate) secondary to physical training has also been postulated (Lesniak & Dubbert, 2001) as a likely mechanism to lower blood pressure.

In agreement with data from non-diabetic populations, physical activity programmes have been shown to lower blood pressure in type 2 diabetic patients. Thus, aerobic training programmes (Yeater et al., 1990), resistance exercise programmes (Castaneda et al., 2002; Dunstan et al., 2002) as well as a combination of aerobic and resistance exercises (Loimaala et al., 2003) have been proven to effectively reduce blood pressure in diabetic subjects. Cauza et al. (2005) reported a similar improvement in blood pressure (both systolic and diastolic) when aerobic training was compared to resistance training. However, other investigations failed to find changes in blood pressure following an exercise programme (Church et al., 2010; Dunstan et al., 2006). It is well established that hypertensive individuals experience a greater reduction in blood pressure than normotensive subjects (Lesniak & Dubbert, 2001). It is worth mentioning that the majority of patients with type 2 diabetes take antihypertensive medications. This explains why some of the studies which failed to demonstrate a change in blood pressure after the exercise training, reported normal baseline blood pressure values (Middlebrooke et al., 2006; Sigal et al., 2007).

Overall, there is evidence that PA programmes trigger improvements in blood pressure, especially in hypertensive individuals, both with and without diabetes. Although aerobic exercises are commonly recommended to lower blood pressure, there is growing

evidence to support the benefits of strengthening exercises on reducing blood pressure in healthy as well as in type 2 diabetic patients. However, some studies failed to find changes in blood pressure following an exercise programme, which suggests that more studies have to be carried out to clarify the role of PA in this cardiovascular risk factor.

2.3.1.3 Lipids profile

Comparisons between sedentary and physically active groups have been used to establish a positive influence of physical activity on blood lipids. Physical active individuals typically exhibit greater HDL and lower triglyceride levels compared to their less active counterparts (ACSM, 2000). A meta-analysis including 25 articles found that aerobic training (longitudinal data) does increase HDL levels, and that exercise duration per session was the most important element to enhance HDL levels in healthy individuals (Kodama et al., 2007). In line with those results, a review of the literature carried out by Durstine et al. (2001) also showed evidence of a positive effect of aerobic training in LDL, triglyceride and total cholesterol (TC) levels, when weekly energy expenditure reached a minimum threshold of 1200 $kcal \cdot week^{-1}$. Unlike aerobic training, the effect of strength training to enhance lipid profiles in healthy individuals has been investigated much less and has provided conflicting results. (Boyden et al., 1993; Elliot et al., 2002).

The effect of physical activity on blood lipids looks less promising in patients with type 2 diabetes compared to healthy individuals. A meta-analysis carried out by Thomas et al. (2006) showed a significant lowering of plasma triglycerides in the exercise intervention groups compared with the control groups. However, there was no significant difference between the exercise and the control group in TC, HDL or LDL. In agreement with this, another study carried out by Sigal et al. (2007), which assessed the effect of different types of physical activity (aerobic, resistance, or combination of both) on lipid values on 251 volunteers with type 2 diabetes, reported no changes in HDL, LDL, TC or triglycerides in any of the exercise groups. However, they reported a trend toward lower triglyceride levels in the resistance training group ($p=0.089$) when compared to baseline levels. The available literature reporting changes in lipid levels due to physical activity in type 2 diabetes does not look very promising. However, more

investigations are needed to better understand the role of physical activity, in particular the different modes of physical activity in changing lipids profile in type 2 diabetes.

Overall, it is well established that physical activity programmes (aerobic training, endurance training and a combination of both) can improve health in patients with type 2 diabetes. Physical activity interventions have been shown to lower glucose levels and blood pressure values. On the other hand, the role of PA to enhance lipids profile in diabetic patients is less clear. Furthermore, aerobic and strengthening exercise programmes appear to trigger comparable improvements in HbA_{1c} and blood pressure levels, whereas a combination of aerobic and resistance exercises may trigger a further reduction of up to 50% in HbA_{1c} than either type of exercise training alone. This leads to the conclusion that a combination of aerobic and resistance exercise may be more efficient to improve health related outcome measures in patients with type 2 diabetes than each type of exercise alone. Since this evidence has been obtained from studies with type 2 diabetic patients with no peripheral neuropathy, these conclusions cannot be generalized to other diabetic groups such as neuropathic patients.

DN carries further health complications than the ones related to type 2 diabetes. The next subsection will explore the association between physical activity and the health problems associated with peripheral neuropathy. Special attention will be paid to the potential role of exercise-based rehabilitation programmes in reducing the risk of foot ulcers in diabetic patients.

2.3.2 Physical activity and peripheral neuropathy

Foot ulceration in diabetic neuropathic patients is a major health problem commonly associated with gait alterations and microvascular impairments. It is therefore essential to investigate the effect of PA, not only on glucose levels and traditional cardiovascular risk factors, but also on outcome measures relevant to foot ulcer formation in this population. In addition to that, QOL, which is increasingly recognized as an important goal for health care, is known to be poorer in subjects with neuropathic complications. It is therefore important to assess whether PA programmes can promote changes in well being in this population. Therefore, this section will investigate the role physical

activity interventions may play to influence gait characteristics and microcirculation, due to their strong association with foot ulcers, as well as QOL.

2.3.2.1 Gait biomechanics

As previously discussed, patients with diabetes in general and neuropathic patients in particular show gait changes when compared to healthy controls, which result in higher foot pressures when walking and consequently higher risk of foot ulcerations (Frykberg et al., 1998; Veves et al., 1991). Sensory loss is believed to be the single most important factor to explain plantar foot pressure (Payne et al., 2002). However, other factors such as reduced joint mobility or muscular weakness are also considered to play an important role in gait abnormalities in this population. In line with this, some investigators have suggested that therapies aiming to improve joint mobility in diabetic patients (Herriott et al., 2004) and/or muscle weakness (Akashi et al., 2008) could improve gait in this population and consequently reduce foot pressures during walking. In the next section, studies that have reported exercise-related changes in 1) sensory loss; 2) limited joint mobility; and 3) muscular weakness and their effect on gait characteristics, will be presented.

2.3.2.1.1 Sensory loss

Balducci et al. (2006) is the only study to investigate the role of exercise training in the progression of neuropathy. Balducci and colleagues carried out a supervised 4 year interventional study on diabetic patients with no signs of neuropathy. They found that low intensity long term aerobic training (4 $h \cdot week^{-1}$) can prevent sensory neuropathy in non-neuropathic diabetic patients. The percentage of diabetic patients who developed sensory neuropathy during the 4 years of the study was significantly higher in the control group (N=47) than in the exercise group (N=31) (28.9% and 6.5% occurrence rate for the control and exercise group, respectively). Although sensory loss has been recognized as the main factor to explain differences in gait in DN patients compared to healthy individuals, no investigation to date has assessed whether sensory neuropathy can be changed through a physical activity programme. It would also be of interest to explore whether other types of exercise can produce beneficial effects on the

progression of neuropathy or whether the progression of neuropathy can only be altered by aerobic training. The ultimate goal would be to investigate whether improvements in sensation could trigger changes in gait characteristics in subjects with DN.

2.3.2.1.2 Limited joint mobility

To date, there is little information available on the treatment of high peak plantar pressure in conjunction with limited joint mobility. One preliminary study found that physical therapy resulted in significant, although temporary, improvements in the mobility of the ankle and foot joints in diabetic neuropathic patients with limited joint mobility (Dijs et al., 2000). In addition to this, Goldsmith et al. (2002) found that an unsupervised range-of-motion therapy, carried out over a 4 week period did reduce peak foot pressures in patients with type 2 diabetes and no neurological complications. They reported that peak plantar pressures during gait decreased on average 4.2% in the treatment group (N=9) while they increased on average 4.4% in the control group (N=10). Despite these interesting results, it should be pointed out that no significant changes were observed in joint mobility when comparing pre- and post-intervention values, which suggests that factors other than ROM could have explained the results. The lack of association between foot pressures and ROM could be also due to the fact that ROM was measured passively and not during the walking task. Spatial-temporal characteristics, such as gait velocity or step length, which are well known to affect foot pressure, were not reported in this study (Goldsmith et al., 2002). Although final conclusions cannot be drawn from this study, it should inspire other researchers to investigate the effect of range-of-motion therapies on plantar pressure distribution patterns in diabetic populations.

Non-enzymatic glycosylation changes in diabetic patients result in greater joint stiffness, which consequently reduces overall joint mobility (Goldsmith et al., 2002). It should be pointed out that the greater the joint stiffness, the greater the force required for a corresponding displacement. Hence, muscle weakness secondary to motor neuropath may further account for the reduction in foot range of motion observed in patients with neuropathic complications, especially during dynamic conditions such as walking (Giacomozzi et al., 2005). In line with this, Mueller et al. (1995) reported a

strong correlation between foot strength levels and ankle dorsi-flexion ROM (r=0.76) when measured actively in 19 subjects with type 2 diabetes. It is therefore reasonable to think that combined flexibility and resistance training may result in greater mobility compared to flexibility exercises alone. In support of this, Fatouros et al. (2002) demonstrated that strength training did improve joint mobility in older adults without diabetes when compared to aerobic alone or no training. Moreover, Herriott et al. (2004) demonstrated that a combination of flexibility and resistance training over an 8 week period produced joint mobility gains in type 2 diabetic patients.

Although there is little evidence for the effect of physical therapy and/or strengthening exercises on foot mobility and foot pressure modification, the data presented above highlights the importance of undertaking further studies to explore whether, or to what extent, foot pressures can be reduced by improving joint mobility.

2.3.2.1.3 Muscle weakness

Muscle weakness has been recognized to play an important role in the gait abnormalities observed in diabetic patients with motor neuropathy. Muscular impairments especially of the foot muscles, have been associated with slower and shorter steps and higher foot pressures, as well as producing a different walking strategy (hip strategy) (Mueller et al., 1994, Uccioli et al., 2001). Although muscle strengthening has been hypothesized as a promising intervention to reduce foot pressures (Akashi et al., 2008) and to improve gait functionality (Gutierrez et al., 2001), the role PA programmes may have on walking biomechanics is still uncertain. Interestingly, patients with type 2 diabetes without neuropathy have also reported significantly lower strength levels when compared to healthy age-matched controls (Andersen et al., 2004), which may partly explain the changes in gait characteristics observed in this population (Yavuzer et al., 2006). More information about gait characteristics in neuropathic and non-neuropathic diabetic patients can be found in Section 2.2.2.1.1.

Exercise programmes have been demonstrated to be an efficient tool to improve strength levels in subjects with diabetes and without neuropathic complications.

Thereby, muscular strength gains have been reported through resistance training (Cauza et al., 2005; Dunstan et al., 2006), combined aerobic and resistance exercises (Loimaala et al., 2003) as well as aerobic exercises alone (Cauza et al., 2005). Thus, it is expected that strengthening exercises, due to the specificity of the training, may trigger the greatest improvements in strength levels than any other type of exercise. Strength training is well known to improve muscle size as well as intramuscular coordination, which result in strength gains (McArdle et al., 2000). However, no study on patients with type 2 diabetes has directly compared the effect of different types of exercises on strength levels.

The effect of strengthening exercises in diabetic neuropathic patients is much less clear than in their non-neuropathic counterparts. The majority of studies assessing resistance training programmes in diabetic patients are carried out on non-neuropathic participants where little is known about the effect of strengthening exercises on this population. In the past, patients with neuromuscular diseases were advised not to exercise because of the fear that too much exercise might produce “overuse weakness” (White et al., 2004). However, new evidence on patients with different neuromuscular diseases suggest that strength training is safe when performed with proper supervision; and that it can reverse any component of disuse weakness as well as potentially improving absolute muscle strength in those with more slowly progressive musculoskeletal diseases (Krivickas, 2003).

There appears to be only one systematic review paper which has investigated exercise based programmes in subjects with peripheral neuropathy (White et al., 2004). White et al. (2006) only found 1 controlled trial comparing the effect of exercise therapy (at least 8 weeks) with no exercise therapy in people with peripheral neuropathy (any type of peripheral neuropathy not just diabetic neuropathy). This trial carried out by Lindeman et al. (1995) reported that long-term resistance training (24 months programme) did improve knee extension strength values in patients with hereditary motor and sensory neuropathy when pre-intervention values were compared to post-intervention values. However, the percentage of improvement was moderate when compared with the strength increases observed in healthy as well as diabetic persons with no neurological complications (Cauza et al., 2005; McArdle et al., 2000) after comparable training programmes. Similar results were reported in a recent study carried out by Allet et al.

(2010), which has been the first investigation assessing strength changes in diabetic neuropathic patients following an 12 week exercise programme. Allet at el. (2010) investigated changes in strength levels following a 12 week exercise programme in an exercise (N=35) and a control group (N=36). The exercise programme in this study consisted of functional strength and endurance exercises (sitting to standing, stair climbing, etc.) in contrast to Lindeman's (1995) study, which controlled weight lifting exercises at a specific % of 1 maximal repetition. Allet et al. (2010) reported small increases in strength at the hip, knee and ankle muscles following the exercise programme in the exercise group compared to the control group. However, only the hip flexors and plantar-flexors reached significant levels. It should be pointed out that in this study muscle strength was measured with a hand-held dynamometer; the reliability of this device is limited by the investigator's ability to hold the dynamometer stationary and by the fact that participants may overpower the testers. The data presented above suggest that although neuropathic patients may have diminished muscular strength gains compared to non-neuropathic counterparts, resistance training could improve strength levels in this population. However, more investigations are needed to understand better the muscular responses to strengthening exercise in diabetic patients with motor neuropathy.

Studies on different clinical populations that suffer from muscular weakness, including elderly people (Rubenstein et al., 2000), have shown that interventions that improve neuromuscular performance are efficient in modifying spatial-temporal gait characteristics (i.e. walking speed or step length). In agreement with this, Brandon et al. (2003) reported that improvements in lower extremity strength levels following 6 months of training were positively associated with mobility performance in subjects with type 2 diabetes (i.e. walking). Interestingly, similar results have been obtained in diabetic patients with neuropathic complications. Thus, Allet et al. (2010) found that a resistance based exercise programme improved walking speed in DN patients when comparing pre- and post-intervention data. Although there is some evidence that strengthening exercise programmes may be effective in changing walking patterns in diabetic patients, only spatial-temporal gait characteristics have been assessed. Further investigations are required to determine the effect of strengthening programmes on other aspects of gait, such as foot kinematics, kinetics or muscular activity in diabetic patients.

Although gait abnormalities in DN subjects are known to contribute to the high rate of foot ulcers that occur in this population, this review of the literature demonstrates that little is known about the effect of PA interventions on changing gait characteristics in DN subjects. It is well established that muscular weakness and limited joint mobility are important factors explaining gait alterations in DN subjects. Therefore, it is reasonable to think that strength training, together with foot range of motion exercise is the most likely physical intervention to trigger changes in gait characteristics in this population. Early evidence suggests that flexibility exercises together with strengthening exercises could improve ROM and strength levels in DN patients. However, only changes in spatial-temporal characteristics has been investigated whereas nobody has yet attempted to assess whether joint mobility and/or muscle strength can alter loading patterns during gait in this population. In addition to that, aerobic training has been shown to produce beneficial effects on the progression of neuropathy, which is considered to be the single most important factor to explain gait alterations in DN subjects. However, the effect of other types of exercise on sensory neuropathy is unknown as well as whether improvements in sensation could lead to changes in gait characteristics in subjects with DN.

2.3.2.2 Microcirculation

It is generally accepted that abnormalities in foot microcirculation may play a significant role in the development of foot ulcers in diabetic neuropathic patients (Korzon-Burakowska & Edmonds, 2006). In addition, impairments in the microvasculature have also been associated with conventional vascular risk factors such as hypertension (Higashi & Yoshizumi, 2004; Tortora & Derrickson, 2006). Thereby, many investigators consider that the attenuation or reversal of microvascular function may be important for the prevention of cardiovascular morbidity and mortality in diabetes (Cohen et al., 2008) as well as in the reparation of tissue breakdown (Korzon-Burakowska & Edmonds, 2006).

Exercise training has been demonstrated to improve microcirculation in healthy subjects. Available data suggests that exercise programmes may improve endothelium-

dependent vasodilatation, but not vascular smooth muscle function in healthy individuals (Higashi et al., 1999). In line with this, Kingwell et al. (1997) observed that 4 weeks of aerobic training did significantly improve resting NO production in young healthy individuals. It is generally believed that the increase in vascular stress resulting from increased exercise-induced blood flow may stimulate the release of NO, which consequently improves endothelial function (Higashi & Yoshizumi, 2004). However, a number of other factors, including reduced oxidative stress secondary to improvements in glycaemic control or hypertension (Higashi & Yoshizumi, 2004; Scott et al., 1999), may also account for the exercise-induced improvements in vascular function. Thereby, it has been reported that an absolute decrease of 1% in HbA_{1c} levels has been associated with a 37% decrease in microvascular complications (Stratton et al., 2000).

Increasing evidence suggests that regular exercise can improve endothelial function in a number of populations in which endothelial dysfunction is common. These include individuals with chronic heart failure (Horning et al., 1996), hypertension (Higashi & Yoshizumi , 2004), or type 1 diabetes (Fuchjager-Mayrl et al., 2002).

Results from exercise interventions in type 2 diabetic patients are controversial. A cross-sectional study carried out by Colberg et al. (2002) reported that chronic exercise (self reported) is associated with enhanced skin blood flow in type 2 diabetes. Interestingly, no differences in maximal levels of NO were found between the exerciser and the sedentary groups, which suggests that exercise improves vascular function by enhancing sensitivity to NO and not necessary due to NO bioavailability (Higashi & Yoshizumi, 2004). Improvements in vascular function secondary to exercise have also been reported during interventional studies. Maiorana et al. (2001) found that 8 weeks of combined aerobic and resistance training improved endothelium dependent vasodilatation, but did not change endothelium independent vasodilatation in type 2 diabetic patients. It should be pointed out that Maiorana investigated endothelium function in the macrocirculation (brachial artery). In accordance with these results, Cohen et al. (2008) found that a 14-month resistance exercise training programme in adults with type 2 diabetes improved endothelial dependent vasodilatation in the microcirculation (skin blood flow). In addition to this, endothelium independent vasodilatation was also shown to be enhanced by the strength training programme. Although, vascular responses seem to be enhanced by physical activity programmes, some investigations failed to find such association. Colberg et al. (2005) investigated the effect of 10 weeks of aerobic training on foot cutaneous blood flow both at rest and

during local heating in patients with type 2 diabetes. This study reported that the defect in perfusion, evident initially in diabetic participants, was not significantly affected by the exercise programme when compared to the healthy group. In line with this, Middlebrooke et al. (2006) reported that 6 months of aerobic exercise did not improve microvascular function, both endothelium dependent or independent vasodilatation, in type 2 diabetes. It is worth mentioning that the interventions that included resistance exercises (Cohen et al., 2008; Maiorana et al., 2001) reported an exercise-related improvement in vascular function, whereas aerobic training interventions did not. Before any final conclusion can be drawn further investigations are required into which exercise modality may be the most efficient to improve microvascular function in type 2 diabetes.

Early evidence suggests that a physical activity intervention may produce beneficial changes in the microcirculation of type 2 diabetic patients without neuropathy. Although it is difficult to determine which type of exercise (aerobic, resistance or a combination of both) is more effective in this matter, strengthening exercises have been linked to changes in the microvasculature of type 2 diabetic patients. It is noteworthy, that all investigations assessing the effect of PA interventions on the microcirculation of type 2 diabetic patients have looked at either resistance vessels or skin microcirculation whereas nobody has investigated muscular microcirculation. In addition to this, the effect of physical activity on oxygen consumption, which may be another aspect of the microcirculation impaired in diabetic individuals (Baldi et al., 2003; Martin et al., 1995), has not been investigated by any study.

A combination of gait and microcirculation alterations is known to be responsible for the high rate of ulcers observed in DN subjects. It is therefore reasonable to think that interventions that can influence these factors may reduce the risk of foot ulcers in this population. It seems that strength training together with foot range of motion exercises, is the most likely intervention to trigger changes in gait characteristics in patients with diabetes. Early evidence suggests that strengthening and foot mobility exercises could improve ROM (Herriott et al., 2004) and influence spatial-temporal characteristics (Allet et al., 2010) in diabetic patients with and without DN. However, nobody has investigated whether joint mobility and/or muscle strength can alter loading patterns during gait in diabetic patients. Furthermore, early evidence suggests that resistance

training may trigger beneficial changes in the microcirculation of type 2 diabetic patients without neuropathy (Cohen et al., 2008). Overall, it appears that a combination of strengthening and foot mobility exercise is the most likely PA intervention to modify gait and microcirculation in diabetic patients. However, the vast majority of investigations assessing PA programmes in diabetic patients have been carried out on subjects with type 2 diabetes whereas little is known about the effect of PA interventions on DN subjects. Therefore, it is very important to investigate whether strength training can improve gait and microcirculation in DN subjects, who are the population at highest risk of developing foot problems.

2.3.2.3 Quality of life

Although glycaemic control, the development of foot complications and mortality represent critical outcomes in people with type 2 diabetes, health-related QOL outcomes are also important (Ali et al., 2010). The value of optimizing QOL is increasingly recognised as it represents an important goal for health care (Rejeski & Mihalko, 2001). The question remains whether QOL is a modifiable risk factor in diabetic patients or just a marker of disease burden. Recently, Harkness et al. (2010) carried out a meta-analysis to indentify psychosocial interventions that improve both physical and mental health in patients with diabetes mellitus. They concluded that although there are efficient treatments to improve both diabetes and mental health, they did not identify types of interventions that consistently provide benefits for both simultaneously.

The evidence provided above demonstrates that PA can influence some aspects of health in patients with diabetes including glucose control and cardiovascular risk factors (Thomas et al., 2006; Sigal et al., 2006). However, little is known on the effect of PA interventions to modify mental health in patients with diabetes. Glasgow et al. (1997) carried out a survey study on 2056 adults with diabetes (type 1 and type 2) to identify, using a multiple regression analysis, factors related to lower QOL. Interestingly, they found that the level of self-reported exercise was the only significant-management behaviour to predict the QOL, after controlling for demographic and medical variables. Similar results were found by Green et al. (2011), who reported an association between self-reported physical activity levels and self-reported QOL in type 2 diabetic patients

(N=2419). Therefore, it seems that participation in physical activity may be associated with an improvement in general well-being and QOL in diabetic patients. However, it is noteworthy that the majority of studies have investigated this issue retrospectively (Glasgow et al., 1997; Green et al., 2011), whereas little is known about whether QOL can be modified in diabetic patients (both with and without neuropathy) through a structured physical activity programme.

Overall, the evidence presented above demonstrates that the vast majority of studies investigating PA interventions on type 2 diabetic patients have been carried out in individuals with no neuropathic complications. In fact after a comprehensive search only 1 study was found in which an exercise programme was carried out on subjects with DN (Allet et al., 2010). This highlights the importance of investigating PA interventions on DN subjects. Nevertheless, it is noteworthy that DN subjects are at risk to develop foot ulcers during weight bearing activities. It is generally believed that the amount of weight-bearing activity among individuals with diabetes is likely to influence the amount of mechanical trauma accumulated by plantar tissues (Cavanagh et al., 1996). This suggests that adaptation of the exercises used is necessary to avoid foot complications (Kanade et al., 2006). Contrary to this idea, Mueller & Maluf (2002) have proposed the "physical stress theory", the basic premise of which is that changes in physical stress (i.e. plantar tissue stress) cause a predictable adaptative response in all biological tissues. According to the physical stress theory, higher cumulative plantar tissue stress may result in an increased stress tolerance, whereas low levels of stress may lead to tissue weakness. In support of this theory, recent studies have indicated that moderate walking does not increase the risk of foot ulcers or reulceration in those with DN (Maluf & Mueller, 2002; LeMaster et al., 2008). More evidence is needed to recommend weight-bearing activities in DN subjects since it may result in devastating consequences in the form of foot ulceration. In line with this interpretation, physical activity guidelines for DN subjects recommend non-weight-bearing activities to decrease the risk of skin breakdown due to excessive stress (Sigal et al., 2006). More studies therefore need to be carried out on DN subjects to find out whether physical activity programmes based on non-weight bearing may increase the risk of ulceration in this population.

2.4 Summary of the literature review

This literature review has attempted to 1) review in detail the pathologies related to DN with particular attention to gait and microcirculation alterations due to their association with foot ulcers; and 2) review the current literature on the effect of PA interventions on the primary pathologies associated with DN. Therefore, this summary of the literature review will be divided into two sections.

Health-related characteristics of DN subjects

The first part of this literature review shows that DN is a complex condition that affects different aspects of health including traditional cardiovascular risk factors, gait, microcirculation and quality of life.

It is well established in the literature that type 2 diabetes is associated with an increased risk of cardiovascular diseases and mortality. It appears that alterations in blood pressure, cholesterol levels and obesity secondary to type 2 diabetes, known as metabolic syndrome, are partly responsible for the 4 – fold increased risk of cardiovascular diseases compared to healthy individuals.

Neuropathy is associated with foot complications. Foot ulceration in diabetic patients with DN is considered a major health problem, often leading to lower-limb amputations and increased mortality rate. It seems that a combination of gait and microcirculatory changes is responsible for the increased risk of foot ulcerations observed in DN subjects.

The evidence presented in this literature review chapter demonstrates that patients with DN present with a variety of gait alterations compared to healthy individuals. Thus, DN subjects show changes in: 1) gait parameters, in the form of spatial-temporal and floor-foot interaction characteristics (COP); 2) foot pressures, especially in the metatarsals region; and 3) muscle activation patterns. This review of the literature shows that, while kinetic and kinematic data have been widely investigated in DN subjects, only a handful of studies have assessed EMG activity in this population. Furthermore early evidence suggests that changes in the onset activation times of the TA and TS have been linked to

changes in kinetic data. This suggests that EMG data should be considered as a potential contributing factor for the higher foot pressures (PP and PTI) observed in DN subjects, especially on the forefoot. This highlights the importance for future studies to study EMG activity patterns in patients with DN.

The evidence presented in this chapter shows that there is a general consensus in the literature that impairments in microcirculation, especially in vasodilatory capacity, are associated with foot ulcer formation in DN subjects. Furthermore, it is well established that vasodilatory abnormalities in patients with diabetes are related to impairment in endothelial dependent vasodilatation, endothelial independent vasodilatation and nerve-axon reflex. In addition to this, functional alterations in the microcirculation have also been reported in response to an exercise bout (exercise stress), which may partly explain the reduced exercise capacity observed in subjects with diabetes. Patients with type 2 diabetes show a diminished exercise-induced vasodilatory capacity compared to healthy individuals, and preliminary evidence suggests that changes in endothelial function may be partly responsible for these alterations. However, all the studies investigating microcirculatory responses to an exercise bout have been carried out in diabetic patients without neuropathic complications. Moreover, there is an ongoing debate about whether the diminished exercise capacity observed in diabetic patients is caused solely by a reduction in oxygen delivery or in combination with a reduction in the muscular oxidative capacity. This highlights the importance to investigate 1) exercise-induced vasodilatory responses in DN patients; and 2) muscular oxygen consumption in patients with diabetes mellitus.

Increasing evidence suggests that diabetes in general and DN in particular, impact on everyday living and consequently diminish health related QOL. Furthermore, it appears that there is a positive correlation between diabetes and its complications and QOL, with subjects with more severe complications showing the poorest QOL. Interestingly, preliminary evidence suggests that QOL could be associated with biomechanical markers; however, more studies need to be carried out to draw any further conclusions.

Overall, it is well known that DN is a complex condition that affects different aspects of health including cardiovascular risk factors, gait, microcirculation and QOL.

Nevertheless, this literature review shows there are still some gaps in the literature that need to be addressed.

Exercise and diabetic peripheral neuropathy

The second part of this literature review highlights the importance of exercise for diabetic patients. There is mounting evidence that physical activity (aerobic, resistance and a combination of both) can produce positive changes in blood sugar levels and cardiovascular risk factors in diabetic patients. However, all the studies that have investigated the effect of PA on traditional cardiovascular risk factors have been carried out on diabetic patients with no neuropathic complications. Thus, findings from patients with diabetes cannot necessarily be generalized to other diabetic groups such as neuropathic patients. This highlights the importance of investigating whether PA can produce beneficial changes in the glucose control and cardiovascular risk factors in patients with DN.

Foot ulceration in DN subjects is a major health problem, often leading to lower-limb amputation and increased mortality rate as well as lower QOL. Therefore, it is essential to investigate the effect of PA, not only on glucose levels and traditional cardiovascular risk factors but also on outcome measures relevant to foot ulcer formation in this population. Muscular weakness and limited joint mobility are important factors when exploring gait alterations in DN subjects. Therefore, it is reasonable to think that strength training together with foot range of motion exercise is the most likely physical intervention to trigger changes in gait characteristics in this population. In line with this argument, some investigators have suggested that therapies which aim to improve joint mobility in diabetic patients and/or muscle weakness could modify gait characteristics in DN subjects, and consequently reduce foot pressures during walking. Early evidence suggests that flexibility exercises together with strengthening exercises could improve ROM in patients with diabetes and strength levels in DN patients. However, only changes in spatial-temporal characteristics has been investigated whereas no study to date has attempted to assess whether joint mobility and/or muscle strength can alter loading patterns during gait in this population.
Beside gait alterations, abnormalities in the microcirculation also play a significant role in the development of foot ulcers in DN subjects. Thus, early evidence suggests that a

physical activity intervention, including strengthening exercises may produce beneficial changes in the microcirculation of type 2 diabetic patients without neuropathy. Interestingly, the evidence presented above suggests that a combination of mobility and strengthening exercises is the most likely intervention to modify both gait and microcirculation. Therefore, it can be speculated that if beneficial changes in gait and microcirculation can be elicited, the risk of foot complications may be reduced. However, to date, no study has attempted to assess the effect of a physical activity programme on gait modification (spatial-temporal characteristics, foot pressures distributions, etc) and microcirculation in DN subjects.

In addition to that, QOL represents an important goal for health professionals and it has been associated with adverse outcomes in people with type 2 diabetes, including poor response to therapy, disease progression and even mortality. Although physical activity appears to improve QOL in healthy individuals, it is unknown whether QOL in diabetic patients can be modified through an exercise programme.

Overall, this literature review chapter shows that there is a gap in the literature when it comes to PA interventions in patients with DN. DN subjects appear to be at greatest risk of developing foot ulcers during weight bearing activities. Thus, it is generally believed that the amount of weight-bearing activity among individuals with diabetes is likely to influence the amount of mechanical trauma accumulated by plantar tissues. This suggests that an adaptation of the exercises used may be necessary to avoid foot complications.

The next chapter outlines the aims, objectives and null hypotheses of this thesis to address the areas identified in the literature review requiring further investigation.

CHAPTER 3

3 Aims, objectives and hypotheses

3.1 Aims of the thesis

This thesis has three main aims:

The first aim was to determine the reliability of some of the methods used in the main part of the present study. This includes three different pilot studies

1) To determine the reliability of near infrared spectroscopy (NIRS) to quantify muscular blood flow and oxygen consumption in the calf muscle simultaneously during a venous occlusion.
2) To develop and ascertain the reliability of a new approach to calculate EMD during isometric contractions.
3) To develop and ascertain the reliability of a new approach to calculate EMD for the plantar-flexor muscles during walking.

The second aim was to investigate differences between DN and healthy individuals in the primary pathologies associated with DN

The third aim was to investigate the effect of a 16-week PA programme, which included strengthening and joint mobility exercises, on identified pathologies associated with peripheral neuropathy in DN subjects. .

On this basis, this thesis includes two main sections: Section 1 contains the three preliminary studies on the reliability of some of the methods used in the main study. Section 2 includes the main study of the thesis and was conducted in two different parts: Part 1 investigated the difference in health-related outcome measures between healthy and DN subjects. Part 2 evaluated the effect of a PA intervention on health-related outcome measures in DN individuals.

3.2 Objectives

3.2.1 Preliminary studies

Near Infrared Spectroscopy (NIRS) reliability study

To test within-day and between-day reproducibility of NIRS when measuring blood flow on the calf muscles (medial gastrocnemious) during venous occlusion.

To test within-day and between-day reproducibility of NIRS when measuring oxygen consumption on the calf muscles (medial gastrocnemious) when using the venous occlusion method.

Electromechanical delay (EMD) determination during isometric contractions.

To test within-day reliability of a new approach to calculate EMD during isometric contractions in healthy and DN subjects.

To compare EMD values during knee extension, knee flexion, plantar-flexion and dorsi-flexion between healthy and DN individuals.

Electromechanical delay (EMD) determination for the plantar-flexor muscles during gait.

To test within-day reliability of a new approach to calculate EMD during gait.

To compare EMD values for the plantar-flexor muscles during gait between healthy and DN individuals.

3.2.2 Main study- Part 1 (Cross-Sectional study)

To investigate differences between healthy and DN subjects in outcome measures related to general health, in the form of cholesterol levels and blood pressure.

To investigate differences between healthy and DN subjects in outcome measures related to gait characteristics, in the form of strength levels, gait parameters, kinetic data and muscular activity patterns.

To investigate differences between healthy and DN subjects in outcome measures related to microcirculation, in the form of muscle blood flow and oxygen consumption, both at rest and in response to an exercise bout.

To investigate differences between healthy and DN subjects in outcome measures related to self reported QOL.

3.2.3 Main study- Part 2 (Intervention study)

To investigate the effect of a 16-week strengthening and joint mobility training programme in general health related outcome measures, in the form of HbA_{1c}, sensory neuropathy, blood pressure, cholesterol levels and obesity.

To investigate the effect of a 16-week strengthening and joint mobility training programme in outcome measures related to gait characteristics, in the form of strength levels, gait parameters, kinetic data and muscular activity patterns.

To investigate the effect of a 16-week strengthening and joint mobility training programme in outcome measures related to microcirculation, in the form of muscle blood flow and oxygen consumption, both at rest and in response to and exercise bout.

To investigate the effect of a 16-week strengthening and joint mobility training programme in outcome measures related to self reported QOL.

3.3 Null Hypotheses

The null hypotheses for the preliminary study and the 2 parts of the main study were:

3.3.1 Preliminary studies

Near Infrared Spectroscopy reliability study

Null Hypotheses 1
There will be moderate reliability (ICC<0.6) between the within-day and between-day scores of blood flow when measured on the calf muscles during venous occlusion.

Null Hypothesis 2
There will be moderate reliability (ICC<0.6) between the within-day and between-day scores of muscle oxygen consumption when measured on the calf muscles during venous occlusion.

EMD determination during isometric contractions

Null Hypothesis 1
There will be moderate reliability (ICC<0.6) between the within-day scores when calculating EMD during isometric contractions in healthy and DN subjects.

Null Hypothesis 2
There will be no differences in EMD values during knee extension, knee flexion, plantar-flexion and dorsi-flexion contractions between healthy and DN individuals.

EMD determination for the plantar-flexor muscles during gait.

Null Hypothesis 1
There will be moderate reliability (ICC<0.6) between the within-day scores when calculating EMD for the plantar-flexor muscles during gait.

Null Hypothesis 2
There will be no differences in EMD for the plantar-flexor muscles during gait between healthy and DN individuals.

3.3.2 Main Study- Part 1.

Null Hypothesis 1
There will be no differences in general health outcome measures in the form of blood pressure and cholesterol levels between the healthy and DN groups.

Null Hypothesis 2
There will be no differences in 1) gait characteristics in the form of gait parameters, foot pressures and muscular activity patterns during a gait task between the healthy and DN groups; and 2) lower limb muscle strength.

Null Hypothesis 3
There will be no differences in microcirculation in the form of capillary blood flow and oxygen consumption both at rest and in response to an exercise bout between the healthy and DN groups.

Null Hypothesis 4
There will be no differences in self reported quality of life in the form of mental health and physical health between the healthy and DN groups.

3.3.3 Main study- Part 2

Null Hypothesis 1
There will be no differences in HbA_{1c}, sensory neuropathy, cholesterol levels and blood pressure before and after the intervention (over time) between the control and the exercise group.

Null Hypothesis 2

There will be no differences in: 1) gait parameters, foot pressures and EMG patterns during a gait task; and 2) lower limb strength levels before and after the intervention between the control and the exercise group.

Null Hypothesis 3

There will be no differences in capillary blood flow and oxygen consumption at rest and in response to an exercise bout before and after the intervention between the control and the exercise group.

Null Hypothesis 4

There will be no differences in self reported QOL before and after the intervention between the control and the exercise group.

CHAPTER 4

4 Preliminary studies

4.1 Reliability of near infrared spectroscopy (NIRS) to quantify muscular blood flow and oxygen consumption in the calf muscle using the venous occlusion method.

4.1.1 Introduction

Skeletal muscles make up approximately 40% of the total body mass, thus representing an important percentage of cardiac output and total oxygen consumption even at rest. Under working conditions, muscle blood flow can increase to 80-85% of cardiac output and oxygen consumption can exceed the resting value 50-fold (McArdle et al., 2000; Tortora & Derrickson, 2006). Thereby, the study of blood flow and oxygen consumption of limbs is of great relevance in exercise physiology and in patients with cardiocirculatory impairments.

The standard for measurement of blood delivery and oxygenation in the arm and leg is the combination of strain-gauge plethysmography and blood gas analysis for determination of blood flow and arteriovenous O_2 difference, respectively (van Whitney, 1953; Cort et al., 1991). However, the volume of interest for both techniques is limited to the total limb, whereas tissues other than the muscle tissue can influence muscle oxygen consumption and blood flow.

NIRS is a non-invasive, continuous, and direct method to determine oxygenation and haemodynamics in tissue. It enables the study of local differences in muscle O_2 consumption and delivery. Furthermore, NIRS enables the measurement of O_2 consumption and blood flow at rest (van Beekvelt et al., 2001a) as well as during exercise (Homma et al., 1996) and it discriminates between normal and pathological states (Boushel et al., 2001; Kooijman, et al., 1997).

Numerous studies have shown the validity of NIRS to determine muscle blood flow (BF) by comparing it against well-established methods such as strain-gauge plethysmography (van Beekvelt et al., 2001b; De Blasi et al., 1994; Homma et al., 1996; Mancini, et al., 1994) or dye dilution in combination with magnetic resonance imaging (Boushel et al., 2000b). Muscle oxygen consumption (mV O_2) measured by NIRS has also shown good agreement with blood gas analysis for determination of arteriovenous O_2 differences (van Beekvelt et al., 2001b; De Blasi et al., 1994; Homma et al., 1996).

Non invasive quantification of mV O_2 and BF using NIRS has become possible by applying an occlusion to control circulation in the limb. BF measurements are carried out when applying a venous occlusion. This method has shown high reproducibility both within one session (van Beekvelt et al., 2001a; van Beekvelt et al., 2002) and between days (De Blasi et al., 1994). The studies reported above investigated the reliability of NIRS for measurements in the upper limb. However little is known about the reproducibility of the NIRS device both within a session and between days when measuring blood flow in the lower limb.

Unlike blood flow measurements, mVO_2 can be measured during both venous (De Blasi et al., 1994; van Beekvelt et al., 2001b) and arterial occlusion (Kragelj et al., 2000; van Beekvelt et al., 2001a). It has been suggested that the venous occlusion (VO) method is to be preferred over the arterial occlusion method because venous occlusion is less inconvenient for the subject, the recovery is much faster, and oxygen consumption and blood flow can be measured simultaneously (van Beekvelt et al., 2002; De Blasi et al., 1997). In addition to this, arterial occlusion, which requires a cuff inflation of at least 30 mmHg above the individual systolic blood pressure for approximately 45 seconds, may not be recommended for patients with hypertension or cardiovascular impairments.

Van Beekvelt and colleagues (2001a) showed high within day reproducibility when resting muscular oxygen consumption was measured on the forearm during arterial occlusion. They reported a coefficient of variation of 16.2 % for the three arterial occlusions carried out during the session. A coefficient of variation slightly higher (17.6%) was reported by the same research group using the same procedures when assessing the reproducibility on three separate days (van Beekvelt et al., 2002). In line

with these results, Kragelj and colleagues (2000) reported a coefficient of variation of 22.3% when oxygen consumption at rest was assessed on the distal part of the foot on four to six different days using arterial occlusion.

Contrary to the arterial occlusion method to assess muscular oxygen consumption, the reproducibility of the "preferable" VO method is less certain. To the best of my knowledge, van Beekvelt et al. (2001b) is the only study, which reports the reproducibility of NIRS for mV O_2 measurements using the VO method. They carried out three VO on the forearm and reported a coefficient of variation of 30.6 % and 25.4% when the inter-optode distance was 35 and 50 mm, respectively. They concluded that the arterial occlusion method was the preferred method to quantify mV O_2 because it offers higher reproducibility compared to the VO method. However, there is only one study investigating the reliability of the "preferable" venous occlusion method and nothing is known about the reproducibility of this technique to assess oxygen consumption in the lower limb.

The purpose of this study was therefore 1) to test within day and between day reproducibility of NIRS when measuring BF on the calf muscles (medial gastrocnemious); 2) to determine whether quantification of mVO_2 by NIRS using VO is reproducible both within a session and between sessions.

4.1.2 Materials and Methods

Subjects

Ten healthy volunteers (5 men, 5 women) participated in this study. The study was approved by the School Ethics Committee, and all subjects gave their written consent. The subject characteristics were 33± 4.3 year in age, 168.1± 6.3 cm in height, and 70.4± 5.3 kg in weight. None of the volunteers were taking any medication that may affect muscle peripheral circulation.

NIRS

NIRS is based on the relative transparency of tissue to light in the near-infrared region, and on the oxygen-dependent absorption changes of haemoglobin and myoglobin. Although there is not a general agreement on the contribution of myoglobin to the near infrared absorption changes (Mancini, 1994), this does not affect the results from the present study, since the current study was interested in the amount of O_2 consumed, regardless of whether it was supplied by haemoglobin or myoglobin. NIRS measurements were performed using the Oxymon MK III (Artinis Medical systems B.V, Zetten, The Netherlands), which generates light at 781 nm and 856 nm. NIRS enables non-invasive continuous measurement of changes in the concentration of oxygenated haemoglobin (HbO) and deoxygenated haemoglobin (HbdO). The sum of HbO and HbdO concentrations reflects the total amount of haemoglobin (tHb), and changes in tHb can be interpreted as changes in blood volume in the tissue.
NIRS measurements were done on the belly of the medial gastrocnemious (MGast) with an inter-optode distance (distance between source and detector) of 40 mm. Waterproof markers on the MGast avoided variation in placement over separate days.

Quantification of BF and mVO_2 was carried out during VO. Venous occlusion causes an increase blood volume by an undisturbed arterial (in) flow and interrupted venous (out) flow (see Figure 4-1). Blood flow can therefore be calculated during venous occlusion from the linear increase of tHb (ΔtHb). Concentration changes of tHB ([Hb]) were expressed in micromolars per second and were converted to millilitres blood per 100 millilitres tissue per minute using an average Hb concentration of 7.5 $mmol \cdot L^{-1}$ for female subjects and 8.5 $mmol \cdot L^{-1}$ for male subjects. The molecular weight of haemoglobin (64.458 $g \cdot mol^{-1}$) and the molecular ration between haemoglobin and oxygen (1:4) were taken into account (Beekvelt et al., 2002; De Blasi et al., 1994). Muscular oxygen consumption was calculated from the linear increase of HbdO (ΔHbdO). Since the venous outflow is blocked the increase in HbdO is thought to be solely due to the O_2 consumed under the assumption the arterial O_2 saturation is near 100%. Concentration changes HbdO were expressed in micromolars per second and converted to $mlO_2 \cdot O_2^{-1} \cdot 100\ g^{-1}$ taking into account that each molecule of haemoglobin

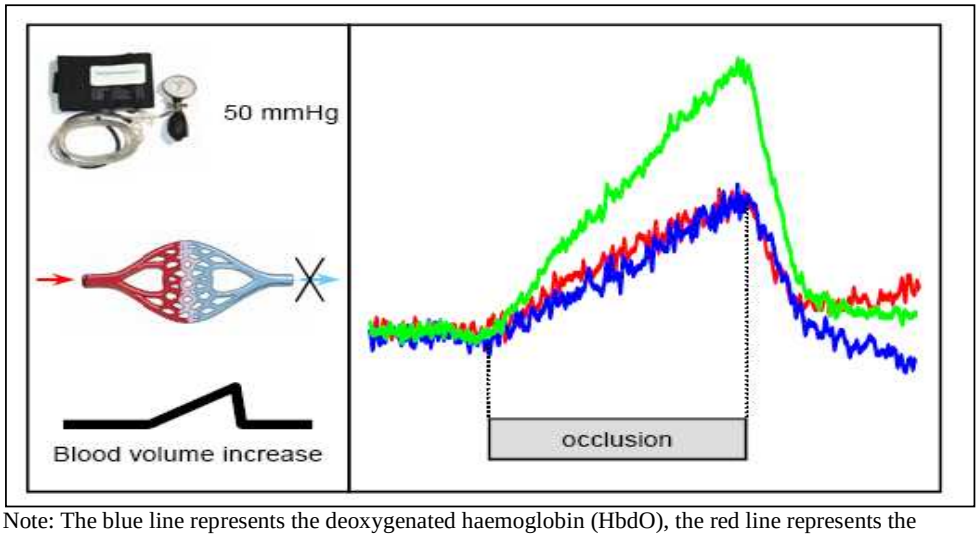
binds for molecules of O2 and that the molar volume of gas is 22.4 L. A value of 1.04 $kg \cdot l^{-1}$ was used for muscle density (Beekvelt et al., 2002; De Blasi et al., 1994).

Equations:

BF= Abs(((ΔtHb*60)/([Hb]*1000)/4))*1000)/10 in $ml \cdot min^{-1} \cdot 100\ ml^{-1}$

mVO_2= Abs(((ΔHbdO*60)/(10*1.04))*4)*22.4/1000 in $mlO_2 \cdot min^{-1} \cdot 100\ g^{-1}$

Figure 4-1. Overview of the venous occlusion method to quantify muscular BF and mVO_2



Note: The blue line represents the deoxygenated haemoglobin (HbdO), the red line represents the oxygenated haemoglobin (HbO) and the green line represents the total haemoglobin content (tHb).

NIRS measurements were carried out on the MGast of the right leg with the subject lying down in a supine position on a KINCOM machine. The right leg was semi-extended (30°± 10 flexion) while the foot rested on a foot dynamometer. The foot was positioned above the heart level and the leg in an upward position to avoid venous pooling of the blood. A pneumatic cuff was placed around the thigh and was used to apply venous occlusion during the test.

After placement of the instruments, the experiment started with a 5 minutes rest period, followed by a VO (50 mmHg). The cuff was maintained inflated for 30 seconds. This procedure was repeated three times with a resting period of 40 seconds between inflations. All subjects attended three times on separate days to test the between days

reproducibility of NIRS to measure muscular BF and mVO_2 using the VO method. NIRS data was processed and analyzed using the Oxysoft 2.1.2 software (Artinis Medical systems B.V, Zetten, The Netherlands).

Statistics

Systematic bias for within day and between day reliability data was assessed by a one-way analysis of variance (ANOVA) for repeated measures. The within-subject variability was calculated as the coefficient of variation (CV) for each subject [(SD/mean)*100]. CV was determined both for the within day as well as for the between days data. The day to day and between days reproducibility was determined by calculating the Intraclass correlation coefficient (ICC). The same statistical tests were applied when assessing the reproducibility of blood flow and muscular oxygen consumption. The level of statistical significance was set at $P \leq 0.05$. All analyses were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

4.1.3 Results

The reproducibility of the NIRS measurements for within day mVO_2 and BF measurements were investigated by means of the repetitions of three venous occlusions. All results for within day reliability measurements are shown in Table 4-1and Table 4-2. No significant differences between within day measurements were observed for BF as well as mVO_2 values ($p=0.535$). In addition to that, the relative variability within subjects (CV) for within days was 10.4% and 14.40% for BF and mVO_2, respectively. An ICC for within day measurements of 0.92 and 0.86 was observed for BF and mVO_2, respectively.

Table 4-1. Within-day reliability: Blood flow

Subject	BF rep 1 ($ml \cdot min^{-1} \cdot 100\ ml^{-1}$)	BF rep2 ($ml \cdot min^{-1} \cdot 100\ ml^{-1}$)	BF rep3 ($ml \cdot min^{-1} \cdot 100\ ml^{-1}$)	Anova p value	CV %	ICC
1	0.492	0.474	0.478		1.98	
2	0.802	0.854	0.993		11.14	
3	0.578	0.527	0.460		11.30	
4	0.895	1.202	0.945		16.25	
5	0.375	0.415	0.300		16.01	
6	0.123	0.110	0.111		6.20	
7	0.414	0.553	0.505		14.32	
8	0.619	0.673	0.766		10.84	
9	0.356	0.362	0.305		9.10	
10	0.607	0.527	0.583		7.12	
Overall	**0.526**	**0.570**	**0.545**	***0.535***	*10.43*	***0.92***

Table 4-2. Within-day reliability: Muscular Oxygen Consumption

Subject	mVO_2 rep 1 ($mlO_2 \cdot min^{-1} \cdot 100\ g^{-1}$)	mVO_2 rep2 ($mlO_2 \cdot min^{-1} \cdot 100\ g^{-1}$)	mVO_2 rep3 ($mlO_2 \cdot min^{-1} \cdot 100\ g^{-1}$)	Anova p value	CV %	ICC
1	0.015	0.013	0.013		7.34	
2	0.025	0.022	0.022		7.63	
3	0.020	0.023	0.024		10.77	
4	0.024	0.018	0.029		22.77	
5	0.029	0.019	0.030		22.30	
6	0.009	0.006	0.006		20.78	
7	0.012	0.011	0.017		19.91	
8	0.035	0.045	0.037		13.83	
9	0.011	0.011	0.010		6.99	
10	0.020	0.024	0.025		11.71	
Overall	**0.020**	**0.019**	**0.021**	***0.442***	***14.40***	***0.86***

The reproducibility of the NIRS measurements for between day mVO_2 and BF measurements were investigated by averaging the repetitions of three venous occlusions on three separate days (average day 1, average day 2 and average day 3).

All results for between day reliability measurements are shown in Table 4-3 and Table 4-4. No significant differences between day measurements were observed for BF as well as mVO_2 values. The relative variability within subjects (CV) for between days was 20.94% and 22.80% for BF and mVO_2, respectively. An ICC for between day measurements of 0.72 and 0.68 was observed for BF and mVO_2, respectively.

Table 4-3. Between-day reliability: Blood flow

Subject	**BF day 1 ($ml \cdot min^{-1} \cdot 100\ ml^{-1}$)**	**BF day 2 ($ml \cdot min^{-1} \cdot 100\ ml^{-1}$)**	**BF day 3 ($ml \cdot min^{-1} \cdot 100\ ml^{-1}$)**	**Anova p value**	**CV %**	**ICC**
1	0.481	0.464	0.390		10.80	
2	0.883	0.882	0.953		4.46	
3	0.522	0.364	0.328		25.44	
4	1.014	1.766	0.887		38.84	
5	0.363	0.371	0.485		16.68	
6	0.114	0.307	0.309		45.76	
7	0.422	0.491	0.542		12.40	
8	0.686	0.480	0.499		20.52	
9	0.341	0.341	0.310		5.46	
10	0.572	0.398	0.328		29.07	
Overall	**0.540**	**0.586**	**0.503**	***0.638***	***20.94***	***0.72***

Table 4-4. Between-day reliability: Muscular Oxygen consumption

Subject	mVO_2 day 1 ($mlO_2 \cdot min^{-1} \cdot 100\ g^{-1}$)	mVO_2 day 2 ($mlO_2 \cdot min^{-1} \cdot 100\ g^{-1}$)	mVO_2 day 3 ($mlO_2 \cdot min^{-1} \cdot 100\ g^{-1}$)	Anova p value	CV %	ICC
1	0.0139	0.012	0.007		32.52	
2	0.023	0.017	0.020		15.27	
3	0.023	0.026	0.021		9.56	
4	0.024	0.022	0.029		14.50	
5	0.026	0.033	0.046		29.45	
6	0.007	0.010	0.014		34.66	
7	0.020	0.014	0.013		25.34	
8	0.039	0.030	0.032		13.80	
9	0.011	0.027	0.015		45.61	
10	0.023	0.026	0.023		7.30	
Overall	**0.021**	**0.021**	**0.022**	***0.911***	***22.80***	***0.68***

4.1.4 Discussion

The main finding in this study was that the venous occlusion method showed good reproducibility when measuring BF and mVO_2 in the calf muscle both within-day and between-days. Data also shows that the relative variability within-subjects, when looking at the SD in relation to the mean, and the ICC values were consistently better for the BF compared to the mVO_2 data both within-day and between-days.

Reproducibility of BF measurements

Blood flow measurements in the calf muscles showed good reproducibility. An ICC value of 0.92 and 0.72 for within-day and between-days demonstrated that the reproducibility of the venous occlusion methods to quantify BF is excellent and substantial, respectively (Landis & Koch, 1977). The relative variability within subjects (CV) was 10.43% and 20.94% for within and between-days respectively. In line with

the results from the present study, de Blasi (1994) reported a coefficient of variation of 10% and 22% when blood flow was calculated in the forearm within the same day and between days, respectively. It should be pointed out that de Blasi (1994) only tested two individuals over three different days to quantify the reproducibility of this method between days. Van Beekvelt and colleagues in two different studies found higher coefficient of variation, 28.6% and 22.4% compared to the results from the current investigation when assessing within day reliability of the NIRS to quantify forearm BF (van Beekvelt et al., 2001b & van Beekvelt et al., 2002).

Previous studies have used NIRS to investigate blood flow delivery in the lower limb in patients with circulatory problems (Kooijman et al., 1997; Mohler et al., 2006). Kooijman et al. (1997) stated that NIRS is an effective noninvasive method for assessing claudication following a walking exercise in patients with peripheral arterial diseases. In addition to this, Mohler et al. (2006) investigated the vasodilatory responses of the calf muscles to physical activity in diabetic patients and they reported significant differences in blood flow delivery when diabetic patients were compared to age matched healthy individuals. Therefore, mounting evidence demonstrates that NIRS is an efficient non-invasive tool to assess muscle circulation in healthy and clinical population. However: 1) the reproducibility of NIRS to quantify BF on different days was uncertain; and 2) the reproducibility of NIRS to assess BF on the lower limb was unknown. This is the first study that shows that NIRS is a reliable tool to 1) quantify blood flow on different days and 2) assess blood flow on the lower limb.

Reproducibility of mV O_2 ***measurements***

There is a debate as to which occlusion method should be used to assess mVO_2 with a NIRS device. It has been suggested that the venous occlusion method should be the preferred option over the arterial occlusion since it is less inconvenient for the subject, the recovery is much faster, and oxygen consumption and blood flow can be measured simultaneously. Arterial occlusion requires a cuff inflation of at least 30 mmHg above the individual systolic blood pressure for approximately 45 seconds, which may not be advisable for patients with circulatory problems.

In the present study it has been shown that venous occlusion is a reliable method to quantify oxygen consumption on the calf muscles. ICC values show an excellent reproducibility for within-day reliability (0.86) and a substantial reproducibility for between-days reliability (0.68).

In disagreement with the results from the current study, van Beekvelt et al. (2001b) suggested that venous occlusion was not a reliable method to assess O_2 consumption using NIRS. They investigated the reproducibility of mVO_2 during three consecutive venous occlusions (within day) and they found significant differences among them by running a one-way analysis of variance for repeated measures. It was therefore concluded that because no differences were expected on the basis of physiological background the venous occlusion method was not reliable to measure mVO_2. Interestingly, Beekvelt et al. (2001b) measured the same muscle (Flexor digitorum superficialis) using two different inter-optode distances, 35 and 50 mm. They found that a distance of 50mm generated reproducible results whereas and distance of 35mm did not. Although the mVO_2 measurements using an inter-optode distance of 50mm were considered reproducible, a CV of 25.4% was reported which is considerably higher that the 14.4% observed in the present study. Near infrared spectroscopy light travels in a "banana shape" from the source to the detector, and the maximum penetration depth is roughly half the inter-optode distance (Homma et al., 1996). Therefore, a possible explanation for the conflicting results observed in Beekvelt´s study, compared to the present investigation, is that an inter-optode distance of 35mm may have not been appropriate to measure the Flexor digitorum superficialis and could have compromised their results. In the present investigation, the NIRS device was applied on the bulk of the MGast using an inter-optode distance of 40mm. Furthermore results from the present investigation (using the venous occlusion method to quantify mVO_2) show a CV of 14.40%, which is comparable with the CV of 16.2% reported by Beekvelt et al. (2001) when assessing mVO_2 using the arterial occlusion method. This confirms that venous occlusion is a reliable method to measure mVO_2.

Expectedly, results from the present investigation show that CV values for muscular O_2 consumption were consistently higher when calculated between days (22.80%) than when measured within the same day (14.40%). However, the CV obtained in the present study for between days is in agreement with CV values reported in the literature when

mVO_2 was determined using the considered “more reliable” arterial occlusion method (Kragelj et al., 2000). Kragelj et al. (2000) reported a CV of 22.3% when O_2 consumption at rest was assessed on the distal part of the foot on four to six different days using arterial occlusion. In addition to that, Van Beekvelt et al. (2002) published a CV of 17.6% when mVO_2 measurements were carried out three times on separate days. Therefore, our results show for the first time that the “preferable” venous occlusion method is reliable to measure mVO_2 in the calf muscle within the same day and between days.

In conclusion, the present results suggest that venous occlusion is a reproducible method both within day and between days to quantify blood flow and muscular oxygen consumption in the MGast using an inter-optode distance of 40 mm.

Reliability studies for “EMD” determination

Electromyography is a non invasive method to measure the electrical signal associated with the contraction of a muscle (Winter et al., 2009). Hence, EMG data provides with useful information about to the timing and relative intensity of muscular function during different conditions, and has played a very important role in the understanding of human movement over the last decades (Perry & Burnfield, 2010). However, the EMG signal refers to the electrical event produced by the muscle and not to the mechanical output (force) (Winter et al., 2009). The time lag between EMG activity and force production is called electromechanical delay (EMD) and it is suggested to include the propagation of the action potential over the muscle membrane, the excitation-contraction coupling process and; the stretching of the series elastic component by the contractile element (Cavanagh & Komi, 1979; Nordez et al., 2009). Therefore, there is little doubt that EMD should be taken into consideration when attempting to associate EMG signal and muscle function. Otherwise the prediction of segmental movement from EMG data could be mistimed and could lead to misinterpretations.

The question remains how to better account for the time delay between muscular activity and force production when attempting to predict muscular function from EMG data. Previous investigations have reported differences in EMD values due to the type of muscle contraction (Cavanagh and Komi, 1979) and movement velocity (Howatson et al., 2009). This suggests that the task used to calculate EMD values should be the same as for the EMG analysis. Since in the present study EMG traces were investigated during gait, it appears ideal to determine EMD for the different muscles under investigation during walking. For this reason a reliability study was carried out to determine the reliability of a novel approach to determine EMD for the plantar-flexor muscles during gait. However, the instrumentation used during gait only allows the measurement of the forces generated by the plantar-flexion (PF) muscles whilst the forces produced by the dorsi-flexion (DF), knee-extension (KE) and knee-flexion (KF) muscles were not be determined. For this reason, another approach to determine EMD values for all the muscle groups during isometric contractions was also investigated. The next sections present the 2 reliability studies carried out prior to the main study to

determine the reliability of two different approaches to investigate EMD 1) during isometric contractions; and 2) during gait.

4.2 Electromechanical delay determination during isometric contractions.

4.2.1 Introduction

When EMD is determined as the time delay from the onset of muscular activity to the onset of force production (definition of EMD), EMG and torque production signals are synchronised at the beginning of the muscular contraction (the timing at the beginning of the muscular output is accurately predicted by the EMG signal). However, if the relationship between tested muscle/s EMG and the total joint moment is not linear then the prediction of muscular output from EMG signal may be inaccurate later on during the contraction. This obviously will compromise the interpretation of the EMG data in relation to movement. Many studies have intended to predict mechanical output on the basis of EMG signal (Amaranti & Martin, 2004; Hof et al., 1987; van Zandwijk, et al., 2000). However, there is not a general consensus on whether there is a linear relationship between EMG and force production even during isometric conditions. Hoff (1997) stated that in isometric conditions there is, usually, a linear relationship between muscle force and EMG data. In contrast, Metral & Cassar, (1981) found a linear relationship between these traces only during low intensity (< 50%) whereas above that intensity the relationship was not linear. This suggests that accounting for EMD does not fully resolve this issue and EMG cannot estimate mechanical output during sustained contractions.

Following this logic, it is reasonable to think that instead of calculating time differences from onset of muscular activity to onset of force production (EMD), the calculation of the mean time differences between EMG and isokinetic data during the whole contraction may be more meaningful in this context. Cross-correlation is a mathematical solution that provides the average time differences between two traces over a period of time. However, to the best of my knowledge, no study has investigated the reliability of this method to assess time differences between EMG data and

isokinetic dynamometer data. For the purpose of this investigation the term “EMD” refers to the average time differences between EMG and force data. However, EMD is usually defined as the time lag between the onset of muscular activity to the onset of mechanical output.

In the main study of the present thesis EMG data was used to investigate muscular activity patterns during gait in healthy subjects, as well as in patients with DN. For this reason the reliability of this new approach to calculate “EMD” was investigated both in healthy and DN subjects. An additional aim of the present study was to investigate “EMD” differences between healthy individuals and patients with DN. To the best of my knowledge no study has investigated whether subjects with DN show differences in EMD values compared to healthy counterparts.

Thus, the aim of this investigation was twofold: 1) to assess the reproducibility of cross-correlation to calculate “EMD” during 3 maximal isometric voluntary contractions in healthy as well as in DN patients; 2) to investigate “EMD” differences between healthy individuals and diabetic patients.

4.2.2 Methods

Subjects

78 volunteers participated in this study of which 53 were diagnosed with DN (DN group) and 25 were healthy (HEALTH group). The study was approved by the Cardiff and Vale NHS Trust Research & Development Office and the South East Wales Local Research Ethics Committee and all subjects gave their written consent.

The subjects characteristics were 62.20± 7.55 years of age, 169.1± 9.8 cm in height, and 93.03± 17.47 kg in body mass for the diabetic group and 57.76± 10.60 years of age, 171.1± 8.6 cm in height, and 78.63± 9.56 kg in body mass for the healthy group.

Procedures

"EMD" was assessed during 3 maximal isometric contractions for the knee extensors, knee flexors, ankle dorsi-flexors and ankle plantar-flexors. Ankle PF and ankle DF measurements were carried out with the subject lying down on a supine position. The right leg was semi-extended (30º± 10 flexion) with the ankle fixed at 15º plantar-flexion. The external malleoli was placed in line with the rotation axis. This position was chosen to maximize the muscular activity in the calf muscles during the PF. KF and KE were tested with the subject seated in an upright position (90º hip flexion) with their knees flexed to 90º and 70º, respectively. The right leg was secured into an instrumented cuff positioned at a point a few centimetres above the ankle joint with a stabilization strap across the femur of the right leg. A seat belt was used to secure the subject in the sitting position and prevent them from altering their position during the data collection. The moment arm distance was recorded and used when processing strength data for all 4 movements.

Prior to data collection, a warm up period was performed. To increase body temperature and therefore reduce the risk of muscular problems such as cramps, participants were asked to ride an indoor bike for 5 minutes at an increasing (moderate) pace. Once, on the isokinetic dynamometer device (on the position described above), they performed 15 contractions for 2 seconds at different levels of intensity (40% and 60%). Data collection consisted in three maximal voluntary contractions (MVC) per movement over a 3 second periods. Each contraction was followed by a 30 second rest period before the next MVC. Altogether 12 MVCs were recorded.

EMG device

EMG data was recorded using surface electromyography (TeleMyo™ 2400T G2 Transmitter, Noraxon Inc., Scottsdale, Arizona, USA) on the VL (knee extersor muscle), biceps femoris (BicFem) (knee flexor muscle), TA (dorsi-fkexir muscle) and TS (plantar-flexor muscle) of the right lower limb. TS EMG activity was calculated as the sum of the MGast, lateral gastrocnemious (LGast) and soleus EMG activity. EMG data was recorded at 1500 Hz using bipolar surface Ag/AgCL electrodes with a conductive area of 10 mm^2 (Kendall Meditrace 230; Tyco Healthcare, Hampshire,

PO13 0AS, UK). The diameter of the electrodes was 18 mm, and the inter-electrode distance was 37 mm. EMG data was collected and saved using the MyoResearch XP Clinical Application software (Noraxon Inc, Noraxon Inc., Scottsdale, Arizona, USA). EMG data processing was performed using a purpose written programme in Matlab (R2007a, Mathworks, Natick, USA). The raw EMG signal was full wave rectified and low pass filtered using a second order Butterworth filter with a 50 Hz cut off frequency to create a linear envelope that was used for further analysis.

Isokinetic dynamometer device

The KINCOM dynamometer (KinCom 125E plus; Chattecx, Oxfordshire, UK) was used to measure maximal isometric strength. The KINCOM dynamometer was connected to The TeleMyo™ 2400R receiver via an input analog channel. Data was collected and saved using the MyoResearch XP Clinical Application software. Further processing of the data was performed using a purpose written programme in Matlab. Further information about the instrumentation set up can be found in Chapter 4 (Section 5.4.1.2).

Calculations

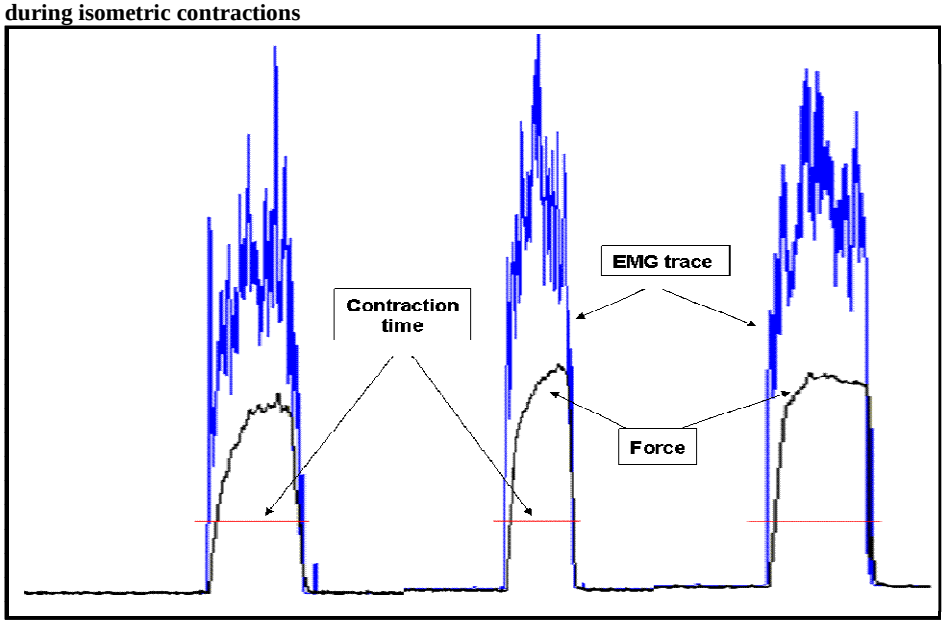
A written-purpose Matlab programme was developed to calculate the time differences between EMG an isokinetic data. The cross-correlation function available in the Matlab programme was used to determine time differences between the traces during the isometric contractions. Thus, VL, BF, TA and TS EMG activity was investigated in relation to isokinetic data during KE, KF, DF and PF measurements, respectively. Figure 4-2 shows an example of both EMG and force traces processed with Matlab.

Statistics

Systematic bias for within-day reliability data was assessed by a one-way ANOVA for repeated measures. The within-subject variability was calculated as the CV for each subject [(SD/mean)*100]. The within-day reproducibility was determined by calculating the ICC. The same statistical tests were applied when assessing the reproducibility during the different types of contraction. In addition to this, an independent t-test was carried out to assess differences in “EMD” values for the

different types of contractions between the DN and HEALTH group. The level of statistical significance was set at $p \leq 0.05$. All analyses were performed with SPSS version 16.0.

Figure 4-2. Example of EMG and Force data processed with matlab to calculate "EMD" values during isometric contractions



Note: The blue line represents the EMG trace and the black line represents the force generated in the KINCOM machine. The red line represents the periods during which the differences between both traces were investigated.

4.2.3 Results

All the results for within-day reliability measurements are shown in Table 4-5. No significant differences between within-day measurements were observed for any of the muscle groups (KE, KF, DF, PF) both in the HEALTH and DN groups. The relative variability within-subjects (CV) was 9.21% during DF, 12.68 % during PF, 13.77 % during KE and 14.96% during KF in the HEALTH group and 13.11% during DF, 13.15% during PF, 15.43% during KE and 16.62% during KF in the DN group. ICC values of 0.68 and 0.62 during DF, 0.75 and 0.65 during PF, 0.61 and 0.64 during KE and 0.62 and 0.60 during KF were observed for the HEALTH and DN groups, respectively.

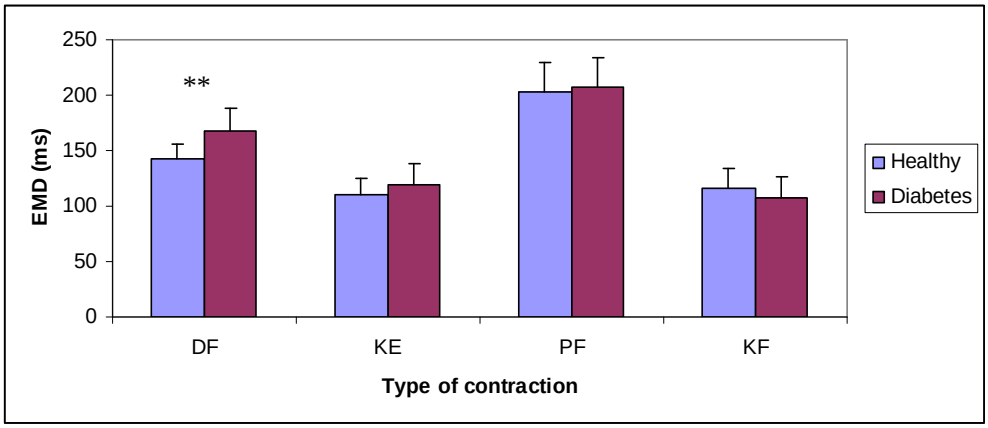
Furthermore, Figure 4-3 presents the results with regard to the differences in "EMD" values for the different group muscles between the HEALTH and the DN group. "EMD" during DF is the only value that significantly differed between both population groups ($p<0.001$). Thus, the DN group reported significantly higher "EMD" values (67.31± 21.19) compared to the HEALTH group (142.24 ± 13.40).

Table 4-5. Within-day reliability for "EMD" calculations during isometric contractions

	Mean over 3 Rep (ms)	**Anova p value**	**CV %**	**ICC**
Dorsi-Flexion				
HEALTH (N=25)	142.24± 13.40 [a]	0.659	9.21	0.68
DN (N=53)	167.31± 21.19 [a]	0.579	13.11	0.62
Plantar-Flexion				
HEALTH (N=25)	204.95± 26.71 [a]	0.426	12.68	0.75
DN (N=53)	206.98± 26.08 [a]	0.101	13.15	0.65
Knee Extension				
HEALTH (N=25)	110.34± 15.10 [a]	0. 759	13.77	0.61
DN (N=53)	119.76± 18.11 [a]	0.572	15.43	0.64
Knee Flexion				
HEALTH (N=25)	116.16± 17.35 [a]	0.625	14.96	0.62
DN (N=53)	107.83± 19.17 [a]	0.735	16.62	0.60

[a] Values are means ± SD.

Figure 4-3. "EMD" values calculated during isometric contractions: Comparison between the HEALTH and the DN group[a]



[a] Mean and standard deviation (SD) (error bar) ; **Significance value is less than 0.01 level (2-tailed).

4.2.4 Discussion

The present investigation demonstrated that cross-correlation is a reproducible (within-day) technique to calculate "EMD" values during 3 maximal isometric voluntary contractions in healthy as well as in subjects with DN. This is the first study that demonstrates that EMD values may be affected by DN.

Reliability

"EMD" values for PF, DF, KE and KF showed substantial within-day reliability both for the healthy and DN group (Landis & Koch, 1977). ICC values in the health group ranged from 0.61 during KE to 0.75 during PF whilst ICC values in the DN group ranged from 0.60 during KF to 0.65 during PF. These results demonstrated that cross-correlation is a reliable approach to calculate average time differences between muscular activity and force production traces over a period of time during maximal isometric contractions. However, the CV values obtained in the present study, which ranged from 9.21% (DF) to 14.96% (KF) in the HEALTH group and from 13.11% (DF) to 16.62% (KF) in the CN group, were substantially higher than the CV values reported by Howatson et al. (2009) when assessing EMD in the elbow flexors during isometric

contractions (CV 3.1%). To the best of my knowledge, Howatson et al. (2009) is the only study that has assessed the reliability of a similar approach to calculate EMD. Furthermore, Howatson et al. (2009) reported EMD values of 58.35 ms while in the present investigation "EMD" values ranged from 110 ms during KE to 210 ms during PF in the HEALTH group. It is likely that methodological differences may explain the diversity in these results. For instance, Howatson and colleagues (2009) calculated EMD as the time differences between EMG onset and force production onset. Since the present study used a longer time frame to determine EMD (whole contraction versus onset), and considering that the relationship between muscular activity and force production may be non-linear at maximal intensities (Metral & Cassar, 1981), it is logical that results from the present study show higher CV compared to Howatson et al. (2009). Furthermore, differences in the approach to determine EMD values (whole contraction versus onset) may also partly explain the greater EMD values found in the present investigation compared to Howatson´s study. Metral & Cassar (1981) investigated the relationship between force and EMG in the forearm (biceps brachii) when working at different intensities. They found a linear relationship between those traces between 0 and 50% of the maximum force while at forces greater than 50% the relationship was non-linear. They reported that the slope of the EMG signal was steeper than the one from the force and this became more obvious at intensities near the maximal force. This finding may partly explain why Hotwatson et al. (2009), who calculated EMD at the onset of the contraction, found lower EMD values compared to the present study, in which "EMD" values were calculated over the whole contraction. This finding is particularly important since it suggests that EMD values calculated at the onset of the contraction may represent EMD values during the whole contraction. This highlights the usefulness of this new approach to calculate "EMD" when attempting to predict muscular function from EMG data during a sustained contraction.

Overall, the present study demonstrated that this new approach to determine the time differences between EMG and force production during the whole contraction is reproducible. Furthermore, this method may result in a more accurate estimation of the mechanical output timing from EMG data during prolonged activities.

EMD differences between healthy and DN subjects

Several investigations have demonstrated differences in EMD values due to gender (Winter & Brookes, 1991), after ligament reconstruction (Kaneko et al., 2002), in response to a training programme (Grosset at al., 2009), and in case of neuropathies (Granata et al., 2000). This is the first investigation reporting that EMD may be affected in patients with diabetic neuropathy. The present study shows that "EMD" values for the TA muscle during DF were significantly higher ($p<0.001$) in the DN group (167.31) compared to HEALTH group (142.24). However, no significant differences were found during PF, KE or KF.

According to Richardson et al. (1992), the first nerve to show electrophysiological alterations due to diabetic motor neuropathy is the fibular nerve, which innervates the TA. This may explain why the TA is the only muscle that shows significant differences when comparing "EMD" values between healthy individuals and patients with DN. This finding in the context of EMG data processing is particularly important since it highlights the importance of calculating individual EMD values in DN subjects when attempting to estimate muscular output from EMG data. For instance, if this finding is related to EMG activity during gait, a longer EMD is expected to bring the activation patterns forward. Since the time lag from activation onset to muscle production is longer muscle activation is expected to occur earlier.

Overall the present investigation demonstrated for the first time changes in EMD values in DN subjects when compared to healthy individuals. This highlights the importance of assessing individual EMD values when attempting to estimate muscular output from EMG data, especially when comparing populations with different EMD values such as DN.

4.3 Electromechanical delay determination for the plantar-flexor muscles during gait

4.3.1 Introduction

Howatson et al. (2008) investigated EMD values during isometric and isokinetic contractions and they found that EMD was somewhat shorter during isometric compared to isokinetic contractions. They reported values of 57± 5.5 ms and 72.3± 8.9 for isometric and slow isokinetic contractions, respectively. In addition to this, Cavanagh & Komi (1979) found that the EMD under eccentric contractions (49.5 ms) was significantly shorter than during concentric movements (55.5 ms). These findings suggest that EMD values determined during isometric conditions may not represent EMD values during more dynamic conditions such as walking.

Some studies looking to estimate muscular forces from EMG recordings have skipped the problem of calculating EMD and focused on the similarity between EMG and joint moment traces (Hof et al., 1987; Metral & Cassar, 1981). It is generally believed that the TS muscle group, which consists of the soleus and gastrocnemious muscles, is the main responsible for the PF of the ankle (Winter, 2009). Furthermore, the ankle moment during the stance phase of gait is believed to be indicative of the torque generating capability for the plantar-flexor muscles (TS) (Winter, 2009). Some studies have linked EMG and kinetic data during gait by synchronising EMG activity patterns with the joint moments obtained when walking over a force platform (Hof et al., 1987 & Metral & Cassar, 1981). However, to the best of my knowledge, no study has investigated the reliability of this approach.

In this study “EMD” was defined as the time delay from the peak muscular activity of the TS to the max vertical GRF generated on the forefoot during the second half of the stance phase. This approach may allow the synchronization of EMG (TS) and kinetics data accurately during gait.

In the main study of this thesis EMG data was measured to investigate muscular activity patterns during gait in healthy subjects and patients with DN. For this reason the reliability of this new approach to calculate “EMD” was investigated both in healthy

and DN subjects. An additional aim of the present study was to investigate “EMD” differences between healthy individuals and patients with DN during gait.

Thus, the aim of this investigation was twofold: 1) to assess the reproducibility of a new approach to calculate the time differences from the peak TS muscular activity to the max GRF during the push off phase of the gait cycle; 2) to compare “EMD” values during gait between healthy individuals and patients with DN.

4.3.2 Methods

Subjects

78 volunteers participated in this study of which 53 were diagnosed with DN (DN group) and 25 were healthy (HEALTH group). The study was approved by the Cardiff and Vale NHS Trust Research & Development Office and the South East Wales Local Research Ethics Committee and all subjects gave their written consent.

The subject characteristics were 62.20± 7.55 years of age, 169.1± 9.8 cm in height, and 93.03± 17.47 kg in body mass for the diabetic group and 57.76± 10.60 years of age, 171.1± 8.6 cm in height, and 78.63 ± 9.56 kg in body mass for the healthy group.

Protocol

Participants were asked to walk barefoot at their self-selected speed on a 9 meter walkway. Before data acquisition, the subjects were instructed to walk freely on the walkway to reproduce their normal gait and to adapt to the laboratory environment. Multiple trials were permitted to allow the subjects to practice walking without visually targeting the platform surface. The testers adapted the subjects starting position to ensure the platform was always hit on the fourth step. Data was collected over 5 trials for the right foot.

EMED platform

Ground reaction forces were measured with the EMED platform (EMED-m, Novel GmbH, Munich, Germany). The platform was positioned level with the floor at the

midpoint of the 9 meter walkway. The platform consists of a matrix of 3840 force transducers that are uniformly distributed in an area of 24 × 38 cm. The platform has a resolution of 4 sensors·cm^{-2} with a sampling frequency of 50 Hz. The sensors have a pressure range from 10 kPa to 1200 kPa.

The data was processed and analysed by Novel Software (Novel 13.3.42, Novel GmbH, Munich, Germany). Using this software the instance of roll over process (in milliseconds) in which the maximal vertical GRF occurs during the push of phase was calculated.

EMG

Muscle activation patterns during walking were recorded using surface EMG (TeleMyo™ 2400T G2 Transmitter) on the MGast, LGast and soleus of the right lower limb. EMG data was recorded at 1500 Hz using bipolar surface Ag/AgCL electrodes with a conductive area of 10 mm^2. The diameter of the electrodes was 18 mm, and the inter-electrode distance was 37 mm. EMG data was collected and saved using the MyoResearch XP Clinical Application software. EMG data processing was carried out using a purpose written programme in Matlab.

Data Processing and Calculations

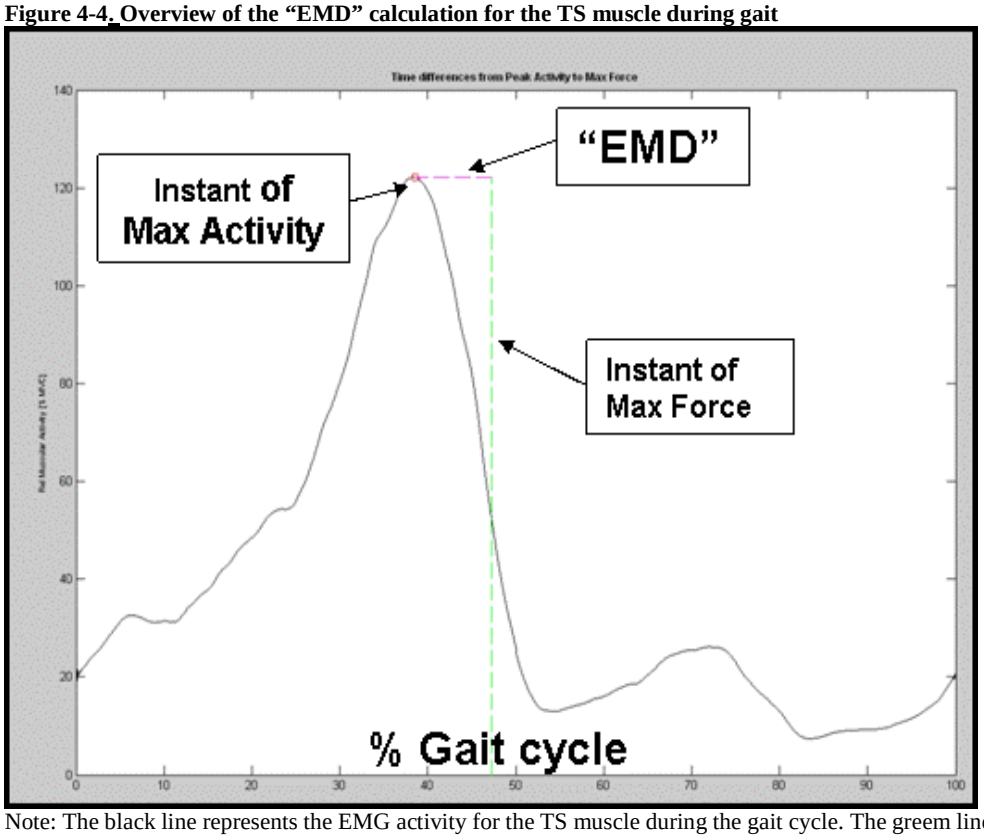
A purpose written Matlab programme was created to calculate the time delay between the peak muscular activity of the TS (calculated as the sum of the MGast, LGast and soleus EMG activity) and the max vertical GRF generated on the forefoot during the second half of the stance phase. As discussed in the introduction, it is believed that the TS muscle group is the main responsible for the PF of the ankle. This suggests that the vertical GRFs during the stance phase are originated from the TS.

The raw EMG signal was full wave rectified and low pass filtered using a second order Butterworth filter with a 50 Hz cut off frequency to create a linear envelope that was used for further analysis. To be able to relate EMG activity to the GC, EMED data and EMG data were collected simultaneously. See Chapter 5 (Section 5.4.1.1) for more information about the instrumentation set up during this task. Therefore, EMG data

could be processed in relation to the heel strike. In addition to that, the instant of the max vertical GRF was calculated by Novel Software.

Figure 4-4 shows a graphical representation of how the time delay between the peak muscular activity of the TS and the max vertical GRF generated on the forefoot during the second half of the stance phase was calculated.

Figure 4-4. Overview of the "EMD" calculation for the TS muscle during gait



Note: The black line represents the EMG activity for the TS muscle during the gait cycle. The greem line shows the instant of the maximal vertical GRF.

Statistics

Systematic bias for within day reliability data was assessed by a one-way ANOVA for repeated measures (5 measurements). The within-subject variability was calculated as the coefficient of variation for each subject [(SD/mean)*100]. The within-day reproducibility was determined by calculating the ICC. In addition to that, an

independent t-test was used to assess group differences in EMD values during PF. The level of statistical significance was set at $p \leq 0.05$. All analyses were performed with SPSS version 16.0.

4.3.3 Results

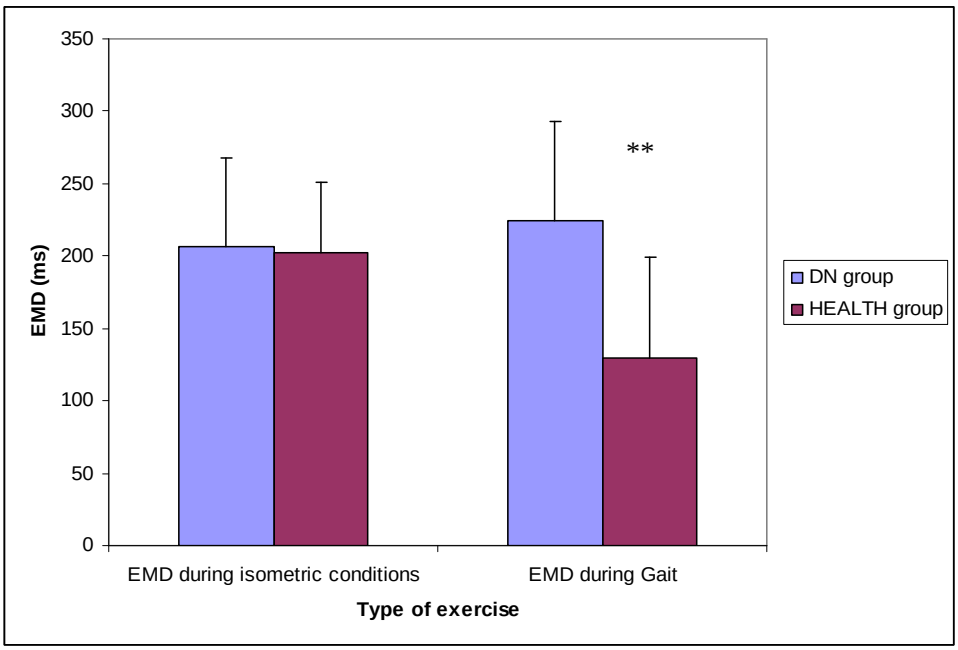
Results for within-day reliability measurements are shown in Table 4-6. No significant differences between within-day measurements were observed both in the HEALTH (p=0.22) and DN (p=0.24) groups. In addition to that the relative variability within subjects (CV) was 19.01% for the HEALTH group and 13.92% in the DN group. An ICC of 0.89 and 0.77 was observed in the HEALTH and DN groups, respectively. Figure 4-5 shows the results from the independent t-test, which investigated differences in EMD values between the HEALTH and the DN group. Thus, results from the present study show significantly higher values in the DN group compared to the HEALTH group when EMD were calculated during gait.

Table 4-6. Within day reliability for "EMD" calculations for the PF muscles during gait

	Mean over 5 Rep (ms)	**Anova p-value**	**CV %**	**ICC**
Peak to Peak				
HEALTH (N=25)	129.28± 69.62 [a]	0.222	19.01	0.89
DN (N=53)	224.77± 67.85 [a]	0.245	13.92	0.77

[a] Values are means ± SD.

Figure 4-5. "EMD" values for the TS muscle calculated both during isometric and gait conditions: Comparison between the HEALTH and the DN group[a]



[a] Mean and SD (error bar); **Significance value is less than 0.01 level (2-tailed).

4.3.4 Discussion

Results from the present investigation demonstrated a good reproducibility of this method to calculate "EMD" during gait. Furthermore, results from the present investigation show that "EMD" values in the plantar-flexor muscles during gait were affected by DN.

Results from the present investigation show that "EMD", calculated as the time delay from the peak muscular activity of the TS to the max GRF generated during the push off, is a reproducible solution to estimate muscular output from EMD recordings. An ICC value of 0.89 and 0.77 for within-day measurements for the HEALTH and DN groups respectively, demonstrated that the reproducibility of this approach to estimate

“EMD” was excellent for the HEALTH group and substantial for the DN group (Landis & Koch, 1977).
Furthermore, the present investigation showed significant differences in “EMD” values between the HEALTH and the DN group. Interestingly, the previous section, which estimated EMD values in the same muscle group during isometric contractions, did not find group differences. Although this is a remarkable finding the understanding of these results go beyond the purpose of this preliminary study. This finding in the context of EMG data processing highlights the importance of assessing individual EMD values when attempting to estimate muscular output from EMG data, especially when comparing populations with different EMD values such as DN.

Overall, the present investigation demonstrated that the synchronization of EMG and kinetics data is a reliable approach to estimate muscular output from EMG data during gait. In addition to that, it appears that EMD values determined during isometric conditions may not represent EMD values during more dynamic conditions such as walking. For this reason during the main study EMD values for the PF muscles will be calculated during gait using the approach tested in this section. However, EMD values for the DF, KE and KF muscles will be calculated during isometric contractions using the method evaluated in the previous section (see Section 4.2), as absence of appropriate force measuring devices prevented DF, KE, and KF moments being calculated.

CHAPTER 5

5 Methods- Main study- Part 1 and 2

This chapter outlines the methodology for Part 1 and 2 of the main study. Part 1 of the main study followed a comparative case-control study design to investigate differences between patients with diabetic peripheral neuropathy (DN) and matched healthy controls (HEALTH) in general health outcome measures, gait characteristics, microcirculation and QOL. Part 2 followed a quasi-experimental test-retest intervention study-design to investigate the effect of a 16-week physical activity programme on general health outcome measures, gait characteristics, microcirculation and QOL in patients with DN.

Part 1 and part 2 of the main study were not independent of one another. Therefore, the volunteers from the DN group from the cross-sectional comparative study were included in the intervention study (see Figure 5-1). Part 1 and 2 followed the same experimental protocol and used the same outcome measures. Therefore, sections on instrumentation, experimental procedures, data processing and ethical considerations apply to both parts whereas sections on the study design, sample size (from recruitment to study completion) and the statistics are presented for each part separately.

5.1 Study design

Part 1

To address the hypotheses for part 1 of the main study, a case-control cross-sectional comparative study design was carried out. This study design is commonly used to compare patients who have a disease or outcome of interest (the “cases”) with patients who do not have the condition, but are otherwise similar (the “controls”) (Rose & Barker, 1978). Furthermore, it allows for potential confounding factors to be controlled by measuring them and making appropriate adjustments in the analysis (Rose & Barker, 1978). In this study, age, height, sex and body mass were identified as potential confounding variables.

Part 2

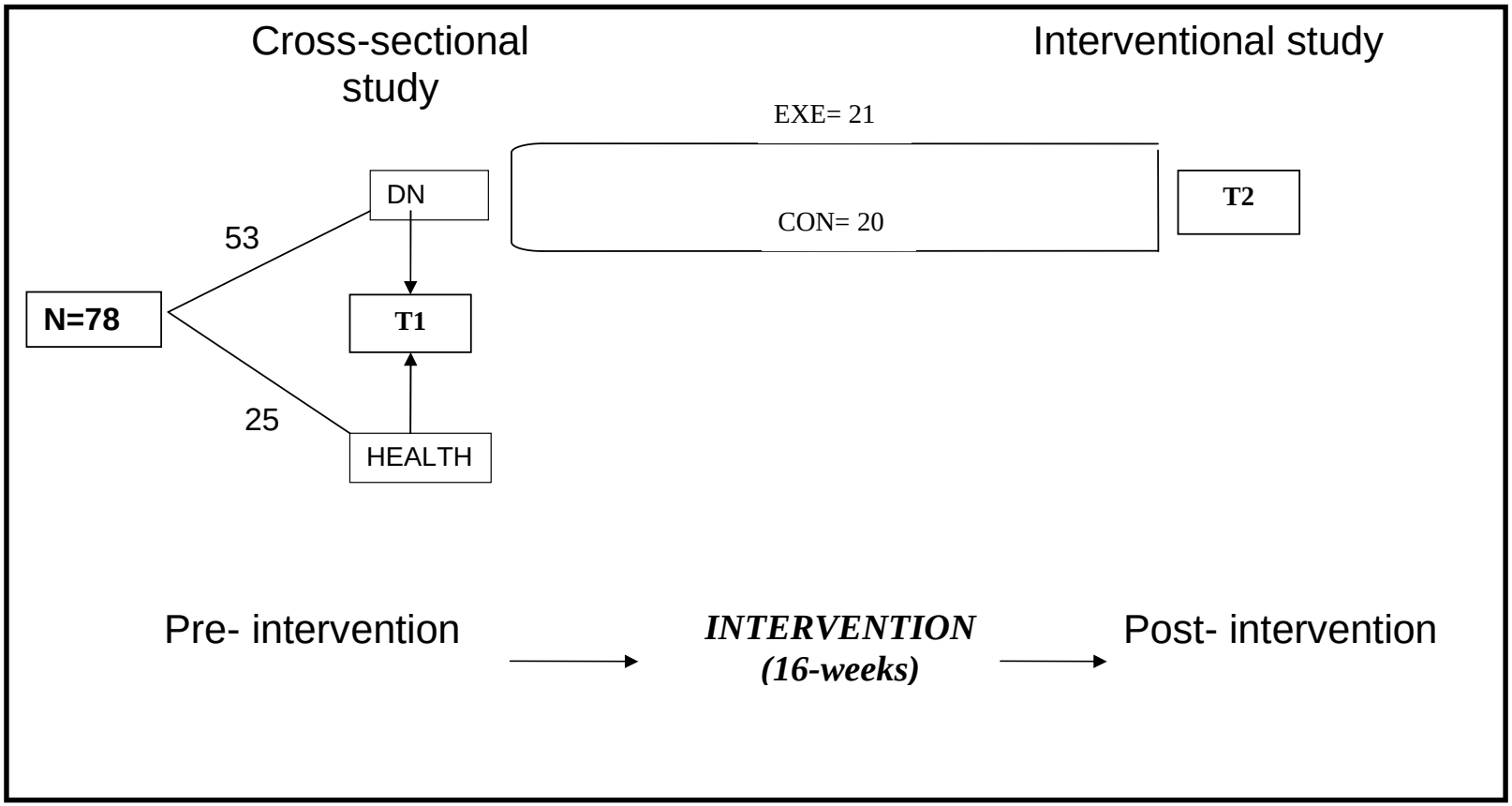
A quasi-experimental, test-retest, interventional study design investigated the effect of a 16-week physical activity programme on the multiple health problems associated with DN including general health, gait and microcirculatory parameters as well as self-reported QOL in DN patients. Two groups were compared over time; an exercise (EXE) group, which participated in the exercise programme, and a control (CON) group, which was not provided with any intervention. Randomisation in the present study was based on the patient's preferences and therefore cannot be called an experimental randomised control trial (RCT). Randomisation based on patient preferences is considered an acceptable randomisation method when patients have strong preferences for one intervention group or the other (Campbell, et al., 2000; Craig, et al., 2008).

Random allocation increases internal validity and minimise the selection and testing bias related to pre-testing subjects with the knowledge of group allocation (Craig et al., 2008). However, the main problem of RCTs is if a substantial proportion of patients refuse the allocation group, and as a consequence differences in outcome may be obscured (Rose & Baker, 1978). In addition to this, it may also result in a high number of drop outs, which may compromise the power of the study (Bratcher et al., 1970). It is noteworthy that previous studies have reported high rates of drop-out in PA intervention in diabetic populations (Thomas et al., 2006). Therefore, a study design which allowed participants the opportunity to choose in which group they wanted to be included was used for the present investigation.

Due to the nature of the study design and since randomisation was based on the patient's preferences, it was impossible to blind the participants to the intervention group they were allocated to. Since the vast majority of the outcome measures were calculated quantitatively by computerised systems, it was not considered necessary to blind the tester with regard to which intervention group participants were allocated.

The subjects were tested on two visits split by a 16-week period. Between the two visits, the EXE group carried out a structured physical activity programme whereas the CON group continued as before.

Figure 5-1. Overview of the study design. ***Population groups:*** **Neuropathic group (DN) and healthy group (HEALTH);** ***Intervention groups:*** **exercise group (EXE) and control group (CON);** ***Tests*****: T1 (pre-intervention measurements) and T2 (Post-intervention measurements).**



5.2 Intervention

The study was a semi-controlled interventional study, in which participants were asked to both attend supervised training sessions and undergo home-based exercises for 16 weeks. All the supervised sessions took place in the outpatients physiotherapy gym at the University Hospital of Wales (UHW). Permission to use the facilities was obtained from the Head of Department.

Each supervised session lasted approximately 1hour and included 10 minutes warm up, 40 minutes resistance training using resistance training machines, and a 10 minute cool-down. During warm-up and cool-down phases flexibility and stretching exercises were included. In addition to this, during cool-down a variety of foot mobility exercises were included for at least 5 minutes. Subjects were asked to perform 2-3 sets of 8-12 repetitions on each machine per session. 5 different exercises were carried out each session to work out all main muscles on the lower limbs. The resistance training protocol was designed to provide progressive increases in volume of approximately 10% every two weeks. However, to minimise the risk of injury and overtraining (Fleck & Kraemer, 1997), volume was reduced by approximately 20% during weeks 7 and 16. In the present study, individual volume was calculated weekly in order to ensure that the appropriate amount of training was completed for each subject. Volume was calculated by adding the amount of kilograms lifted per muscle group per week. Training intensities during weeks 1-8 were set up at 60-80 % of 1RM (percentage of a one repetition maximum contraction), whereas intensities during weeks 9-16 weeks were programmed at 70-80 of 1RM. 1-RM testing was repeated at week 8 to establish a new baseline. All subjects were required to perform each repetition in a slow, controlled manner, with a rest of 75-120 seconds between sets.

Home based exercises included 2 sets of 12 repetitions targeting the major muscle groups on the upper body. These exercises were carried out using a resistive exercise band (Thera-band, UK). In addition to this a variety of joint mobility exercises targeting all the main joints were also included in the home based programme (see Appendix 2). All the participants were required to exercise at least 70% of all the sessions in order to be included for analysis. In order to control the home-based training programme, each

participant was provided with a diary which they were asked to fill out daily. Every week each participant was asked to bring the diary so the home-based session could be inspected and therefore controlled. However, it was observed early in the intervention that the dairy was not a reliable tool to control home exercises. For instance, some participants who reported in the dairy that they successfully completed the home exercises did not remember the exercises or had lost the resistance exercise band. For this reason home exercises were left optional for the participants. Therefore, the inclusion criterion for the participants to be included in the analysis was at least 70% of all the gym based sessions.

5.3 Subjects

The characteristics of the subjects who were invited to participate in the study are specified below.

5.3.1 Inclusion Criteria

The inclusion criteria for the subjects with diabetic peripheral neuropathy were:

- Diabetic peripheral neuropathy (inability to detect 10 g monofilament in at least one of four plantar areas)
- Age (45 – 70 years old)
- Type 2 diabetes + non insulin dependent
- All the participants should be capable of walking independently to perform their activities of daily life without a walking aid
- Corrected vision 20/20

The inclusion criteria for the healthy group were:

- No diabetes
- Age (45 – 70 years old)
- All the participants should be capable of walking independently to perform their activities of daily life without a walking aid

5.3.2 Exclusion Criteria

The participation criteria were framed to exclude individuals with conditions that could affect the outcome measures under investigation independently of DN. Since one of the aims of the study was to investigate gait characteristics, people with foot deformities, which are known to affect gait parameters, were excluded from the study. Similarly, people with peripheral vascular diseases or taking medications that could affect circulation were also excluded from the present investigation since they may have an effect on the microcirculation. In addition to this, to avoid any adverse effect during either the data collection or the physical activity intervention subjects suffering from cardiac or lung diseases, uncontrolled blood pressure, current ulcers or other neuropathic complications were also not invited to participate in this study. People with high levels of physical activity were excluded from the study since physical activity may have a direct effect on many of the outcome measures under investigations including general health microcirculation, gait and metal health. Subjects involved in PA programmes are less sensitive to long-term exercise-induced adaptations, which may compromise the results from the intervention study. A more detailed description of the exclusion criteria can be found below.

The exclusion criteria for the subjects with diabetic peripheral neuropathy were:

- Foot deformities
    - Charcot foot
    - Osteomyelitis
- Ulcers
    - Previous ulcers must be prior to 3 months before the study starts.
    - All previous ulcers must have been superficial (no bone, tendons or cartilage must have been affected)
- Peripheral vascular diseases
    - Patients with a history or any symptoms of peripheral vascular disease (pain in calf after walking 100 yards)
- Severe cardiac or lung diseases
    - History of cardiac events (i.e. heart attack, stroke, angina pectoris)
    - History or clinical evidence of cardiac abnormality (complications) (i.e. arrhythmias, heart blockage)

    - Patients taking medications that may affect the cardiovascular system (i.e. vaso-active agents).
    - Severe lung diseases
- Other neuropathic complications
    - Retinopathy
        - Glaucoma (High blood pressure)
    - Nephropathy
        - Clinically diagnosed nephropathy
        - Serious history of kidney failure (dialysis)
    - Patients with severe painful forms of diabetes peripheral neuropathy
- Patients with high blood pressure not controlled by medication
- Patients with any major neurological and/or musculo-skeletal impairment other than those resulting from diabetic foot complications
- People who underwent regular physical exercise

The exclusion criteria for the healthy group were:

- Peripheral vascular diseases
    - Patients with a history or any symptoms of peripheral vascular disease (pain in calf after walking 100 yards)
- Severe cardiac or lung diseases
    - History of cardiac events (i.e. heart attack, stroke, angina pectoris)
    - History or clinical evidence of cardiac abnormality (complications) (i.e. arrhythmias, heart blockage)
    - Patients taking medications that may affect the cardiovascular system (i.e. vaso-active agents).
    - Severe lung diseases
- Patients with high blood pressure not controlled by medication
- Patients with any major neurological and/or musculo-skeletal impairment
- People who underwent regular physical exercise

5.3.3 Recruitment Strategy

Eligible patients for the DN group were identified by the referring clinician[1] and given a brief outline of the study for their consideration. Clinicians were contacted through two main routes: 1) Podiatric clinics and 2) Wound healing clinics in the Cardiff & Vale Trust. At the podiatric clinics clinicians were contacted via the departmental director, Mr Scott Cowley, whereas at the wound healing clinics clinicians were contacted via the clinical director, Professor Keith Harding.

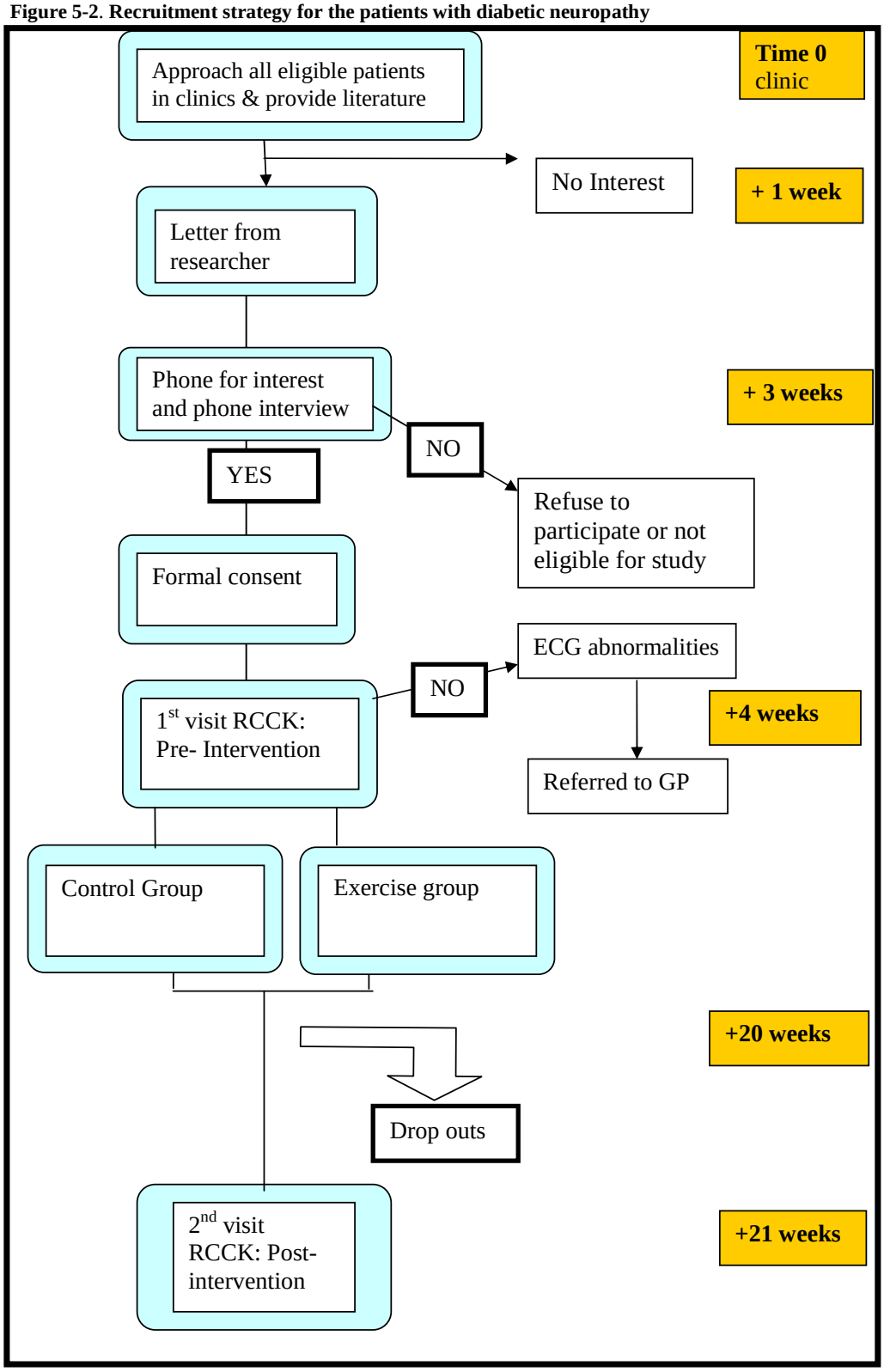
Patients who were in agreement were then contacted by the researcher. The first contact was done by correspondence in which an invitation letter together with an information sheet explaining the characteristics of the study were attached (Appendix 3 and Appendix 4, respectively). They were offered up to two weeks time to decide whether they were interested in participating in the study, and if interested, in which group (EXE or CON) they would like to be included (see Section 5.1 to find out more details about the randomisation process). After this period participants were contacted by phone to ask them about their decision; if positive an interview was held on the phone to confirm whether or not the participant was suitable for the study (see Appendix 5). If the interview was successful, an appointment for them to visit the Research Centre for Clinical Kinaesiology (RCCK) laboratory at Ty Dewi Sant (Heath Park Campus, Cardiff) was arranged. Once the appointment was made, a confirmation letter was mailed to each participant, together with the informed consent sheet (Appendix 6 and Appendix 7, respectively). During this first visit to the RCCK, an electrocardiogram (ECG) at rest was carried out on the participants interested in participating in the exercise programme. If the ECG at rest was within normal limits, which was determined by a qualified anaesthetist who reviewed the ECGs, participants were invited to participate in the exercise programme. On the other hand, if the ECG was not within normal limits the participant was referred to their GP following consultation with the clinical supervisor. After the first visit, participants belonging to the CON group were contacted again in 16 weeks to arrange the second visit to the RCCK. Further information about the recruitment strategy can be found in the flow chart presented in Figure 5-2.

[1] Note: Clinical data was used by the referring clinician to identify suitable participants (i.e. type of neuropathy, peripheral vascular diseases, history of cardiac or lung diseases, foot deformity, etc)

One of the exclusion criteria for the study was severe (or a history of) cardiac diseases. However, an ECG on the exercise group was carried out to confirm that participants did not suffer from any heart condition that could be worsened by the exercise programme.

The healthy control group was contacted: 1) from social clubs in the Cardiff & Vale Trust catchment area; 2) from university staff members; and 3) from the DN group. Participants from the DN group were asked whether any family member without history of diabetes would be interested in participating in the study as a healthy control.

Figure 5-2. Recruitment strategy for the patients with diabetic neuropathy



5.3.4 Sample size: From the recruitment stage to the study completion

Initial statistical analysis consultation determined that for part 1 of the main study 60 subjects in the DN group and 30 subjects in the HEALTH group were needed. No additional recruitment was required for part 2 of the main study since volunteers who participated in the intervention study were obtained from the DN group in part 1(see Figure 5-1 for more information about the study design). Information about how sample size was determined can be found in Section 5.6.1.

5.3.4.1 Part 1 of the main study

To achieve those numbers recruitment was extended for a 2-year period (from April 2008 to March 2010). This period included the recruitment of patients with diabetic peripheral neuropathy as well as the healthy group. Data collection was carried out over 16 months (from January 2009 until April 2010). The time from the start of recruitment (April 2008) to the start of data collection (January 2009) was spent in creating a pool of participants (DN group) who were interested and suitable for the study. Prior to the start of recruitment, this part of the study was expected to be completed within 12 months. This time frame was constructed based on experience from previous research at the Research Centre for Clinical Kinaesiology (RCCK) with the same population. However, due to difficulties in the recruitment this time frame had to be extended for another 12 months.

During those two years a total of 425 patients with diabetic neuropathy were approached in clinics (by clinicians), provided with information about the study (information sheet) and agreed to be contacted by researchers. Following this first contact, all participants were contacted by phone to find out about their decision. If interested, an interview over the phone was carried out to make sure they were suitable for the study. 325 of those 425 candidates (76%) refused to participate or were not eligible for the study. If the interview was successful, an appointment for them to visit the RCCK laboratory at Ty Dewi Sant (Heath Park Campus, Cardiff) was arranged. The main motives to refuse to participate in the study were: 1) not interested in research and 2) lack of time due to work or family commitments. The main causes to be excluded

from the study were: 1) type I diabetes (insulin dependents); 2) history of heart problems (heart attack or angina); 3) walking difficulties (walking aids); and 4) regular physical activity.

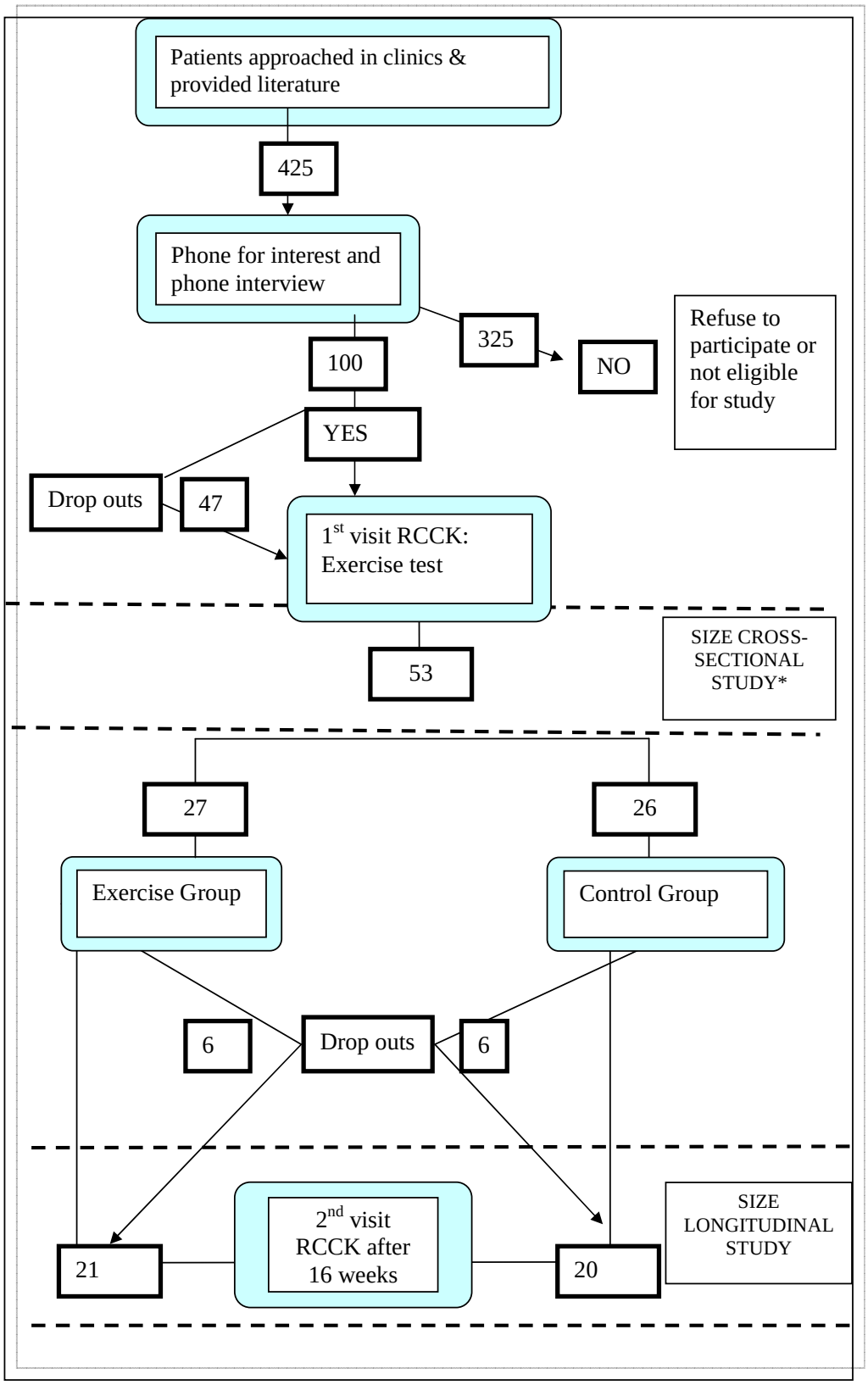
Once the appointment was made a confirmation letter was mailed to each participant, together with the informed consent sheet. Out of the 100 confirmation letters, sent to as many individuals, at the end only 58 attended. 5 out of those 58 had to be excluded from the study. 2 people were excluded due to ECG abnormalities, 1 person due to uncontrolled hypertension and 2 people since they were undertaking regular physical activy. The subjects who did not attend the appoinment were recontacted to find out the reasons for their absence and to give them the opportunity to rearrange another appoinment if still interested. Therefore, 53 participants with diabetic neuropathy visited the RCCK for the first visit and were included in the DN group for the cross-sectional study. In summary out of 425 persons who were invited to participate in the study, only 53 participated in part 1.

The healthy group was recruited in two different time frames. Ten healthy individuals were measured together with our first DN patients, and the remaining 15 volunteers were measured at the later part of the study. The reason for leaving half of the HEALTH group for the end of the study was to match both groups, DN and HEALTH, on weight, height, age and gender since those variables may influence the outcome measures investigated in the present study. However, it was impossible to succesfully match both groups on weight. Due to the characteristics of our DN group, whose mean age and weight were 62 and 93 kg respectively, it was imposible to find sufficient individuals with those characteristics and no further health issues (i.e. diabetes or heart problems). See results Chapter (Section 6.1.1) for more information about the participants characteristics. In summary 53 subjects were included in the DN group while 25 individuals were included in the HEALTH group. See Figure 5-3 for more information about the recruitment process.

5.3.4.2 Part 2 of the main study

Of the 53 individuals included in the DN group, 27 subjects were then included into the EX group while the CON group was composed of 26 individuals. During the 16-week period between visits, 6 participants dropped out from each group. Then, 21 subjects completed the exercise programme in the EXE group and 20 individuals completed the 2 visits to the RCCK as controls, which resulted in a 22 and 23% of drop outs for the CON and the EXE groups, respectively. See Figure 5-3 for more information about the recruitment process.

Figure 5-3. Sample size from recruitment stage to study completion



5.4 Data collection

To test the study hypotheses data was collected on four different domains named general health, gait characteristics, microcirculation and quality of life. Table 5-3 provides an overview of the different measurements carried out during data collection and how they relate to the different domains investigated in the present study. As mentioned earlier, data collection did not differ between the cross-sectional and intervention studies and for this reason this section applies to both studies. This section will start providing some specific information about some of the instrumentation set up carried out during the main tasks of the data collection. Thereafter, the procedures carried out during the whole data collection will be explained.

5.4.1 Instrumentation set up

Data collection included three main tasks, during which 1) gait measurements, 2) strength measurements and 3) microcirculation measurements were carried out. During these tasks data was collected simultaneously with different equipments. This section will provide details of the specific instrumentation set up used during each task.

5.4.1.1 Instrumentation set up during gait

During gait analysis, foot-floor interaction and muscular activity data were collected simultaneously. Foot-floor interaction parameters during barefoot walking were assessed using a pressure platform (EMED, Novel) while muscular activity on the lower limb (TA, TS, VL and BicFem) was measured using a wireless 8 channel EMG device (TeleMyo™ 2400T G2 Transmitter). To be able to accurately relate EMG data to the phase of the GC it was necessary to link both sets of data via a synchronisation box (TeleMyo™ 2400R G2 receiver, Noraxon Inc., Scottdale, Arizona, USA). See Figure 5-4 for a picture of the instrumentation set up.

TeleMyo™ 2400R G2 is a receiver with 8 channels of analogue input, which allows the synchronisation of data coming from different sources. The EMG device was connected wirelessly using WIFI technology to the Telemyo 2400 G2, which was also linked to a

computer where data was saved using the MyoResearch XP Clinical Application software. At the same time, the EMED platform was also connected via a USB cable to the TeleMyo™ 2400R G2 receiver. EMED platform sent a signal to the TeleMyo™ 2400R G2 receiver when heel strike on the pressure platform occurred. Heel strike was identified when vertical GRF in the EMED platform exceeded 5 Newtons. The number 5 in Figure 5-4 shows the data display in the EMG computer during gait. Thus, the top 6 rows (channels) display EMG activity of 6 different lower limb muscles while the bottom row displays the exact moment in time where the heel strike occurred. As a result EMG data can be analysed in relation to the heel strike. In addition to that, the EMED platform was also connected via USB cable to a different laptop, where EMED data was processed and analysed by Novel Software.

5.4.1.2 Instrumentation set up during strength measurements

The KINCOM dynamometer was used to measure maximal isometric strength. During these measurements EMG data was also collected so peak EMG activity could also be determined. Peak EMG activity is normally used to normalise EMG signal to the peak activity during maximal effort (Perry & Burnfield, 2010). Normalisation is a necessary step during EMG data processing to be able make statements about the relative intensity of an EMG signal. In order to be able to synchronise force and EMG data the following instrumentation set up was carried out:

The KINCOM machine was connected with a cable to the TeleMyo™ 2400R G2 receiver. Simultaneously the TeleMyo™ 2400R G2 received EMG data wirelessly via WIFI and was also linked via a USB cable to the EMG computer, where EMG and KINCOM data were visualised and saved using the MyoResearch XP Clinical Application software. The number 4 in Figure 5-5 shows the data display in the EMG computer. Thus, the top 6 rows (channels) display EMG activity of 6 different lower limb muscles while the bottom row displays the force generated in the KINCOM machine. Therefore, EMG data could be analysed in relation to amount of force produced during the MVC.

5.4.1.3 Instrumentation set up during microcirculation measurements

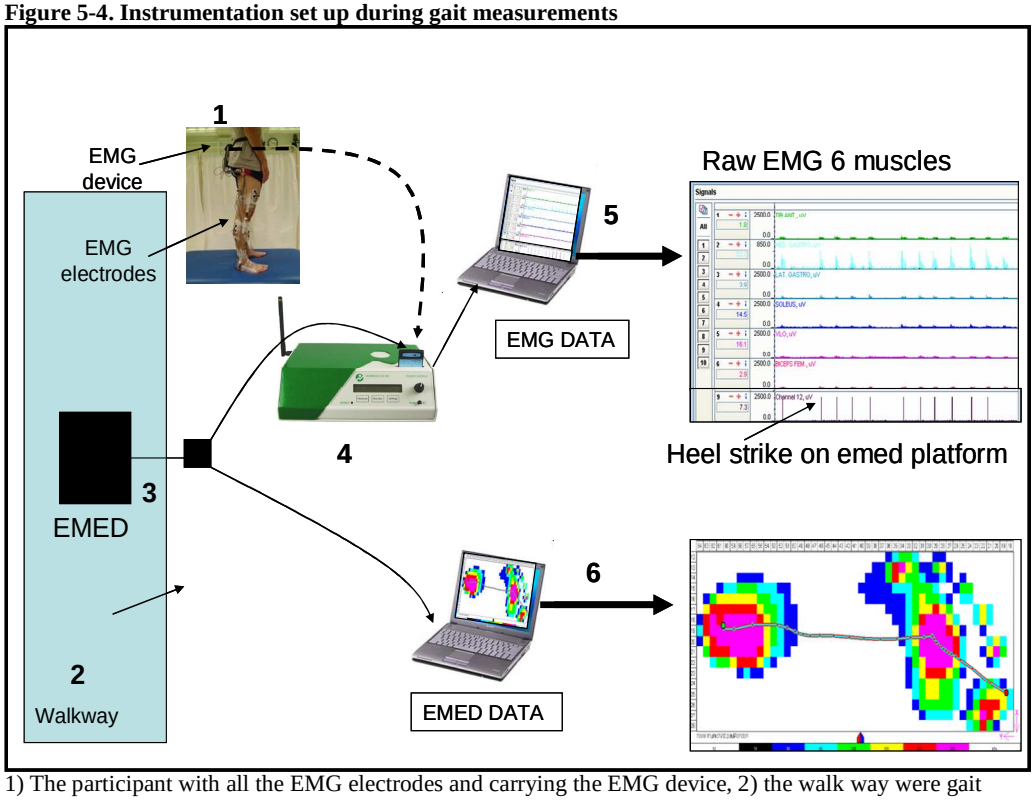
Muscular microcirculation was assessed using a NIRS device (Oxymon MK III). Measurements using NIRS were carried out in the MedGast at rest and during an exercise protocol, which included 14 submaximal isometric contractions with the calf muscle on the KINCOM machine (plantar-flexion at 50% of MVC value). However, since plantar-flexion moment is the sum of the moments generated by the different plantar-flexion muscles (especially the soleus, LGast and MGast), with only the KINCOM data it is impossible to determine the amount of work coming from each of those muscles separately. Van Beekvelt et al. (2002) demonstrated that muscular microcirculation during an exercise bout, measured by NIRS, was dependent on the intensity of the work of the specific muscle under investigation. Thus, in the present investigation muscular activity on the MGast during the exercise protocol was measured together with microcirculation data on the same muscle. Hence, microcirculatory responses during the exercise protocol could be related to the amount of work coming from the same muscle. To allow the NIRS and the EMG electrodes to be placed on the same muscle, the EMG electrodes were placed slightly lateral to the medial-lateral centre of the muscle belly. As seen in Figure 5-6, the position of the electrode was still on the muscle belly and did not change throughout the whole measurement. In order to be able to synchronise: 1) force (KINCOM data); 2) EMG data; and 3) NIRS data, the following instrumentation set up was carried out (see Figure 5-5 for a picture of the instrumentation set up).

The KINCOM machine was connected with a cable to the Telemyo 2400 G2. Simultaneously the Telemyo 2400 G2 received EMG data wirelessly via WIFI and was also linked via a USB cable to the EMG computer, where EMG and KINCOM data were visualised and saved using the MyoResearch XP Clinical Application software. The number 4 in Figure 5-5 shows the data display in the EMG computer. Thus, the top 6 rows (channels) display EMG activity of 6 different lower limb muscles (channel 2 corresponds to the MedGast) while the bottom row displays the force generated in the KINCOM machine. Therefore, EMG data could be analysed in relation to amount of force produced by the plantar-flexor muscles during the isometric contractions. This

part of the instrumentation set up is the same as explained above for the strength measurements.

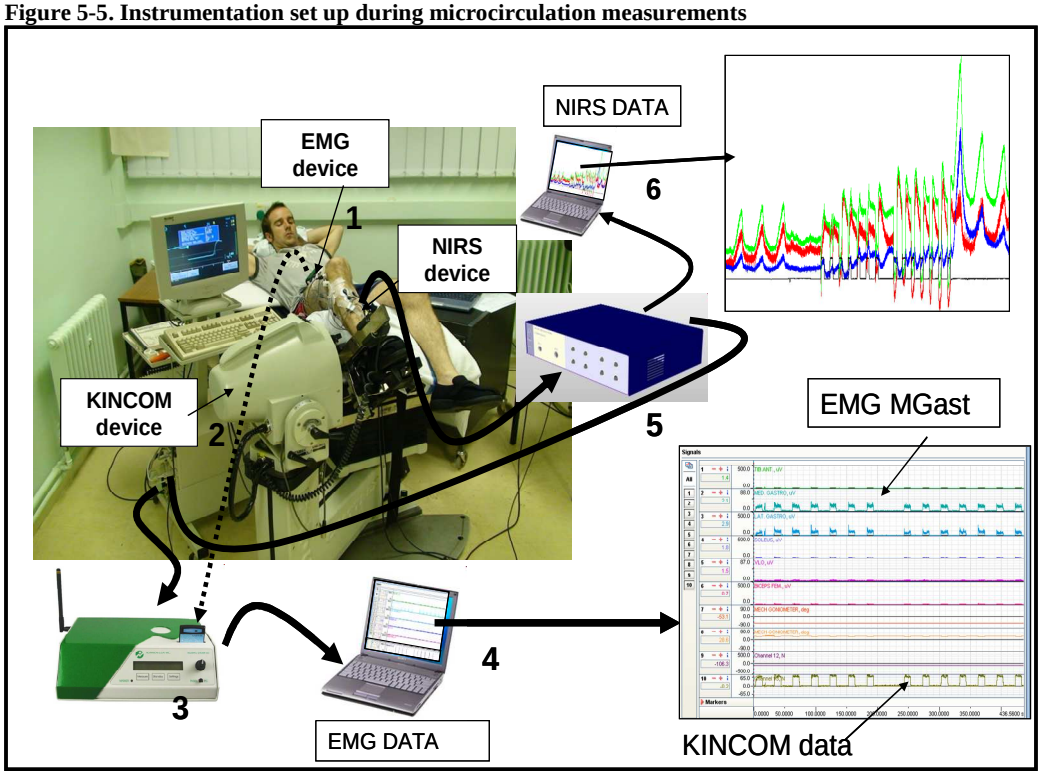
The KINCOM machine was also connected with a cable to the NIRS device. This device has 16 channels of analogue input so additional traces can be added to the NIRS data. KINCOM data and NIRS data were then inspected and saved together using the Oxysoft 2.1.2 software. Number 6 Figure 5-5 shows the data display in the NIRS computer. The green, red and blue traces represent total haemoglobin content, oxygenated haemoglobin and deoxygenated haemoglobin respectively, while the black trace at the bottom of the graph represents the mechanical output coming from the KINCOM machine.

Figure 5-4. Instrumentation set up during gait measurements



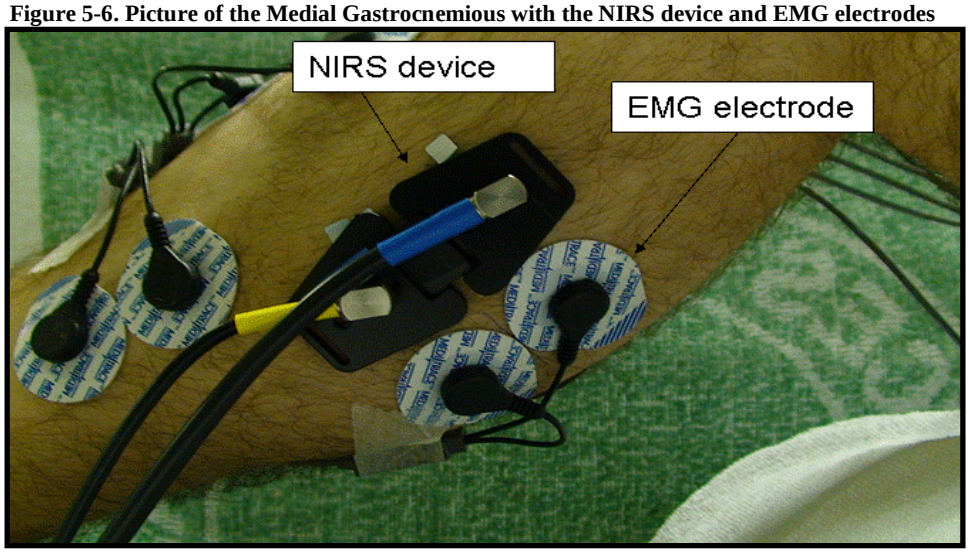
1) The participant with all the EMG electrodes and carrying the EMG device, 2) the walk way were gait measurements took place. 3) EMED platform in the centre of the walk way; 4) the synchronization box (Telemyo 2400 R G2); 5) laptop 1 with the “EMG” software; and 6) laptop 2 with the “EMED” software.

Figure 5-5. Instrumentation set up during microcirculation measurements



1) Subject carrying EMG device and NIRS device; 2) KINCOM machine; 3) the synchronization box (Telemyo 2400 R G2); 4) laptop 1 with the "EMG" software; 5) NIRS machine; and 6) laptop 2 with the "NIRS" software.

Figure 5-6. Picture of the Medial Gastrocnemious with the NIRS device and EMG electrodes



5.4.2 Summary of the procedures

All experimental testing was carried out at the research Centre for Clinical Kinaesiology (RCCK). A summary of the procedures followed at each data collection session is presented in this section. Further details of specific methods can be found at sections referred to in the flow chart (see Figure 5-7).

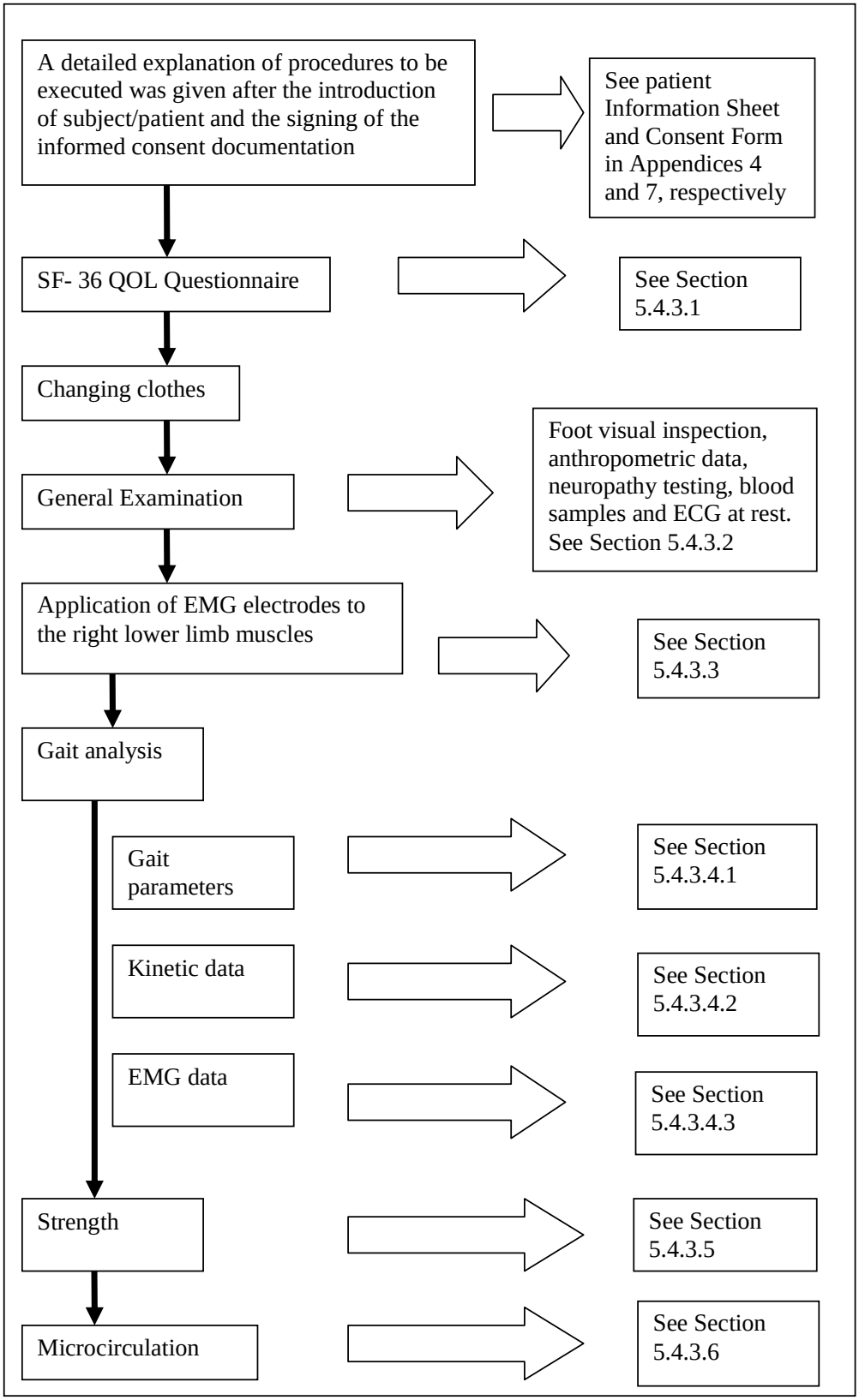
Prior to the measurement day, subjects received a confirmation letter in which they were requested not to do unusual physical activity for 24 hours before the appointment and to refrain from caffeine containing drinks on the day of the measurements. At arrival at the RCCK they were welcomed and given a detailed explanation of procedures to be executed. They were also encouraged to re-read the information sheet and ask anything they wanted about the study. After this informative introduction, they were asked to read and sign the consent form. Once the required consent documentation had been completed participants were asked to fill in the SF-36 questionnaire as a measure of self reported quality of life (Luscombe et al., 2000).

Then a general examination was conducted. This included foot visual inspection, anthropometric (height, body mass and body fat %), peripheral neuropathy (touch pressure sensation and vibration perception) and blood pressure measurements. In addition blood samples (to analyse cholesterol and sugar levels) and a 12-lead ECG at rest were both taken on the diabetic group only.

Thereafter, skin was prepared to reduce skin impedance during EMG recordings and EMG electrodes were placed on the following muscles: TA, MGast, LGast, Soleus, VL and BicFem. Prior to that, skin was prepared to reduce skin impedance. Gait analysis was carried out after. Spatial and temporal parameters of gait were recorded using a digital video at 25Hz while foot-floor interaction parameters were assessed using the EMED platform system. During gait EMG was used to assess muscular activity patterns on the TA, TS (MGast, LGast and soleus), VL and BicFem (see Section 5.4.1.1 for more information about the instrumentation set up during gait).

Muscle strength was tested isometrically using the KINCOM dynamometer on the right knee extensor, knee flexor, and ankle dorsi-flexor and ankle plantar-flexor muscles. EMG data was recorded during the strength testing and this was used to calculate peak muscular activity (see Section 5.4.1.2 for more information about instrumentation set up during strength measurements). Microcirculation measurements were carried out afterwards. Blood flow and muscle oxygen consumption were assessed on the calf muscle (MGast) using a NIRS device. These measurements were performed at rest and after an exercise protocol, which included 14 submaximal isometric contractions with the calf muscle (plantar-flexion at 50% of MVC value calculated during muscle strength test). During the exercise protocol EMG data and NIRS data were collected simultaneously to quantify the effect of muscular activity on the muscular physiological responses (see Section 5.4.1.3 for more information about the instrumentation set up during microcirculation measurements).

Figure 5-7. Overview of the data collection procedures



5.4.3 Explanation of the Procedures

5.4.3.1 Questionnaire

The SF-36 questionnaire was used to assess health related quality of life. This questionnaire has been widely used and validated to assess wellbeing in different populations including diabetic patients (Jacobson et al., 1994; Luscombe et al., 2000).

The SF-36 comprises 36 questions that measure eight dimensions of health: physical functioning, role limitations due to physical health, bodily pain, general health, vitality, social functioning, role limitations due to emotional health, and mental health. In addition to the dimension scores two summary scales, which assess the overall mental and overall physical function, were calculated.

Answers to each question were scored and transformed to a 0-100 scale. Then, the average for each dimension and summary scale (sum of the transformed scores from each item divided by the number of items belonging to that dimension or summary scale) was computed using Excel 2003 (Microsoft Office Excel 2003, Microsoft Corporation, USA).

5.4.3.2 General examination

5.4.3.2.1 Foot Visual inspection

All participants were inspected for foot deformities (i.e. Charcot foot, claw toes, and hammer toes) by the clinicians before being referred to the study. Only subjects with no severe foot deformity were given the opportunity to participate in the study.

Feet were inspected visually by the researcher to confirm that foot deformity was not present prior to collecting foot-floor interaction data during gait. Static inclination of first phalanx at the metatarsophalangeal joint in relation to the longitudinal axis of the first metatarsal head was measured with a hand goniometer to confirm no foot

deformities (claw toes). Foot deformity was defined as an angle higher than 10 degrees. Myerson and Shereff (1989) defined an extension of 5 degrees of the metatarsophalangeal joint as a foot deformity (measured on cadaver feet). Since structural changes are related to aging independently of DN (Caselli et al., 2003) and the sample of the present study was expected to be relatively elderly a broader definition of foot deformity was chosen for the present study.

5.4.3.2.2 Anthropometric data

Height in metres was measured when standing barefoot using a wall attached height measure (Seca 222 telescopic wall mounted measuring rod; Seca Ltd, Medical scales and measuring systems, Birmingham, B5 5QB, UK). Body mass was measured in kilograms using a digital weighing scale (Seca 888 digital weighing scales; Seca Ltd, Medical scales and measuring systems, Birmingham, B5 5QB, UK). Body mass measurements were carried out with the subject wearing shorts and a t-shirt.

Body fat percentage was measured using the Harpenden Skinfold Calliper (Baty International, Victoria Road, Burgess Hill, West Sussex RHI5 9LR). The four site system, which requires measuring skin fold at the following sites: biceps, triceps, subscapular and suprailiac, was the selected protocol. These measurements were converted to an estimated body fat percentage by the Siri equation (Siri, 1961). Three measurements were taken per site. If repeated measurements varied by more than 1mm, the test was repeated. Numerous researchers have demonstrated the validity and reproducibility of this technique to predict body fat percentage (Patterson, 1992).

5.4.3.2.3 Peripheral neuropathy

The subjects recruited for the study were already clinically established cases of diabetic peripheral neuropathy. Touch pressures sensation using the Semmes-Weinstein monofilament, and vibration perception using a neurothesiometer, were used to confirm the presence of diabetic peripheral neuropathy in these patients.

5.4.3.2.4 Touch pressure sensation

Semmes-Weinstein monofilament (Bailey Instruments Ltd, Manchester, UK) is known to be a valid and reliable clinical tool to test peripheral neuropathy (Collins et al., 2010). The S-W monofilament is a set of 20 pressure-sensitive nylon filaments attached to a penholder. Each monofilament is a piece of nylon line of a precise diameter that is applied end-on-to the skin until the line begins to bend (see Figure 5-8), providing a reproducible, metered sensory stimulus (Kumar et al., 1991).

Cutaneous sensation was measured by the 5.07 monofilament. The Semmes–Weinstein 5.07 monofilament exerts 10 grams of force when bowed into a C-shape against the skin for one second. Patients who cannot detect application of the 10 g monofilament are considered to have lost protective sensation against foot ulceration (Olmos et al. 1995).

Diabetic peripheral neuropathy was defined as inability to perceive the 5.07 (10 g) S-W monofilament (loss of protective sensation) (Kumar et al., 1991) in at least one of the 4 plantar areas tested in this study (heel, 1st metatarsal head, 5th metatarsal head and hallux).

The four sites were tested in the prone lying position and the subject needed to perceive 80% of the trials to be graded as the sensation present over that site.

5.4.3.2.5 Vibration sensation

Vibration sensation was measured using a neurothesiometer (Bailey Instruments Ltd, Manchester, UK), which has being widely accepted as a valid and reliable method (Cassella et al., 2000) to assess diabetic peripheral neuropathy. The neurothesiometer is considered the preferable method to assess vibration sense due to the ease and rapidity of testing by this method (Brill & Perkins, 2002). In addition to that, this method provides the very specific threshold above which sensation can be perceived by the subject (vibration perception threshold), while other methods such as the commonly

used tuning fork (128Hz) can only assess whether sensation is perceived above an established cut off point (128 Hz).

Vibration perception threshold (VPT) was measured over the 1st metatarsal head with the subject in prone lying position with the knee flexed 90º (see Figure 5-8). This position was chosen to blind the participant. The head of the device was placed on the 1st metatarsal head with the neurothesiometer turned off. After a few seconds (randomly to avoid the learning effect) the vibration was increased constantly from 0 until the vibration was perceived by the participant. Five trials were performed and the average represented the final vibration score (in volts). If repeated measurements varied by more than five volts the test was repeated.

5.4.3.2.6 Blood pressure

Blood pressure (BP) was assessed by an automatic blood pressure device (Microlife BP A100 Plus, 60 Cranmer Terrace; London; SW17 0QS). This device has been proven to be reliable and valid (Stergiou et al., 2006) and has been recommended by the British Hypertension Society.

Blood pressure was measured from the right arm. Participants were placed in a sitting position with both arms resting comfortably on a table. Subjects were required to rest in that position for two minutes before the measurements started. Three consecutive measurements were taken automatically by the device and the average of those three readings was used as the final score. In addition to systolic and diastolic BP, resting heart rate (HR) was also measured by this device.

5.4.3.2.7 Blood Samples

Finger-prick blood samples were taken in this study to measure: HbA_{1c} and cholesterol levels. HbA_{1c} was measured using the DCA Vantage™ Analyzer (Siemes Healthcare, Camberley, Surrey, GU16 8QD). DCA Vantage has been proven to be a valid and reliable portable device to assess HbA_{1c} rapidly (Carter et al., 1996).

HbA_{1c} has been chosen to monitor blood sugar level because it provides information about glucose control history contrary to normal blood tests which determine instant glucose levels. In addition to this, these measurements can be carried out on site and it does not require prior fasting. Subjects were asked to perform different physical tasks during the data collection. For this reason, a fasting test was not considered appropriate since it may increase the risk of hypoglycaemia in diabetic patients.

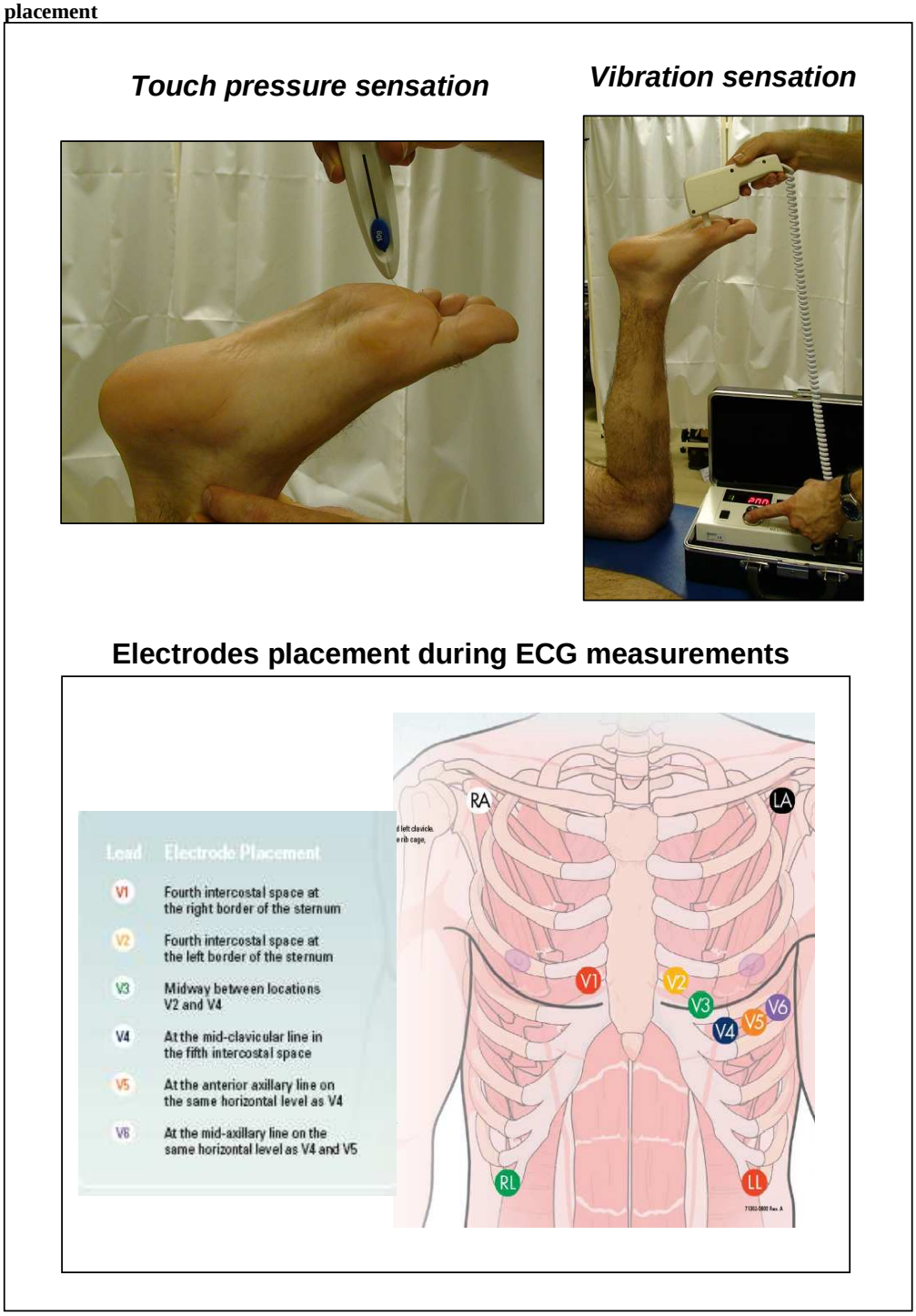
Cholesterol levels (TC, HDL and LDL) were measured with the Cholestech´s LDX system (Cholestech Corporation, Inverness Medical, UK). This device has become very popular both in clinical settings and research studies due to the prompt and accurate results. Cholestech´s LDX has shown very good correlation ($r>0.8$; $p<0.001$) when compared to well established laboratories (Parikh, et al., 2009).

5.4.3.2.8 ECG at rest

A rest 12-lead electrocardiogram was performed on all the subjects included in the intervention programme using an ESAOTE P80 (ESAOTE S.P.A, Florence, Italy) device. Diabetic individuals have a 4-fold increased risk of cardiovascular diseases compared to healthy people (McCallum & Fisher, 2006). The ECG test was therefore carried out to confirm that the participants in the exercise group did not suffer from any heart condition that could be worsened by the exercise programme.

The resting ECG was taken by the researchers, who were trained by a qualified clinician at the ECG department at the University Hospital of Wales. The skin was shaved where necessary and cleaned with alcohol free wipes before the application of the electrodes, in order to ensure good electrode-skin contact. Electrode placement was carried out following the American Heart Association (AHA) recommendations (Kossmann, et al., 1967) (see Figure 5-8).

Figure 5-8. Touch pressure measurements, vibration sensation measurements and ECG electrodes placement



5.4.3.3 Preparation for data collection using surface electromyography (EMG)

The subject was positioned in a sitting position with legs extended on the plinth. The skin was shaved where necessary and cleaned with alcohol free wipes before application of the electrodes, in order to ensure good electrode-skin contact. Good electro-skin contact improves the EMG recordings by reducing electrical interference and noise. Ag/AgCL electrodes with a conductive area of 10 mm^2 were applied to VL (quadriceps), BicFem (hamstrings), TA, MGast, LGast and soleus of the right lower limb according to the European recommendations for surface electromyography (Zipp, 1982). The electrode application position was determined by palpation (during muscular contraction) along with the use of accurate anatomical landmarks to ensure that the electrode was applied to the muscle belly. The reference electrode was placed in the medial part of the tibia, which is considered to be electrically inactive. The distances between agonist and antagonist bipolar electrodes were greater than 10 cm. It is, therefore, unlikely that EMG cross-talk could have contaminated agonist or antagonist signals (Mullany et al., 2002). As a precaution against any possible signal contamination, the electrodes were applied in a parallel orientation to the muscle fibres being tested, mid-way and central along the muscle belly. The diameter of the electrodes was 18 mm, and the inter-electrode distance (centre to centre distance between conductive areas of 2 bipolar electrodes) was 37 mm.

In order to minimise the risk of artefacts due to cable movement, the pre-amplifier cables were attached to the subject's skin using adhesive tape (Micropore; 3M Healthcare, D-41453 Nuess, Germany). Once the electrodes were placed and the cables fixed, the raw signal was visually inspected for any distortion of the signal using the MyoResearch XP Clinical Application software. EMG data was acquired using a wireless 8 channel EMG device (TeleMyo™ 2400T G2 Transmitter).

NIRS and EMG data were collected simultaneously on the MGast during a plantar-flexion exercise protocol (see Section 5.4.1.3 for further information about instrumentation set up during this procedure). Therefore, it was necessary to place the EMG electrodes slightly lateral to the medio-lateral centre of the muscle belly (Figure

5-6). The position of the electrode was still on the muscle belly and did not change throughout the whole measurements.

As it was necessary that the subjects were not encumbered by numerous EMG cables and fatigued by a lengthy data collection, a practical approach of collecting the EMG data from the right leg of each individual was chosen.

5.4.3.4 Gait analysis

Gait analysis was carried out during barefoot conditions. To protect the foot during the experiments the subjects only removed the shoes at the walkway. Special attention was paid not to allow any participant to step out from the walkway without shoes on. Prior to each measurement the walkway was cleaned with an alcohol free wipe to minimise any potential hazard to the bare foot.

Participants were asked to walk barefoot at their self-selected speed on a 9 meter walkway. The platform to analyse foot-floor interaction (EMED-m) was positioned level with the floor at the midpoint of the 9 m walkway (see Figure 5-9). Self-selected gait speed is considered to be the most efficient walking speed for an individual and has been found to be an appropriate predictor of function and disability (Guralnik et al., 2000)

Before data acquisition, the subjects were instructed to walk freely on the walkway to reproduce their normal gait and to adapt to the laboratory environment. Multiple trials were permitted to allow the subjects to practice walking without visually targeting the platform surface. The testers adapted the subjects starting position to ensure the platform was always hit on the fourth step. Data was collected over 5 trials for each foot (five successful trials with the left foot followed by five successful trials with the right foot). The EMED system has demonstrated overall reliability when 3 to 5 walking trials are used (McPoil et al., 1999). During this task gait characteristics, kinetic data and muscular activity characteristics were evaluated.

5.4.3.4.1 Gait parameters

Spatial-temporal characteristics

Spatial and temporal parameters of gait were recorded using the Sony digital video camera at 25Hz (Sony digital Camcorder, DSR-PD1P, STYLUS, Cardiff, UK). During the gait task, only four trials (two in which the right foot hit the platform and two in which the left foot hit the platform) were recorded and saved on a DV tape for further analysis.

The camera position was perpendicular to the walkway at a distance of 3.5 meters. The video clip was downloaded onto a computer and processed using the SiliconCoach software (SiliconCoachPro 6.0, SiliconCoach, London, UK). The time difference between the 3 consecutive heel strikes was used to calculate the step and stride times. The frame capturing the heel strike was identified and saved for further analysis using Matlab. A purpose written programme in Matlab was used to calculate gait velocity, cadence and stride length. To compute spatial parameters two parallel sticks (1 meter long each) were positioned on both sides of the walk way (See Figure 5-9).

Centre of pressure parameters

Foot-floor interaction parameters were measured with the EMED platform. This system has been proven to be an accurate method to quantify foot-floor interaction characteristics (Putti et al., 2008).

The platform consists of a matrix of 3840 force transducers that are uniformly distributed in an area of 24 × 38 cm. The platform has a resolution of 4 sensors·cm^{-2} with a sampling frequency of 50 Hz. The sensors have a pressure range from 10 kPa to 1200 kPa.

The data was processed and analysed by Novel 13.3.42 software. With this software it is possible to customise the data analysis by selecting specific areas of the foot (masks) and parameters you are interested in. Then, individual masks were created for each trial subdividing the foot into 9 masks: heel, midfoot, metatarsals, 1st metatarsal head, 2nd metatarsal head, 3rd, 4th and 5th metatarsal heads, bit toe, 2nd toe and 3rd, 4th and 5th

toes. These masks were determined automatically by the Novel software after applying the PRC-mask on all files. After the automatic masking, visual inspection of all the trial was carried out. If the mask did not look correct it was corrected manually to ensure the mask was covering the right area. Although the PRC-mask did determine 9 masks (regions) automatically, only 3 areas were used for further analysis: heel, metatarsals and big toe (see Figure 5-10).

Centre of pressure parameters were investigated. Although COP parameters are kinetic data (COP is the point of application of the GRFs) it provides descriptive information about gait in general and the roll over process in particular. For this reason COP data was included in this section together with spatial-temporal characteristics. Total distance travelled by COP gives information about the transverse plane foot mobility during the GC. In addition to that, velocity of the COP within different foot regions may provide information about the roll over process throughout the stance phase. Thus, total distance travelled by the COP, velocity of the COP at the heel, metatarsal heads and hallux were measured (Figure 5-10 shows an example of COP data).

5.4.3.4.2 Kinetic data

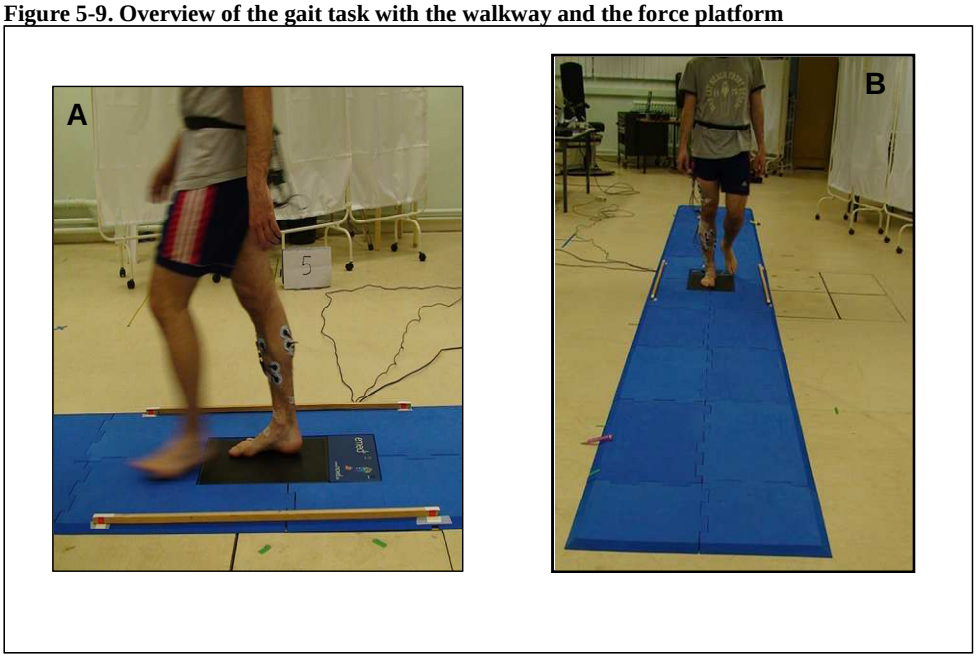
Kinetic data was measured with the EMED platform. (See previous section for details about the EMED platform). Same as for COP parameters (Section 5.4.3.4.1) the data was processed and analysed by Novel Software and 3 areas were used for further analysis: heel, metatarsal heads and big toe.

Foot plantar pressures are known to be an important contributing factor for plantar tissue damage in DN subjects (Frykberg et al., 1998), Although there is an ongoing debate on which kinetic variables predict foot problems in this population, there is little doubt that PP which accounts for the highest stress generated under the sole of the foot (or foot region) and PTI which represents the magnitude and duration of the stress, are important contributing factors (Mueller & Maluf, 2002; Uccioli et al., 2001; Veves et al., 1992). For this reason, PTI and PP were examined in this study. In addition to that other parameters related to PP and PTI such as contact area (CA), maximal force (Max Force) and contact time (CT) (both in milliseconds and as % of the stance phase) were

also investigated. These parameters related to peak pressure and PTI may provide some extra information to better understand PP and PTI values. Therefore, PTI, PP, CA, Max Force, CTms and CT% during the whole stance phase were calculated for each of the areas under investigation (heel, metatarsals and hallux).

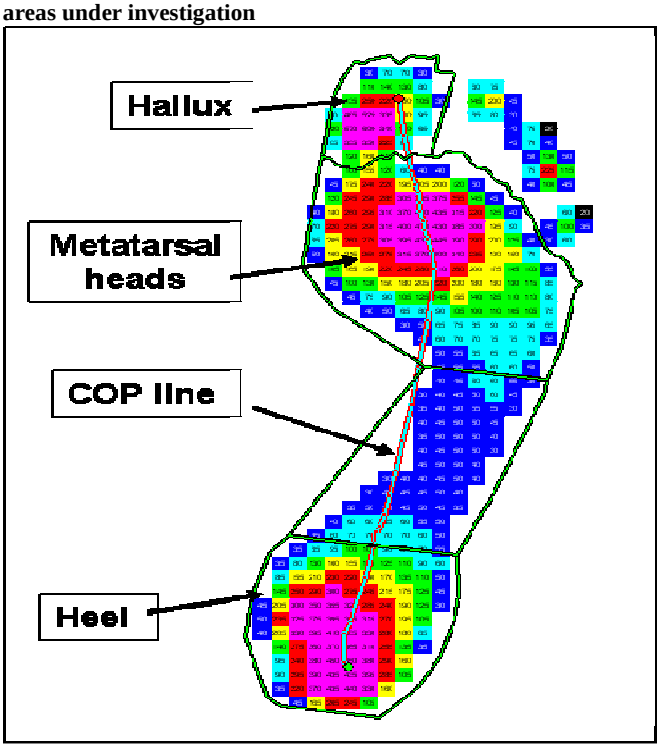
Push off is known to be the phase of the GC where the forefoot, which is the foot area more likely to develop ulceration (Boulton, 1994), undergoes the highest pressures. Then further analysis was carried out to investigate loading patterns on the forefoot area during this critical phase of the GC. In this context forefoot was defined as the foot area that covered the metatarsal heads and the toes. The new forefoot mask was created manually and it included the masks metatarsal heads and toes, which were previously created by the novel software. Push off was defined as the time period from 40% to 100% of the stance phase (Perry & Burnfield, 2010). Therefore PP and Max Force were analysed for this new created mask and time frame. The instant of Max Force and the instant of PP during the push off phase in relation to the full roll over process (% ROP) were also investigated. They may provide some information about the exact time that the highest stress occurs in the forefoot during the push off phase. Although, arch index provides information about the structural characteristics of the foot and it is therefore not a kinetic variable, some investigators have stated that arch index is an important predictor for foot pressures (especially under the metatarsal heads) (Morag & Cavanagh, 1999). For this reason arch index is included into this section together with kinetic data.

Figure 5-9. Overview of the gait task with the walkway and the force platform



A. 1 meter long sticks to determine spatial parameters; B. EMED platform in the middle of the walkway.

Figure 5-10. Example of EMED data with the COP line and the foot areas under investigation



5.4.3.4.3 Surface electromyography during gait

EMG data was recorded at 1500 Hz using a wireless 8 channels EMG device (TeleMyo™ 2400T G2 Transmitter). EMG data was collected and saved using the MyoResearch XP Clinical Application software. Further processing of the EMG signal was performed using a purpose written programme in Matlab. Next, the steps followed to process and analyse EMG data are explained.

The raw EMG signal was full wave rectified and low pass filtered using a second order Butterworth filter with a 50 Hz cut off frequency to create a linear envelope that was used for further analysis. To be able to relate EMG activity to the GC, EMED data and EMG data were collected simultaneously. (See Section 5.4.1.1 for more information about the instrumentation set up during this task). Therefore, EMG data could be processed in relation to the heel strike (See picture 4 from Figure 5-4). Furthermore to be able to relate EMG data not only to the heel strike but to the rest of the GC, the duration of the stance phase, which was calculated by the Novel 13.3.42 software, was taken into account when processing the EMG signal. Then, if the instant of the heel strike and the duration of the stance phase are known the instant of the push off can also be determined. It has been stated that stance phase normally represents 62% of the GC while swing phase accounts for the remaining 38% (Perry & Burnfield, 2010). Therefore, the swing phase of the GC was estimated based on these percentages. This approach was used in the present investigation to relate EMG data to the GC (stance phase and swing phase).

Thereafter each step was normalised to 100% of the GC. 0% represents the heel strike and 100% represents the end of the swing phase. Regarding to the normalisation of the EMG amplitude, EMG is usually normalised to EMG registered during a maximum effort test (Perry & Burnfield, 2010). In the present investigation some participants from the DN group showed muscular activity values during gait up to 600% of the highest EMG activity recorded during MVCs (see Section 5.4.3.5 and Section 5.4.1.2 for more information about MVC measurements and instrumentation set up during this task). This shows that EMG data normalised to % MVC was not a valid approach to use with the patients with DN in the present study. It was then decided to normalise EMG signal

to the peak activity during the GC, which is an acceptable and widely used normalisation technique during dynamic conditions (Burden, 2010).

EMG data was collected throughout the entire gait task. However only the steps in which the right foot hit the EMED platform were analysed (5 steps in total). To help identify the correct step a sheet was used during data collection in which the correct heel strikes were written down.

For each of the six muscles recorded, an average EMG trace for each subject was obtained by getting an average across all five trials. For a more comprehensive interpretation of the results, it was decided to analyse the three plantarflexor muscles (MGast, LGast and soleus) as a single muscle, TS. Then, EMG activity was investigated for TS, TA, VL (quadriceps), BicFem (hamstrings). EMG signal was then investigated 1) over the entire GC; 2) per phase of the GC; and 3) during the push off phase.

Firstly, the % of time each muscle was kept active during the whole process was determined. Perry & Burnfield (2010) suggested a cut off point of 5% of the peak activity to define muscular activity. Then, muscle activity was calculated when the average EMG trace was above 5% of the peak activity during gait (Figure 5-11 shows an example of EMG data processed with Matlab to determine % of time EMG trace was kept above 5% of the peak activity).

Secondly, the mean EMG activity (normalised) was calculated within selected phases of the GC. According to Perry & Burnfield (2010) the GC is composed of 7 phases: loading (0-10% of the GC), mid-stance (10-30% of the GC), terminal swing (30-50% of the GC), pre-swing (50-60% of the GC), initial swing (60-73%), mid swing (73-87%) and terminal swing (87-100% of the GC). Since foot problems are related to the stance phase of the GC, initial swing and mid swing were not investigated in the present study. Therefore, average activity was calculated only for 5 phases (Figure 5-12 displays an example of EMG output processed with Matlab to calculate mean EMG activity throughout the different phases of the GC).

Numerous studies have reported excessive foot pressures on the forefoot during push off in patients with diabetic neuropathy (See Chapter 2 for more information about foot

pressure in neuropathic patients). It was therefore decided to create time windows to investigate TS and TA EMG data around the push off phase during the GC. The time window for the TS covered the time from the peak activity during the push off phase (around 50% GC) to the initiation of that activity. The time window for the TA covered the time from the peak activity during the initial swing phase to the initiation of that activity. Hence, a time frame window was created for the TA and TS muscles to investigate: 1) when the peak activity occurred in each window, expressed as % of the GC; and 2) the time delay from the onset of the activity, which was defined as 5% of the peak activity during gait, to the peak muscular activity. This measure was calculated in milliseconds. (See Figure 5-13 for more information on how the windows were defined as well as the calculations carried out within each window).

EMG signal refers to the electrical event produced by the muscle and not to the mechanical output (force) (Winter et al., 2009). The time lag between EMG activity and force production is called electromechanical delay. Therefore, it has been proposed that EMD should be taken into consideration when processing EMG data, so muscle function during dynamic conditions such as walking can be investigated in relation to movement (Amaranti & Martin, 2004). Since in the current study we were not interested in when EMG activity was present but when muscle output from that activity was produced, EMG data was processed taking in consideration EMD. Two different approaches were followed in this study to determine EMD. Thus, one method was used to determine EMD for dorsi-flexors, knee extensors and knee flexor muscles, while a second method was used to quantify EMD for the plantar-flexor muscles. The reliability of the EMD measurements used in the present study were not well established prior to the start of the study. For this reason two reliability studies were carried out before the main study started, which demonstrated that these methods were reliable to quantify EMD. See Chapter 4 (Section 4.2 and Section 4.3) for more information about these reliability studies.

Figure 5-11. Example of EMG data to quantify the amount of time each muscle was kept active during the gait cycle

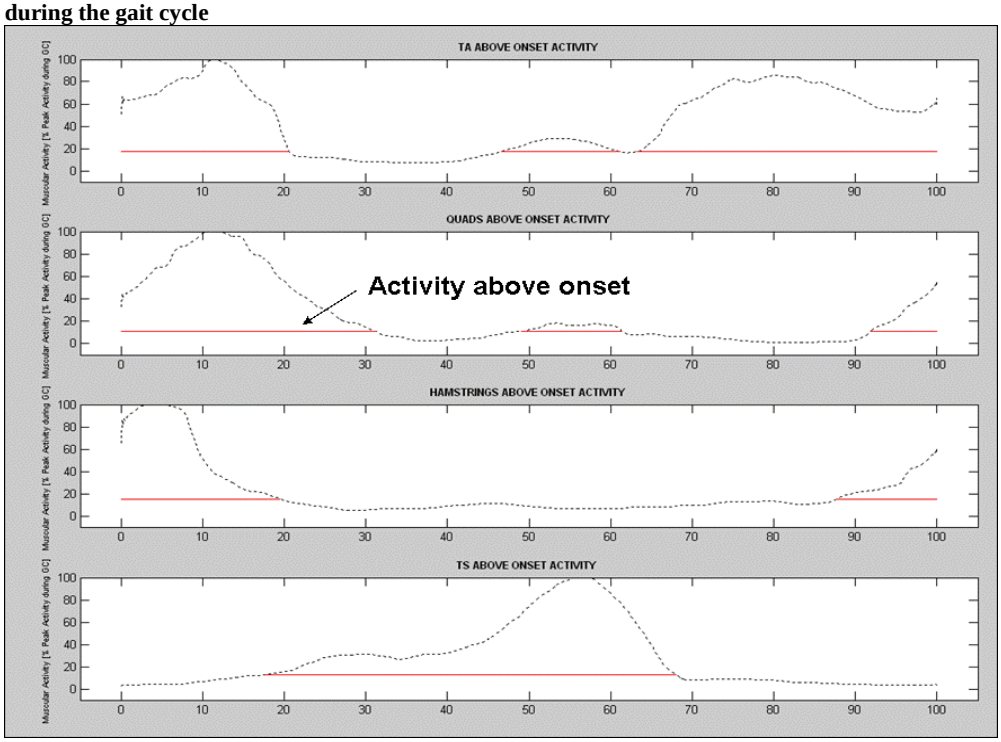


Figure 5-12. Example of EMG activity throughout the different phases of the gait cycle

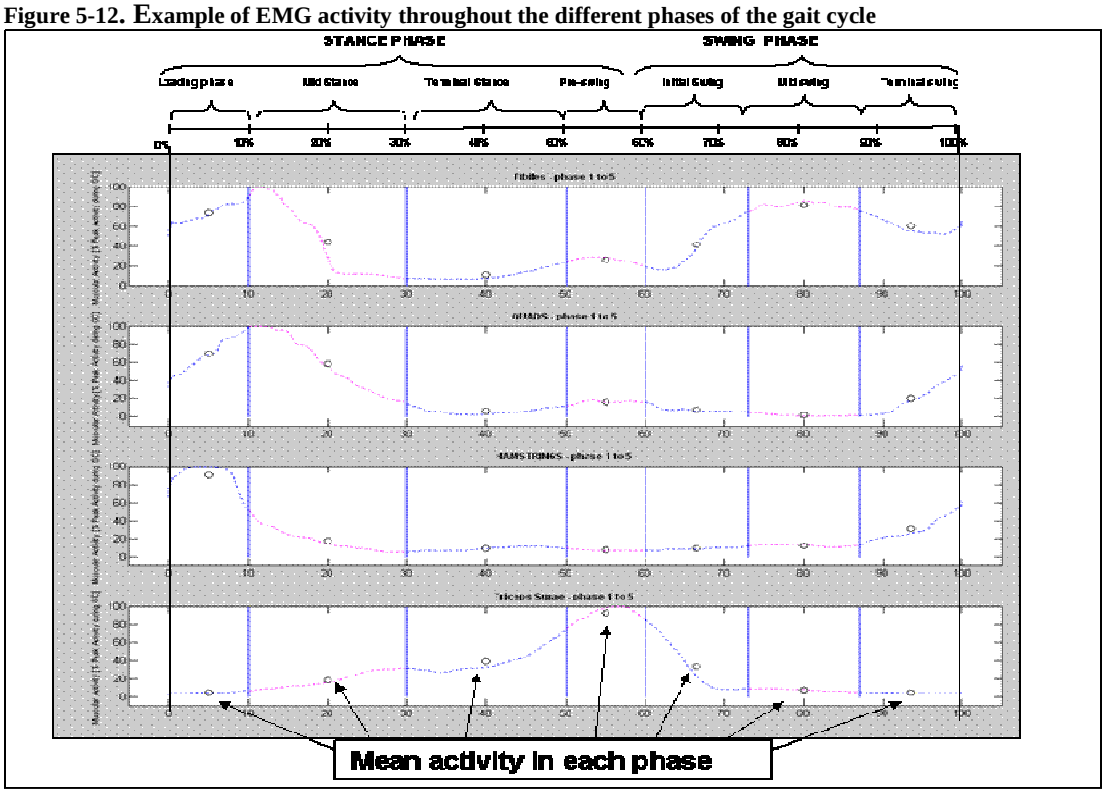
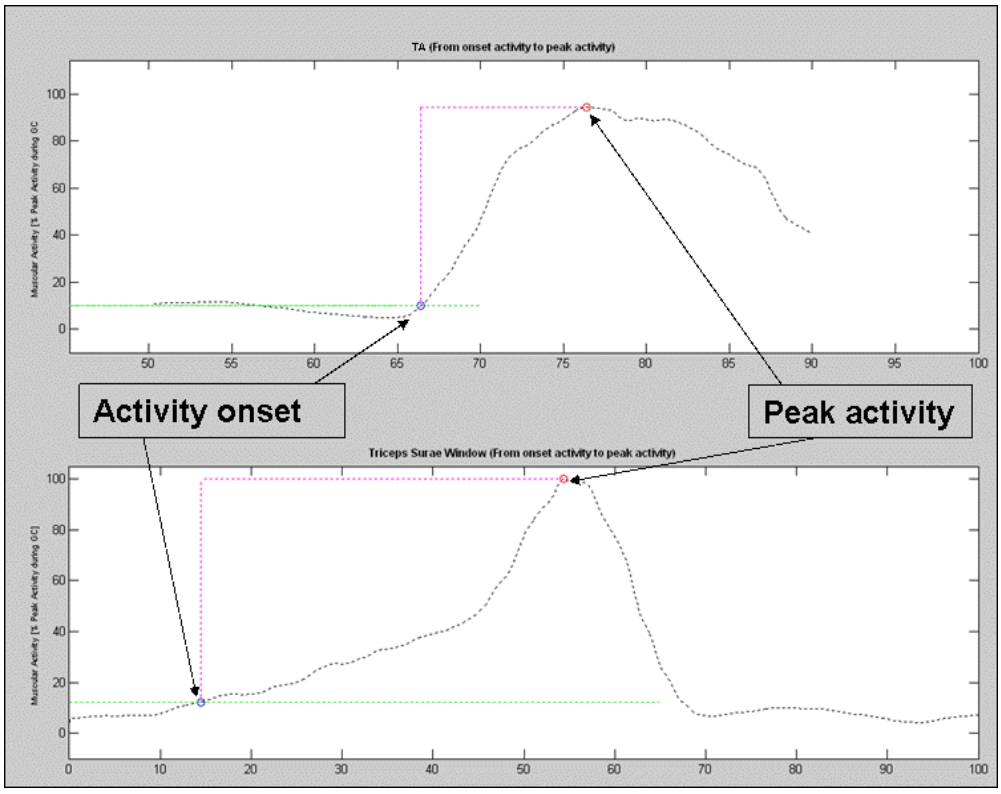


Figure 5-13. Example of how the time window was determined to calculate time related EMG variables for the TA and TS muscles



5.4.3.5 Muscle strength

Muscle weakness secondary to DN is considered an important factor in explaining gait abnormalities in this population (Mueller et al., 1994; Giacomozzi et al., 2002). It has been hypothesised that gains in strength levels in this population may change gait characteristics in DN patients. For this reason strength levels were measured in part 1 and part 2 of this study.

The KINCOM dynamometer was used to measure maximal isometric strength. This device has been shown to be accurate and reliable in these measurements (Farrell & Richards, 1986). Muscle strength was assessed isometrically for the ankle plantar-flexors, ankle dorsi-flexors knee extensors and knee flexors. Ankle plantar-flexion and

ankle dosi-flexion measurements were carried out with the subject lying down on a supine position. The right leg was semi-extended (30º±10 flexion) with the ankle fixed at 15º plantar-flexion. The external malleoli was placed in line with the rotation axis. This position was chosen to maximise muscular activity in the calf muscles during the plantar-flexion.

Knee flexion and knee extension were tested with the subject seated in an upright position (90º hip flexion) with their knees flexed to 90º and 70º, respectively. The right leg was secured into an instrumented cuff positioned at a point a few centimetres above the ankle joint with a stabilisation strap across the femur of the right leg. A seat belt was used to secure the subject in the sitting position and prevent them from altering their position during the data collection. The moment arm distance was recorded and used when processing strength data for all 4 movements.

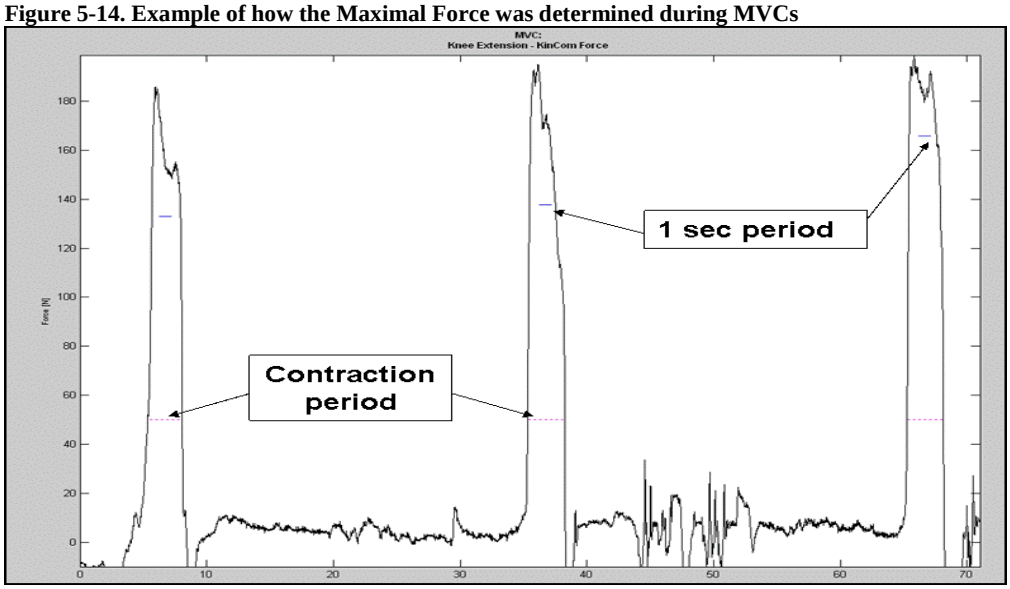
Prior to data collection, a warm-up period was performed. To increase body temperature and therefore reduce the risk of muscular problems such as cramps, participants were asked to ride an indoor bike for 5 minutes at an increasing (moderate) pace. Then, participants were taken to the KINCOM machine, where (on the positions described above), they performed 15 contractions for 2 seconds at different levels of intensity (40% and 60%). During these contractions, EMG was used as a feedback tool for the tester to ensure participants were doing the movement correctly (contractions was observed in the correct muscle) (see Section 5.4.1.2 for more information about the instrumentation set up). This tool was especially useful during plantar-flexion. When subjects were doing the movement at a high intensity, they tended to recruit the whole leg which resulted in a shift in activity from the calf muscles (soleus, MGast and LGast) to the quadriceps. By providing live feedback participants achieved better movement awareness which resulted in better muscle isolation.

Data collection consisted of three maximal isometric voluntary contractions per movement lasting 3 seconds per contraction. Each contraction was followed by a 30-second rest period before the next MVC. All together 12 MVCs were recorded.

Data collected in the KINCOM dynamometer was saved in a laptop using the MyoResearch XP Clinical Application software (see Section 5.4.1.2 for more

information about the instrumentation set up). Further processing of the data was performed using a purpose written programme in Matlab. Thus, maximal force was defined as the mean activity during the middle of the three seconds contraction periods (Figure 5-14 display an example of how maximal force was defined during MVCs). The highest of these three means (one per MVC) was considered the maximal force for that specific movement. The moment arm distance for the different movements was recorded and the joint moment used to quantify maximal strength. EMG data during MVCs was used to normalise the EMG data collected during gait to maximal voluntary contraction (see Section 3.4.4. for more information about EMG data normalization during gait).

Figure 5-14. Example of how the Maximal Force was determined during MVCs



5.4.3.6 Microcirculation measurements

Microcirculation measurements were carried out on the skeletal muscle using NIRS. NIRS is a non-invasive optical and direct method to determine oxygenation and hemodynamics in tissue. This technique is based on the relative transparency of tissue to light in the near-infrared region, and on the oxygen-dependent absorption changes of haemoglobin and myoglobin. It enables non-invasive continuous measurements of changes in the concentration of HbO and HbdO. The sum of HbO and HbdO

concentrations reflects the total amount of haemoglobin (tHb), and changes in tHb can be interpreted as changes in blood volume in the tissue.

Numerous studies have proven the validity of NIRS to determine muscle BF and mVO_2 by comparing it against well-established methods such as strain-gauge plethysmography or the Fick method (De Blasi et al., 1994; Mancini et al., 1994; van Beekvelt et al., 2001b). Data collection was performed using the Oxymon MK III. This device has been proven to be a reliable and valid method to investigate local muscle O_2 consumption and blood flow, both in resting and exercising muscle (van Beekvelt et al., 2001b; van Beekvelt et al., 2002).

Quantification of muscle oxygen consumption and blood flow was performed by applying a VO to control circulation in the limb (see Figure 4-1 in Chapter 4). Venous occlusion causes an increase blood volume by an undisturbed arterial (in) flow and interrupted venous (out) flow. Blood flow can therefore be calculated during venous occlusion from the linear increase of tHb. Muscular oxygen consumption is calculated from the linear increase of HbdO. Since the venous outflow is blocked, the increase in HbdO is thought to be solely due to the O_2 consumed under the assumption the arterial O_2 saturation is near 100%. NIRS measurements in our study were done on the belly of the MGast with an inter-optode distance of 40 mm. The reliability of the method to measure blood flow and O_2 consumption in the lower limb was not well established prior to the start of this study. For this reason a reliability study was carried out before the main study started, which demonstrated that this method is reliable both within-day and between-days to quantify blood flow and oxygen consumption in the calf muscle. See Chapter 4 (Section 4.1) for more information about this reliability study.

NIRS measurements were carried out with the subject lying down on the KINCOM machine (in the same position as during the ankle plantar-flexion measurements) (see Section 5.4.3.5) (see Section 5.4.1.3 for information about the instrumentation set up during these measurements). This position was chosen to ensure the heart position was not above the limb position. The opposite may result in venous pooling and therefore may compromise the quality of the measurements. A pneumatic cuff was then placed around the right thigh and was used to apply venous occlusion during the test. Venous occlusion was performed at 50 mmHg and maintained for 30 seconds. A resting period

of 40 seconds was allowed between inflations. In total, six venous occlusions were carried out throughout the NIRS measurements, 3 during resting conditions and 3 following an exercise protocol. The exercise protocol consisted of two sets of seven isometric plantar-flexion contractions at 50% of the MVC, separated by a one minute resting period. Contractions were maintained for 10 seconds. A 20-second resting period was allowed between contractions.

To control the intensity of the contractions, the maximal force produced during plantar-flexion MVC was computed and the 50% was calculated (see Section 5.4.3.5 for more information about how MVC was carried out). This value was entered on the KINCOM machine. The KINCOM machine has got an option by which continues feedback (current force and target forces) can be displayed on the screen (see Figure 5-16).

NIRS data was processed and analysed using the Oxysoft 2.1.2 software. Oxygen consumption as well as blood flow in the MGast was measured to investigate muscle microcirculation during three different conditions: 1) circulation at rest; 2) circulatory responses to exercise and; 3) circulatory recovery from exercise.

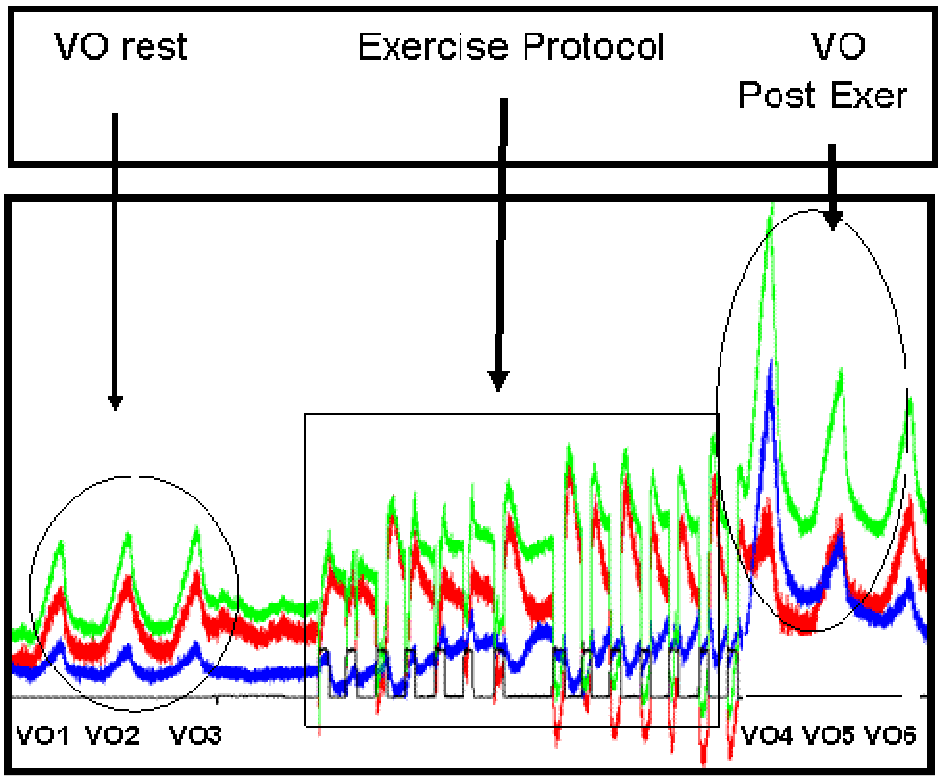
Blood flow was calculated during venous occlusion from the linear increase of tHb. Concentration changes of tHb were expressed in micromolars per second and were converted to $ml \cdot min^{-1} \cdot 100ml^{-1}$ using an average Hb concentration of 7.5 $mmol \cdot L^{-1}$ for female subjects and 8.5 $mmol \cdot L^{-1}$ for male subjects. The molecular weight of haemoglobin (64.458 $g \cdot mol^{-1}$) and the molecular ration between haemoglobin and oxygen (1:4) were taken into account. Muscular oxygen consumption was calculated from the linear increase of HbdO. Concentration changes HbdO were expressed in micromolars per second and converted to millilitres O_2 per minute per 100 grams. A value of 1.04 $kg \cdot L^{-1}$ was used for muscle density (van Beekvelt et al., 2002).

Circulation at rest was presented in absolute values. Blood flow values were then expressed in $ml \cdot min^{-1} \cdot 100ml^{-1}$ and oxygen consumption in $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$. Resting BF and mVO_2 were calculated as the average of the three values acquired during the first three VOs. Responses to exercise were calculated as the % of change in BF and mVO_2 from resting condition values (average values obtained during VO1,VO2 and VO3) to post-exercise values (values obtained during VO4). Recovery from exercise

investigated the % of recovery in BF and mVO_2 from VO4 (first VO after the exercise protocol) to VO5 (second VO after the exercise protocol finished). (See Figure 5-15 for more details about the microcirculation measurements).

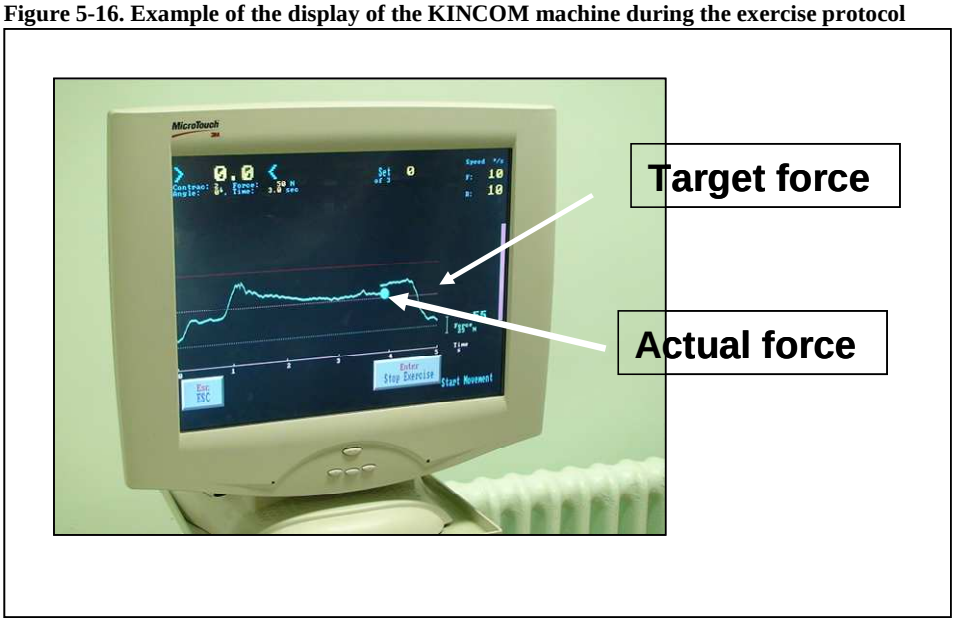
During the exercise protocol, force and EMG were collected simultaneously (see Section 5.4.1.3 for more information about instrumentation set up). This enabled us to relate NIRS data (blood flow and oxygen consumption in the MGast) to the amount of work done by the MGast during the exercise protocol. Even though force was controlled during the exercise protocol, the amount of work coming specifically from MGast was unknown. EMG data during the exercise protocol was calculated using Matlab and was processed in relation to the maximal activity obtained during the plantar-flexion MVCs (strength test). Then, the mean activity during the 14 contractions (10 seconds each) was determined.

Figure 5-15. Example of the microcirculation data obtained from the NIRS device.



Note: The blue line represents the deoxygenated haemoglobin, the red line represents the oxygenated haemoglobin and the green line represents the total haemoglobin content.

Figure 5-16. Example of the display of the KINCOM machine during the exercise protocol



5.4.4 Summary of the outcome measures in relation to the aims of the study.

Table 5-3 shows an overview of the outcome measures investigated in the present investigation and how they relate to the different domains under investigation.

5.5 Ethical Considerations

General ethical consideration

The study was approved by the Cardiff and Vale NHS Trust Research & Development Office and the South East Wales Local Research Ethics Committee (See copy of approval letters in Appendix 8). All work undertaken complied with the Research Governance Framework for Health and Social care in Wales and Cardiff University Research Governance Framework. Patients were free to withdraw from the study at any time without a given reason, and without medical care or legal rights affected.

Ethical issues related to data collection

The participants were required to wear shorts and a t-shirt during the data collection. Changing facilities were available on site. During skin-folds measurements participants were required to lift the t-shirt so the skin folds were accessible to the researcher. During the ECG measurements, in order to place the chest electrodes in the right position participants were requested to take the t-shirt off. Women were also asked to unfasten their bra. A towel was placed on the chest of the women participants during the whole duration of the ECG recordings to protect their privacy. In addition to that, in order to respect the dignity of the participants all the measurements were carried out by someone of the same gender as the subject, a male researcher for men participants and a female research for women participants.

Since DN subjects are at risk of developing foot problems, special attention was paid in protecting their feet throughout the entire data collection session. Participants were provided with a pair of comfortable and adjustable shoes (Classic xtra, Chaussures Pulman, Saint Paul Les Dax, France) that they wore throughout the data collection. When measurements required barefoot standing (gait analysis, weight and height measurements) a mat or walkway was placed on the foot to avoid direct contact between the foot and the floor at any time during the whole session.

Ethical issues related to data storage

All data was collected, processed and analysed at the Research Centre for Clinical Kinaesiology, department of Physiotherapy at Cardiff University. To ensure anonymity all participants were allocated a unique subject code. All collected data was then kept anonymous and saved under the subject code in password protected computers. Any written data as well as the video taken from the gait analysis were kept in a key-locked cabin and only the researcher (PhD student) had access to the key.
Cardiff University was/is the data controller. In this capacity, the School of Healthcare Studies Cardiff University will retain all data collected during the research for 15 years, in accordance with the Data Protection Act (1998).

Ethical consideration of the exercise programme

(This section only applies to the part 2 of the main study).

The exercise programme was designed and supervised by the researcher, who is a qualified exercise scientist. The exercise programme was planned to be performed both in the gym and at home. It was thought that encouraging people to exercise by themselves will improve their confidence and determination to integrate physical activity as part of their daily lives which will extend the benefits of physical activity beyond the duration of this study. During the first fortnightly gym sessions special attention was paid to teach participants; 1) how to exercise by themselves; and 2) how to reduce the risk of any hypoglycaemic events caused by the exercise bout (i.e. controlling glucose levels before and after training). After that period subjects were provided with a guide which included the exercises they had to do at home (see Appendix 2).

There are some exercise-induced risks associated with the interventional study. Even though the strictness in the inclusion/exclusion criteria may minimise these risks, there were still some threats secondary to peripheral neuropathy, which need to be taken into consideration. Foot problems and hypoglycaemic episodes are the most common risks associated with exercise training. To reduce the risk of foot complications the amount of weight bearing exercises were reduced to a minimum and only non-impact exercises were used. In addition to that, all participants were encouraged to closely examine their feet after each training session to prevent sores and were required to use proper footwear. To reduce the risk of hypoglycaemic episodes, subjects were advised before each session to check their sugar levels. If sugar levels were below 100 $mg \cdot dL^{-1}$ participants were provided with a carbohydrate snack (Flood & Constance, 2002). The information sheet included a section in which the potential risks of the study were addressed. Moreover, participants were encouraged to ask about their concerns before accepting to take part in the study.

5.6 Statistical consideration

5.6.1 Sample size determination

Prior to the beginning of the study, the sample size and power calculation were assessed for the primary outcome measures. The primary outcome measures in the present investigation were PP and microcirculation due to their association with foot ulceration in subjects with DN. Two power calculations were performed, 1) to determine the numbers for the cross-sectional study and 2) to determine the numbers for the intervention study. Details on the power calculations for the cross-sectional and intervention studies are provided below.

5.6.1.1 Cross-Sectional

Effect size for peak pressure

Data from previous research investigating group differences in forefoot peak pressures between healthy and subjects with DN were used for the calculations. Forefoot peak pressures were chosen since most of the foot ulcers in subjects with DN occur in this foot region. Pitei et al. (1999) reported mean values of 242± 25.1 kPa and 204.6± 37.8 kPa for subjects with DN and healthy individuals, respectively. These values indicate a standard difference of 1.18 (Bratcher et al., 1970). For this power calculation, a more conservative estimate of the standard difference of 1 was used.

Effect size for microcirculation

Data from previous research investigating group differences in vasodilatory changes in the microcirculation between healthy and subjects with DN were used for the calculations. Colberg et al. (2005) reported mean values of 119.7± 12 units and 91.6± 14.9 units for healthy individuals and patients with type 2 diabetes, respectively. These values indicate a standard difference of 2 (Bratcher et al., 1970). For this power calculation an effect size of 2 was used.

Study Power calculation

Two groups namely DN and HEALTH were compared in the cross-sectional study. Minimum sample size was calculated using a standardised difference of 1 (which reflects the lower of the two effect sizes reported above), and a power of 0.90 (α= 0.05). Each group therefore needed a minimum of 22 participants.

5.6.1.2 Intervention study

The main objectives of this research study were: 1) to determine whether a physical activity programme based on strengthening exercises can modify gait characteristics in patients with diabetic neuropathy and 2) to examine whether a strength training programme can improve circulation in this population.

Effect size for chances in strength levels

An important aim of this investigation was to increase strength levels in a sample of patients with diabetic neuropathy. Therefore, available literature was investigated for changes in strength levels due to a physical activity programme in patients with diabetic neuropathy. Since prior to the beginning of the present investigation there was no information available on the effect of a PA programme on strength levels on subjects with DN, the effect size calculation was carried out on individuals with type 2 diabetes and no neuropathic complications. Cauza et al. (2005) carried out a 4 months resistance training programme on 22 individual with type 2 diabetes. They reported changes in lower limb strength levels from 113.6± 7.8kg to 167.9± 9.7kg that indicate a standardised difference of 8.7 (Bratcher et al., 1970). For this power calculation an effect size of 8 was used.

Effect size for chances in circulation parameters

An important aim of this study is to explore the effects of resistance training on microcirculation in a diabetic population with peripheral neuropathy. Therefore, available data in the literature was investigated for changes of microcirculation parameters through a strength training intervention. Vasodilatory responses to local heat

were chosen for the power calculations since they indicate adaptations in microcirculation. Colberg, et al. (2006) carried out 8 weeks of strengthening exercises in a group of 10 subjects with type 2 diabetes. They reported pre-intervention values of 91.6+14.9 units and post-intervention values of 102.9± 16.9 that indicate a standardised difference of 1 (Bratcher et al., 1970). For this power calculation an effect size of 1 was used.

Study power calculation

Two groups namely EXE and CON were compared based on physiological outcomes. Minimum sample size was calculated using a standardised difference of 1 (which reflects the lower of the two effect sizes reported above) and a power of 0.90 (α= 0.05). Each group therefore needed a minimum of 27 participants. However, due to fact that some studies reported high rates of drop-out in physical activity interventions with diabetic populations (Thomas et al., 2006), which could drastically affect the power of the study, it was attempted to recruit about 30 participants per group to minimise this risk, whilst aiming to limit the drop-out as much as possible by motivating participation.

Part 1 and part 2 of the main study were not independent to one another. Therefore, the volunteers from the DN group from the cross-sectional study were included in the intervention study either as exercisers (EXE group) or controls (CON group) (see Figure 5-1). The intervention study required a larger number of subjects with DN (30 in each group, totalling 60 subjects with DN) than needed for the cross-sectional study (only 22 individuals required). This resulted in a higher number of subjects for the DN group than needed based on the power calculation.

5.6.2 Statistical analysis

The present investigation includes two different studies, a cross-sectional study, and an intervention study. Table 5-1and Table 5-2 include a summary of the statistical analysis followed for the cross-section and longitudinal studies, respectively. SPSS 16.0 software was used for all statistical analyses of the data.

5.6.2.1 Cross sectional study

Normality and equal variance of the data were assessed to allow for the appropriate choice of statistical test (Field, 2009; Portney & Watkings, 2009). Histograms and Q-Q plots were used to inspect the distribution of the data visually (skewness and kurtosis), whereas the Shapiro Wilk test was used to confirm whether the data was normally distributed. Since the Shapiro Wilk test is more appropriate for small sample sizes (<50) compared to the Kormogorov-Smirnow test (Elliot & Woodward, 2010), the Shapiro Wilk test was used in the present study. In addition to this, to further explore the distribution of the variables, the values of skewness and kurtosis were recorded. Homogeneity of variance was tested using Levene's test. Pearson's correlations and in cases where normality was not shown the non-parametric Kendall´s tau-b was used to identify possible confounding variables. Kendall's tau-b was chosen instead of Spearman's correlation coefficient since Kendall's statistic is considered a better estimate of the correlation in the population compared to Spearman's coefficient (Howell, 2006). Independent t-test and in cases where normality was not shown the non-parametric Mann Witney U test was used to assess between group differences. When confounding variables were identified group differences were investigated using analysis of covariance (ANCOVA), which allows a group comparison after controlling for the confounding variables. ANCOVA assumes that data is normally distributed, therefore, in the case where normality was not shown data transformation was carried out. Square root transformations for positive skew data and reverse score transformations for negative skew data were chosen (Field, 2009). After the data was transformed and prior to carrying out ANCOVA it was checked via histograms, Q-Q plots and the Shapiro Wilk test that the data was normally distributed.

Significance was set at an alpha level of 0.05. Data was considered to show a trend when alpha level was <0.1. Descriptive statistics were presented in the form of means and standard deviations when the data was normally distributed and in the form of medians and ranges when the assumption of normality was violated. Variables that were not normally distributed can be identified in the results Chapter since: 1) they were presented as median and range; and 2) they were analyzed with a non parametric test. When a non parametric test was used to analyse the data, it was stated in the table with

the results. Transformed data was presented in the form of original means and standard deviations not to lose the meaning of the actual units.

The two groups were matched on marginal distributions for age, body mass, sex and height as those variables would potentially affect the findings in the present study. Since matching for body mass was not possible the effect of body mass on the data was explored. Thereafter when body mass was identified as a confounding variable ANCOVA was used to control for the effect of body mass in the analysis.

Table 5-1. Overview of the statistical analyses carried out for the cross-sectional study.

<table>
<tr><th>Procedure</th><th colspan="3">Statistical test</th></tr>
<tr><td>Normality check</td><td colspan="3">Q-Q plot, Histogram and Shapiro-Wilk test</td></tr>
<tr><td rowspan="2">Correlations (confounding variables)</td><td colspan="2">Normality assumed</td><td>Normality NOT assumed</td></tr>
<tr><td colspan="2">Pearson’s Correlation (Parametric)</td><td>Kendall’s tau-b (Non Parametric)</td></tr>
<tr><td rowspan="3">Group differences</td><td colspan="2">Normality assumed</td><td>Normality NOT assumed</td></tr>
<tr><td>No confounding identified</td><td>Confoundings identifed</td><td rowspan="2">Mann Witney U (Non Parametric)</td></tr>
<tr><td>Independent t-test</td><td>ANCOVA*</td></tr>
<tr><td colspan="4">* In the case where normality was not shown data transformation was performed</td></tr>
</table>

5.6.2.1.1 Further data exploration

After performing the statistical analyses to test the hypotheses of the cross-sectional study, it was considered of interest to explore the possible variables that may explain those results. The main objective of the cross-sectional study was to understand the differences between healthy and neuropathic patients when investigating “health

characteristics" from a multidimensional comprehensive approach. Further to this information, it was interesting to explore the factors that may better account for those group differences as well as whether those factors play a similar or a different role on the diabetic neuropathic patients when compared to the healthy counterparts. It is noteworthy that only outcome measures that have been identified in the literature to influence the dependent variable were explored. In the results chapter and prior to the presentation of the exploratory data the variables that were investigated and the theoretical reasons behind that choice were explained.

The main analysis of the exploratory data was carried out using bivariate correlations. Normality and equal variance of the data were assessed to allow for the appropriate choice of statistical test. Histograms and Q-Q plots were used to inspect the distribution of the data visually, whereas the Shapiro Wilk test was used to confirm whether the data was normally distributed (Field, 2009). Then, Pearson's correlations were used when the data was normally distributed whereas Kendall's tau-b was used when normality was not shown (Howell, 2006). Significance was set at an alpha level of 0.05. The exploratory analysis of the data was intended to give an indication of the variables that may contribute to the main outcome measures investigated within the cross-sectional study. For instance, it was explored whether sensory neuropathy or muscle weakness, which are considered two important factors responsible for the gait differences between healthy and DN subjects (Mueller et al., 1994; Payne et al., 2002), were important contributing factors in the present investigation.

5.6.2.2 Intervention study

Two groups namely CON and EXE were compared over time to assess the effect of a resistance training programme on cardiovascular risk factors, gait characteristics, circulation and quality of life. Adaptations to the exercise programme were calculated using a 2 way Mixed ANOVA design. The analysis of interest was the interaction of group*time. Prior to running the analysis for ANOVA, normality of the data was inspected as described in the section above. Since for 2 way Mixed ANOVA there is no non-parametric counterpart, no alternative test was carried out in the present study when the data broke the assumption of normality (Field, 2009). Although 2 way Mixed

ANOVA assumes that data are normally distributed, it is well known that ANOVA is robust to violations of the assumption of normality of the data (Field, 2009; Howell, 2006). Thus, it is believed that normality of the data do not seriously affect the results. For this reason and to reduce the risk of losing the "meaning" of the actual units due to data transformation, variables that were not normally distributed were not transformed. 2 way Mixed ANOVA also assumes that the data do not violate the assumption of sphericity. However, at least three means are required for sphericity to be an issue (Field, 2009) and in the present study only two means (pre-intervention and post-intervention) were analysed.

Hence, pre- and post-intervention data sets were entered in the within subjects box whereas the intervention groups (CON and EXE) were entered for the between subjects analysis in the SPSS 16 software. The covariates used throughout the intervention results section were carried over from the cross sectional results sections. No post hoc tests or contrast were carried out since only two means were (pre-intervention and post-intervention) were analyzed.

Significance was set at an alpha level of 0.05. Data was considered to show a tendency when alpha level was <0.1. Descriptive statistics were presented in the form of means and standard deviations.In addition to this, F values and degrees of freedom were also displayed. Please note that the F values only relate to the group*time interaction effect.

Table 5-2. Overview of the Statistical analyses carried out for the intervention study

Procedures	**Statistical Test**	
Normality check	Q-Q plot, histogram and Shapiro- Wilk test	
Group*Time interaction	**Confounding NOT identified in the Cross-Sectional study**	**Confounding IDENTIFIED in the Cross-Sectional study**
	2 way Mixed ANOVA	Covariate was entered in the 2 way mixed ANOVA analysis

Table 5-3. Overview of the outcome measures investigated in the present study

Domain	*Measurement*	*Instrument*	*Outcome measure*
General Health	Cholesterol	LDX system	Total cholesterol
			LDL
			HDL
	Blood Pressure	Microlife BP	Systolic Blood Pressure
			Diastolic Blood Pressure
	Glucose Control	DCA 2000	HbA_{1c}
	Neuropathy	Neurothesiometer	VPT
		Monofilaments	N/A (only for confirmation of DN)
Gait	Strength	KINCOM	Peak Moment
	Gait parameters	Video Camera	Gait velocity
			Step length
			Step time
		EMED Platform	Distance COP
			Velocity COP
			Arch Index
	Kinetic data	EMED Platform	Peak Pressure
			PTI
			Contact Area
			Contact time
			Max Force
			Instance Max Force
			Instant Max Pressure
	Muscular Activity	NORAXON EMG device	% peak activity per gait phase
			% GC muscle active
			Time to peak (push off phase)
			Instant Peak Activity (Push off)
Microcirculation	Muscular microcirculation	NIRS	Muscular Blood flow
			Muscular Oxygen Consumption
Quality of Life	Self-Reported Quality of life	SF-36 Questionnaire	Physical Health
			Mental Health

CHAPTER 6

6 Results

The present investigation includes two different studies, a cross-sectional study (Part 1 of the main study), and an intervention study (Part 2 of the main study). The cross-sectional study investigated differences between patients with DN and healthy controls whereas the intervention study analysed the effects of a 16-week semi-controlled strength training programme on subjects with peripheral neuropathy. As mentioned in the methods chapter both studies investigated the same outcome measures, which included parameters related to general health, gait, microcirculation and QOL These are considered the primary pathologies associated with DN.

This chapter was therefore divided into two sections named cross-sectional study and intervention study. Therefore, section 1 presents the results related to the hypotheses for the part 1 of the main study and section 2 presents the results related to the hypotheses for the part 2 of the main study.

6.1 CROSS-SECTIONAL STUDY

The aim of this cross-sectional study was to investigate differences in the primary pathologies associated to diabetic peripheral neuropathy between healthy and DN subjects. Therefore, after the demographic characteristics of the sample groups are presented, results of the outcome measures relating to general health, gait, microcirculation and QOL are displayed in different sections in this chapter (see Table 6-1). These results compose the main analysis. Each section begins by exploring the effect of previously identified possible confounders (in the literature) on the dependent variable. Thereafter, based on this exploration the decision was made to which analysis would be appropriate. Further information about the statistical approach used during the cross-sectional study can be found in the Chapter 5 (Section 5.6.2.1). In addition to that, the last section of the results includes an exploratory analysis which provides some additional information regarding the factors that may account for the group differences investigated in the main analysis.

Table 6-1. Overview of the results presented in cross-sectional study

DOMAIN	*Measurement*		*Outcome measure*	
General Health	Cholesterol		TC	
			LDL	
			HDL	
	Blood Pressure		Systolic Blood Pressure	
			Diastolic Blood Pressure	
	Glucose Control		HbA_{1c}	
	Obesity		Body mass	
			Body fat %	
	Neuropathy		VPT	
Gait	Strength		Peak Moment	
	Gait parameters	Spatial-temporal	Gait velocity	
			Step length	
			Step time	
		COP parameters	Distance COP	
			Velocity COP	
	Kinetic data		Peak Pressure	Heel
				Metatarsals
				Big toe
			PTI	Heel
				Metatarsals
				Big toe
	Muscular Activity		% peak activity per gait phase	
			% GC muscle active	
			Time to peak (push off phase)	
			Instant Peak Activity (Push off)	
Microcirculation	Muscular microcirculation		Muscular Blood flow	Rest
				Post-Exercise
				Recovery
			Muscular Oxygen Consumption	Rest
				Post-Exercise
				Recovery
Quality of Life	Self-Reported Quality of life		Physical Health	
			Mental Health	

6.1.1 Subjects characteristics

The two groups were matched on marginal distributions for age, height and gender. Table 6-2 presents the demographic features of the subjects belonging to the 2 groups (DN and HEALTH). No statistical differences were shown between the groups regarding to age, height or gender. However, both groups differed significantly in body mass (<0.01). Mean± standard deviation was 93.03± 17.47 kg and 78.63± 9.56 kg for DN and HEALTH groups, respectively. Since matching for body mass was not possible the effect of body mass on the outcome measures was explored prior to the main analysis.

Table 6-2. Subject characteristics for the HEALTH and DN group[a]

Variable	**Group**		**Inferential statistical results**
	DN (N=53)	HEALTH (N=25)	*Case*Control comparison*
Age (years)	62.20± 7.55	57.76± 10.60	t(76)=1.851, p=0.068
Mass (kg)	93.03± 17.47	78.63± 9.56	***t(76)=3.846, p<0.001 *****
Height (m)	1.69± 0.98	1.71± 0.86	t(76)=-0.939, p=0.350
Sex Male (n) Female (n)	 34 (64%) 19 (36%)	 16 (64%) 9 (36%)	NA

[a] Values are means ± SD; ** Significance value is less than 0.01 level (2-tailed).

6.1.2 General Health

This section investigates characteristics related to general health. Thus, blood pressure, body fat% and lipids profile were explored.

Table 6-3 shows the medications that were taken by the DN group at the time of the measurements and that could have affected some of the group comparisons discussed later. Thus, Table 6-3 shows that all the subjects in the DN group were taking

medications against hypertension, hyperglycaemia and hypercholesterolemia. In addition to this, 7 subjects (13%) were taking antidepressants.

Table 6-3. List of medications taken by the DN group

Treatment	Medication	Number of patients taking specific medications	Number of patients taking medications for specific treatment
Hypertension	• ACE inhibitor (i.e. Ramipril, Lisinopril, Perinpopril, Losartan or Valsartan	48 (92%)	52 (100%)
	• Thiazide diuretic (i.e. Bendroflumethiazide	6 (11%)	
Hyperglycaemia	• Metformin	51 (98%)	52 (100%)
	• Repaglinide	3 (5%)	
Hypercholesterolemia	• Statin (i.e. Simvastatin, atorvastatin)	52 (100%)	52 (100%)
Antidepressant	• Amitriptyline (i.e. tryptomer, Lentizon)	7 (13%)	7 (13%)

Main results: Comparison of general health related outcome measures between the DN and the HEALTH group

Information with regards to the risk factors associated with cardiovascular diseases is presented in Table 6-4. DN group showed significantly higher values in systolic blood pressure, heart rate at rest and body fat % compared to the HEALTH group. Diastolic blood pressure did not differ significantly between the groups. Since cholesterol levels were only measured in the DN group Table 6-5 presents cholesterol levels in the DN group. For informative reasons current recommendations from the National Cholesterol Education Programme (NECP) regarding LDL, HDL and TC (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 1993) were also presented.

Table 6-4. Cardiovascular risk factors: Comparison between the HEALTH and the DN group

Variable	Group		Inferential statistical results
	DN (N=53)	HEALTH (N=25)	*Case*Control comparison*
Systolic blood pressure (mmHg)	136.00 (53.00)	127.00 (62.00)	***0.032* (NPT)***
Diastolic blood pressure (mmHg)	79.77± 9.06 [a]	83.24± 8.80[a]	t(76)=-1.590, p=0.116
Body fat %	37.74± 6.32[a]	31.41± 6.40 [a]	***t(76)=4.111, p<0.001****
Heart rate at rest (beats·min $^{-1}$)	76.73± 12.42 [a]	70.76± 10.36 [a]	***t(76)=2.085, p=0.040****

[a] Values are means ± SD; [b] Values are medians with range in parentheses; * Significance value is less than 0.05 level (2-tailed); ** Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test.

Table 6-5. Cholesterol levels: Values in the DN group in relation with current recommendations

Variable	Group	
	DN (N=53)	Recommendations from NECP
HDL (mmol·L^{-1})	1.11± 0.31[a]	>0.9
LDL mmol·L^{-1})	1.83± 0.73[a]	<3.4
TC (mmol·L^{-1})	3.81± 0.94 [a]	<5.2

[a] Values are means with SD in parentheses.

6.1.3 Gait biomechanics

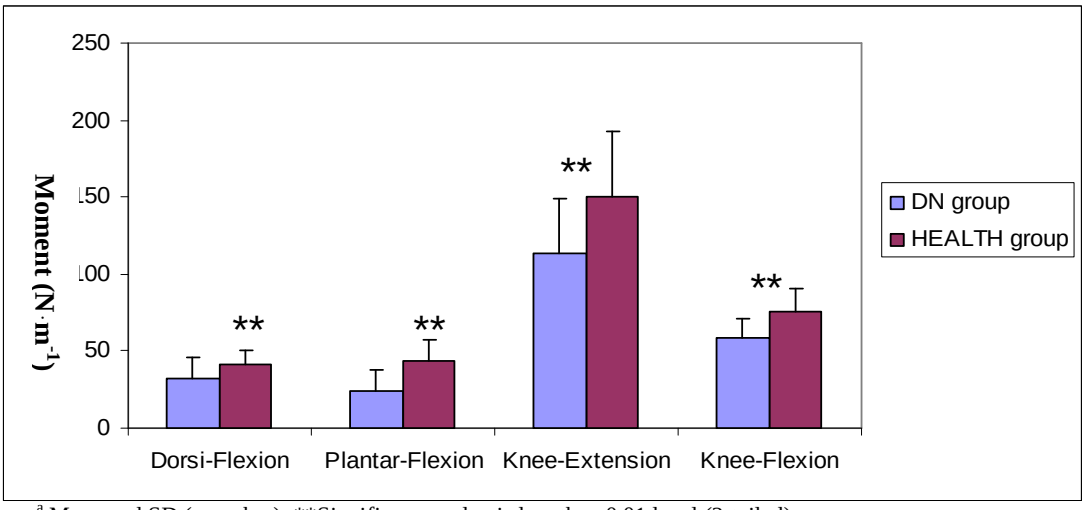
Gait was investigated from different perspectives. This intended to provide a more comprehensive analysis of walking in patients with diabetic neuropathy. This section starts presenting results of the strength levels in the lower limb muscles. Although strength is not a gait-related outcome measure, these results are included in this section due to the close association that exists between muscle weakness and gait alterations in

individuals with DN (Mueller et al., 1994). See Chapter 2 (Section 2.2.2.1) for more information about the way muscle weakness can affect gait in DN subjects. Thereafter three more subsections are included in which outcome measures related to gait parameters, kinetic data and muscular activity data during gait are presented.

6.1.3.1 Isometric strength

Maximal strength levels during isometric testing for the DN and HEALTH groups are presented in Figure 6-1. Results show that the DN group was significantly weaker in all muscle groups compared to HEALTH group.

Figure 6-1. Strength levels: Comparison between the HEALTH and the DN group [a]



[a] Mean and SD (error bar); **Significance value is less than 0.01 level (2-tailed)

6.1.3.2 Gait parameters

Possible confounders for spatial-temporal and COP parameters

Since body mass was not sufficiently controlled it was explored as a potential confounder variable for spatial-temporal parameters. Table 6-6 shows that body mass did not correlate significantly with any of the spatial-temporal parameters. However,

both groups showed a trend toward longer step times and slower cadence in subjects with larger body mass ($p<0.06$).

Body mass was also explored as a possible confounder variable for COP measurements. Thus, body mass appeared to be positively correlated to the total distance travelled by the centre of pressure in the DN group. This correlation was not present in the HEALTH group ($p=0.013$). However, there was a trend toward a positive correlation between the total distance travelled by the COP and body mass in the HEALTH group ($p=0.081$). Apart from that, no other significant correlation was observed between body mass and all the other floor-foot interaction parameters (velocity of COP at the heel, forefoot and hallux).

Beside body mass, gait velocity was also explored as a possible confounding variable for COP measurements. Since some of the COP variables are velocity related it was important to explore the effect of gait velocity on those variables. Gait velocity was found to be positively correlated to the velocity of the COP at the heel in both groups ($p<0.15$) and not to the velocity of the COP at the forefoot or hallux either in the DN or HEALTH groups. Table 6-7 shows the effect of body mass and gait velocity on COP outcome measures

In the main analysis, body mass and gait velocity were therefore handled as follows: body mass was controlled for when analysing the total distance travelled by the COP whilst gait velocity was controlled for when analysing the velocity of the COP at the heel.

Table 6-6. Spatial-Temporal parameters: Possible confounding variables (Bivariate correlation)

		Step time	Step length	Cadence	velocity
Body Mass	N=53 (DN group)	r=0.267 p=0.055	r=-0.131 p=0.928	r=-0.271 p=0.052	r=-0.165 p=0.243
	N=25 (HEALTH group)	r=0.381 p=0.060	r=-0.112 p=0.593	r=-0.386 p=0.056	r=-0.113 p=0.589

Table 6-7. Floor-foot interaction parameters: Possible confounding variables (Bivariate correlation)

		COP (total distance)	COP velocity Heel	COP velocity Forefoot	COP velocity Hallux
Velocity	N=53 (DN group)	N/A	***r=0.560*** ***p<0.001***	τ=-0.049 p=0.615 (NPT)	r=0.049 p=0.738
	N=25 (HEALTH group)	N/A	***r=0.480**** ***p=0.015***	r=-0.402 p=0.052	r=0.135 p=0.519
Body Mass	N=53 (DN group)	***r=0.339**** ***p=0.013***	r=0.06 p=0.968	r=-0.269 p=0.054	r=0.082 p=0.569
	N=25 (HEALTH group)	r=0.356 p=0.081	r=0.205 p=0.325	r=-0.134 p=0.534	r=0.280 p=0.175

* Significance value is less than 0.05 level (2-tailed); ** Significance value is less than 0.01 level (2-tailed).

Main results: Comparison of gait parameter outcome measures between the DN and the HEALTH group

Group differences in gait parameters are shown in Table 6-8 and Table 6-9. The HEALTH group reported significantly quicker and longer steps, higher cadence and higher walking velocity when compared to DN group. With regard to the COP parameters the velocity of the COP at the heel was the only dependent variable that differed significantly between the groups. Hence, the HEALTH group showed slower velocity of the COP at the heel when compared to the DN group (p=0.07). A trend toward longer distance travelled by the COP in the HEALTH group compared to the DN groups was also observed (p=0.084). No group differences were observed in all the other floor-foot interaction parameters (p>0.05). Overall, the data shows that the main group differences in gait parameter refer to spatial-temporal parameters.

Table 6-8. Spatial-temporal parameters: Comparison between the HEALTH and the DN group [a]

Variable	Group		Inferential statistical results
	DN (N=52)	HEALTH (N=25)	*Case*Control comparison*
Step time (sec)	0.55± 0.05	0.51± 0.02	***t(75)=3.299, p<0.001*** **
Step length (m)	0.61± 0.07	0.71± 0.06	***t(75)=-5.466, p<0.001*** **
Cadence (steps·min^{-1})	109.86± 9.81	117.22± 6.71	***t (75)=-3.386, p=0.001*** **
Velocity (m·sec^{-1})	1.13± 0.18	1.39± 1.64	***t(75)=-6.003, p<0.001*** **

[a] Values are means±SD; ** Significance value is less than 0.01 level (2-tailed).

Table 6-9. COP parameters: Comparison between the HEALTH and the DN group

Variable	Group		Inferential statistical results	
	DN (N=53)	HEALTH (N=25)	*Case*Control comparison*	
			without covariate	with covariate (ANCOVA)
Distance travelled by the COP (cm)	24.49± 2.00[a]	24.72± 1.50 [a]	N/A	$F_{(1,75)}$=3.064, p= 0.084[1]
Velocity COP at the heel (m·sec^{-1})	0.47± 0.12 [a]	0.485± 0.11 [a]	N/A	***$F_{(1,73)}$=7.827, p=0.007*** *[2]***
Velocity COP at the metatarsals (m·sec^{-1})	0.27 (0.25) [b]	0.28 (0.16) [b]	0.140 (NPT)	N/A
Velocity COP at the hallux (m·sec^{-1})	0.81± 0.29 [a]	0.752± 0.21 [a]	t(74)=0.899, p= 0.372	N/A

[a] Values are means ± SD; [b] Values are medians with range in parentheses; [1]Analysis of covariance with body mass as a covariate; [2]Analysis of covariance with gait velocity as a covariate; ** Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test.

6.1.3.3 Kinetic data

Due to the high association between plantar pressures and foot problems peak pressure and pressure time integral were the primary outcome measures under investigation. In addition to this, parameters related to PP (i.e. CA, Max Force and arch index) and PTI

(i.e. CT) were also investigated. Kinetic data was investigated in three different foot areas, the heel, the metatarsal heads and the hallux. Figure 6-2 shows an example of the distribution of the plantar pressures in a healthy individual with normal foot pressures and in a DN subject with abnormal loading patterns.

In addition to this, peak pressure, Max Force, the instant of peak pressure and the instant of Max Force were investigated under the forefoot (metatarsals and toes) during the push off phase. These results may provide some valuable information about the amount of stress the forefoot undergoes during the most critical phase of the GC.

6.1.3.3.1 Primary outcome measures: Peak Pressure and Pressure Time Integral.

Comparison of peak pressure and pressure time integral parameters between the right and the left foot for the HEALTH and DN groups are presented in Table 6-10. This Table shows that there were no significant differences in PP and PTI variables between the right and the left foot in the DN group and in the HEALTH group. This justifies that further analysis on the kinetic parameters were done only on the right foot.

Figure 6-2. Example of foot pressure distribution of a DN subject with loading abnormalities (left picture) and a healthy individual (right picture)

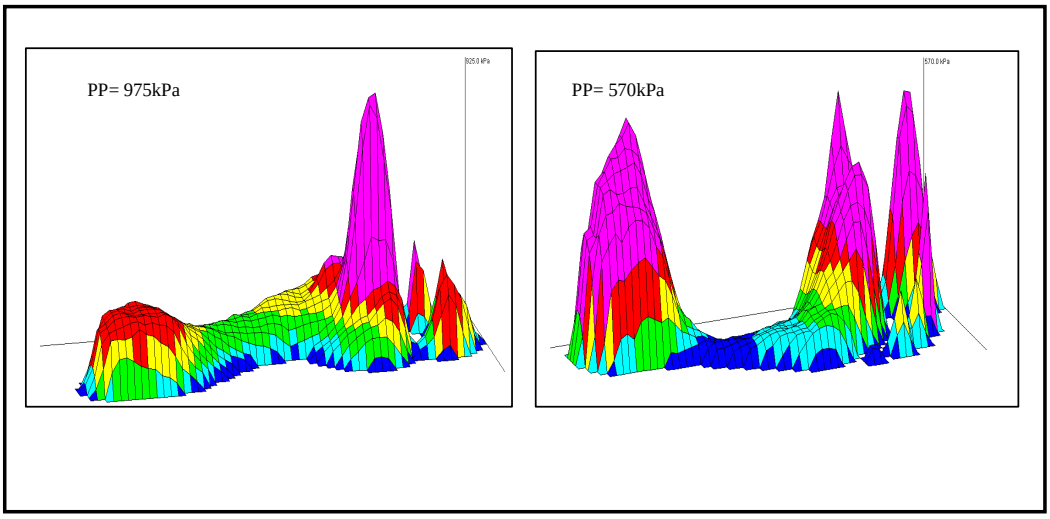


Table 6-10. Foot pressures: Comparison between the right and left foot in the HEALTH (N=25) and DN group (N=53)

Variable	Group	Foot		Inferential statistics results
		Right	Left	*Right*Left comparison*
PP Heel (kPa)	HEALTH	546.60± 155.89 [a]	535.32± 137.88 [a]	t(24)=0.271, p=0.788
	DN	450.60± 136.25 [a]	444.67± 117.87 [a]	t(76)=0.239, p=0.811
PP Metatarsals (kPa)	HEALTH	647.36± 230.68 [a]	642.04± 213.00 [a]	t(24)=0.085, p=0.933
	DN	779.45± 270.14 [a]	797.16± 264.05 [a]	t(76)=-0.341, p=0.733
PP Hallux (kPa)	HEALTH	575.92± 240.09 [a]	618.88± 306.13 [a]	t(24)=-0.552, p=0.583
	DN	405.00 (1275.0)[b]	403.00 (1207,0)[b]	p=0.943 (NPT)
PTI Heel (kPa·sec)	HEALTH	99.93± 24.26 [a]	102.16± 26.59 [a]	t(24)=0.194, p=0.758
	DN	110.64± 34.56 [a]	111.90± 32.11 [a]	t(76)=-0.194, p=0.836
PTI Metatarsals (kPa·sec)	HEALTH	145.42 (328.80)[b]	151.66 (794.00)[b]	p=0.776 (NPT)
	DN	206.94 (318.86)[b]	222.92 (430.08)[b]	p=0.543 (NPT)
PTI Hallux (kPa·sec)	HEALTH	100.35± 48.00[a]	114.81± 64.42[a]	t(24)=-0.207, p=0.372
	DN	68.42 (258.70)[b]	79.32 (310.86)[b]	0.836 (NPT)

[a] Values are means ± SD; [b] Values are medians with range in parentheses; NPT. Non parametric test.

Possible confounders for peak pressure and pressure time integral

Since group matching for body mass was not possible in the present study and it is known that it may affect foot pressures (Cavanagh et al., 1991), body mass was explored as a potential confounding variable for PP and PTI parameters. The effect of gait velocity on PP and PTI was also investigated since its influence in PP and PTI has been stated in the literature (Morag & Cavanagh, 1999).

Table 6-11 presents the correlations between PP and PTI outcome measures and the potential confounders. Gait velocity was the only variable that was significantly related to PP or PTI data. Thus, gait velocity was positively correlated to PP at the heel both in the DN (p=0.012) and the HEALTH (p=0.016) groups whereas gait velocity was negatively correlated to PTI at the heel in the DN group (p=0.01) and not in the HEALTH group (p=0.553). Contrary, PP and PTI both at the metatarsals and hallux were not significantly correlated to either velocity or body mass. Body mass did not seem to be related to PP and PTI at the heel either. Therefore, in the main analysis gait velocity was accounted for when analysing PP at the heel.

Table 6-11. Peak pressure and Pressure time integral: Possible confounding variables (Bivariate correlation)

		PP Hallux	PP Metatarsals	PP Heel	PTI Hallux	PTI Metatarsals	PTI Heel
Velocity	N=53 (DN group)	τ=0.032 p=0.740 (NPT)	r=-0.128 p=0.364	***r=0.350* p=0.012***	τ=0.047 p=0.625 (NPT)	τ=-0.087 p=0.360 (NPT)	***r=-0.353* p=0.010***
	N=25 (HEALTH group)	r=0.332 P=0.105	r=-0.066 p=0.754	***r=0.488* p=0.016***	r=0.246 p=0.235	τ=-0.140 p=0.327 (NPT)	r=-0.125 p=0.553
Body Mass	N=53 (DN group)	τ=0.114 p=0.228 (NPT)	r=-0.182 p=0.192	r=0.017 p=0.906	r=-0.126 p=0.184 (NPT)	τ=-0.015 p=0.872 (NPT)	r=-0.181 p=0.195
	N=25 (HEALTH group)	r=-0.163 p=0.436	r=-0.113 p=0.591	r=0.183 p=0.392	r=-0.129 p=0.538	τ=0.178 p=0.215 (NPT)	r=-0.182 p=0.383

* Significance value is less than 0.05 level (2-tailed); NPT. Non parametric test.

Main results: Comparison of peak pressure and pressure time integral parameters between the DN and the HEALTH group

Group differences in peak pressures are shown in Table 6-12. The HEALTH group showed significantly higher foot pressure values than the DN group at the hallux (p=0.033) whereas the DN group reported significantly higher values at the metatarsals compared to the HEALTH group (p=0.03). Group differences in PP at the heel were not significant after controlling for the effect of gait velocity (p=0.481).

Table 6-12. Peak pressure: Comparison between the HEALTH and the DN group

Variable	Group		Inferential statistical results	
	DN (N=53)	HEALTH (N=25)	*Case*Control comparison*	
			without covariate	with covariate (ANCOVA)
Peak Pressure Heel (kPa)	453.63± 136.36[a]	546.60± 155.89 [a]	N/A	$F_{(1,75)}$ =0.500, p= 0.481[1]
Peak Pressure Metatarsals (kPa)	779.45± 270.14 [a]	647.36± 230.68 [a]	***t(76)=2.211, p=0.030****	***N/A***
Peak Pressure Hallux (kPa)	405.00 (1275.00) [b]	548.00 (1020.00)	***p=0.033** (NPT)*	***N/A***

[a] Values are means ± SD; [b] Values are medians with range in parentheses; [1]Analysis of covariance with gait velocity as a covariate; * Significance value is less than 0.05 level (2-tailed); NPT. Non parametric test.

Table 6-13 shows that when comparing PTI parameters between the HEALTH and the DN group, the only significant group difference is observed at the metatarsal area. The DN group obtained median values (range) of 206.94 (318.86) kPa·sec compared to the 145.42 (328.80) kPa·sec reported by the HEALTH group (p=0.01).

Overall, these results show that the metatarsal area is the foot region in which the greatest PP and PTI differences occur between healthy and DN individuals.

Table 6-13. Pressure Time Integral: Comparison between the HEALTH and the DN group

Variable	Group		Inferential statistical results
	DN (N=53)	HEALTH (N=25)	*Case*Control comparison without covariate*
PTI Heel (kPa·sec)	110.64± 34.56[a]	99.93± 24.26[a]	t(76)=1.391, p=0.168
PTI Metatarsals (kPa·sec)	206.94 (318.86)[b]	145.42 (328.80)[b]	***p=0.01** (NPT)***
PTI Hallux (kPa·sec)	68.42 (258.70) [b]	101.42 (328.80) [b]	p=0.156 (NPT)

[a] Values are means ± SD; [b] Values are medians with range in parentheses; ** Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test.

6.1.3.3.2 Secondary outcome measures: Parameters related to Peak Pressure and Pressure Time integral

1) Parameters related to peak pressure

Peak pressure is defined as the force per unit area. Therefore it is important to investigate vertical GRFs and CA outcome measures to better understand PP values. Structural characteristics are also known to play an important role in PP. For this reason, arch index, which measures the height of the foot arch, was also analysed and included in this section.

Possible confounders for contact area

Body mass is likely to affect CA. In fact some investigations have found an association between these two variables (van Deursen, 2004). For this reason, body mass was explored as a possible confounder for CA. Table 6-14 displays the correlations between CA in different foot areas and body mass for both the DN and HEALTH groups

separately. A strong positive correlation between body mass and CA on different foot areas was observed for both groups. The only parameter that did not reach significant levels when related to body mass was CA at the hallux and this non significant correlation was only observed in the HEALTH group (p=0.235). Due to the high correlation that is expected between body mass and forces (GRFs), Max Force (vertical GRF) was normalised to body mass ($N \cdot kg^{-1}$).

Results from the present study show that CA is highly dependent on body mass. For this reason, body mass was controlled for when comparing CA between the healthy and DN groups.

Table 6-14. Contact area: Possible confounding variables (Bivariate correlation)

		CA All foot	CA Metatarsals	CA Heel	CA Hallux
Body Mass	N=53 (DN group)	***r=0.724*** ***p<0.001***	***r=0.770*** ***p<0.001***	***r=0.642*** ***p<0.001***	***r=0.301**** ***p=0.028***
	N=25 (HEALTH group)	***r=0.439**** ***p=0.028***	***r=0.410**** ***p=0.042***	***r=0.618*** ***p<0.001***	r=0.235 p=0.258

* Significance value is less than 0.05 level (2-tailed); ** Significance value is less than 0.01 level (2-tailed).

Main results: Comparison of CA and Max Force between the DN and the HEALTH group

Table 6-15 presents data on the parameters that are related to peak pressure. It shows that the only significant differences in CA between groups were found at the hallux (p=0.005). Table 6-15 also shows that there are significant group differences in the Max Forces (normalized to body mass) measured on the different foot areas. The DN group reported significantly higher Max Force values ($N \cdot kg^{-1}$) at the metatarsals compared to the HEALTH group (p=0.018) whereas the HEALTH group showed significant higher Max Force values ($N \cdot kg^{-1}$) at the heel (p<0.001) as well as at the hallux (p=0.002). The results also show significant group differences in the structure of the foot as demonstrated by the higher foot arch observed in the DN compared to the HEALTH group (p=0.048). Overall, these results show that Max Force is the main factor

explaining the differences in PP between the DN and the HEALTH group in the different foot regions under investigation.

Table 6-15. Parameters related to Peak Pressure: Comparison between the HEALTH and the DN group[a]

Variable	Group		Inferential statistical results	
			*Case*Control comparison*	
	DN (N=53)	HEALTH (N=25)	without covariate	with covariate (ANCOVA)
Contact Area (cm^2)				
Metatarsals	49.20± 6.14	45.33± 5.21	N/A	$F_{(1,75)}$=0.010, p=0.92[1]
Heel	33.82± 4.52	32.11± 3.37	N/A	$F_{(1,75)}$=0.811, p=0.371[1]
Hallux	8.91± 1.92	9.83± 1.99	N/A	***$F_{(1,75)}$=8.210, p=0.005****[1]
Max Force ($N \cdot kg^{-1}$)				
Metatarsals	9.34± 1.09	8.72± 1.04	***t(76)=2.411, p=0.018****	
Heel	7.05± 0.97	7.75± 0.78	***t(76)=-3.915, p<0.001***	
Hallux	1.37± 2.06	2.06± 0.80	***t(76)=-3.112, p=0.002***	
Arch Index	0.20± 0.06	0.18± 0.046	***t(76)=2.011, p=0.048****	

[a] Values are means ± SD; * Significance value is less than 0.05 level (2-tailed); ** Significance value is less than 0.01 level (2-tailed); [1]Analysis of covariance with body mass as a covariate.

2) Parameters related to Pressure Time Integral

PTI, calculated as the area under the peak pressure curve, represents the magnitude (pressure) and duration of the plantar stress (Mueller & Maluf, 2002). Therefore, the contact times in the different foot regions were analyzed. Since contact times, when measured in milliseconds, are dependent on gait velocity, contact times were normalized to the percentage of the stance phase.

Main results: Comparison of contact times between DN and HEALTH groups

Table 6-16 shows that there are significant differences between the HEALTH and the DN group in the amount of time the metatarsals and the heel areas contact the floor during the GC. The DN group reported higher contact times (% of the roll over process) at the metatarsals ($p<0.001$) and heel areas ($p<0.001$) compared to the HEALTH group. The contact time spent on the hallux area did not differ significantly between both groups ($p=0.251$).

Table 6-16. Parameters related to PTI: Comparison between the HEALTH and the DN group [a]

Variable	Group		Inferential statistical results
	DN (N=53)	HEALTH (N=25)	*Case*Control comparison without covariate*
Contact Time (% ROP)			
Metatarsals	84.99± 2.75	82.10± 3.04	***t(76)=4.181, p<0.001***
Heel	62.23± 7.44	55.19± 8.51	***t(76)=3.722, p<0.001***
Hallux	52.32± 16.47	56.57± 11.65	t(76)=-1.152, p= 0.251

[a] Values are means ± SD in parentheses; ** Significance value is less than 0.01 level (2-tailed).

6.1.3.3.3 Kinetic parameters during the push off phase

Main results: Comparison of kinetic parameters between DN and HEALTH groups during the push off phase

Table 6-17 presents data during the push off phase, which is thought to generate the highest stress on the sole of the forefoot. No significant differences were observed when comparing time related variables between the HEALTH and the DN group. Therefore, both groups reported very similar results when investigating the instant during the roll over process in which PP ($p=0.547$) as well as Max Force ($p=0.957$) were produced. Interestingly, no significant group differences were observed in PP at the forefoot

(metatarsals and toes) during the push off. Even though, the DN group obtained higher PP mean values (855.36± 259.08 kPa) compared to the HEALTH group (772.69± 209.97 kPa), those differences did not reach significant levels (p=0.161). Regarding to the amount of vertical GRF generated during push off, the DN group showed significantly higher values compared HEALTH groups (p<0.001). However, those differences disappeared after controlling for body mass (p=0.811). Thus, the DN and HEALTH groups reported a mean value of 10.83± 0.82 $N \cdot kg^{-1}$ and 10.88± 0.82 $N \cdot kg^{-1}$, respectively.

Table 6-17. Peak pressure and Max Force parameters during the push off phase: Comparison between the HEALTH and the DN group

Variable	Group		Inferential statistical results
	DN (N=53)	HEALTH (N=25)	*Case*Control comparison without covariate*
PP (kPa)	855.36± 259.08 [a]	772.69± 209.97 [a]	t(76)=1.416, p=0.161
Instant PP (% ROP)	83.24 (25.54) [b]	80.00 (23.92) [b]	0.547 (NPT)
Max Force (N)	999.38± 178.16 [a]	868.30± 126.66 [a]	***t(76)=3.85, p<0.001***
Instant of Max Force (%ROP)	77.38 (8.38) [b]	76.94 (19.90) [b]	0. 957 (NPT)
Max Force Normalised ($N \cdot kg^{-1}$)	10.83± 0.82 [a]	10.88± 0.82 [a]	t(76)=-0.24, p=0.811

[a] Values are means ± SD; [b] Values are medians with range in parentheses; ** Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test.

In summary, when comparing foot pressures between the HEALTH and the DN group, the most noticeable differences occurred underneath the metatarsal heads. Thus, results from the present study showed significantly higher PP and PTI values at the metatarsals area in the DN group when compared to the HEALTH group. Interestingly, no significant group differences in PP or Max Force ($N \cdot kg^{-1}$) at the forefoot (which

included the metatarsal heads and the toes) were observed during the push off phase.

6.1.3.4 Surface Electromyography

This section presents the results obtained from the EMG measurements. Firstly, the group differences in mean muscular activity during the differences phases of the GC are presented. Thereafter, time related variables are shown, which include: 1) the percentage of time each muscle was kept activity during the whole GC; 2) the instant of the GC at which TS and TA activity peaked during and after the push off phase, respectively; and 3) time lag from the onset to peak activity during and after the push off phase for the TS and TA muscles, respectively.

Main results: Comparison of mean activity during different phases of the gait cycle between DN and HEALTH groups

EMG data during the GC is displayed in Figure 6-3. On the top of the picture the different phases of the GC as well as their duration in relation to the GC can be observed. EMG data traces from the different muscles (TS, TA, VL and BicFem) as well as the results Table shown at the bottom of the picture have been presented in relation to the phases of the GC. Figure 6-3 shows the average EMG values during a GC for the DN (blue line) and HEALTH (red line) groups. EMG values are presented for the TA, TS, quadriceps (VL) and hamstrings (BicFem) muscles. The Table at the bottom of Figure 6-3 displays the differences in mean activity for the TA, TS, quadriceps (QUADS) and hamstrings (HAMS) during each phase of the GC when the DN group was compared to HEALTH group. Note that no statistical analysis has been carried out to investigate group differences in muscular activity during the initial swing and mid-swing phases of the GC. EMG data is presented as percentage of the peak activity during the GC.

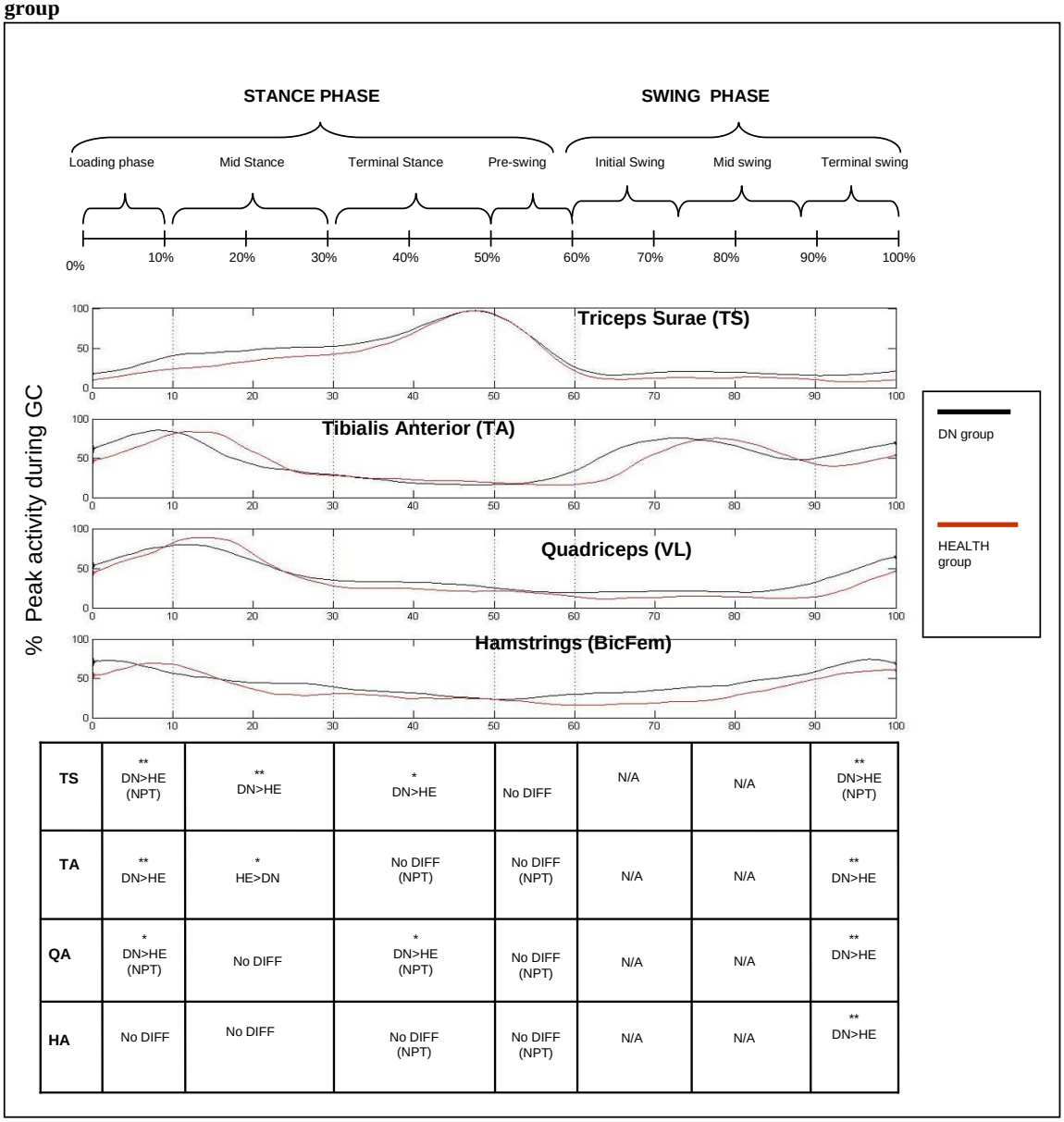
During the loading phase (0-10% of GC) the DN group reported significantly higher mean values than the HEALTH group for TS (28.6± 13.36% vs. 17.35± 9.14%), TA (77.11± 11.07% vs. 63.77± 18.03%) and QUADS (68.95± 19.82% vs. 64.03± 14.70%) while no significant differences were observed for the HAMS (67.25± 18.31% vs.

63.00± 22.19%). The same trend was observed for the TS during the mid-stance phase (10-30% of GC), in which the DN group showed higher mean values ($p<0.001$) than the HEALTH group (47.63± 12.63% vs. 33.94± 11.78%). Unlike TS, TA activity during this phase was significantly higher ($p<0.05$) in the HEALTH compared to the DN group (57.21± 14.92% vs. 47.63± 16.99%). Muscular activity patterns for QUADS and HAMS during the mid-stance did not differ considerably between groups. During the terminal stance phase (30-50% of GC), the DN group showed significantly higher ($p<0.05$) mean values for the TS (75.62± 9.33% vs. 70.18± 8.80%) and QUADS (31.98± 14.48% vs. 23.02± 17.85%) compared to the HEALTH group while no significant differences were observed for TA or HAMS. During the pre-swing phase no significant group differences were observed for any of the four muscles ($p>0.15$). The analysis of muscular activity levels during the last phase of the GC (Terminal swing) showed significant group differences for all 4 muscles. TS, TA QUADS and HAMS activity levels were consistently higher in the DN group compared to the HEALTH group. Significance values were lower than 0.01 for all four muscles.

Main results: Comparison of time related EMG variables between DN and HEALTH groups

Table 6-18 presents time related EMG data. Parameters under the heading % GC muscle active refer to the amount of time (as percentage) each muscle is kept activity during the whole GC. The other two sets of results refer to a specific time window around the push off phase for the TA and TS. More information about the time windows for those muscles can be found in the Chapter 5 (Section 5.4.3.4.3).

Figure 6-3. EMG activity patterns during the GC: Comparison between the HEALTH and the DN group



TS	** DN>HE (NPT)	** DN>HE	* DN>HE	No DIFF	N/A	N/A	** DN>HE (NPT)
TA	** DN>HE	* HE>DN	No DIFF (NPT)	No DIFF (NPT)	N/A	N/A	** DN>HE
QA	* DN>HE (NPT)	No DIFF	* DN>HE (NPT)	No DIFF (NPT)	N/A	N/A	** DN>HE
HA	No DIFF	No DIFF	No DIFF (NPT)	No DIFF (NPT)	N/A	N/A	** DN>HE

* Significance value is less than 0.05 level (2-tailed); ** Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test.

When investigating muscular activity patterns during the whole GC, results from the current study showed that the DN group maintained the TS, QUADS and HAMS muscles active during the GC significantly longer (% GC) than the HEALTH group ($p<0.05$). However, no statistical group differences were observed when looking at the TA.

When assessing time related outcome measures during the specific time window around the push off phase for the TA and TS muscles, results from the present study show that the DN and HEALTH groups did not differ significantly in the instant of the GC TA and TS peaked. When looking at the time delay from the onset of muscular activity to the peak activity, longer time delays were observed in the DN group compared to the HEALTH group for the TA ($p=0.026$) and the TS ($p<0.001$). Therefore activation was started earlier in the GC for the DN group for the TA and TS muscles compared to the HEALTH group.

Results from the present study showed significant differences in EMG activity patterns between the DN and the HEALTH group. Thus, the DN group showed significantly higher EMG activity compared to the HEALTH group as demonstrated by: 1) higher mean activity values throughout different phases of the GC; 2) higher amount of time the muscles were activity during the GC; and 3) earlier muscular activation of the TA and TS muscles.

Table 6-18. Time related EMG variables: Comparison between the HEALTH and the DN group

Variable	Group		Inferential statistical results
	DN (N=52)	HEALTH (N=23)	*Case*Control comparison without covariate*
% GC muscle active			
TS	67.46± 11.15[a]	59.04± 9.11[a]	***t(74)=3.101, p=0.003***
TA	75.33± 9.69[a]	72.78± 12.16[a]	t(74)=0.956, p=342
QUADS	68.46± 12.01[a]	59.68± 14.51[a]	***t(74)=2.700, p=0.009***
HAMS	70.29± 15.22[a]	62.16± 17.63[a]	***t(74)=2.001, p=0.049****
Time From Onset To Peak Activity (ms)			
TS	75.86± 12.43[a]	61.36± 12.73[a]	***t(74)=4.552, p<0.001***
TA	25.21 (83.58) [b]	20.93 (19.98) [b]	***p=0.026* (NPT)***
Instant of peak activity (% GC)			
TS	47.50 (10.70) [b]	47.65(4.70) [b]	0.323 (NPT)
TA	72.95 (38.30) [b]	77.35 (22.30) [b]	0.201 (NPT)

[a] Values are means ± SD; [b] Values are medians with range in parentheses; * Significance value is less than 0.05 level (2-tailed); ** Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test.

6.1.4 Microcirculation

Muscular BF and mVO_2 were investigated during different conditions: at rest, in response to an exercise bout and during recovery from an exercise bout.

Possible confounders for microcirculation variables

Since body composition was not controlled body mass and body fat% were explored as potential confounder variables for microcirculatory data. Table 6-19 shows that during resting conditions body mass was positively correlated to blood flow and oxygen

consumption in the DN group ($p<0.05$), while those correlations did not reach significant levels in the HEALTH group ($p>0.05$). Body fat% was not significantly correlated to either resting BF or resting mVO_2. Table 6-20 shows that body mass and body fat% did not correlate significantly to BF or mVO_2 variables during the other 2 conditions.

Table 6-19. BF and mV O_2 at rest: Possible confounding variables (Bivariate correlation)

		BF at rest	mVO_2 at rest
Body Fat %	N=50 (DN group)	τ=0.176 p=0.072	τ=-0.014 p=0.891 NPT
	N=25 (HEALTH groups)	τ=-0.100 p=0.484	τ=-0.276 p=0.052 NPT
Body Mass	N=50 (DN group)	***τ=0.335**** ***p=0.001*** ***NPT***	***τ=0.239**** ***p=0.018*** ***NPT***
	N=25 (HEALTH groups)	r=-0.128 p=0.542	τ=-0.003 p=0.981 NPT

* Significance value is less than 0.05 level (2-tailed); ** Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test.

Beside body mass and body fat%, the effect of MGast muscular activity on microcirculation during the exercise bout was also explored. Microcirculatory responses to an exercise bout will depend on the intensity of the exercise. For this reason the intensity of the exercise bout was set up constantly at 50% of the individual's MVC. However, even thought force was controlled during the exercise protocol the amount of work coming specifically from the MGast (which is the muscle under investigation) was not controlled. Therefore, the possible effect of the specific amount of work carried out by the MGast on the microcirculatory responses was investigated.

Table 6-20 shows that EMG activity (normalised to the peak activity during MVC) during the exercise bout (measured on the MGast) appeared to be positively correlated

to the microcirculatory responses to exercise (measured also in the MGast). Thus, the muscular oxygen consumption response to exercise (calculated as the percentage of change from baseline to post-exercise values) was significantly correlated to EMG activity in the DN (p=0.01) and HEALTH groups (p<0.001). Furthermore, the change in BF in relation to the exercise bout was correlated to EMG activity in the DN group (p=0.002). However, this correlation did not reach significant levels in the HEALTH group (p=0.180). Table 6-20 also shows that the microcirculatory recovery from the exercise bout (blood flow and muscular oxygen consumption) did not appear to be related to the activity level of the MGast during the exercise protocol both in the DN group and in the HEALTH group.

Table 6-20. BF and mVO_2 in relation to the exercise protocol: Possible confounding variables (Bivariate correlation)

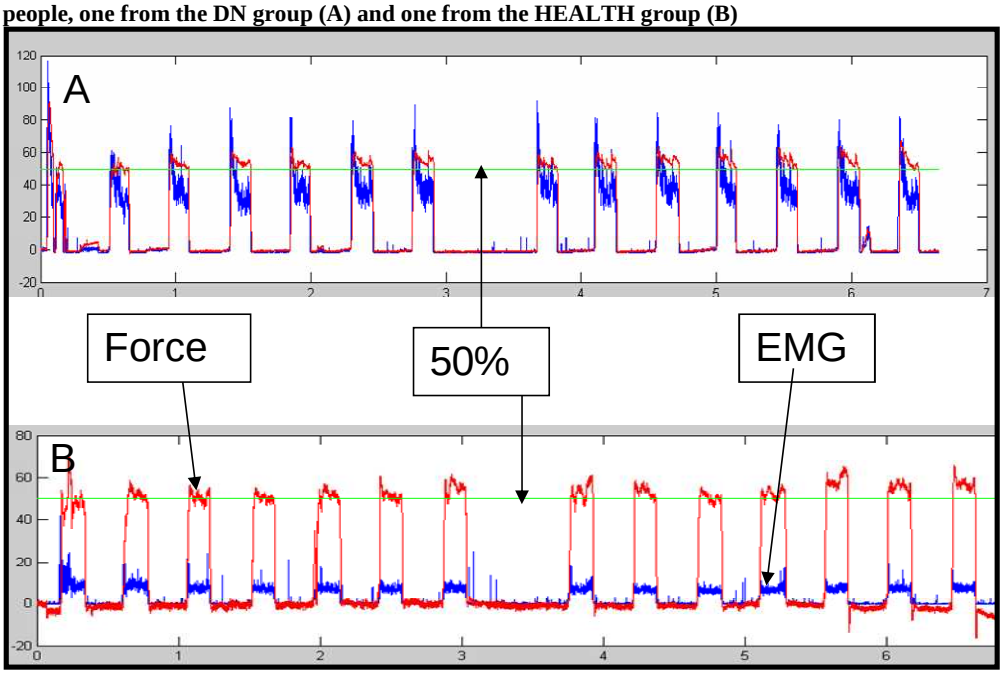
		BF PreVsPost %	BF Recovery %	mVO_2 PreVsPost %	mVO_2 Recovery %
Body Fat %	N=53 (DN group)	τ=-0.169 p=0.098 NPT	r=0.110 p=0.467	τ=-0.127 p=0.225 NPT	r=0.271 p=0.056
	N=24 (HEALTH group)	τ=-0.100 p=0.484 NPT	τ=-0.167 p=0.254 NPT	r=0.073 p=0.607	r=-0.070 p=0.624
Body Mass	N=53 (DN group)	τ=-0.125 p=0.222 NPT	r=-0.006 p=0.968	τ=-0.135 p=0.199 NPT	r=-0.311 p=0.053
	N=24 (HEALTH group)	τ=-0.077 p=0.591 NPT	τ=-0.302 p=0.052 NPT	r=-0.199 p=0.341	r=-0.180 p=0.388
Muscular Activity	N=53 (DN group)	***τ=0.320** p=0.002 NPT*	r=-0.035 p=0.817	***τ=0.338** p=0.001 NPT*	r=0.084 p=0.589
	N=24 (HEALTH group)	τ=0.196 p=0.180 NPT	τ=-0.043 p=0.771 NPT	***r=0.664** p<0.001*	r=-0.108 p=0.615

** Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test.

In the main analysis, body mass and EMG activity were therefore handled as follows: body mass was accounted for when comparing resting BF and mVO_2 between the groups while EMG was entered as a covariate when comparing group differences for BF and mVO_2 responses to the exercise bout.

Furthermore, muscular activity during the exercise bout was significantly higher (mean ± SD) in the DN group compared to the HEALTH group (40.26± 21.88 vs. 28.54± 12.20, p=0.017). Figure 6-4 shows a typical example of the force and EMG traces during the exercise protocol in two different subjects, one from the DN group and one from the HEALTH group.

Figure 6-4. Example of the force and EMG traces during the exercise protocol in two different people, one from the DN group (A) and one from the HEALTH group (B)



Note: The red trace represents the force generated in the KINCOM machine whilst the blue trace represents the EMG activity. The green line shows the force targeted during the exercise bout (50% of MVC).

Main results: Comparison of microcirculatory related variables between the DN and the HEALTH group

Table 6-21 presents data on the comparison of microcirculatory related outcome measures during different conditions between the DN and the HEALTH group. It should be noted that the main analysis for the different microcirculatory related outcome measures was carried out with 46 subjects in the DN group and 24 subjects in the HEALTH group whereas all the other analyses were carried out with 53 in the DN group and 25 in the HEALTH group. Some individuals suffered from muscle cramps during the exercise protocol and the exercise had to be stopped. This explains the reduction in the numbers compared to the other analyses.

During resting conditions, no significant differences were observed when comparing either BF ($p=0.167$) or mVO_2 ($p=0.535$) between the DN and the HEALTH group. With regard to the microcirculatory responses to exercise, results from the present study showed significant lower exercise-induced vasodilatation in the DN group compared to the HEALTH group ($p=0.003$) as demonstrated by the smaller percentage of change in BF from baseline to post-exercise conditions. Group differences were also significant when comparing the percentage of change of mVO_2 from the baseline to the post-exercise condition between the DN and the HEALTH group ($p<0.034$). Table 6-21 also shows that the DN group recovered from the exercise bout significantly slower than the HEALTH group. Thus, significant group differences were observed when comparing BF recovery ($p<0.004$) and mVO_2 recovery ($p<0.033$) after 70 seconds.

Overall, results from the present study show that the DN group had reduced microcirculatory responses to an exercise bout compared to the HEALTH group and that the recovery from the exercise was slower in the DN compared to the HEALTH group.

Table 6-21. Microcirculation parameters: Comparison between the HEALTH and the DN group[a]

Condition	Variable	Group		Inferential statistical results	
				*Case*Control comparison*	
		DN (N=46)	HEALTH (N=24)	without covariate	with covariate (ANCOVA)
At rest	BF ($ml \cdot min^{-1} \cdot 100g^{-1}$)	0.60± 0.28[a]	0.64± 0.26[a]	N/A	$F_{(1,67)}$=1.951, p=0.167[1 3]
	mVO_2 rest ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)	0.024± 0.012[a]	0.025± 0.012[a]	N/A	$F_{(1,67)}$=0.389, p=0.535[1 3]
Responses to exercise protocol	BF pre vs. post exercise (% change)	112.40± 75.15[a]	154.18± 68.36[a]	N/A	***$F_{(1,67)}$=9.633, p=0.003**** [2 3]
	mVO_2 pre vs. post exercise (% change)	301.42± 228.86[a]	346.78± 253.60[a]	N/A	***$F_{(1,65)}$=4.683, p=0.034**** [2 3]
Recovery from exercise protocol	BF recovery after 70 sec (%)	74.99 (114.08)[b]	91.05 (76.67)[b]	***p= 0.004** NPT***	N/A
	mVO_2 recovery after 70 sec (%)	77.77± 22.08[a]	89.83± 22.11[a]	***t(65)=-2.17; p=0.033****	N/A

[a] Values are means with SD in parentheses; [b] Values are medians and range in parentheses * Significance value is less than 0.05 level (2-tailed); **Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test; [1]Analysis of covariance with body mass as a covariate; [2]Analysis of covariance with muscular activity (EMG) as a covariate; [3] Data transformed not to break the assumption of the normal distribution of the data when using parametric tests.

6.1.5 Quality of life (SF-36)

Table 6-22 displays the group differences in the different dimensions measured by the quality of life questionnaire. Thus, significantly lower scores were reported by the DN group in every single dimension compared to the HEALTH group (p<0.001).

Table 6-22. Quality of life dimensions: Comparison between the HEALTH and the DN group[a]

Variable	Group		Inferential statistical results
	DN (N=53)	HEALTH (N=25)	*Case*Control comparison without covariate*
Physical Function	70.00 (100.00)	95.00 (20.00)	***p<0.001 ** NPT***
Role Physical	75.00 (100.00)	100.00 (0.00)	***p <0.001 ** NPT***
Body Pain	70.00 (100.00)	100.00 (55.00)	***p <0.001 ** NPT***
General Health	60.00 (90.00)	85.00 (60.00)	***p <0.001 ** NPT***
Vitality	55.00 (90.00)	70.00 (45.00)	***p <0.001 ** NPT***
Social Function	100.00 (100.00)	100.00 (45.00)	***p =0.001 ** NPT***
Role Emotional	100.00 (100.00)	100.00 (0.00)	***p <0.001 ** NPT***
Mental Health	84.00 (100.00)	88.00 (52.00)	***p <0.001 ** NPT***
Physical Health Overall	66.25 (92.50)	93.75 (42.50)	***p <0.001 ** NPT***
Mental Health Overall	80.37 (95.00)	90.00 (37.50)	***p <0.001 ** NPT***

[a] Values are medians with range in parentheses; ** Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test.

6.1.6 Summary of the main results for the cross-sectional study

Table 6-23 provides a summary of the results in relation to the null hypotheses for part 1 of the main study.

The null hypothesis 1 relating to differences in general health between the DN and the HEALH group was rejected for the systolic blood pressure but was accepted for the diastolic blood pressure. The DN group demonstrated higher systolic blood pressure compared to the HEALTH group.

The null hypothesis 2 relating to differences in gait characteristics between the DN and the HEALTH group was rejected for spatial-temporal characteristics, foot pressures under the metatarsal heads, and EMG parameters but it was accepted for COP parameters and foot pressures at the heel. Compared to the HEALTH group, the DN group demonstrated higher PP and PTI under the metatarsal heads, slower gait velocity (gait velocity, shorter steps and slower cadence) and overall higher EMG activity. Furthermore, null hypothesis 2 relating to differences in strength levels between the DN and the HEALTH group was rejected for all the muscle groups. The DN group was significantly weaker in all the muscle groups compared to the HEALTH group.

The null hypothesis 3 relating to differences in microcirculation during different conditions between the DN and the HEALTH group was rejected for non-resting conditions but it was accepted for resting conditions. The DN group demonstrated smaller BF and mVO_2 responses to an exercise protocol and slower BF and mVO_2 recovery from the exercise protocol compared to the HEALTH group.

The null hypothesis 4 relating to differences in QOL between the DN and the HEALTH group was rejected for physical and mental health. Compared to the HEALTH group, the DN group demonstrated significantly lower scores in all the physical and mental dimensions of the SF 36 QOL questionnaire.

Table 6-23. Summary of the results in relation to the main analysis of the cross-sectional study.

Domain	*Measurement*		*Outcome measure*		*p<0.05*
General Health	Cholesterol		TC		NOT APPLICABLE
			LDL		NOT APPLICABLE
			HDL		NOT APPLICABLE
	Blood Pressure		Systolic Blood Pressure		*↑
			Diastolic Blood Pressure		N/S
Gait	Strength		Peak Moment		*↓
	Gait parameters	Spatial-temporal	Gait velocity		*↓
			Step length		*↓
			Step time		*↓
		COP parameters	Distance COP		N/S
			Velocity COP		*↑
	Kinetic data		Peak Pressure	Heel	N/S
				Metatarsals	*↑
				Big toe	*↓
			PTI	Heel	N/S
				Metatarsals	*↑
				Big toe	N/S
	Muscular Activity		% peak activity per gait phase		*↑
			% GC muscle active		*↑
			Time to peak (push off phase)		*↑
			Instant Peak Activity (Push off)		N/S
Microcirculation	Muscular microcirculation		Muscular Blood flow	Rest	N/S
				Post-Exercise	*↓
				Recovery	*↓
			Muscular Oxygen Consumption	Rest	N/S
				Post-Exercise	*↓
				Recovery	*↓
Quality of Life	Self-Reported Quality of life		Physical Health		*↓
			Mental Health		*↓

* Significance value is less than 0.05 level (2-tailed); N/S no significant differences; ↑↓ reflect the DN group in relation to the HEALTH group.

6.1.7 Exploratory study

This section explores the variables that may better account for the group differences observed in the cross-sectional study. Thus, this section explores outcome measures that are likely to have influenced gait characteristics, microcirculation and QOL in the present investigation. At the start of each section there is a brief explanation of the variables under investigation as well as the theoretical reasons behind that choice.

Bivariate correlations (two tailed) were used to relate those parameters. Thus, Pearson's correlation coefficient was used when data sets were normally distributed whereas Kendall's correlation coefficient was used when variables were not normally distributed. Note that significance was set at an alpha level of 0.05 and no Bonferroni corrections, to reduce the chances of type I error, were applied. Correlations were carried out separately for the DN and HEALTH groups.

6.1.7.1 Gait Biomechanics

Gait parameters (spatial-temporal and COP parameters) and peak pressure parameters (PP and PTI) were used to explore gait characteristics. In relation to gait parameters, sensory neuropathy and muscular weakness, especially of the distal leg muscles, are believed to influence spatial temporal characteristics (Yavuzer, et al., 2006) and COP parameters (Uccioli et al., 2001). For this reason, the bivariate relationships between both VTP and muscular strength (at the dorsi-flexor and plantar-flexor muscles) and gait parameters were investigated. In relation to peak pressures, the literature has identified structural changes (i.e. arch index) (Giacomozzi et al., 2005), sensory neuropathy (Frykberg et al., 1998) and muscle weakness, especially of the distal leg muscles (Akashi et al., 2008), as contributing factors for the changes in foot pressures between healthy and DN subjects. For this reason the bivariate relationships between VPT, arch index and muscular strength at the dorsi-flexor and plantar-flexor muscles and both PP and PTI were investigated. Results on the exploratory analysis for gait parameters and foot pressures are presented.

Gait parameters

Table 6-24 investigates the relationships between gait parameters (spatial-temporal and COP parameters) and sensory (measured by vibration perception threshold) and strength levels. Sensory neuropathy was positively correlated to step time ($p=0.029$) whist sensory neuropathy was not related to step length or gait velocity. VPT also showed a weak positive correlation with the total distance travelled by the centre of pressure ($\tau=0.270$; $p=0.005$).

When assessing the relationships between strength levels and spatial-temporal characteristics, plantar-flexor muscle strength appeared to be correlated to gait velocity, step time and step length. Therefore, individuals who reported higher PF moment seemed to walk quicker, with longer and quicker steps. Interesting, these associations were only present in the HEALTH group ($p<0.05$), whereas no correlation was found between the PF moment and any of the spatial-temporal parameters in the DN group.

The strongest correlation between lower limb muscle strength and COP variables was found between the DF moment and the distance travelled by the COP. Thus, longer distance travelled by the COP was observed in participants who reported higher DF moment values. This significant correlation was observed both in the HEALTH ($r=0.545$; $p=0.008$) and in the DN group ($r=0.533$; $p<0.001$). The only other significant correlation was found between DF strength levels and the velocity of the COP at the heel. However, this negative correlation was only observed in the DN group ($p=0.038$) and not in the HEALH group ($p=0.910$).

Table 6-24. Gait parameters: Exploratory data (Bivariate correlation)

		Gait velocity	Step Time	Step Length	COP (total distance)	COP velocity (Heel)
VPT	N=53 (DN group)	r=-0.081 p=0.398 NPT	***τ=0.212** **p=0.029*** ***NPT***	r=0.040 p=0.676 NPT	***τ=0.270** **p=0.005*** ***NPT***	r=-0.24 p=0.807 NPT
PF Moment	N=53 (DN group)	r=0.179 p=0.203	r=-0.086 p=0.542	r=0.156 p=0.268	r=0.140 p=0.308	N/A
	N=24 (HEALTH group)	***r=0.575***** ***p=0.005***	***r=-0.479** **p=0.024***	***r=0.467** **p=0.028***	***r=0.409*** ***p=0.059***	N/A
DF Moment	N=53 (DN group)	N/A	N/A	N/A	***r=0.533***** ***p<0.001***	***r=-0.288** **p=0.038***
	N=24 (HEALTH group)	N/A	N/A	N/A	***r=0.545***** ***p=0.008***	r=-0.017 p=0.910

* Significance value is less than 0.05 level (2-tailed); ** Significance value is less than 0.01 level (2-tailed); NPT. Non parametric test.

Foot pressures

Table 6-25 explores the relationships between plantar pressure outcome measures at the heel and metatarsal regions and sensory neuropathy (measured by vibration perception threshold), strength levels (dorsi-flexors and plantar-flexors) and foot arch characteristics.

Sensory neuropathy (VPT) did not appear to be correlated to peak pressures or pressure time integral variables both at the heel and at the metatarsals areas ($p>0.05$). Similar results were obtained when relating arch index with kinetic variables. Therefore, arch index did not show significant correlations with peak pressure or pressure time integral variables both at the heel and at the metatarsal areas ($p>0.05$). Strength levels seemed to be related to some foot pressure outcome measures. DF moment was correlated to foot pressures at the metatarsal region. Hence, people who reported higher strength levels at

the dorsi-flexor muscles also reported lower PTI values at the metatarsal region. Although there was a tendency in both groups ($p<0.089$), this negative correlation only reached significant levels in the DN group ($p<0.047$). In addition to that, the HEALTH group also reported a strong negative correlation ($r=0.0492$; $p<0.05$) between DF moment and peak pressure at the metatarsal region.

Table 6-25. Foot pressures: Exploratory data (Bivariate correlation)

		Peak Pressure Heel	Peak Pressure Metatarsals	PTI Heel	PTI Metatarsals
VPT	N=53 (DN group)	τ=0.054 p=0.701 NPT	τ=-0.01 p=0.994 NPT	τ=0.134 p=0.160 NPT	τ=0.127 p=0.364 NPT
Arch Index	N=53 (DN group)	r=-0.206 p=0.139	r=-0.151 p=0.280	r=-0.166 p=0.233	r=-0.233 p=0.102
	N=24 (HEALTH group)	r=0.372 p=0.067	r=0.030 p=0.885	r=-0.080 p=0.704	r=-0.069 p=0.741
PF Moment	N=53 (DN group)	N/A	r=-0.004 p=0.977	N/A	r=-0.291 p=0.632
	N=24 (HEALTH group)	N/A	r=-0.160 p=0.297	N/A	r=-0.291 p=0.189
DF Moment	N=53 (DN group)	r=0.041 p=0.773	r=0.171 p=0.221	r=-0.103 p=0.461	***r=-0.274**** ***p=0.047***
	N=24 (HEALTH group)	r=-0.164 p=0.467	***r=-0.492**** ***p=0.020***	r=-0.372 p=0.088	r=-0.371 p=0.089

* Significance value is less than 0.05 level (2-tailed); NPT. Non parametric test.

6.1.7.2 Microcirculation

Muscular blood flow and muscular oxygen consumption during different conditions were used to explore microcirculation. Previous investigations have linked impairments in blood flow and oxygen consumption to sensory neuropathy (Dinh & Veve, 2004) and hyperglycaemia (Tooke et al., 2000) in patients with diabetes. For this reason, the bivariate relationships between VPT and HbA_{1c} and both blood flow and mVO_2 were explored.

Table 6-26 explores the relationships between microcirculation and sensory neuropathy (measured by vibration perception threshold), glycaemic control and systolic blood pressure. Blood sugar level appeared the only variable that correlated significantly with microcirculation variables. Thus, higher HbA_{1c} related to diminished blood flow and muscular oxygen consumption recovery from exercise ($p<0.05$). On the other hand, VPT and BP did not seem to correlate with any of the microcirculatory variables ($p>0.05$).

6.1.7.3 Quality of Life

Some investigations have suggested an association between QOL and physiological markers in diabetic patients (Wikblad et al., 1996). For this reason one of the objectives of this exploratory study was to investigate whether outcome measures linked to DN such as sensory neuropathy, glycaemic control and obesity, were associated with any of the dimensions of the 36-SF questionnaire.

Table 6-27 shows the relationships between quality of life dimensions assessed by the 36-SF questionnaire and glycaemic control, sensory neuropathy and obesity (referred to as body mass index). VPT and HbA_{1c} correlations were only carried out on the DN group while correlations involving BMI were done in both groups. Sensory neuropathy (measured by vibration perception threshold) was not found to be correlated to any of the QOL dimensions as well as to the overall mental and physical health scores ($p<0.05$). In contrast, HbA_{1c} was found to be negatively correlated to physical function ($p=0.025$) and mental health ($p=0.047$) dimensions as well as to the overall mental

health score (p<0.032). In addition to that, a tendency toward lower scores in the role emotional were shown in DN patients with higher HbA_{1c} (p<0.052). In addition to that, both groups reported that people with higher BMI scored lower in the general health dimension (p<0.041).

Table 6-26. Microcirculation: Exploratory data (Bivariate correlation)

		Blood flow at rest	mVO_2 at rest	Blood flow After exercise	mVO_2 After exercise	Blood flow recovery	mVO_2 recovery
VPT	N=53 (DN group)	τ=-0.091 p=0.357 (NPT)	τ=-0.011 p=0.943 (NPT)	τ=0.121 p=0.240 (NPT)	τ=0.113 p=0.283 (NPT)	τ=0.027 p=0.859 (NPT)	τ=-0.202 p=0.068 (NPT)
HbA_{1c}	N=53 (DN group)	r=0.040 p=0.785	τ=0.086 p=0.409 (NPT)	τ=0.153 p=0.144 (NPT)	τ=0.178 p=0.097 (NPT)	***r=-0.266* p=0.011***	***r=-0.219* p=0.044***
BP (systolic)	N=53 (DN group)	τ=0.062 p=0.669 (NPT)	τ=0.004 p=0.971 (NPT)	τ=-0.003 p=0.977 (NPT)	τ=-0.115 p=0.199 (NPT)	τ=-0.59 p=0.698 (NPT)	r=-0.170 p=0.275
	N=24 (HEALTH groups)	r=-0.184 p=0.378	τ=-0.187 p=0.191 (NPT)	τ=0.127 p=0.375 (NPT)	r=-0.248 p=0.233	τ=-0.055 p=0.519 (NPT)	r=-0.033 p=0.876

* Significance value is less than 0.05 level (2-tailed); NPT. Non parametric test.

Table 6-27. Psychology: Exploratory data (Bivariate correlation)

		Physical Function	Role Physical	Body Pain	General Health	Vitality	Social Function	Role Emotional	Mental Health	Mental Health Overall	Physical Health overall
VPT	N=53 (DN group)	τ=0.019 p=0.847 (NPT)	τ=-0.092 p=0.381 (NPT)	τ=0.052 p=0.599 (NPT)	τ=0.044 p=0.655 (NPT)	τ=-0.070 p=0.473 (NPT)	τ=-0.097 p=0.350 (NPT)	τ=0.041 p=0.705 (NPT)	τ=0.089 p=0.366 (NPT)	τ=-0.015 p=0.878 (NPT)	τ=0.008 p=0.933 (NPT)
HbA_{1c}	N=53 (DN group)	***τ=-0.224* p=0.025 (NPT)***	τ=0.055 p=0.573 (NPT)	τ=0.066 p=0.509 (NPT)	τ=-0.079 p=0.432 (NPT)	τ=-0.130 p=0.193 (NPT)	τ=-0.065 p=0.538 (NPT)	τ=-0.207 p=0.053 (NPT)	***τ=-0.196* p=0.047 (NPT)***	***τ=-0.208* p=0.032 (NPT)***	τ=-0.058 p=0.544 (NPT)
BMI	N=53 (DN group)	τ=-0.125 p=0.198 (NPT)	τ=0.055 p=0.573 (NPT)	τ=-0.075 p=0.573 (NPT)	***τ=-0.218* p=0.025 (NPT)***	τ=-0.118 p=0.224 (NPT)	τ=-0.025 p=0.811 (NPT)	τ=-0,018 p=0.871 (NPT)	τ=-0,039 p=0.694 (NPT)	τ=-0.044 p=0.640 (NPT)	τ=-0.047 p=0.623 (NPT)
	N=24 (HEALTH groups)	τ=-0.156 p=0.325 (NPT)	τ=-0.094 p=0.579 (NPT)	τ=-0.131 p=0.402 (NPT)	***τ=-0.275* p=0.041 (NPT)***	τ=-0.004 p=0.981 (NPT)	τ=-0.063 p=0.707 (NPT)	τ=-0.071 p=0.677 (NPT)	τ=-0.135 p=0.374 (NPT)	τ=-0.203 p=0.160 (NPT)	τ=-0.054 p=0.708 (NPT)

* Significance value is less than 0.05 level (2-tailed); NPT. Non parametric test

6.2 INTERVENTION STUDY

The aim of this study was to evaluate the effects of a 16-week strength training programme on identified pathologies associated with peripheral neuropathy in DN subjects. Adaptations to the exercise programme were calculated by comparing pre-and post-intervention data between groups (EX and CON) (2 way Mixed ANOVA design). It should be noted that the covariates used throughout this results section are carried over from the cross sectional results sections. Information about the statistical approach used to analyse the intervention study can be found in Chapter 5 (Section 5.6.2.2.) Results from the intervention study are presented in different sections, namely demographic characteristics of the sample groups, general health, gait biomechanics, microcirculation and QOL. Table 6-29 shows an overview of the data presented in this section.

6.2.1 Subjects Characteristics

Table 6-28. Subject characteristics for the EXE and the CON group

Variable	Group		Inferential statistical results
	EXE (N=20)	CON (N=21)	*Groups comparison*
Age (years)	60.85± 7.56[a]	64.95± 5.76[a]	t(40)=-1.941, p=0.059
Body Mass (kg)	91.78± 18.77[a]	95.44± 15.26[a]	t(40)=-0.683, p=0.499
BMI ($kg \cdot m^{-2}$)	31.52± 5.48[a]	33.16± 5.72[a]	t(40)=-0.936, p=0.355
Body fat (%)	37.81± 6.87[a]	38.63±6.23[a]	t(40)=-0.399, p=0.692
VPT (V)	17.00(40.00)[b]	14.00(47..50)	p=0.256 (NPT)
Height (m)	1.70± 0.97[a]	1.69± 0.86[a]	t(40)=0.129, p=0.898
Sex Male (n) Female (n)	 14 (66%) 7 (34%)	 13 (65%) 8 (35%)	NA

[a] Values are means ± SD; [b] Values are medians with range in parentheses; NPT. Non parametric test.

Table 6-29. Overview of the results presented in intervention study

DOMAIN	*Measurement*		*Outcome measure*	
General Health	Cholesterol		TC	
			LDL	
			HDL	
	Blood Pressure		Systolic Blood Pressure	
			Diastolic Blood Pressure	
	Glucose Control		HbA_{1c}	
	Obesity		Body mass	
			Body fat %	
	Neuropathy		VPT	
Gait	Strength		Peak Moment	
	Gait parameters	Spatial-temporal	Gait velocity	
			Step length	
			Step time	
		COP parameters	Distance COP	
			Velocity COP	
	Kinetic data		Peak Pressure	Heel
				Metatarsals
				Big toe
			PTI	Heel
				Metatarsals
				Big toe
	Muscular Activity		% peak activity per gait phase	
			% GC muscle active	
			Time to peak (push off phase)	
			Instant Peak Activity (Push off)	
Microcirculation	Muscular microcirculation		Muscular Blood flow	Rest
				Post-Exercise
				Recovery
			Muscular Oxygen Consumption	Rest
				Post-Exercise
				Recovery
Quality of Life	Self-Reported Quality of life		Physical Health	
			Mental Health	

The two groups (EXE and CON groups) were matched on marginal distributions for age, height, mass, neuropathy and sex. Table 6-28 presents the demographic features of the subjects belonging to the 2 groups. No statistical differences were shown between the groups regarding to age, height, body mass, body fat%, VPT and gender.

6.2.2 General Health

Table 6-30 presents the results of the effect of the intervention programme on body composition, cholesterol levels, blood pressure, foot sensation and resting heart rate. Body composition, calculated as BMI and body fat %, was reduced in the EXE group when compared to the CON group over time. However, only body fat % reached significant levels (p=0.045) when both groups were compared over time. Although BMI changes over time showed a tendency toward lower values in the EXE group compared to the CON group, those differences did not reach significant levels (p=0.078). Table 6-30 also shows that there were significant group*time interactions in systolic blood pressure (p=0.032), resting heart rate (p=0.044) and vibration sensation (p=0.027). Thus, the EXE group significantly lowered blood pressure, resting heart rate and vibration perception threshold over time when compared to the CON group. In contrast, no group differences were obtained over time in cholesterol levels [HDL (p=0.391), LDL (0.850) and TC (0.508)], diastolic blood pressure (p=0.149) and HbA_{1c} values (p=0.739).

Table 6-30. General health: Comparison between the EXE and the CON group over time[a]

Variable	Group				Inferential statistics results
	Control Group (N=21)		Exercise Group (N=20)		*2 way Mixed ANOVA (Group*Time interaction)*
	Pre-Int	Post-Int	Pre-Int	Post-Int	
BMI ($kg \cdot m^{-2}$)	31.31± 5.54	31.30± 5.29	33.16± 5.72	32.65± 5.89	$F_{(1,39)}$=3.284, p=0.078
Body fat (%)	37.91± 6.72	37.92± 5.99	38.63± 6.23	37.28± 6.53	***$F_{(1,39)}$=4.288, p=0.045****
Systolic blood pressure (mmHg)	134.10± 11.80	134.35± 17.02	142.0± 11.25	135.75± 8.90	***$F_{(1,38)}$=4.974, p=0.032****
Diastolic blood pressure (mmHg)	78.75± 0.12	78.45± 10.35	80.90± 7.43	77.35± 8.96	$F_{(1,39)}$==2.181, p=0.148
Vibration Threshold (V)	18.77± 14.62	20.20± 15.35	21.52± 13.37	18.40± 13.14	***$F_{(1,39)}$=5.267, p=0.027****
Heart rate at rest ($beats \cdot min^{-1}$)	76.15± 3.05	77.25± 14.94	77.45± 11.45	74.00± 9.71	***$F_{(1,39)}$=4.301, p=0.044****
TC ($mmol \cdot L^{-1}$)	3.74± 0.80	3.78± 0.65	3.82± 0.94	3.72± 0.84	$F_{(1,39)}$=0.447, p=0.508
HDL ($mmol \cdot L^{-1}$)	1.20± 0.29	1.20± 0.30	1.02± 0.29	1.07± 0.26	$F_{(1,39)}$=0.739, p=0.395
LDL ($mmol \cdot L^{-1}$)	1.95± 0.54	1.94 ± 0.59	1.67± 0.79	1.63± 0.80	$F_{(1,39)}$=0.036; P=0.850
HbA_{1c} (%)	7.63 ± 1.76	7.20± 0.81	7.61± 1.06	7.32± 0.81	$F_{(1,39)}$=0.113; p=0.739

[a] Values are means ± SD; * Significance value is less than 0.05 level (2-tailed).

6.2.3 Gait biomechanics

This section starts presenting the results of the effect of the exercise programme on lower limb strength levels. Thereafter three more subsections were included in which outcome measures related to gait characteristics, kinetic data and muscular activity data during gait were presented.

6.2.3.1 Isometric strength

Table 6-31 shows strength levels for the CON and the EXE group before and after the 16 weeks intervention. There were no significant group*time interactions in any of the muscles groups under investigations. KE, KF, DF and PF reported p values of 0.115, 0.310, 0.166 and 0.118, respectively.

Table 6-31. Strength levels: Comparison between the EXE and the CON group over time [a]

Variable	Group				Inferential statistics results
	Control Group (N=21)		Exercise Group (N=20)		2 way Mixed ANOVA (Group*Time interaction)
	Pre-Int	Post-Int	Pre-Int	Post-Int	
Knee-Extension ($N \cdot m^{-1}$)	349.35± 99.87	382.44± 313.13	313.13± 82.56	394.14± 104.99	$F_{(1,39)}$=2.594, p=0.115
Knee-Flexion ($N \cdot m^{-1}$)	172.20± 36.84	170.41± 34.37	183.11± 34.52	189.09± 36.34	$F_{(1,39)}$=1.061, p=0.310
Dorsi-Flexion ($N \cdot m^{-1}$)	166.14± 73.52	165.54± 68.18	159.16± 70.43	182.86± 63.37	$F_{(1,39)}$=1.989, p=0.166
Plantar-Flexion ($N \cdot m^{-1}$)	96.28± 42.06	129.36± 37.40	129.56± 78.66	127.99± 41.09	$F_{(1,39)}$=2.560, p=0.118

[a] Values are means ± SD.

6.2.3.2 Gait parameters

Spatial temporal parameters together with COP parameters are presented in this section. Based on the exploration for possible confounders carried out in the cross-sectional study (see Section 0), body mass was accounted for when comparing total distance travelled by the COP between groups over time, while gait velocity was entered as a covariate when comparing group differences over time in the velocity of the COP at the heel.

Table 6-32 presents the changes in gait parameters over time when comparing the EXE and the CON group. No significant changes or trends were reported in any of the spatial-temporal parameters under investigation when both groups were compared over time. Therefore, it appears that the exercise programme did not trigger noticeable changes in gait velocity (p=0.818), step length (p=0.404), step time (p=0.799) or cadence (p= 0.636).

When assessing COP parameters, the only significant differences over time were observed in the velocity of the COP at the heel. Thus, the EXE group reported slower velocity of the COP at the heel (p=0.035) when compared to the CON group over time. All the other COP parameters did not show any significant group*time interactions.

Table 6-32. Gait parameters: Comparison between the EXE and the CON group over time [a]

Variable	Group				Inferential statistical results
	Control Group (N=21)		Exercise Group (N=20)		*2 way Mixed ANOVA* (Group*Time interaction)
	Pre-Int	Post-Int	Pre-Int	Post-Int	
Spatial-temporal data					
Step time (sec)	0.553± 0.047	0.557± 0.045)	0.544± 0.066	0.549± 0.043	$F_{(1,39)}$=0.066, p=0.799
Step length (m)	0.614± 0.072	0.615± 0.061	0.624± 0.084	0.637± 0.084	$F_{(1,39)}$=0.711, p=0.404
Cadence (steps·min^{-1})	108.71± 9.48	108.22± 8.41	111.36± 12.03	109.70± 7.72	$F_{(1,39)}$=0.228, p=0.636
Velocity (m·sec^{-1})	1.11± 0.163	1.11± 0.157	1.16 ± 0.21	1.16± 0.19	$F_{(1,39)}$=0.054, p=0.818
Foot floor interaction					
Distance travelled by the COP (cm)	24.37± 2.28	24.48± 1.96	24.57± 2.18	24.80± 2.07	$F_{(1,39)}$=0.270, p=0.606 [1]
Velocity COP at the heel (m·sec^{-1})	0.44± 0.12	0.45± 0.10	0.50± 0.10	0.46± 0.099	***$F_{(1,39)}$=4.729, p=0.035**[2]***
Velocity COP at the Forefoot (m·sec^{-1})	0.277± 0.055	0.268± 0.056	0.273± 0.046	0.275± 0.039	$F_{(1,39)}$=1.067, p=0.308
Velocity COP at the hallux (m·sec^{-1})	0.812± 0.338	0.868± 0..346	0.874± 0.329	0.874± 0.350	$F_{(1,39)}$=0.921, p=0.343

[a] Values are means ± SD; [1] Analysis of covariance with body mass as a covariate; [2] Analysis of covariance with gait velocity as a covariate; * Significance value is less than 0.05 level (2-tailed).

6.2.3.3 Kinetic data

This section presents the results of the effect of the exercise programme on the primary outcome measures, PP and PTI. Thereafter, results of the parameters related to PP and PTI are shown.

6.2.3.3.1 Primary outcome measures: Peak Pressure and Pressure Time Integral.

Changes over time in PP are shown in Table 6-33. The only foot area that showed significant changes over time when the EXE group was compared to the CON group was PP at the heel (p=0.017). Thus, the results show significantly higher PP values over time in the EXE compared to the CON group. No significant group differences or trends were observed at the metatarsals and hallux regions.

Changes over time in PTI both for the CON and EXE groups are shown in Table 6-34. No significant changes were reported in any foot area when both groups were compared over time. However, there was a trend towards higher PTI values over time in the EXE group, both at the heel (p=0.075) and hallux (p=0.094), when compared to the CON group.

Table 6-33. Peak pressure values: Comparison between the EXE and the CON group over time [a]

Variable	**Group**				**Inferential statistical results**
	Control Group (N=21)		Exercise Group (N=20)		*2 way Mixed ANOVA* (Group*Time interaction)
	Pre-Int	Post-Int	Pre-Int	Post-Int	
PP Heel (kPa)	475.05± 196.40	454.33± 198.23	431.60± 94.76	455.40± 126.81	***$F_{(1,39)}$=6.274, p=0.017**[1]***
PP Metatarsals (kPa)	852.55± 260.93	826.88± 266.31	765.1± 281.85	774.35± 282.30	F(1,39)=1.878, p=0.178
PP Hallux (kPa)	523.33± 351.79	505.83± 342.15	489.89± 290.57	525.15± 329.15	F(1,39)=2.507, p=0.121

[a] Values are means ± SD; [1] Analysis of covariance with gait velocity as a covariate; * Significance value is less than 0.05 level (2-tailed).

Table 6-34. PTI values: Comparison between the EXE and the CON group over time[a]

Variable	Group				Inferential statistical results
	Control Group (N=21)		Exercise Group (N=20)		*2 way Mixed ANOVA* (Group*Time interaction)
	Pre-Int	Post-Int	Pre-Int	Post-Int	
PTI Heel (kPa·sec)	119.34± 45.84	118.28± 58.29	104.75± 82.56	112.65± 104.99	$F_{(1,39)}$=3.348, p=0.075
PTI Metatarsals (kPa·sec)	260.69± 95.96	259.10± 104.27	222.00± 76.71	230.82± 79.18	$F_{(1,39)}$=1.768, p=0.191
PTI Hallux (kPa·sec)	93.20± 68.62	89.83± 68.18	88.15± 57.15	101.03± 76.45	$F_{(1,39)}$=2.938, p=0.094

[a] Values are means ± SD.

6.2.3.3.2 Secondary outcome measures: Parameters related to peak pressure and pressure time integral

Since the intervention did not seem to trigger changes in plantar pressures, apart from PP at the heel, parameters related to PP and PTI were not expected to change either. However, these analyses were carried out to confirm that there were no group differences over time.

Table 6-35 and Table 6-36 present data on the parameters related to peak pressure and pressure time integral for both groups over time, respectively. Table 6-35 confirmed that there were not significant group differences in the parameters related to peak pressure (Max Force, CA and arch index) over time. Similar results were found with regard to the parameters related to PTI (results presented in Table 6-36). Thus, group differences over time did not reach significant values when comparing the amount of time that the heel, metatarsals and hallux remained in contact with the floor during the stance phase of the GC. However, there was a trend toward higher contact times at the heel in the EXE group over time compared to the CON group (p=0.082).

Table 6-35. Parameters related to Peak pressure: Comparison between the EXE and the CON group over time[a]

Variable	Group				Inferential statistical results
	Control Group (N=21)		Exercise Group (N=20)		2 way Mixed ANOVA (Group*Time interaction)
	Pre-Int	Post-Int	Pre-Int	Post-Int	
Max Force ($N \cdot Kg^{-1}$)					
Heel	7.13± 0.91	6.97± 0.99	7.00± 1.06	7.06± 0.98	$F_{(1,39)}$=0.725, p=0.399
Metatarsals	9.27± 0.98	9.25± 0.94	9.30± 1.16	9.48± 1.07	$F_{(1,39)}$=2.791, p=0.103
Hallux	1.38± 0.67	1.42± 0.60	1.38± 0.71	1.49± 0.79	$F_{(1,39)}$=0.486, p=0.447
Contact Area (cm^2)					
Heel	33.41± 4.96	33.43± 5.14	34.63± 4.24	34.48± 4.08	$F_{(1,39)}$=0.486, p=0.490
Metatarsals	47.24± 5.82	47.18± 6.26	49.90± 49.83	48.83± 6.52	$F_{(1,39)}$<0.001, p=0.983
Hallux	8.40± 1.76	8.70± 1.42	9.15± 1.79	9.38± 1.58	$F_{(1,39)}$=0.075, p=0.786
Arch Index	0.191± 0.074	0.194± 0.080	0.212± 0.057	0.202± 0.069	$F_{(1,39)}$=2.634, p=0.113

[a] Values are means ± SD.

Table 6-36. Parameters related to PTI: Comparison between the EXE and the CON group over time [a]

Variable	Group				Inferential statistical results
	Control Group (N=21)		Exercise Group (N=20)		2 way Mixed ANOVA (Group*Time interaction)
	Pre-Int	Post-Int	Pre-Int	Post-Int	
Contact Time (ms)					
Heel	465.55± 97.88	453.57± 103.61	431.57± 95.77	447.57± 9.75	$F_{(1,39)}$=3.200, p=0.082
Metatarsals	611.77± 66.54	614.44± 66.46	601.47± 81.09	612.42± 72.35	$F_{(1,39)}$=0.355, p=0.555
Hallux	359.33± 102.85	376.88± 92.69	380.44± 119.85	390.88± 123.47	$F_{(1,39)}$=0.147, p=0.704

[a] Values are means ± SD.

6.2.3.3.3 Kinetic parameters during the push off phase

In line with the results presented in the previous sections (gait related results), the intervention did not trigger any changes in PP (p=0.616) or Max Force (p=0.441) (normalised and non-normalised to body mass) values during the push off when the DN and the EXE groups were compared over time. The same trend towards no group differences over time was observed when assessing time related parameters during the push off phase. Thus, the instant of the peak pressure and the instant of the Max Force during the push off did not show significant group differences when comparing pre- and post-intervention values. These results are presented in Table 6-37.

Table 6-37. Pressure and Force related parameters during push off: Comparison between the EXE and the CON group over time [a]

Variable	Group				Inferential statistical results
	Control Group (N=21)		Exercise Group (N=20)		2 way Mixed ANOVA (Group*Time interaction)
	Pre-Int	Post-Int	Pre-Int	Post-Int	
Peak Pressure (kPa)	901.55± 263.66	867.61± 244.17	851.36± 268.37	852.89± 277.91	$F_{(1,39)}$=0.256, p=0.616
Instant of Peak Pressure (% ROP)	80.86± 6.95	79.26± 7.47	82.66± 5.43	81.59± 6.44	$F_{(1,39)}$=0.056, p=0.814
Max Force (N)	930.46± 161.19	949.35± 163.23	1019.65 ± 156.58	1006.01± 170.24	$F_{(1,39)}$=0.608, p=0.441
Instant of Max Force (% ROP)	77.20± 1.84	76.57± 2.50	77.05 ± 2.27	77.37± 1.92	$F_{(1,39)}$=2.114, p=0.154
Max Force Normalized ($N \cdot Kg^{-1}$)	10.69± 0.96	10.91± 1.25	10.69 ± 0.83	10.68± 1.19	$F_{(1,39)}$=0.324, p=0.572

[a] Values are means ± SD.

6.2.3.4 EMG

This section presents pre- and post-intervention data with regard to the EMG measurements for the EXE and the CON group. The mean muscular activity during the differences phases of the GC is presented first. Thereafter, time related EMG variables are shown, including: 1) the percentage of time each muscle was kept activity during

the whole GC; 2) percentage of the GC at which TS and TA activity peaked during and after the push off phase, respectively; and 3) time lag from the onset to peak activity during and after the push off phase for the TS and TA muscles, respectively.

Figure 6-5 shows pre-intervention (blue line) and post-intervention (red line) EMG activity during a GC. EMG data was presented for the TA, TS, (MGast + LGast + Soleus), Quadriceps (VL) and Hamstrings (BicFem) muscles. EMG data traces from the EXE group are displayed at the top of the figure while traces from the CON group are displayed below. The table at the bottom of Figure 6-5 presents the EMG differences between the EXE and the CON group over time throughout the phases of the GC. It should be pointed out that no statistical analysis has been carried out to investigate muscular activity during the initial swing and mid-swing phases of the GC.

During the loading phase the only significant difference between the EXE and the CON group was observed for the HA ($p<0.03$). This muscle showed a decrease in muscular activity in the CON group when compared to the EXE group over time. In addition, there was a trend toward a decrease in TA activity in the CON group when compared to the EXE group over time ($p=0.062$). No significant group differences were observed during the loading phase when TS and QUADS were compared over time.

The intervention programme did not change muscular activity patterns during the mid stance phase of the GC in any of the muscles under investigation (TS, TA, QUADS, HAMS) ($p>0.05$).

The 2 way Mixed ANOVA analysis for the terminal stance phase revealed a trend towards a decrease in TS activity ($p=0.058$) and QUADS activity ($p=0,091$) over time in the EXE group compared to the CON group. No significant group differences were observed when comparing TA and HA during this phase over time.

During the pre-swing phase, no statistical differences between groups were observed when comparing EMG data over time. However, TS and TA show a trend toward changes when the EXE and the CON group were compared over time. Thus, the EXE group showed a trend toward increased TS activity over time when compared to the

CON group (p=0.098), whereas a trend in the opposite direction was observed when looking at the TA (p=0.092).

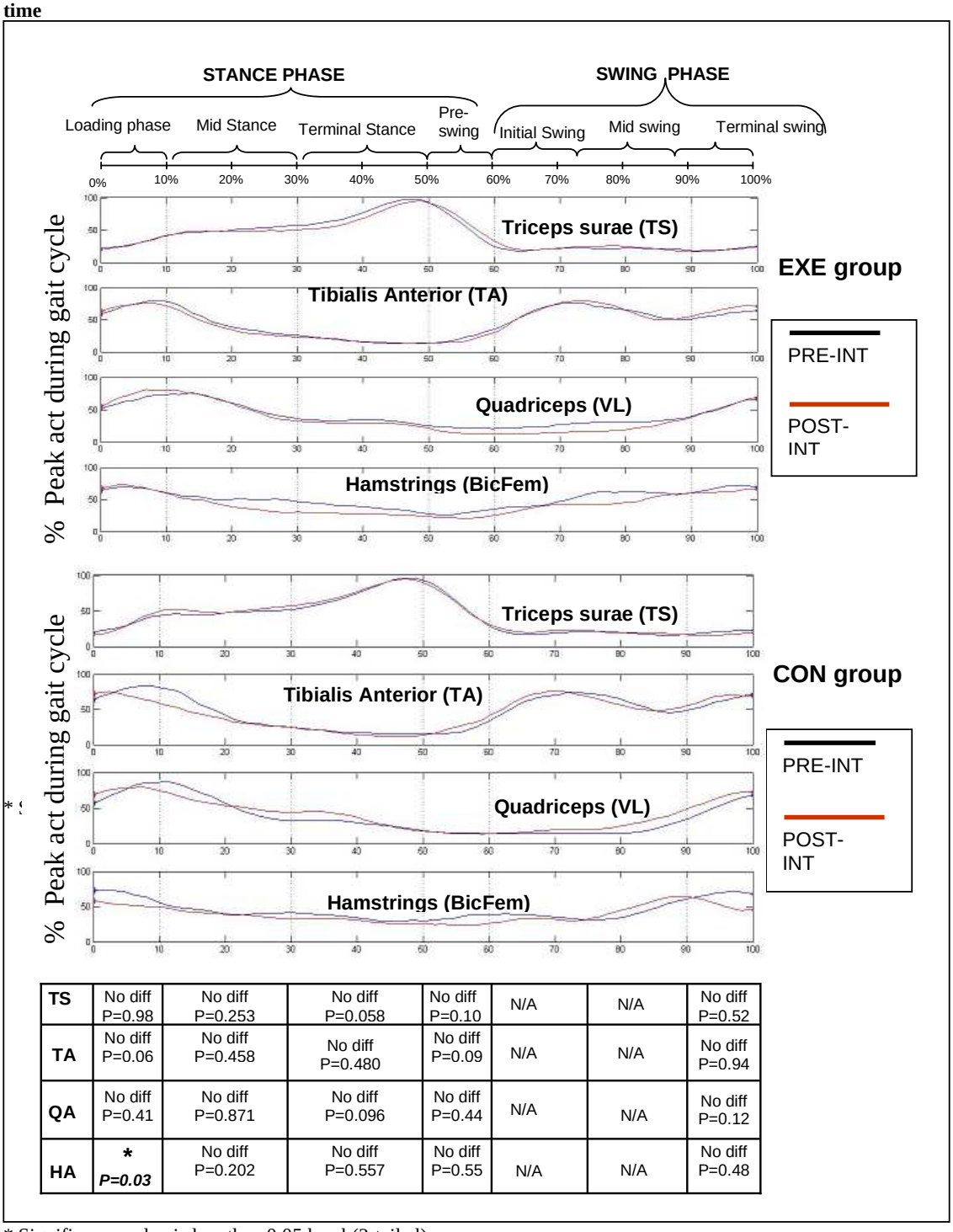
No significant group differences during the terminal swing phase were observed when assessing EMG activity over time in any of the muscle groups under investigation (p>0.12).

Overall, results from the present study show that 16 weeks of strengthening and mobility exercises did not influence muscular activity patterns substantially throughout the different phases of the GC in DN subject.

Results from the time related EMG data for the EXE and the CON group over time is presented in Table 6-38. When investigating muscular activity patterns during the whole GC, results from the present study show no significant group* time interaction in the amount of time (as percentage) each muscle was kept activity during the GC. Similar results were obtained when investigating time related parameters for the TA and TS muscles during push off phase (see Chapter 5, Section 5.4.3.4.3 for more information about the time windows for the TA and TS muscles). Thus, no group differences over time were observed both in the time lag from activation onset to peak activity for the TA (p=0.694) and the TS muscle (p=0.404) and in the instant that TA (p=0.158) and TS (p=0.136) peaked.

Overall, EMG activity patterns did not seem to change secondary to a PA programme. This is consistent with the lack of changes in gait parameters and kinetic data presented above.

Figure 6-5. EMG patterns during the GC: Comparison between the EXE and the CON group over time



TS	No diff P=0.98	No diff P=0.253	No diff P=0.058	No diff P=0.10	N/A	N/A	No diff P=0.52
TA	No diff P=0.06	No diff P=0.458	No diff P=0.480	No diff P=0.09	N/A	N/A	No diff P=0.94
QA	No diff P=0.41	No diff P=0.871	No diff P=0.096	No diff P=0.44	N/A	N/A	No diff P=0.12
HA	* ***P=0.03***	No diff P=0.202	No diff P=0.557	No diff P=0.55	N/A	N/A	No diff P=0.48

* Significance value is less than 0.05 level (2-tailed);

Table 6-38. Time related EMG parameters during gait: Comparison between the EXE and the CON group over time [a]

Variable	Group				Inferential statistical results
	Control Group (N=19)		Exercise Group (N=19)		2 way Mixed ANOVA (Group*Time interaction)
	Pre-Int	Post-Int	Pre-Int	Post-Int	
% GC active above threshold					
TS	67.85± 11.24	67.11± 9.96	69.40± 11.58	66.07± 10.15	$F_{(1,36)}$=1.023, p=0.312
TA	75.08± 9.66	74.02± 10.91	72.95± 9.93	71.34± 10.32	$F_{(1,36)}$=0.037, p=0.850
QUADS	67.51± 12.31	71.49± 12.75	67.79± 9.01	67.1± 11.76	$F_{(1,36)}$=0.398, p=0.532
HAMS	70.38± 17.65	67.07± 14.94	74.50± 12.60	68.96± 14.59	$F_{(1,36)}$=0.146, p=0.705
Time From Onset To Peak Activity (ms)					
TS	78.44± 10.32)	77.37± 8.28)	75.06± 16.08)	72.78± 18.19)	$F_{(1,36)}$=0.157, p=0.694
TA	28.65± 15.22	28.82± 13.26	25.42± 9.43	23.53± 8.36	$F_{(1,36)}$=0.714, p=0.404
Instant of Peak Activity (%GC)					
TS	47.30± 1.18	47.11± 2.30)	46.94± 2.38)	47.45± 1.34)	$F_{(1,36)}$=2.082, p=0.158
TA	74.18± 6.56	71.54± 7.40	71.23± 4.22	71.37± 5.95	$F_{(1,36)}$=2.326, p=0.136

[a] Values are means ± SD.

6.2.4 Microcirculation

Muscular blood flow and oxygen consumption were investigated during different conditions: at rest, in response to an exercise bout and during recovery from an exercise

bout. According to the exploration for possible confounders carried out in the cross-sectional study (see Section 6.1.4), body mass was accounted for when comparing resting BF and mVO_2 between the groups over time whilst EMG was entered as a covariate when comparing group differences over time for BF and mVO_2 responses to the exercise bout. It should be noted that the sample size for the statistical analysis of the microcirculatory related outcome measures was 17 subjects in the CON group and 17 subjects in the EXE group whereas all other analysess were carried out with 21 in the CON group and 20 in the EXE group. Some individuals suffered from cramps during the exercise protocol and the exercise had to be stopped. This explains the reduction in the numbers compared to the other analyses.

Table 6-39 presents pre- and post-intervention blood flow and oxygen consumption data obtained on the MGast for the EXE and CON groups during different protocols. When investigating group differences over time in resting microcirculation, the 2 way Mixed ANOVA analysis revealed no group difference over time in blood flow ($p=0.193$) or muscular oxygen consumption ($p=0.222$).
Table 6-39 also shows that there were no significant group differences over time with regard to the BF or mVO_2 responses to the exercise protocol ($p>0.05$). However, there was a trend toward higher vasodilatory (BF) responses over time in the EXE group compared to the CON group ($p=0.092$). The only significant group difference over time, when assessing microcirculation, was observed during the recovery condition. BF and mVO_2 were assessed 70 seconds after the exercise protocol finished and the % of recovery was calculated in relation to resting values. Even though BF recovery over time did not vary significantly between groups ($p=0.244$), significant group differences ($p=0.040$) were observed when comparing the % of mVO_2 recovery over time. Furthermore, the EXE group reported a significant faster mVO_2 recovery over time compared to the CON group.

In summary results from the present study show that the exercise programme did trigger some changes in the microcirculation of subjects with DN. Thus, 1) there is a trend toward higher vasodilatation in response to the exercise bout over time in the EXE group compared to the CON group and 2) mVO_2 recovery after 70 seconds was significantly quicker over time in the EXE compared to the CON group.

Table 6-39. Microcirculation parameters: Comparison between the EXE and the CON group over time[a]

Condition	Variable	Group				Inferential statistical results
		Control Group (N=17)		Exercise Group (N=17)		2 way Mixed ANOVA (Group*Time interaction)
		Pre-Int	Post-Int	Pre-Int	Post-Int	
Rest	BF ($ml \cdot min^{-1} \cdot 100g^{-1}$)	0.563± 0.20	0.565± 0.227	0.571± 0.153	0.513± 0.172	$F_{(1,32)}$=1.771, p=0.193[1]
	mVO_2 ($mlO_2 \cdot min^{-1} \cdot 100g^{-1}$)	0.0284± 0.016	0.0286± 0.033	0.0225± 0.011	0.0266± 0.012	$F_{(1,32)}$=1.548, p=0.222[1]
Responses to Exercise Protocol	BF (% of Change)	119.18± 88.15	119.73± 82.87	105.04± 55.61	136.05± 67.37	$F_{(1,32)}$=3.023, p=0.092[2]
	mVO_2 (% of Change)	307.26± 246.98	263.42± 173.99	276. 78± 176.35	237.46± 127.85	$F_{(1,32)}$=0.055, p=0.816[2]
Recovery from Exercise Protocol	BF (% of Recovery)	75.29± 26.99	72.91± 21.87	61.94± 23.07	75.26± 34.79	$F_{(1,32)}$=1.410, p=0.244
	mVO_2 (% of Recovery)	82.26± 23.08	80.06± 12.63	75.38± 23.12	94.51± 18.08	***$F_{(1,32)}$=4.604; p=0.040****

[a] Values are means with SD in parentheses; [1]Analysis of covariance with body mass as a covariate; [2]Analysis of covariance with muscular activity (EMG) as a covariate; * Significance value is less than 0.05 level (2-tailed).

6.2.5 Quality of life

The effect of the intervention on self reported quality of life is presented in Table 6-40 The 2 way Mixed ANOVA analysis revealed significant group differences in two of the dimensions under investigation, Vitality ($p<0.001$) and Mental health ($p=0.027$), as well as in the overall Mental Health Score ($p=0.013$). All the three variables reported higher values in the EXE group over time compared to the CON group. Although all the other dimensions, except from body pain, obtained higher scores over time in the EXE group compared to the CON group, none of these scores reached significant values ($p>0.05$).

Table 6-40. Quality of life: Comparison between the EXE and the CON group over time[a]

Variable	Group				Inferential statistical results
	Control Group (N=18)		Exercise Group (N=21)		2 way Mixed ANOVA (Group*Time interaction)
	Pre-Int	Post-Int	Pre-Int	Post-Int	
Physical Function	69.44± 24.00	71.11± 24.04	63.33± 22.49	72.49± 22.61	$F_{(1,37)}$=1.029, p=0.317
Role Physical	61.11± 44.73	66.66± 46.17	68.02± 30.84	85.71± 30.17	$F_{(1,37)}$=0.858, p=0.360
Body Pain	69.02± 21.66	64.02± 31.49	75.47± 20.64	74.64± 20.75	$F_{(1,37)}$=0.344, p=0.561
General Health	60.83± 19.72	59.72± 15.57	52.61± 18.61	56.90± 16.54	$F_{(1,37)}$=1.367, p=0.250
Vitality	59.72± 21.17	52.77± 24.20	49.28± 18.72	66.90± 17.78	***$F_{(1,37)}$=21.994, p<0.001*****
Social Function	82.08± 20.93	77.77± 30.67	87.38± 93.57	93.57± 10.20	$F_{(1,37)}$=2.675, p=0.110
Role Emotional	77.77± 34.29	74.07± 37.14	85.71± 29.00	93.65± 22.65	$F_{(1,37)}$=2.233, p=0.144
Mental Health	81.77± 11.74	77.77± 15.29	78.19± 15.36	83.42± 12.41	***$F_{(1,37)}$=5.325, p=0.027****
Physical Health Overall	64.82± 22.17	65.38± 26.47	62.76± 14.82	70.08± 17.69	$F_{(1,37)}$=1.452, p=0.236
Mental Health Overall	73.95± 17.19	70.60± 22.54	73.59± 15.17	82.93± 11.51	***$F_{(1,37)}$=6.823, p=0.013****

[a] Values are means ± SD; * Significance value is less than 0.05 level (2-tailed); ** Significance value is less than 0.01 level (2-tailed).

6.2.6 Summary of the main results for the intervention study.

Table 6-41 provides a summary of the results in relation to the null hypotheses for part 2 of the main study.

The null hypothesis 1 relating to the effect of 16 weeks of strengthening and mobility exercises on general health when comparing the EXE and CON groups was rejected for the systolic blood pressure, body composition and sensory neuropathy but was accepted for the diastolic blood pressure and cholesterol levels. The EXE group showed a reduction in systolic blood pressure, body fat percentage and VPT values over time compared to the CON group.

The null hypothesis 2 relating to the effect of the PA programme on gait characteristics was rejected for the velocity of the COP at the heel and for the peak pressure at the heel but was accepted for all the other gait parameters and for the plantar pressures at the metatarsals and hallux. Compared to the CON group the EXE group increased foot pressures at the heel (p values for PTI at the heel only reached tendency levels) and reduced the velocity of the COP at the heel over time. Furthermore the null hypothesis 2 relating the effect of the PA programme on strength levels was accepted for all the muscle groups.

The null hypothesis 3 relating to the effect of 16 weeks of strengthening and mobility exercises on microcirculation was rejected for mVO_2 recovery from the exercise protocol and accepted for all the other variables. Compared to the CON group, the EXE group recovered mVO_2 values after the exercise protocol quicker over time. Although no statistically significant group*time interactions were revealed for the other dependent variables, changes in blood flow from baseline to post-exercise levels (vasodilatation) showed a tendency toward higher values over time in the EXE group compared to the CON group ($p=0.092$).

The null hypothesis 4 relating to the effect of the PA programme on QOL was rejected for mental health and accepted for physical health. Compared to the CON group the EXE group showed higher overall mental health scores over time.

Table 6-41. Summary of the results in relation to the main analysis of the intervention study

AIM	*Measurement*		*Outcome measure*		*p<0.05*
General Health	Cholesterol		TC		N/S
			LDL		N/S
			HDL		N/S
	Blood Pressure		Systolic Blood Pressure		*↓
			Diastolic Blood Pressure		N/S
	Glucose Control		HbA_{1c}		N/S
	Obesity		Body mass		N/S
			Body fat %		*↓
	Neuropathy		VPT		*↓
Gait	Strength		Peak Moment		N/S
	Gait parameters	Spatial-temporal	Gait velocity		N/S
			Step length		N/S
			Step time		N/S
		COP parameters	Distance COP		N/S
			Velocity COP		*↓
	Kinetic data		Peak Pressure	Heel	*↑
				Metatarsals	N/S
				Big toe	N/S
			PTI	Heel	*↑ (trend)
				Metatarsals	N/S
				Big toe	N/S
	Muscular Activity		% peak activity per gait phase		N/S
			% GC muscle active		N/S
			Time to peak (push off phase)		N/S
			Instant Peak Activity (Push off)		N/S
Microcirculation	Muscular microcirculation		Muscular Blood flow	Rest	N/S
				Post-Exercise	*↑ (trend)
				Recovery	N/S
			Muscular Oxygen Consumption	Rest	N/S
				Post-Exercise	N/S
				Recovery	*↑
Quality of Life	Self-Reported Quality of life		Physical Health		N/S
			Mental Health		*↑

* Significance value is less than 0.05 level (2-tailed); N/S no significant differences; ↑↓ reflect changes over time in relation to the exercise programme.

CHAPTER 7

7 Discussion

Diabetic neuropathy is a very complex condition that carries multiple complications, including cardiovascular diseases, foot ulceration secondary to gait and microcirculatory changes, and poor QOL. The aim of the study was twofold. In part 1 the aim was to investigate the differences between DN and healthy individuals in the primary pathologies associated with DN. This aimed to broaden the understanding of the health problems linked to this condition and to compare the findings in this study to previously published data. In part 2 the aim was to investigate the effect of a 16 week PA programme, which included strengthening and joint mobility exercises, on identified pathologies related to DN. This aimed to explore for the first time whether these health problems can be influenced by 16 weeks of resistance training in DN subjects. This study also includes 3 preliminary investigations which explored the reliability of some of the methods used in the main part of the study. The results of the Preliminary studies have been discussed in Chapter 4. This chapter discusses the results of Part 1 of the main study, followed by the discussion of the results obtained in Part 2.

7.1 Part 1 – Cross-Sectional study

Part 1 was a cross-sectional study that investigated differences between DN and healthy individuals in the primary pathologies associated to DN, including general health, gait, microcirculation and QOL. Therefore, part 1 of the main study found significant differences in general health, gait characteristics, microcirculation and QOL when the DN group was compared to the HEALTH group. The discussion of the results from the cross-sectional study is presented in the same order as in the literature review and results chapter. Then, general health outcome measures are discussed first, followed by gait, microcirculation and QOL parameters.

7.1.1 General Health

It is well established that type 2 diabetes increases the risk of cardiovascular diseases secondary to alterations in traditional cardiovascular risk factors such as blood pressure and cholesterol levels. The present study found that the DN group had significantly higher systolic blood pressure compared to the HEALTH group. Interestingly, the differences in systolic blood pressure were seen even though most of the DN patients were taking antihypertensive agents (see Table 6-3 for more information about the medications taken by the DN group) and subjects with uncontrolled blood pressure were not invited to participate in the study. Hypotensive medications may also explain why diastolic blood pressure was within normal range in the DN group (79.77± 9.06 mmHg). This also explains why no group differences in diastolic blood pressure were observed. Cholesterol levels were not measured in the HEALTH group, so no group comparison was carried out on these parameters. However, DN subjects in the present study did not show abnormal cholesterol levels when compared to current recommendations (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 1993). Thus the DN group reported cholesterol values of 3.81± 0.94 mmol·L^{-1}, 1.83± 0.73 mmol·L^{-1} and 1.11± 0.31 mmol·L^{-1} for TC, LDL and HDL, respectively whilst current guidelines recommend values of <5.2 for TC, <3.4 for LDL and >0.9 for HDL (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 1993). The fact that the majority of the DN subjects were taking anti-cholesterol medications can explain these results (see Table 6-3 for more information about the medications taken by the DN group).

In addition to the risk of cardiovascular diseases, another major problem linked to DN is foot ulceration. It seems clear that a combination of gait biomechanics and microcirculatory changes are responsible for the increased risk of foot ulcerations observed in this population. Results from the group differences in gait characteristics and microcirculation obtained in the present study are discussed next.

7.1.2 Gait biomechanics

In the present study gait was investigated from different perspectives to develop a comprehensive understanding of the walking characteristics in patients with DN. Most of the investigations analyzing gait characteristics in neuropathic patients have been interested in kinetic data, which is believed to predict the risk of foot ulceration in this population (Boulton et al., 1983; Guldemond et al., 2006) and gait parameters (Giacomozzi, 2005), which provide descriptive information about the overall gait characteristics (i.e. gait velocity). However, EMG activity, which provides insight into how muscles are activated to generate movement, has not been widely investigated in this population during gait tasks. The assessment of muscle activities in the lower limb may be useful for interpreting and clarifying changes in kinetic and gait parameters. Thus, the present study investigated differences in all three aspects of gait in DN patients compared to healthy individuals.

The present study found a number of alterations in gait parameters, kinetic data and EMG activity patterns in the DN group compared to the healthy control group. Overall, DN gait was characterized by shorter and slower steps, higher foot pressures under the metatarsal heads and higher muscular activity compared to the healthy group. To follow the same order as the literature review, hypothesis and results section, gait parameters are discussed first, followed by kinetic data and EMG data.

7.1.2.1 Gait parameters

The results from the present study show significant differences in all the spatial-temporal, and in some of the COP outcome measures under investigation between the DN and HEALTH groups. Thus, DN subjects walked with smaller step length, lower cadence and velocity and longer step times compared to the healthy individuals. In addition to that, the DN group showed a trend toward smaller distance travelled by the COP during the stance phase and a significantly quicker foot drop following the heel strike as demonstrated by the faster velocity of the COP at the heel. The possible underlying factors explaining the changes in 1) spatial-temporal characteristics and 2) COP parameters in the diabetic group are discussed.

Finding from the present study are in agreement with previous investigations that have compared spatial-temporal characteristics between healthy and DN subjects (Courtemanche et al., 1996; Yavuzer et al., 2006). It has been proposed in the literature that both sensory neuropathy (van Deursen & Simoneau, 1999; Yavuzer et al., 2006) and motor neuropathy (Mueller et al., 1994) may be responsible for the changes in spatial-temporal characteristics observed in DN subjects. Next a discussion of the possible effect sensory neuropathy and motor neuropathy may have had on the changes in spatial-temporal characteristics observed in the present investigation.

It has been hypothesized that sensory feedback plays a role in adjusting step-to-step limb trajectories to maintain balance during locomotion (Gandevia & Burke, 1992). Then the loss of this feedback information that results from DN may lead to loss of stability in these patients during gait. Consequently, this may increase the need for producing a more cautious gait in this population. This idea is supported by previous investigations that have assessed stability in DN subjects by measuring the distance travelled by the COP during different conditions (Simmons et al., 1997b; Simoneau et al., 1994). It is assumed that stability is negatively related to the distance travelled by the COP during a fixed period of time. Therefore, subjects with poorer stability are expected to show higher distances and vice versa. Numerous studies have reported a high correlation between the severity of DN and COP distance in this population (Simmons et al., 1997b; Simoneau et al., 1994), which demonstrates that DN may produce instability in this population. Furthermore, the level of neuropathy has also been associated with step times (negative correlation), gait velocity (negative correlation) and step length (negative correlation) during gait (Yavuzer, et al., 2006). Results from the present investigation show that the degree of neuropathy (quantified by VPT measurements) was positively correlated with step times in the DN group, which is in line with the results presented above. It is therefore possible that the changes in spatial temporal characteristics observed in the DN group in the present investigation are secondary to lack of stability in this population.

However, in the present study other spatial-temporal characteristics such as gait velocity or step length did not show any relationship with sensory neuropathy. It is likely that instability, which is believed to produce changes towards a more cautious gait, is not only dependent on plantar cutaneous sensation (van Deursen et al., 1998b). This may explain why gait velocity or step length were not correlated to sensory

neuropathy in the present study. The somatosensory system appears to be the biggest contributor of feedback for postural control (van Deursen & Simoneau, 1999). This sensory system is composed of several different muscle, joint, and cutaneous mechanoreceptors. The information from these receptors is integrated in the central nervous system to produce a sensation of joint position and movement (Gandevia & Burke, 1992). It has therefore been hypothesized that instability in DN subjects may be the result of a loss of peripheral sensory receptor function in the lower legs and cannot be attributed exclusively to loss of plantar cutaneous sensation (van Deursen et al., 1999). Van Deursen et al. (1998b) carried out a study on 10 young healthy, 15 DN subjects and 15 age-matched controls to investigate muscle spindles function in ankle movement perception in DN subjects compared to young healthy and age-matched healthy controls. Muscle spindles are a type of proprioceptors which are sensitive to changes in muscle length (since they are located in parallel with the contractile fibres) (Tortora & Derrickson, 2006). In this study, muscle spindle function was assessed by measuring the movement perception threshold during muscle vibration (which is known to particularly stimulate the muscle spindle primary endings and therefore introduces a bias into the muscle spindle output). They found that neuropathic subjects had the highest movement amplitude of all the groups, and more interestingly, muscle vibration had much less of an effect on their performance. These findings led the authors to conclude that muscle spindle function is impaired in DN subjects (van Deursen et al., 1998b). In line with these results, other studies have reported an impairment of the sensory function of the foot to perceive movement (van den Bosch et al., 1995; Son et al., 2009). Therefore, it appears that reduced postural stability in persons with diabetic neuropathy cannot be attributed exclusively to loss of plantar cutaneous sensation. This may explain why plantar cutaneous sensation in the present investigation was not significantly correlated to all the spatial-temporal changes in the DN group.

Beside sensory neuropathy, an efferent/motor deficit, which is identified by muscle weakness, may further explain the difference in walking characteristics between the DN and healthy groups. The results in the present study confirmed that neuropathic patients suffered from muscular weakness secondary to neuropathy. All the muscle groups under investigation (plantar-flexors, dorsi-flexors, knee-extensors and knee-flexors) showed significantly lower strength levels in the DN compared to the healthy group. It has been hypothesized that if patients with DN are unable to generate sufficient moments about the ankle during walking, they will take shorter steps and walk more

slowly than individuals without peripheral neuropathy (Mueller et al., 1994). The present study supports this idea when investigating healthy individuals without neurological complications. Gait velocity, step time and step length were significantly correlated with plantar-flexion strength in the healthy group. Increased plantar-flexor strength appeared to enhance the ability of the plantar-flexor muscles to push off and resulted in larger steps, shorter step times and quicker walking velocity. On the other hand, ankle strength levels did not correlate to any of the spatial-temporal parameters in the DN group. This suggests that ankle weakness is not likely to be the main factor for more cautious walking strategy observed in our DN subjects.

Overall, results from the present study seem to suggest that in DN subjects, the loss of peripheral sensory receptor function may be the main responsible factor for the more cautious walking strategy observed in this population. Numerous studies have reported that patients with diabetes and peripheral neuropathy have a high incidence of falls during walking (Dingwell & Cavanagh, 2001; Macqilchrist et al., 2010; Richardson & Hurvitz, 1995) and a low level of perceived safety (Cavanagh et al., 1992; Simoneau et al., 1994). Therefore, it is likely that risk avoidance, fear of falling, and lack of confidence are the main factors that influence postural control and gait behaviours in this population. On the other hand, spatial-temporal characteristics in healthy subjects seem to be dependent on the plantar-flexion strength level.

Beside alterations in the spatial-temporal outcome measures, the present study showed changes in some COP parameters (i.e. COP distance and velocity of the COP at the heel) in the DN group compared to the HEALTH group. Results from the present study show that weakness of the DF muscles may partly explain the alterations in the COP parameters observed in the DN group compared to the HEALTH group. On the one hand, weakness of the dorsi-flexor muscles may result in a reduced dorsi-flexion angle during heel strike, which provokes the foot to contact the floor with the most anterior part of the heel (Giacomozzi et al., 2002). This interpretation is supported by the present study that shows a significant correlation between strength levels at the dorsi-flexor muscles and COP distances both in the DN and HEALTH groups. Therefore, it appears that strength of the dorsi-flexor muscles is related with the articular mobility of the tibiotalar joint during heel strike. Therefore stronger individuals may approach the floor with the posterior part of the heel (more dorsi-flexion) resulting in a larger distance travelled by the COP. This may explain why distance travelled by the COP

tended to be shorter in the DN patients. On the other hand, weakness of the dorsi-flexor muscles may also contribute to the quicker drop of the forefoot after the heel strike. This hypotheses is well supported by the comprehensive analysis presented Perry & Burnfield, (2010) that explains the role that specific muscles of the leg have during the stance phase of walking. They state that between heel contact and 10% stance, dorsal flexors act eccentrically. Because DN subjects show muscle weakness of these muscle group, especially TA (Schoenhaus et al., 1991), there is a general lack of foot control in the heel strike phase, which results in a flat-footed approach. This interpretation is supported by the results from the present study that show a correlation between DF strength levels and velocity of the COP at the heel. Interestingly this correlation was only significant in the DN group, which suggests that only severe (clinical) weakness compromise the normal drop of the foot after heel strike.

Another possible explanation for the reduced displacement of the COP along the foot surface is a feeling of instability secondary to neuropathy (Cavanagh et al., 1992; Simoneau et al., 1994). This may lead to a change in walking behaviour so that the body mass centre is positioned in a neutral position, directly above the foot during the whole GC (Katoulis, et al., 1997). This mechanism observed in DN subjects to improve stability may explain the reduced displacement of the COP along the foot during the stance phase of gait (Giacomozzi et al., 2002). Contrary to this, in the present investigation DN subjects with more severe neuropathy tended to show larger COP distances. This association does not rule out the theory that instability secondary to neuropathy may result in a more secure gait characterized by reduced displacement of the COP. Instead, it suggests that other confounding variables (i.e. age, body mass, gait velocity, etc.) may have influenced this correlation. Moreover, correlations in the present study should be interpreted with caution as the result may be due to insufficient sample size.

Overall, results from the present investigation show some alterations in spatial-temporal and COP parameters in patients with DN compared to healthy controls. It is likely that the feeling of instability secondary to DN is partly responsible for some of the changes in walking characteristics observed in this population. Furthermore, results from the current investigation also show that muscular weakness, especially of the DF, should be included among causes that lead to changes in floor foot interaction parameters in DN subjects. This finding suggests that interventions to improve strength levels in the lower

limb, especially of the DF muscles, could possible influence gait characteristics in DN subjects. Nevertheless, changes in walking characteristics cannot explain the high rate of ulcers observed in diabetic neuropathic patients. Group differences in PP and PTI, which are considered a substitute measure to determine risk of foot ulceration (Frykberg et al., 1998; Veves et al., 1992) are discussed next.

7.1.2.2 Kinetics data

Results from the current study show significant differences in foot loading patterns between the DN and HEALTH groups. Thus, the most noticeable differences were observed under the metatarsal heads, where the DN group showed significantly higher PP and PTI values compared to the HEALTH groups. Group differences were also observed at the hallux, where the HEALTH group showed significantly higher PP compared to the DN group. On the other hand, no group differences in PP and PTI at the heel region as well as in the PTI at the hallux were observed. Furthermore results from the present investigation demonstrated the importance of the hallux to reduce foot pressures at the metatarsal region during the push off phase. This section starts discussing foot pressures (PP and PTI) at the metatarsal heads, which is the foot region more likely to develop foot complications (Boulton, 1994), followed by the foot pressures at heel and foot pressures at the hallux. In addition to this, the last part of this section discusses kinetic data on the forefoot during the push off phase, which is the phase of the GC where highest plantar stress occurs and is therefore more likely to damage the DN foot.

Results from the present study, in accordance with the vast majority of previous investigations (Boulton et al., 1987; Caselli et al., 2002; Pitei et al., 1999), found higher PP on the metatarsal region in the DN group compared to the healthy group. Thus, different factors have been proposed in the literature to explain the higher pressures at the metatarsal region observed in subjects with DN compared to healthy individuals. These include body mass, sensory neuropathy, structural changes and foot ROM. The possible effect of each of these factors in the present investigation is discussed.

Increased body mass, which is common in subjects with type 2 diabetes, increases the magnitude of the GRFs during gait and standing, but it also increases the areas of contact between foot and ground (van Deursen, 2004). In support of this the present

results show a strong correlation between contact area and body mass in both groups, especially in the DN group. This may explain why body mass in the present study was not correlated to peak pressures in any foot region. Although some investigations have reported a weak relationship between the two variables (Cavanagh et al., 1991), it appears that weight cannot explain the differences in forefoot foot pressures found in the current study between the HEALTH and the DN group.

Sensory neuropathy has also been proposed to increase forefoot PP in DN subjects. For instance, Caselli et al. (2002) demonstrated an association between the level of neuropathy and forefoot peak plantar pressures. They investigated regional plantar foot pressures in 248 subjects, who were divided into 4 different groups in relation to their level of neuropathy (no neuropathy: mild neuropathy; moderate neuropathy; and severe neuropathy). They found a progression in the magnitude of the peak pressures along with the severity of the neuropathy. Similar results were found by Boulton et al. (1987) who reported higher peak pressures in the group of diabetic subjects with neuropathic complications (N=16) compared to the diabetic group without neuropathy (N=28) and the control group (N=41). In this context other studies did not find significant correlations between sensory neuropathy and peak pressures (Sacco et al., 2000; Mason et al., 1989). Sacco et al. (2000) assessed neuropathy levels through the determination of sensitive chronaxie (electrodiagnostic evaluation) on 2 forefoot areas (medial forefoot and lateral forefoot). Sensitive chronaxie is defined as the minimum time of an electrical pulse required to excite a sensitive nerve. They looked for relationships between the sensitive chronaxie and peak plantar pressure in corresponding selected areas and they did not find any correlations between the two. In line with these results, in the current study sensory neuropathy was not correlated to PP at the metatarsal heads. These findings suggest that loss of sensation may not have influenced forefoot peak pressures in the current study. However, it cannot be ruled out that the assessment of neuropathy in the present study via VPT, which limits the assessment of nerve function to the A beta-fibre function, (van Schie et al., 2004), may not be ideal for the overall quantification of neuropathy. For instance, Payne et al. (2002) and Caselli et al. (2002), who found a correlation between neuropathy and high forefoot pressures, quantified neuropathy based on a variety of tests including, VPT, the presence or absence of ankle reflex and temperature perception, which may provide with a better overall quantification of the sensory nerves function (A fibres, C fibres, mechano receptors, etc).

Structural changes secondary to hyperglycaemia, such as Charcot foot, claw toes and hammer toes, have been proposed to increase foot pressures in diabetic patients (Mueller et al., 2005; Rahman et al., 2006; Robertson et al., 2002). In the present study in order to reduce to effect of these structural changes in foot pressures, all participants were inspected for foot deformities and only subjects with no severe structural changes were included in the study. However, results from the current study show that the arch index was significantly higher in the DN group compared to HEALTH group, which agrees with previous investigations (D'Ambrogi et al., 2002; Duffin et al., 2002; Giacomozzi et al., 2005). It is believed that the thickening of the plantar fascia secondary to non-enzymatic glycosylation of the collagenous may cause the development of a foot fixed in a cavus configuration, with a high longitudinal arch for the entire stance period (D´Ambrogi et al., 2003; D´Ambrogi et al., 2005; Giacomozzi et al., 2005). Plantar fascia, despite being a passive structure, actively contributes to counteract the GRFs acting on the metatarsal heads by absorbing forces at the midtarsal joints during the heel strike and the load transfer from rearfoot to forefoot (Hicks, 1954). In support of this idea, D'Ambrogi et al. (2003) found an inverse correlation (r=-0.52) between the thickness of plantar fascia and vertical forces under the metatarsal heads in a group of 61 diabetic patients with different degrees of neuropathic complications. Moreover, other authors have demonstrated using a regression analysis that arch index is an important predictor for forefoot plantar pressures during barefoot gait in healthy individuals (Cavanagh et al., 1997; Morag & Cavanagh, 1999). Results from the current study showed that there was not a direct correlation between arch index and peak pressures at the metatarsals region. However, lack of correlation between these two does not necessary mean that the high foot arch did not influence forefoot pressures in the present study since there are a number of variables such as ROM or sensory neuropathy that could have compromised this association. Moreover, the lack of correlations in the present study should be interpreted with caution as the result may be due to insufficient sample size. Therefore, it is likely that a higher foot arch secondary to a thickness of the plantar fascia could partly explain the higher forefoot pressures observed in the DN compared to the HEALH group in the present investigation.

Limited ROM has been also associated with increased foot pressures in patients with diabetes mellitus (Fernando et al., 1991; Frykberg et al., 1998). It is well established

that glycosylation plays an important role for the limited ROM of the foot and ankle joints in diabetic patients (Frykberg et al., 1998; Simmons et al., 1997a; Yavuzer et al., 2006; Zimny et al., 2004). Abnormally high sugar levels promote glycosylation of proteins and the consequent accumulation of advanced glycosylation end-products in most human tissues (Rahman et al., 2006: Yavuzer, et al., 2006). Limited dorsi-flexion of the ankle has been associated with abnormal foot pressures under the metatarsal heads particularly when the tibia rolls over the foot during the late stance phase of gait (Mueller et al., 1989; Salsich et al., 2005). It is therefore possible that limited ROM of the foot and ankle joints in the DN group in the present study could be another factor responsible for the higher foot pressures observed in this population. However, an important limitation of the present investigations is that due to technical difficulties ankle ROM could not be measured, and therefore it cannot be demonstrated whether reduced ROM was a contributing factor the higher PP observed in the DN group.

Overall, the present study showed higher peak pressures at the metatarsals region in the DN compared to the HEALTH group. It is likely that changes in the foot secondary to hyperglycaemia, in the form of high foot arch and limited ROM may be responsible for the high foot pressures observed in the DN group compared to the HEALTH group. On the other hand, other factors such as body mass or sensory neuropathy did not seem to explain the differences in peak pressures at the metatarsal heads found in the current study between the DN and HEALTH groups. PTI values at the metatarsal heads will be discussed next.

Recent investigations have looked at PTI values since they represent both, the magnitude and duration of plantar loading through the stance phase and have therefore been postulated to be a better predictor for foot ulceration compared to peak pressures (Mueller & Maluf, 2002). In agreement with previous investigations, PTI values on the forefoot were significant higher in the DN group compared to the HEALTH group (D'Ambrogi et al., 2005; Sacco et al., 2009; Turner et al., 2007). Results from the present study demonstrated that higher PTI values at the metatarsal region in the DN group resulted from higher peak pressures (discussed above) as well as from longer duration of the plantar loading in this foot region. In agreement with the results from the present study, previous investigations found that DN patients spent longer period of time at the metatarsals heads compared to healthy individuals (Courtemanche et al., 1996). The reasons for the higher foot pressures at the metatarsal heads region were

discussed above: the possible reasons for the longer duration of the plantar loading at this foot region are discussed next.

It has been proposed that a higher foot arch, which is characteristic of the DN group in the current investigation, makes the foot less adaptable to the floor during the foot-floor interaction (Giacomozzi et al., 2005). As a result, most of the plantar surface makes early contact with the ground, and propulsion is poorly effective. In support of this interpretation, the present study, which agrees with the majority of previous studies (Courtemanche et al., 1996; Zimny et al., 2004) found that DN patients spent a longer period of time at the heel and at the metatarsal heads and less time at the toes compared to the HEALTH group. The loading time differences between groups in the current study remained statistically significant even after normalization with respect to stance phase duration, thus indicating that the increase of the overall foot loading time during stance is not homogeneously distributed among foot sub-areas. These results suggest an early loading of the metatarsals in the DN group, which explains the prolonged forefoot contact time and consequently increase in PTI at the forefoot in this group when compared to the HEALTH group. However, the present investigation did not find a correlation between foot arch and metatarsal PTI values, which suggests that other factors are more likely to explain the increased loading times at the metatarsals region in the DN group compared to the HEALTH group in the present study. Thus, results from the current study suggest that muscular function may play an important role in the higher forefoot PTI values observed in the DN group. On the one hand, results from the present study show an early activation of the TS muscles in the DN group compared to the HEALTH group, which may also explain the early contact of the forefoot with the ground. To the best of my knowledge, Kwon et al. (2003) has been the only investigation demonstrating that DN activate the TS earlier compared to healthy individuals (a detailed explanation about the causes that may lead DN subjects to fire the TS early will be presented when discussing EMG data). On the other hand, results from the current study show that DF strength levels were inversely correlated to forefoot PTI values in the DN group. It is therefore likely that the weakness of the DF muscles in the DN group may result in an early forefoot contact with the ground in this population, demonstrated by the quicker velocity of the COP at the heel discussed earlier. Interestingly, the correlation between peak DF moments and forefoot PTI was only significant for the DN group, which suggests that only severe weakness compromise the normal drop of the foot after the heel strike.

Overall, results from the present investigation suggest that while structural changes are the main factors responsible for the high peak pressures observed in DN subjects compared to healthy individuals, muscular function appears to play an important role in the longer duration of the plantar loading at the metatarsal heads region. It is therefore reasonable to think that strength gains in the DF muscles in subjects with DN could delay the drop of the foot after the heel strike. Consequently, this could reduce PTI at the metatarsal heads, which is the foot region most likely to suffer from foot ulcers (Caselli et al., 2002).

Previous investigations have reported lower loads on the heel in DN subjects compared to healthy individuals (Bacarin et al., 2009; Uccioli et al., 2001). It has been proposed that higher foot arch, weakness of the DF muscles as well as reduced dorsi-flexion mobility (Giacomozzi et al., 2002; Giacomozzi et al., 2005) cause subjects with DN to approach the floor with the most anterior part of the heel, which results in a minimum heel strike. In support of this theory some studies have reported lower peak vertical GRFs at the heel during landing (Katoulis et al., 1997; Uccioli et al., 2001). Contrary to this interpretation the present study did not find any significant group differences in regard to the magnitude of the stress at the heel. Furthermore, results from the current investigation show a strong association between PP at the heel and gait velocity, which agrees with previous investigations (Morag & Cavanagh, 1999). In fact, absolute PP values at the heel were significantly higher in the HEALTH group compared to the DN group (data not presented in the results section), but those differences disappeared when velocity, which was higher in the HEALTH group, was entered in the analysis as a covariate. It should be noted that some of the interventions, which reported reduced GRF at the heel in the DN groups did not control gait velocity (which is normally reduced in DN subjects) (Katoulis et al., 1997; Uccioli et al., 2001). In addition, some of the studies that did control gait velocity did not find significant group differences in vertical GRFs (Sacco et al., 2009; Yavuzer et al., 2006) or PP (Bacarin et al., 2009) at the heel between DN and healthy individuals. These results suggest that further investigations are warranted to clarify group difference between health and DN subjects in heel PP when velocity is accounted for.

Results from the present study also show that the heel region in the DN group spent more percentage of time in contact with the ground compared to the HEALTH group,

which agrees with previous investigations (D' Ambrogi et al., 2005; Sacco et al., 2009). It is believed that higher foot arch and weakness of the PF muscles lead to a less effective propulsion, which is characterized by a higher contribution of the heel during the push off phase (Giacomozzi et al., 2005; Mueller et al., 1994). However, PTI at the heel in the DN group did not show significant changes compared to the HEALTH group. The few studies assessing PTI in DN subjects have investigated regional PTI in relation to the main phases of the GC. Thus, it appears that PTI at the heel are reduced during the heel strike (D'Ambrogi et al., 2005) and increased during push off (D'Ambrogi et al., 2005; Sacco et al., 2009). It is not surprising then that overall PTI at the heel during the stance phase did not show significant group differences in the present study.

In addition to foot pressures at the metatarsals and heel regions the present study also investigated foot pressures at the hallux. In agreement with previous studies (Boulton et al., 1987; Uccioli et al., 2001; Veves et al 1991), it was found that the DN group developed significantly lower foot pressures under the hallux compared to the HEALTH group. This finding has been linked to severe deformities of the toes typical of the most advanced stages of diabetic motor neuropathy (Schoenhaus et al., 1991). However, this justification cannot fully explain the results from the previous study since all participants were inspected for foot deformities and only subjects with no severe structural changes were included in the study. It is well established that DN subjects have reduced ROM at the metatarso-phalangeal joint (Fernando et al., 1991; Zimny et al., 2004), which may compromise the distribution of the load under the toes. It is therefore possible that changes in the mobility at the metatarso-phalangeal joint together with mild foot deformities secondary to motor neuropathy may explain the lower PP at the hallux observed in the DN group compared to the HEALTH group in the present investigation. However, since ROM at the metatarso-phalangeal joint was not measured it is impossible to draw any final conclusion regarding the exact contribution of the metatarso-phalangeal joint mobility in the present study. It is also possible that sensory neuropathy may have contributed to this finding in the present study. It has been proposed earlier that loss of stability secondary to sensory neuropathy may result in a reduction of the COP progression along the longitudinal axis (Giacomozzi et al., 2002). Consistent with this idea the present study found that the contact area at the hallux was also significantly reduced in the DN group, which demonstrates the reduced contribution of more distal part of the forefoot during the

stance phase in DN patients. However, stability was not measured in the present study. Therefore it is only a speculation that a feeling of instability may have contributed to the reduded contact area under the hallux observed in the DN group. Overall, it is likely that structural changes (including reduced ROM) secondary to hyperglycaemia and feeling of instability secondary to sensory neuropathy may explain the lower contribution of the toes during the stance phase in the DN compared to the HEALTH group in the present investigation.

The present investigation has provided insight regarding how the overall foot loading distribution is in DN subjects compared to healthy individuals. The metatarsal heads appears to be the foot region that suffers most of the stress during walking in the DN group. On the other hand the heel and hallux regions showed no group differences and higher pressures in the HEALTH group, respectively. The present study also investigated forefoot loading during the push off phase, which may provide insight regarding how foot loading is distributed during the most demanding phase of the GC. These results are discussed next.

Results from the present study highlight the importance of the hallux to reduce foot pressures at the metatarsal region during the push off phase. Since pressure relates to the amount of vertical GRF per area unit to which the force is applied, the same force applied to a smaller area will result in higher pressures. In the present investigation, and in agreement with the majority of the literature (D'Ambrogi et al., 2003; Giacomozzi et al., 2005; Uccioli et al., 2001) the peak vertical GRF under the metatarsal heads (normalized to body mass) was significantly higher in the DN group compared to the HEALTH group. Interestingly, the peak vertical GRF under the whole forefoot (metatarsal and toes) during the push off did not differ between groups. This finding suggests that although the magnitude of the vertical GRF is comparable between the DN and HEALTH group, the load was distributed differently in the DN and the HEALTH group. Thus, the DN group appears to direct most of that vertical GRF to the metatarsal region whilst the HEALTH group seems to distribute that load more homogenously throughout the whole foot (metatarsal area and toes). Furthermore, the peak pressure when considering the whole forefoot area (metatarsals and toes) did not show significant differences between the DN and HEALTH groups. These results demonstrate that the contribution of the toes during the push off is very important to reduce the magnitude of vertical GRFs under the metatarsals. In support of this idea the

present study found that the contact area and the peak pressures under the big toe were significantly reduced in the DN group compared to the HEALTH group (discussed above). This demonstrates the reduced contribution of this foot region during the push off phase in patients with DN.

Overall, results from the present study demonstrate that the metatarsal heads region is the foot area that suffers most of the stress during walking. This explains why this is the foot area in which most of the ulcers develop (Boulton, 1994; Mueller et al., 2005). On the one hand, it is likely that structural changes such as high foot arch or reduced metatarso-phalangeal mobility may have contributed to the higher foot pressures at the metatarsal heads region observed in the DN subjects. On the other hand, muscular function changes, such as weakness of the DF muscles and PF activation patterns, seemed to have contributed to the higher amount of time the metatarsals region spend in contact with the ground in the DN group. Longer contact time obviously increases the overall stress applied over that foot region. Furthermore, the present study demonstrated that the contribution of the hallux during the push off is very important to reducing the magnitude of the loads under the metatarsal heads. Actually, the lack of contribution of this foot region during the push off is likely to partly explain the group differences in foot pressures at the metatarsal heads observed in the present study. Structural changes and feeling of instability are likely contributing factors for the reduced loading at the hallux observed in the DN subjects. Beside foot gait parameters and foot pressures the present study investigated muscular activity patterns during the GC. These results are discussed below.

7.1.2.3 EMG data

Results from the present investigation demonstrated significant differences in muscular activation patterns when the HEALTH group was compared to the DN group. Thus, the DN group showed an earlier muscular activation onset for the TA and TS muscles and a higher overall muscular activity for the TS, QUADS and HAMS compared to the HEALTH group. Furthermore, the present investigation found an association between EMG activity patterns and loading patterns.

Most of the studies assessing gait characteristics in diabetic patients have investigated kinetic or kinematic data, whereas the underlying functional factors that may be driving these changes are not well understood. EMG provides insight how muscles are

activated to generate movement. However, since EMG is an expression of neuromuscular control depending on nerve conduction, it is potentially affected by neuropathy. EMG signal refers to the electrical event produced by the muscle prior to contraction and not to the mechanical output (force) (Winter, 2009). The time lag between muscle activation (EMG signal) and muscle force production is known as EMD (Cavanagh & Komi, 1979) and it is crucial to take it into consideration when attempting to estimate muscle function timing (mechanical output) from EMG data . The fact that the current intervention accounted for EMD values may explain why the activation patterns in relation to the different phases of the GC presented in this study are more shifted to the right (activation occurs later) compared to standard EMG patterns reported in the literature for healthy subjects (Perry & Burnfield, 2010). For example, Perry & Burnfield (2010) reported that TA stays active from heel strike to the end of the loading phase (10% GC) in healthy individuals, while the present study found TA to be active until half way throughout the mid stance phase.

The preliminary studies number 2 and 3 presented in Chapter 4 demonstrated for the first time that EMD was longer in DN subjects compared to HEALTH individuals. Longer EMD is expected to bring the activation patterns forward. Since the time lag from activation onset to muscle production is longer, muscle activation is expected to occur earlier. This highlights the importance of calculating individual EMD values when attempting to present EMG data in relation to muscular mechanical function, especially when comparing populations with different EMD values such as DN. It seems this is the first study that has investigated EMG patterns during gait in subjects with DN whilst taking into account alterations in electromechanical delay when processing EMG data. However, it is noteworthy that although previous investigations did not report any action to control for EMD, they presented similar EMG patterns compared to the present study (Akashi et al., 2008; Mueller et al., 1994). This suggests that previous investigations may have used signal processing to correct for EMD without reporting it in the methods.

Results from the present study show that the peak activity for the TS and TA (during the end of the stance phase and early swing phase, respectively) occurred at the same time in the DN and HEALTH groups. Interestingly, both muscles show an earlier activation in the DN compared to the HEALTH group. Similar results were found by Kwon et al. (2003) who found an earlier activation onset for the TS and TA in the DN

group compared to the HEALTH group. However, Kwon et al. (2003) only found significant differences for the TS. It is possible that DN subjects adopt an anticipatory strategy to efficiently produce force at the right moment in time. Gutierrez et al. (2001) demonstrated a decrease in the ability to rapidly develop torque about the ankle in a group of 6 DN subjects compared to 6 diabetic individuals without neuropathy. Force generation in this study was determined from the rate of change of the ground reaction force vector in the lateral direction (measured with a force platform) during 1) a rapid lateral loss of balance and 2) a quick voluntary inversion movement of the ankle. The results showed that DN subjects were able to produce about half the rate of torque compared to a healthy group in both conditions. Although the reasons for this slower force production are not fully understood, evidence from animal studies suggests that the fast-twitch fibres (type 2) of the muscles are highly sensitive to the loss of strength due to atrophy (Bishop & Milton, 1997). Therefore, a generalized DN is likely to cause distal loss of fast twitch muscle fibres, which may result in slower force production by the muscles, especially the distal ones. This limitation to generate force quickly might require DN subjects to activate the muscles earlier as a mechanism to overcome the slower rate of force production and ensure the peak force is produced at the right moment in time. It is therefore reasonable to think that changes in the neuromuscular function in DN individuals may obligate this population to develop an early muscular activation to ensure mechanical output is produced at the right time.

Furthermore, the present investigation demonstrated an association between muscular activity patterns during the initial phase of the GC and foot plantar pressures under the metatarsal heads. Alongside the earlier activation of the TS by the DN group, which has been discussed above, the current investigation found an earlier cessation of the TA following heel strike in the DN group compared to the HEALTH group. Thus, an early cessation of the TA activity, together with an early activation of the TS may result in an early contact of the forefoot with the ground. This may also explain why contact times and consequently PTI values under the metatarsal heads were higher in the DN group in the present study compared to the HEALTH group. Results from the present study then suggest that changes in TA and TS activity patterns following heel strike could also play an important role in the pathogenesis of foot complications in DN individuals. Previous studies investigating TA activity during the early stance phase of the GC have been controversial. Some investigations have reported a delayed peak activity of the TA muscle during the initial phase of the GC (Abboud et al., 2000; Sacco & Amadio,

2003). Akashi et al. (2008) did not find any group differences in activation times for the TA during the initial phase of the GC. The present study found an early activation cessation in the DN group. It should be noted that these studies have used different population groups, which may partly explain the different results. Similar to the present study, Sacco & Amadio (2003) and Akashi et al. (2008) recruited DN subjects with no other previous foot complications whereas Abboud et al. (2000) included patients with diabetes mellitus who were not assessed for neuropathy. However, studies using similar populations and comparable methodological approaches have also reported conflicting results (Sacco & Amadio, 2003; Akashi et al., 2008). Dingwell & Cavanagh (2001) demonstrated an increased walking variability in DN subjects, which may make EMG data in this population more difficult to interpret and could explain the discrepancies among the results from different studies presented above. Gait dynamics evolve over multiple strides, where each stride depends on multiple previous strides (Hausdorff et al., 2001). Thus, Kang & Dingwell (2009) quantified EMG dynamics over consecutive strides in healthy young individuals (N=17) as well as in older adults (N=17), which are well known to exhibit greater step variability while walking, like DN subjects. They proved that older adults exhibited greater interstride variability of muscle activation patterns during gait. Therefore, increased interstride variability, together with different gait tasks (6 meters walk, 10 meters walk or treadmill walk), may partly explain the discrepancies between the studies presented above. Nevertheless, results from the present study appear to suggest that an early TS activation onset and early cessation of the TA activity may partly explain why contact times and consequently PTI values under the metatarsal heads region were higher in the DN group compared to the HEALTH group.

Furthermore, results from the present study, in agreement with previous investigations (Kwon et al. 2003; Petrofsky et al., 2005a), show that the overall EMG activity in all the muscles was higher in the DN group compared to the HEALTH group, and that those differences were significant for the TS, HAMS and QUADS muscles. Higher coactivation of muscles suggests higher co-contraction between agonist and antagonist muscles. Co-contraction has been observed as a task independent strategy that is believed to be used to stiffen the joint and enhance stability (Benjuya et al., 2004; Manchester et al., 1989). Results from the present study show higher activation of all the muscles during the terminal swing phase in the DN group compared to the HEALTH group, which demonstrate a simultaneous activation of agonist and

antagonist muscles (co-contraction). Furthermore, results from the present study suggest that the joint stiffening strategy is present not only at the ankle as previously thought (Kwon et al. 2003; Petrofsky et al., 2005a) but also at the knee joint. It is likely that the feeling of instability associated with sensory neuropathy may result in higher co-contraction in this population. The group differences in muscle activity observed in this study cannot be attributed to gait speed differences, since walking speed has been positively correlated with EMG activity levels (Chung & Wang, 2010) and the DN group in the present study walked significantly slower compared with the HEALTH group. This strengthens the results from the present study and demonstrates that co-contraction was present in the DN group. It is also possible that muscle weakness may have contributed to the higher EMG activity observed in the DN group. Since DN are significantly weaker compared to the HEALTH group, higher muscular activity may need to be generated to make sure adequate moment is produced. However, it is unlikely this is the reason for the group differences in overall EMG activity in the present investigation since EMG data was normalized to the peak activity during gait and not to the peak activity during MVCs. It is also possible that the anticipatory strategy proposed above may have contributed to these results. Thus, an early muscular activation may result in longer activation times and therefore higher EMG activity during the GC.

Overall, results from the present study show EMG pattern alterations in the DN when compared to the HEALTH group. Thus, it appears that DN subjects adopt an anticipatory gait strategy to ensure an adequate moment is generated at the right moment in time. Although, the exact reason/s behind this adaptation is/are unclear, it is possible that this is a compensatory mechanism to overcome the slower rate of force production observed previously in DN subjects (Gutierrez et al., 2001). Furthermore it seems that changes in EMG patterns at the early phases of the GC may contribute to the higher pressures at the metatarsal heads observed in the DN group. Thus, an early TS activation onset and early cessation of the TA activity may partly explain why contact times and consequently PTI values under the metatarsals region were higher in the DN group in the present study compared to the HEALTH group. In addition to that, the present study show higher overall EMG activity in the DN group compared to the HEALTH group. It is likely that co-contraction secondary to the feeling of instability associated with DN and the anticipatory strategy mentioned above may partly explain the higher overall EMG activity observed in the DN group.

Summary of gait biomechanics

Overall, the present investigation showed alterations in all the different aspects of gait in the DN group compared to the HEALTH group. Thus, DN gait was characterized by shorter and slower steps, higher foot pressures under the metatarsal heads and higher muscular activity compared to the healthy group. It appears that structural changes (including limited ROM) and loss of stability secondary to neuropathy are the main contributing factors responsible for the alterations in gait parameters and kinetic data observed in the DN group in the present study. However, the present study also demonstrated that muscular function in the form of muscular activity patterns and muscular weakness should be considered among the main causes that lead DN subjects to change their walking strategy. For instance, weakness of the DF muscles in the DN group was associated with an early forefoot contact with the ground and consequently with higher forefoot PTI values in this group. Interestingly, muscular weakness together with joint mobility are modifiable conditions. Therefore, it would be interesting to evaluate whether changes in these condition may result in beneficial changes in gait characteristics in DN subjects.

7.1.3 Microcirculation

In addition to changes in gait characteristics, impairments in the microcirculation are also known to play an important role in the pathogenesis of foot ulcers in patients with DN (Dinh & Veves, 2005, Tooke, 2004). The present study investigated muscular microcirculation (capillary) during different condition to evaluate both blood flow and oxygen consumption 1) at rest; 2) in response to muscle contractions (stress condition); 3) recovery from the stress condition in DN subjects when compared to healthy individuals. Furthermore, this is the first study that has investigated microcirculatory responses to and from an exercise bout based on plantar-flexion contractions in DN individuals. Results from the current study, which are in line with previous investigations with subjects with type 2 diabetes mellitus (Kingwell et al., 2003; Scheuermann-Freestone et al., 2003), show diminished functional responses in blood flow and oxygen consumption during conditions 2 and 3 in the DN group compared to the HEALTH group. No significant group differences were found during resting conditions in either blood flow or oxygen consumption values. Discussion of the results

will be presented as follows: Microcirculation at rest, microcirculatory responses to muscle contraction and microcirculatory recovery from the muscle contractions

7.1.3.1 Microcirculation at rest

Results from the present study show that at baseline both muscular uptake and blood flow did not differ between the HEALTH and the CON group. Previous investigations assessing resting blood flow in patients with DN reported increased values in the DN group compared to the healthy controls (Flynn et al., 1988; Urbancic-Rovan et al., 2004). However, those investigations assessed cutaneous blood flow contrary to the present investigation that investigated muscular blood flow. It is known that the skin microcirculation is rich in arteriovenous shunts (Fagrell et al., 1999). The arteriovenous shunts are innervated by sympathetic nerves; the presence of diabetic neuropathy with sympathetic denervation may lead to the opening of these shunts with blood flow bypassing the skin capillaries (Korzon-Burakowska & Edmonds, 2006; Schramm et al., 2006). Therefore, the fact that the present investigation assessed muscular blood flow may explain the discrepancies in the results. Regarding the baseline muscular uptake, it seems this is the first investigation reporting muscular oxygen consumption values in patients with DN. Furthermore, it is noteworthy that the absolute values reported in the present investigation, both for mVO_2 and blood flow, are slightly lower than previous values reported in the literature for healthy individuals during NIRS measurements. For example, van Beekvelt et al. (2001b), van Beekvelt et al. (2002) and De Blasi et al. (1997) published mVO_2 values of 0.009± 0.003 $mlO2 \cdot min^{-1} \cdot 100g^{-1}$, 0.14± 0.02 $ml02 \cdot min^{-1} \cdot 100g^{-1}$ and 0.06± 0.002 $mlO2 \cdot min^{-1} \cdot 100g^{-1}$, respectively whereas in the present study the DN and HEALTH groups reported mVO_2 values of 0.0246± 0.012 $ml02 \cdot min^{-1} \cdot 100g^{-1}$ and 0.0251± 0.012 $mlO2 \cdot min^{-1} \cdot 100g^{-1}$, respectively.
Similarly, van Beekvelt et al. (2001a) and van Beekvelt et al. (2001b) reported BF values of 1.28± 0.82 $ml \cdot min^{-1} \cdot 100g^{-1}$ and 0.72± 0.32 $ml \cdot min^{-1} \cdot 100g^{-1}$, respectively for the 0.60± 0.28 $ml \cdot min^{-1} \cdot 100g^{-1}$ and 0.64± 0.26 $ml \cdot min^{-1} \cdot 100g^{-1}$ found in the present study for the DN and HEALTH groups, respectively. Although it is not clear why the present study showed lower vales compared to previous investigations, it is likely that methodological differences may partly explain these differences. Different studies have used: 1) different muscles; 2) different inter-optode differences (distance between source and detector) and 3) different differential path-length factor [used to correct for scattering of photons in the tissue (van Beekvelt et al., 2001b)]. Overall the present

investigation showed that resting microcirculation in the form of muscular blood flow and oxygen consumption did not differ in the DN compared to the HEALTH group. However, it is well known that most critical functional changes in DN subjects occur during stress conditions (Tooke et al., 1995). Results on microcirculatory responses to an exercise bout are discussed below.

7.1.3.2 Microcirculatory responses to muscle contraction

The current study found impaired capillary responses in the DN group in the form of blood blow and oxygen consumption compared to the healthy group in response to an exercise bout. The exercise bout consisted of 2 sets of 7 isometric contractions of 10 seconds of duration at 50% of the MVC. Changes in blood flow will be discussed first whilst changes in oxygen consumption will be discussed thereafter.

Previous investigations found reduced blood flow responses to an exercise bout in subjects with type 2 diabetes when compared to healthy individuals, which has been associated with the reduced exercise capacity observed in diabetic patients (Kingwell et al., 2003; Mohler et al., 2006; Petrofsky et al, 2005b; Scheuermann-Freestone et al., 2003). Although similar results were expected in different diabetic populations, this is the first study to demonstrate impairments in the ability to increase blood flow in the microcirculation during an exercise bout in DN subjects.

The reduced percentage of change in blood volume observed in the current study likely reflects a decrease in the exercise-induced vasodilatation capacity of capillaries in the skeletal muscle of the lower extremity in the DN group. Patients with diabetes, and especially individuals with peripheral neuropathy, are known to have microvascular alterations, including endothelial dysfunction (Pitei et al., 1997; Schramm et al., 2006). Endothelial-dependent vasodilatation is thought to contribute to exercise hyperaemia (Higashi & Yoshizumi, 2004). Boushel et al. (2002) demonstrated that a combined inhibition of NO and prostaglandins, which are considered the most important endothelium-derived vasodilator substances, reduced muscle blood flow during exercise in healthy individuals (up to 50%). In line with this finding, Mekus and colleagues (2004) carried out a very similar study and also concluded that protanoids and NO appear to play important roles in elevating skeletal muscle blood flow during exercise. It has been proposed that an increase in vascular stress resulting from increased

exercise-induced blood flow may induce the endothelial cells to release substances such as NO and prostaglandins, which allow blood flow to locally increase (Higashi & Yoshizumi, 2004). Kingwell et al., 2003 was the first study providing evidence that impaired endothelium-dependent vasodilatory responses limits blood flow during exercise in patients with type 2 diabetes. Evidence presented above suggests that impairments in the endothelial function in the DN group may be the main factor responsible for the group differences in the blood volume percentage of change observed in the current study. Moreover, it should be noted that in the present study BMI and % of body fat were significantly greater in the DN group compared to the HEALTH group and obesity is known to be associated with endothelial dysfunction via numerous complex mechanisms (Caballero, 2003). Thus, it is possible that a greater BMI and/or body fat percentage in the DN group may have contributed to the reduced exercise-induced vasodilatation observed in this population. However, this association between obesity (as determined by either BMI or body fat percentage) and endothelium function was not supported by the current study, since BMI and body fat % were not correlated with exercise induced changes in blood flow.

Although impairments in exercise–induced vasodilatation are normally associated with endothelial dysfunction (Kingwell et al., 2003), it is possible that other mechanisms may have also contributed to the results found in the present investigation with DN patients. For instance, reduced cardiac output due to neuropathic related changes in the ANS (autonomic neuropathy) may also reduce exercise-induced blood flow in this population (Petrofsky et al., 2005b). Although the cardiac muscle initiates its own electrical impulses, which is set by the sino-atrial node at about 80 times per minute (Wilmore & Costill, 2004), neural influences superimpose the inherent rhythm of the myocardium. These influences originate in the cardiovascular centre and flow through the sympathetic and parasympathetic components of the ANS (Tortora & Derrickson, 2006). Those neural influences interact together adjusting HR in response to the action of other mechanisms such as, ventilation, blood pressure control, thermoregulation and renin-angiotensin system (Winsley, 2002). The net effect of sympathetic and parasympathetic autonomic modulation is to increase and decrease the HR respectively (Tortora & Derrickson, 2006). It is well known that neuropathy may alter autonomic regulation in this population (Howarka, et al., 1997; Loimaala, et al., 2000; Loimaala, et al., 2003) due to the damage, especially, to the parasympathetic nervous system. Petrofsky et al. (2005b) demonstrated that heart rate responses during isometric exercise

were diminished in subjects with type 2 diabetes compared to healthy controls, which suggests changes in the autonomic function prior to clinical presentation of neuropathic complications. Since the increase in heart rate during isometric exercise is due to a reduction in vagal tone, the reduced heart rate response observed by Petrofsky et al. (2005b) is likely to be due to impairments in the parasympathetic function in this population. It is therefore possible that alterations in the ANS may have affected HR response during the isometric exercise in the DN group in the present study (lower cardiac output), which may result in reduced vasodilatory response to the exercise bout. In line with this idea, results from the current intervention show significantly higher resting heart rates in the DN group compared to the HEALTH group. Since a predominance of vagal autonomic modulation will lower resting heart rate, results from the present study match previous data on impaired parasympathetic function in people with type 2 diabetes. It is therefore possible that changes in the ANS may have also contributed to the diminished exercise-induced vasodilatory capacity observed in the DN group. However, further studies are required to confirm (or quantify) whether changes in the autonomic nervous system in diabetic subjects with undiagnosed diabetic autonomic neuropathy are important contributing factors to the reduced exercise-induced vasodilatory responses observed in this population.

Overall, the present investigation found impairments in the exercise-induced vasodilatation in DN subjects. It is believed that endothelial-dependent vasodilatation contributes to exercise hyperaemia, which suggests that endothelial function may have played an important role in the reduced blood volume percentage of change in the DN group in the present study. Moreover, results from the present study also suggest that central alterations in the ANS are likely to have contributed to the results found in the present study via reductions in cardiac output during the exercise bout. Further investigations are warranted to understand better the underlying mechanisms in the impaired exercise-induced vasodilatation in DN subjects. Whilst there is agreement in the literature that oxygen delivery during stress conditions is affected in subjects with diabetes, (which the present investigation demonstrated for the first time in DN subjects) there is controversy among published papers on whether muscular oxygen consumption (which refers to the use of the delivered oxygen), is altered in patients with diabetes. Next, results from the current investigation, which investigated for the first time muscular oxygen consumption in response to an exercise bout in DN subjects, will be discussed.

Results from the present study show that the mVO_2 percentage of change (from baseline to post-exercise values) was significantly lower in the DN group compared to the HEALTH group. This finding suggests that muscular oxygen uptake was also impaired in the DN group. Consistent with the results found in the present study Baldi and colleagues (2003) demonstrated that arteriovenous oxygen differences, which assess the muscular oxidative capacity, was reduced in type 2 diabetic subjects compared to healthy controls when working at maximal and submaximal intensities (70% VO_{2max}). However, there is an ongoing debate about whether the reduced muscular oxidative capacity observed in various studies with subjects with type 2 diabetes (Baldi et al., 2003), is solely caused by a reduction in oxygen delivery (Regensteiner et al., 1998) or in combination with a reduction in the muscular oxidative capacity (Martin et al., 1995). The author of the current investigation believes that the reduced mVO_2 responses to the exercise bout in the DN group in the present study are, at least partly, due to impairment in the muscular oxidative capacity in this group. The reasons that led the author to conclude this are presented below.

The exercise protocol chosen for the present investigation was set up at submaximal intensities (see Section 5.4.3.6 in Chapter 4 for more information about the exercise protocol and how it was controlled). When working at submaximal intensities and if blood flow is restricted, an increase in the amount of oxygen uptake by the muscle may be expected to compensate for the impaired oxygen delivery. In line with this idea, Nyberg et al. (2010) demonstrated that muscle blood flow and oxygen delivery can be markedly reduced without affecting muscle oxygen uptake during moderate-intensity exercise, suggesting that blood flow does not limit muscle oxygen uptake when working at low intensities in young healthy individuals (25± 4 years). This study measured the leg oxygen consumption during a 3.5 minute one-legged knee-extensor exercise under two conditions: without (control) and with arterial infusion of inhibitors of NO (N-monomethyl-L-arginine) and prostanoids (indomethacin). Increased activity of several mitochondrial enzymes has been found in muscles of patients with peripheral vascular diseases (Bylund et al., 1976; Janson et al., 1988). For example, Janson et al. (1988) investigated side-differences in muscle metabolic characteristics in patients with unilateral arterial disease and demonstrated increased activities of enzymes involved in the oxidative metabolism in the claudication leg compared to the healthy leg. These adaptations in limbs suffering from peripheral vascular diseases suggest compensation

for impaired oxygen delivery. This idea is further supported by a study carried out by Mohler et al. (2006). They assessed the hemodynamic response to exercise in calf muscles in patients with type 2 diabetes, peripheral vascular diseases, or both using NIRS. They found that the presence of peripheral vascular diseases was associated with a significant increase in percentage deoxygenation during the exercise bout, which suggests that impairments in circulation during submaximal exercises may result in a higher amount (%) of desaturation of haemoglobin in the tissue. According to this logic, it is likely that changes in the muscle oxidative capacity in the DN group in the present study may be partly responsible for the reduced oxygen uptake observed in this group during the moderate intensity exercise protocol. In further support of this interpretation, there is some evidence that metabolic abnormalities may have implications in the oxidative capacity in diabetic patients (Scheuermann-Freestone et al., 2003; Sivitz, 2010). Type 2 diabetic individuals have increased type IIb-to-type I fibre ratio (Marin et al., 1994) and lower oxidative enzyme capacity (Simoneau & Kelley, 1997; Vondra et al., 1977) than nondiabetic subjects. In the present study, it is therefore reasonable to hypothesize that impairments in the skeletal muscle oxidative capacity in the DN group may have resulted in less O_2 being consumed following the submaximal exercise bout in the DN group relative to the HEALTH group.

Contrary to the results from the present study, some investigations that used NIRS did not find significant differences in exercise-induced muscular deoxygenation when comparing healthy and diabetic individuals (Bauer et al., 2007; Mohler et al., 2006). However, both studies reported higher deoxygenation values in the control group compared to the diabetic group, although those differences did not reach significant levels. It is likely that differences in the subjects characteristics of the diabetic groups could partly explain why the present study was more sensitive when identifying group differences (diabetes vs. healthy) compared to previous studies. Firstly, the diabetic group in the present study was composed of subjects with peripheral neuropathy while Bauer et al. (2007) and Mohler et al. (2006) recruited diabetic patients without neuropathic complications. Although it has been demonstrated that oxidative capacity is reduced in subjects with type 2 diabetes (Baldi et al., 2003), a longer duration and/or poorer control of diabetes (>HbA_{1c}), which is associated with DN (Pirart, 1978), may be expected to have a greater negative effect on muscle function. Secondly, the diabetic group from the current study was more obese and older compared to the other two studies and it is known that age and BMI are associated with mitochondrial function

(Rabol et al., 2010). However, the most important reason for the conflicting results between the present study and the other two investigations is likely to be methodological. NIRS measures local muscular oxygenation. Thus, it is important to determine the specific amount of work that the muscle under investigation is contributing to the whole workout. In the present study NIRS data and EMG data were measured in the same muscle simultaneously. This procedure was followed in order to be able to relate NIRS data (blood flow and oxygen consumption in the MGast) to the amount of work done by the MGast during the exercise protocol (see Section 5.4.1.3 for more information about the instrumentation set up). Thus, the percentage of change in the mVO_2 was correlated to the average EMG activity (normalized to MVC) in the MGast during the 14 isometric contractions that composed the exercise bout. As expected and in agreement with previous investigations (van Beekvelt et al., 2002) the current study found that there was a significant correlation between EMG activity and muscular oxygen consumption both in the DN and HEALTH groups. More interestingly, results from the present investigation only showed group differences when EMG activity was entered in the analysis as a covariate. This highlights the importance of assessing local muscular workout when attempting to investigate local oxygen consumption. This may explain why Bauer et al. (2007) and Mohler et al. (2006), who only assessed total workout, did not find significant differences in exercise-induced muscular deoxygenation when comparing healthy and diabetic individuals. However, further studies are warranted in this context to provide insight how muscular oxidative capacity is affected in DN subjects.

The present investigation has demonstrated for the first time that 1) muscular oxygen delivery is restricted in the lower limb (calf muscle) of DN subjects in response to an exercise bout; and 2) the muscular oxygen uptake is also diminished in the calf muscle of DN subjects, likely due to impairments in the muscular oxidative capacity. Therefore, it seems that not only the delivery of oxygen to the muscle is restricted in patients with DN following an exercise stress, but also the ability of the muscle to use that oxygen. Results regarding the recovery of the microcirculation following an exercise bout will be discussed next.

7.1.3.3 Microcirculatory recovery from the muscular contractions

The present study found that the DN recovered slower compared to the HEALTH group. Since the DN showed reduced microcirculatory responses in the form of BF and mVO_2 to the muscular contractions, it is not surprisingly that percentage of recovery after 70 seconds both BF and mVO_2 was also reduced in the DN group. Therefore, it appears that the slower BF and mVO_2 recovery in the DN group in the present investigations is secondary to impairments in the ability of the microcirculation to response to the exercise bout.

In summary the present study demonstrated that microcirculatory function is affected in DN subjects compared to health individuals, especially under stress conditions. Thus, BF and mVO_2 absolute values at rest did not differ in the DN and the HEALTH group whilst BF and mVO_2 percentage of change 1) in response to the exercise bout and 2) from the exercise bout were significantly reduced in the DN group compared to the exercise group. Furthermore, it appears that the most critical changes in the DN group occurred in response to the exercise bout as demonstrated by the fact that alterations in the recovery capacity of the DN group were secondary to abnormalities in vasodilatory and oxygen uptake responses to the muscular contractions. It is likely that changes in the endothelial function are the main responsible factor for the reduced vasodilatory capacity observed in the DN group. However, it is also possible that reduced cardiac output secondary to changes in the ANS have also contributed to this finding. Furthermore the present study found that not only oxygen delivery was reduced in the DN group but also the ability of the muscle to uptake oxygen, which suggests changes in the muscle oxidative capacity. This is an important finding since previous studies using the NIRS device failed to find significant differences in deoxygenation values between the healthy and subjects with type 2 diabetes. Moreover, the results from the present study highlight the importance of assessing local muscular workout when attempting to investigate local oxygen consumption with the NIRS device.

It is commonly accepted that gait alterations and microcirculatory changes are responsible for the high rate of foot ulcers observed in DN subjects. Results from the present investigation demonstrated alterations in gait characteristics, including higher foot pressures under the metatarsal heads, and impairments in the microcirculation in response to a physical stress (exercise stress) when comparing the DN and the

HEALTH group. These findings confirm that the DN group in the present study was at considerably higher risk to develop foot problems compared to the healthy controls. Beside the foot complications associated with diabetes neuropathy, increasing evidence suggest that diabetes in general and DN in particular has an important impact on people's QOL (Price, 2004). The results on the differences in QOL between the DN and the HEALTH group are discussed next.

7.1.4 Quality of Life

Results from the present study, in agreement with previous investigations (Ali et al., 2010), show that all the dimensions of the SF-36 questionnaire were significantly lower in the DN compared to the HEALTH group. Furthermore, early evidence suggests that QOL in subjects with diabetes could be associated with physiological markers related to the disease. For instance, the severity of diabetic complications has been associated with poor health-related quality of life (Currie et al., 2006). However, results from the current study failed to find a relationship between the severity of sensory neuropathy measured with VPT and any dimension of QOL. On the other hand, HbA_{1c} was correlated to two different SF-36 domains, namely physical function and mental health, as well as with the overall mental health score. This association between blood glucose and QOL has been previously found in patients with type I diabetes (Wikblad et al., 1996) and painful neuropathy (Galer et al., 2000). In addition, BMI was negatively correlated with general health both in DN and HEALTH groups. Hypertension and cholesterol levels were not correlated to any of the SF-36 domains, a not altogether surprising finding since these two conditions are asymptomatic in the majority of patients. The majority of the DN subjects were taking medications to control both blood pressure and cholesterol levels, which could also have influenced their association with QOL. Consistent with the results from the present study some investigations have found no association between blood pressure and QOL (Lloyd et al., 2001; UKPDS, 1998).

Overall, findings from the present study support early evidence that 1) QOL is largely affected in DN subjects; and 2) poorer QOL in DN subjects could be associated with some physiological markers, such as HbA_{1c} or BMI. However, more studies are needed to understand the relationship between physiological and psychological markers and QOL in diabetic patients in general and DN subjects in particular.

7.1.5 Summary of the results for the cross-sectional study

The aim of this cross-sectional study was to investigate differences in the primary pathologies associated to DN when comparing healthy and DN subjects. This study demonstrates that DN carries a complex set of complications. Thus, the DN group in the present study showed alterations in general health, in the form of blood pressure, in different aspects of gait including spatial-temporal characteristics, foot pressures and muscular activity patterns, in microcirculation, especially during stress conditions, and in QOL when compared to healthy individuals.

In the present study special attention was paid to investigate differences in gait characteristics and microcirculation between DN and healthy subjects, due to their association with foot ulceration in this population. On the one hand, changes in gait characteristics are considered a common mechanism by which tissue damage may occur in diabetic patients with peripheral neuropathy. On the other hand, microcirculatory impairments are thought to disable the diabetic neuropathic foot to respond to injury and infection in the usual manner. The main findings in relation to gait biomechanics and microcirculation are presented next.

The present investigation, in agreement with previous studies, found group differences in all the aspects of gait under investigation which included gait parameters (spatial-temporal characteristics and COP parameters), foot pressures and muscular activity patterns. Gait parameters in the present study were characterized by a slower gait velocity and a shorter distance travelled by the COP during the roll over process. Overall results from the present investigation suggest that these changes are related to lack of stability secondary to DN. Therefore it is likely that risk avoidance, fear of falling, and lack of confidence are the main factors that lead DN subjects to develop a more cautious gait strategy. Beside changes in gait parameters the present study also shows alterations in the loading patterns in the DN when compared to the HEALTH group. The most noticeable group differences were observed under the metatarsal heads, where the DN group showed significantly higher PP and PTI values compared to the HEALTH group. It is therefore not surprising that the metatarsal area is the foot region in which most of the ulcers occur in DN subjects. Results from the present investigation suggest that muscular function (including muscular weakness) appear to play an important role in the longer duration of the plantar loading at the metatarsals

region. In fact, in the present study DF strength levels were inversely correlated to forefoot PTI in the DN group. It is therefore likely that weakness of the DF muscles in the DN group may have resulted in an early forefoot contact and more prolonged forefoot contact with the ground in this population. Furthermore, the present study demonstrated that the contribution of the hallux during the push off phase is very important to reduce the magnitude of loads under the metatarsal heads. In fact the lack of contribution of this foot region during this phase of the GC is likely to partly explain the group differences in foot pressures at the metatarsal heads observed in the present study. Structural changes and feeling of instability are likely contributing factors for the reduced loading at the hallux observed in the DN subjects.

The vast majority of studies assessing gait characteristics in DN patients have investigated kinetic or kinematic data, whereas only a handful of studies have investigated EMG activity patterns during gait in this population. Moreover, all the previous studies processed EMG data without taking into account EMD. It seems that this is the first time that EMG patterns during gait were investigated in DN subjects whilst taking into account individual EMD values when processing EMG data. Data from the present study show different EMG patterns in the DN when compared to the HEALTH group. On the one hand, it appears that overall EMG activity during the whole GC is higher in the DN group compared to the HEALTH group. It is possible that co-contraction of agonist and antagonist muscle is a compensatory mechanism in this population to improve stability. On the other hand, it seems that EMG activity patterns throughout the different phase of the GC differ between DN and healthy individuals. For instance, the present study found an early activation of the TS and TA muscles in the DN group compared to the HEALTH group whilst the peak activity occurred at the same time in both groups. This suggests that DN subjects adopt an anticipatory strategy to efficiently produce force at the right moment in time. It is likely that a slower force production rate in DN subjects may be at least partly responsible for this compensatory mechanism. The present study demonstrated that an early activation of the TS muscle is linked to higher PTI values at the forefoot. Overall, the present investigation, as expected, showed alterations in different aspects of gait in the DN subjects compared to the healthy control. It seems that structural changes, including limited ROM, muscular weakness and sensory neuropathy are the main factor responsible for the changes in gait characteristics discussed above. Interestingly, muscular weakness together with joint mobility are modifiable conditions and it would

be interesting to evaluate whether changes in these conditions may result in beneficial changes in gait characteristics in DN subjects.

The present study investigated for the first time microcirculatory responses to an exercise bout based on plantar-flexion contractions in DN individuals. Results from the current study, which are in line with previous investigations in subjects type 2 diabetes, show diminished 1) microcirculatory (blood flow and oxygen uptake) responses to the plantar-flexion contractions; and 2) microcirculatory (blood flow and oxygen uptake) recovery from the plantar-flexion contractions. It appears that impairments in the exercise-induced vasodilatation in DN patients are associated with changes in the endothelial function. Furthermore, the present study suggest that central alterations in the autonomic nervous system are likely to have contributed to the results found in the present study via reductions in cardiac output during the exercise bout. Although there is general consent in the literature that vasodilatation is impaired in diabetic patients, there is an ongoing debate whether the capacity of the muscle to uptake oxygen is also altered in this population. The present investigation seems to suggest that the ability of the muscle to uptake oxygen is also affected in the DN group. However, the exact reasons behind this finding are not fully understood. Furthermore, the present investigation demonstrated the importance of assessing local muscular workout when attempting to investigate mVO_2 responses to an exercise bout. Since NIRS measures local muscular oxygenation, it is important to determine the specific amount of work that the muscle under investigation is contributing to the whole workout. Thus, the current study demonstrated that local mVO_2 responses in the MGast to an exercise bout were dependent on the amount of work done by the specific muscle and not necessarily by the amount of work carried out by the whole leg. Since in the present study the DN showed reduced microcirculatory responses to the exercise bout in the form of BF and mVO_2, it is not surprising that recovery of both BF and mVO_2 was also reduced in the DN group. Overall, the present study demonstrated that microcirculatory responses to an exercise bout are diminished in DN subjects compared to healthy individuals. Early evidence suggests that PA interventions may produce positive changes in the microcirculation of subjects with type 2 diabetes. However, it is still unknown whether PA interventions could influence microcirculation in DN subjects.

In addition to foot ulcerations there are further health problems associated with diabetes in general and DN in particular. Thus, it is well established the association between

diabetes and both cardiovascular diseases and poor QOL. On the one hand, it is thought that alterations in traditional cardiovascular risk factors such as blood pressure or cholesterol levels are responsible for the increased risk of cardiovascular diseases in this population. Results from the present investigation show that DN had higher BP (despite taking hypotensive medications) compared to the HEALTH group. On the other hand, increasing evidence suggests that diabetes may have an impact on everyday living and consequently diminish health related QOL. Furthermore, some investigations have suggested an association between QOL and physiological markers (Wikblad et al., 1996). Findings from the present study support early evidence that 1) QOL is severely affected in DN subjects; and 2) poorer QOL in DN subjects could be associated with physiological markers, such as HbA_{1c} or BMI.

Overall, the present investigation has demonstrated that DN is a very complex condition which affects different aspects of health. The question remains whether the primary pathologies associated with DN, namely traditional cardiovascular risk factors, alterations in gait, impairments in the microcirculation (in particular during stress conditions) and QOL can be modified by rehabilitation programmes. Part 2 of the discussion chapter discusses the results from the effect of a 16-week PA programme on the whole range of primary pathologies associated with DN.

7.2 Part 2 – Intervention study

Part 2 was an intervention study that investigated the effects of 16 weeks of strengthening and mobility exercises on identified pathologies associated with DN. Results from this intervention study documents for the first time the beneficial effects of a 16-week resistance training programme on sensory neuropathy in patients with peripheral neuropathy. Results from the present investigation demonstrate that a well controlled strengthening training programme does have beneficial effects on the microcirculation, obesity, blood pressure as well as on QOL in patients with diabetic neuropathy. On the other hand, the exercise programme did not seem to have a substantial effect on gait characteristics including spatial-temporal, plantar pressures or EMG parameters, HbA_{1c} or strength levels. Importantly, it should be noted that, no adverse effects related to the intervention were reported in any of the volunteers who participated in the physical activity programme.

The discussion of the results of the intervention study is presented in the same order as in the previous chapters. Thus, general health outcome measures are discussed first followed by gait, microcirculation and QOL parameters.

7.2.1 General Health related to DN

This section discusses the results of the intervention programme on outcome measures related to general health in patients with DN. This includes the effect of the PA programme on parameters linked to: 1) diabetic neuropathy, such as sensory neuropathy and motor neuropathy; 2) diabetes control such as HbA_{1c}; and 3) cardiovascular risk factors, such as cholesterol levels, blood pressure or obesity. Results from the present investigation show that 16 weeks of strengthening exercises produce positive changes in sensory neuropathy and cardiovascular risk factors (i.e. blood pressure and obesity). On the other hand, the intervention did not seem to trigger substantial changes in HbA_{1c} and strength levels. Discussion of these results will be presented as follows: DN related outcome measures in the form of sensory neuropathy and motor neuropathy (strength levels), diabetes control (HbA_{1c}) and cardiovascular risk factors in the form of blood pressure, obesity and cholesterol levels.

7.2.1.1 Sensory neuropathy

One of the most striking findings of the present study was that 16 weeks of strength training improved sensory neuropathy in DN subjects. To the best of my knowledge this is the first investigation that has demonstrated that physical activity can reverse sensory neuropathy in DN subjects, which obviously may have tremendous clinical implications in this population. Balducci et al. (2006) is the only investigation that has previously assessed the effect of physical activity on neuropathy. They carried out a 4-years intervention study on diabetic patients with no signs of neuropathy and they found that low intensity long-term aerobic exercise training prevented diabetic patients from developing both motor and sensory neuropathy. The percentage of diabetic patients who developed motor and sensory neuropathy during the 4 years of the study was significantly higher in the control group (17% and 29.8% occurrence rate for motor and sensory neuropathy, respectively) than in the exercise group (0% and 6.5% occurrence rate for motor and sensory neuropathy, respectively). Results from the present investigation demonstrated that a physical activity programme can not only prevent the progression of diabetic neuropathy in individuals with no neuropathic complications, as demonstrated by (Balducci et al., 2006) but can also reduce sensory neuropathy in DN subjects. Currently there is no effective treatment available to prevent or treat neuropathy, except for tight control of blood glucose (Yagihashi et al., 2011). Thus, some investigations have found that intensive insulin treatments, which lower HbA_{1c}, can decrease the incidence of neuropathy by 60% (Diabetes Control and Complications Trial Research Group, 1993) and improve nerve conduction velocity and VPT (Ohkubo et al., 1995) in patients with type 2 diabetes. However, the absence of statistically significant changes on HbA_{1c} following both the present and Balducci's intervention suggest a possible direct and local effect of exercise on peripheral nerves on diabetic individuals. Next possible exercise-induced changes that could have influenced sensory nerves in the present investigations are discussed.

Nerve function depends on adequate blood flow and blood flow is known to be diminished in patients with diabetes. This association has led some investigators to believe that vascular problems may lead to peripheral nerve damage (Cameron & Cotter, 1994). In addition, vasodilatory treatment has been demonstrated to restore endoneurial blood flow and improve nerve conduction velocities in diabetic rats (Maxfield et al., 1993). Physical activity programmes have been shown to improve

circulation in patients with diabetes via an enhancement in the endothelial function (endothelial dependent vasodilatation) (Higashi & Yoshizumi, 2004). It is generally believed that the increase in vascular stress resulting from increased exercise-induced blood flow may stimulate the release of NO, which consequently improves endothelial function (Higashi & Yoshizumi, 2004). Thus, improvements in the circulation secondary to physical activity training could be a mechanism through which endoneurial blood flow may be restored, and in turn might lead to improved sensory nerves. Results from the present investigation show a trend toward greater muscular vasodilatory responses in the EXE compared to the CON groups following the exercise programmes. However, the fact that the present study only found a trend toward improved vasodilatory responses followed the exercise programme suggests that this interpretation should be taken with caution. Thus, further investigations are warranted to confirm whether exercise-induced improvements in the circulation (blood flow) can influence sensory nerves in DN subjects.

Beside changes in the circulation, changes in the mitochondria is another mechanism by which exercise training could have influenced sensory nerves in the present study. There is accumulating evidence indicating that mitochondrial degeneration is involved in the pathogenesis of DN (Leinninger et al., 2006). In most eukaryotic cells (cells with a nucleus), including neurons, mitochondria are essential in managing oxidative stress and providing energy by generating adenoside triphosphate (ATP) through oxidative phosphorylation (Lehmann et al., 2011). In neurons ATP is required for axonal transport and maintenance of ionic gradients for generation of action potentials and synaptic activity (Kwong et al., 2006). Reactive oxygen species (ROS), which are generated at low levels during the normal mitochondrial respiratory chain, are known to produce mitochondrial dysfunction at higher levels, which in turn may lead to mitochondrial degeneration (Figueroa-Romero et al., 2008; Leinninger et al., 2006). Hey-Mogensen et al. (2010) demonstrated that mitochondrial ROS release was reduced and muscle oxidative function was improved in 13 type 2 diabetes patients in response to a 10-weeks aerobic training programme. This finding suggests that physical training could potentially improve nerves function (reverse neuropathy) via beneficial mitochondrial changes. Interestingly, results from the present investigation demonstrate that a 16-week strength training programme enhanced mVO_2 recovery following an exercise bout in DN patients. Since the current investigation did not find changes in the exercise-induced muscular oxygen consumption in response to the exercise programme,

it appears that the intervention produced changes not in the capacity of the muscle to uptake oxygen (i.e. changes in the muscle fibre type), but in the efficiency of its usage. It is therefore possible that changes in the oxidative capacity of the mitochondria may be responsible for the more efficient use of oxygen following the intervention, which could explain the beneficial changes in the sensory nerves. However, more studies are needed on DN subjects to better understand 1) the effect of strength training on mitochondrial function; and 2) the association between improvements in mitochondrial function and sensory neuropathy.

Overall, it appears that vascular changes secondary to the exercise programme may be responsible for the unexpected improvements in sensory neuropathy observed in the present study. It is possible that exercise-induced changes in the endothelial function could be a mechanism through which endoneurial blood flow may be restored, and in turn might lead to improved sensory nerves. However, it should be stated that the present study only found a trend toward greater muscular vasodilatory responses in the EXE compared to the CON groups following the exercise programmes. It is therefore uncertain at this stage whether changes in the circulation may be partly responsible for the results found in the present study. On the other hand, it is also possible that changes in the mitochondrial function could explain the beneficial changes in the sensory nerves observed in the current study. In support of this interpretation, the present study found a more efficient use of oxygen in the EXE group following the intervention, which could be secondary to changes in the oxidative capacity of the mitochondria. Regardless of the exact mechanism behind this finding, the present study found for the first time that 16 weeks of strengthening exercises influenced sensory neuropathy in DN subjects. It is worth mentioning that sensory neuropathy in the current study was quantified by VPT measurements whilst conduction velocity measurements of the sensory nerves, which is considered the gold standard to assess nerve function, were not conducted. Future studies should assess sensory nerve function directly to understand better the effect of PA on sensory neuropathy. Nevertheless, this new finding should encourage researchers to investigate the therapeutic tool of PA to improve sensory neuropathy in DN subjects. The next section discusses the effect of the intervention on strength levels. Due to the close link between motor neuropathy and muscular weakness in DN patients, strength results are discussed under the heading motor neuropathy.

7.2.1.2 Motor neuropathy

Results from the present study show that 16 weeks of strengthening exercises did not produce substantial changes in muscular strength levels in DN patients. Thus, no significant gains in strength levels were observed in any of the muscle groups under investigation, dorsi-flexors, plantar-flexors, knee flexors and knee extensors. It is most likely that the lack of effect of the resistance training programme on strength levels in the DN subjects in the present investigation could be related to motor neuropathy.

Diabetes related motor neuropathy can be defined as the process in which segmental demyelination combined with axonal degeneration of motor nerve fibres limits the peripheral efferent stimulation of skeletal muscles in the lower and upper extremities (Greene et al., 1997).Thus, a general improvement in nerve function (sensory and motor), which is suggested by the improved sensation discussed above, might be expected to trigger similar changes in motor nerves, resulting in strength gains in response to the exercise programmes. Numerous studies on subjects with diabetes have shown the efficiency of strength training to improve strength levels in this population with no neuropathic problems (Cauza et al., 2005; Dunstan et al., 2006). However, the present study shows evidence for the first time that motor function was not improved with 16 weeks of resistance training in diabetic patients with moderate neuropathy. Some investigations have demonstrated using magnetic resonance imaging (MRI) techniques to quantify the muscle mass, a substantial loss in muscle tissue in DN subjects (Bus et al., 2002; Brash et al., 1999). Brash et al. (1999) and Suzuki et al. (2000) found significant correlations between motor nerve conduction velocities and muscle atrophy, which suggests that changes in muscle tissue are secondary to motor nerve dysfunction. Therefore, it is possible that muscle atrophy secondary to neuropathy may partly explain the lack of effect of the 16 weeks of strengthening exercises in the DN patients in the present investigation.

To the best of my knowledge there is only one previous investigation assessing muscle strength changes in DN patients following a PA programme. Allet et al. (2010) carried out a 12-week intervention (twice weekly) based on function-orientated strengthening exercises. Contrary to the present investigation, they reported changes in strength values (measured with a hand held dynamometer) in response to the exercise programme. However, the percentage of improvement was moderate compared with

strength increases found in healthy elderly persons after comparable training program (Rubenstein et al., 2000), and only the hip flexors and plantar-flexors reached significant levels. It is most likely that differences in the severity of neuropathy may explain why Allet´s study using a shorter and less intense intervention was more efficient to modify strength levels compared to the present investigation. Notably, Allet et al. (2010) included subjects with significantly milder DN (VPT<4) compared to the present study (VPT=21.52-+13.37 in the EXE group). This early evidence suggests that interventions to change muscle function in diabetic patients may be more efficient in the early stages of the condition before substantial muscle tissue is lost. Although more studies are required to draw any final conclusions about the ability of DN subjects to improve muscular strength, this finding stresses the importance of strengthening exercises in early DN subjects. It is noteworthy that these two studies have used different methods to assess muscular strength, which may also explain the discrepancy of the results. Thus, Allet' study used a hand-held dynamometer, the reliability of which is limited, in contrast to the present study, which assessed for the first time strength changes in DN subjects with an isokinetic dynamometer.

However, this explanation is likely to apply more to the distal muscles (mainly foot muscles), and it is improbable that a substantial loss of muscle tissue occurred in the more proximal muscles (knee muscles) of the DN subjects from the present study (who did not suffer from severe neuropathy). Andersen et al. (1997) demonstrated in a well conducted study a distal-to-proximal gradient of muscle atrophy (measured with MRI) from cross sections in the proximal lower leg, mid lower leg, and distal lower leg in DN subjects. Furthermore, Andersen et al. (1997) found that muscle area in the proximal lower leg is unchanged in DN subjects with severe neuropathy. Therefore, it appears that there must be another explanation for the lack of exercise-induced changes in strength levels in the present study. Next, the neuromuscular adaptations that were expected from the resistance training programme used in the present investigation will be explained. Additionally, theoretical reasons why adaptations may have been elicited in the present study with DN subjects will be proposed.

It is well documented that gains in muscle strength following a resistance training programme occur from neural and muscular adaptations. Thus, neural factors account for the majority of the strength gains over the first 5 weeks of workouts. Thereafter, muscle fibre adaptations become progressively more important to strength improvement

(McArdle et al., 2010). It is noteworthy that the present investigation started with very low workloads, which slowly and progressively increased throughout the 16 weeks of the programme. It is likely that the workloads targeted during the first few weeks (3-4 weeks) might not have reached a sufficient threshold for adaptations to occur. However, it was considered very important to improve participant's confidence towards the programme and minimize the discomfort associated with the first workouts in sedentary people (delayed-onset muscle soreness). Nevertheless, the exercise programme had a sufficient duration to produce both neural and muscular adaptations so it is unlikely that the lack of strength gains can be associated to the characteristics of the intervention.

Changes associated with neural adaptations result in more efficient recruitment of motor units and increase in their firing rate (McArdle, 2010). Thus, the learned recruitment of additional motor units, which may respond in a synchronous (the coincident timing of impulses from 2 or more motor units) fashion may contribute to enhance the muscle's ability to generate more force without changes in the cross-sectional area of the muscle. Motor neuropathy is characterized by loss of motor units and a compensatory collateral reinnervation to preserve muscle strength (Andersen et al., 1998; Fleckenstein, 1993) Therefore, it is likely that a reduction in the number of motor units available secondary to DN may limit the ability of the muscle to recruit simultaneously different motor units to increase muscle force. This obviously may result in less strength gains via neural adaptations in this population. Increases in muscular strength following a resistance training programme are also associated with changes in the cross-sectional area of the muscle. The fact that in the present study BMI did not change significantly following the intervention whereas body fat percentage was significantly reduced suggests increases in muscle mass. However, unexpectedly, those changes in body composition did not relate to gains in muscular strength. A limitation of the study is that a muscular cross-sectional area was not determined. Studies with other musculo-skeletal conditions have reported impairments in neuromuscular transmission or abnormal contractile properties in reinnervated muscle fibres (Dengler et al., 1990), which leads to the assumption that similar abnormalities might occur in DN subjects. Thus, more studies are needed on DN subjects to better understand the underlying factors limiting their ability to improve muscular function after 16 weeks of high-intensity resistance training.

It should be noted that strength gains are related to the specificity of the training exercises (McArdle et al., 2010). Thus, an isometrically trained muscle shows greatest strength improvement when measured isometrically. The same principle applies to muscles trained during dynamic conditions, where the greatest improvements in strength will be observed when measured dynamically. It is worth mentioning that the training programme was based on dynamic exercises whereas strength measurements were carried out isometrically. It is possible that this limitation in the methodology of the present study could have affected the sensitivity of the measurements to identify strength changes following the exercise programme. Thus, it is important that future studies measure strength levels taking into account the specificity of strength-training responses.

In summary, the present study has demonstrated for the first time that 16 weeks of strengthening exercises did not produce strength gains in any of the muscle groups under investigation in the DN group. However, it is unclear why DN subjects in the present study did not improve strength levels following the exercise programme. On the one hand, it is possible that muscular atrophy secondary to motor neuropathy may have resulted in reduced neural and muscular adaptations to the exercise programme. On the other hand, impairments in neuromuscular transmission or abnormal contractile properties in reinnervated muscles, as demonstrated in other neuro-skeletal conditions could have also contributed to the results. It is noteworthy that the sample size in the present investigation did not reach the numbers warranted by the power calculation. Consequently this may have affected the power of the study. In addition, strength levels were assessed isometrically whereas the exercise programme was based on dynamic exercises. This could also have affected the sensitivity of the measurements to identify strength changes following the exercise programme. Thus, further studies are required to draw any final conclusion about the muscular adaptability of patients with DN to strength training programmes. Beside, changes in outcome measures related to DN, it was also very important to assess whether PA could influence glucose control in DN patients. Results on the effect of the intervention on HbA_{1c} values are discussed.

7.2.1.3 HbA_{1c}

The current investigations did not find that 16 weeks of strengthening and mobility exercises carried out twice weekly changed HbA_{1c} levels in DN subjects. It is well established that physical activity can improve glucose control in patients with type 2 diabetes, which obviously is one of the main targets of any health-related intervention in this populations. A meta-analysis carried out by Thomas et al. (2006), which included 13 studies, reported a clinically significant reduction in HbA_{1c} when the exercise group (N=185) was compared to the non exercise group (N=176). In addition, numerous investigations have demonstrated the positive effect of strength training to lower HbA_{1c} in individuals with type 2 diabetes (Castaneda, et al.2002; Cohen et al., 2008; Dunstan et al., 2002). It has been proposed that PA interventions may increase the availability of the protein glucose transporter type 4 (GLUT4) (insulin-responsive glucose transporter) and enhance the glucose transport into the muscle (Holten et al., 2004).

Contrary to these findings in subjects with type 2 diabetes, the PA programme in the present study did not seem to produce significant changes in HbA_{1c} levels in DN patients. It should be noted that Hb HbA_{1c} A1c levels decreased in the EXE group by -0.3% from baseline to post-intervention values which is comparable with other investigations which used similar exercise programmes (Cohen et al., 2008; Dunstan et al., 2008). However, similar HbA_{1c} reductions were observed in the CON group, which explains why group differences were not significant in the present study. In the current study, it was not attempted to minimize hypoglycaemic medication changes, which could have resulted in substantial changes in hypoglycaemic medication in the CON group. It is noteworthy none of the subjects from the exercise group changed medications during the 16 weeks that the programme lasted. However, no information was recalled in the present study about changes in hypoglycaemic medications in the CON group. It is therefore possible that medication changes could at least partly explain the improvement in HbA_{1c} levels observed in the CON groups over time. It is possible that other factors may have also contributed to the lack of significant changes in HbA_{1c} levels following the interventions in the present study. These are discussed.

Some investigations have found that exercise-induced improvements in glycaemic control were greater among persons with higher baseline HbA_{1c} (Dunstan et al., 2008;

Sigal et al., 2007). Sigal and colleagues (2007), who carried out an interventional study with 251 participants, found that decreases in HbA_{1c} levels were greatest for participants with a baseline HbA_{1c} higher than 7.5%. The subjects in the present study who participated in the intervention had a baseline HbA_{1c} of 7.61± 1.06 %, which is relatively low and may explain why participants in the current study were less sensitive to HbA_{1c} changes compared to other investigations with comparable exercise programmes and higher baseline HbA_{1c} levels (Castaneda et al., 2002; Cauza et al., 2005; Dunstan et al., 2002). In support of this argument, other investigations which reported low HbA_{1c} baseline values failed to find exercise-induced changes in HbA_{1c} (Ibañez et al., 2005; Middlebrooke, et al., 2006)

Another reason for the lack of effect of the present intervention on HbA_{1c} levels could be the fact that the frequency of the gym based training sessions was twice per week. The effect of a single bout of exercise on insulin sensitivity lasts 24-72 hours depending on the duration and intensity of the activity (Wallberg-Henriksoon et al., 1998). Because the duration of increased insulin sensitivity is generally no longer than 72 hours, physical activity guidelines for subjects with type 2 diabetes recommend that there should not be more than 2 consecutive days without exercise training (at least 3 times per week) (Sigal et al., 2006). It is therefore likely that 2 sessions per week of controlled exercises were not enough to reduce HbA_{1c} in the present study in DN subjects with low baseline HbA_{1c} levels.

Overall, the present investigation found that 16 weeks of twice weekly strengthening and mobility exercises did not produce significant changes in HbA_{1c} levels in DN. It is likely that factors such as low baseline HbA_{1c} levels, changes in hypoglycaemic medications or the weekly frequency of the sessions may explain the results from the present investigation. However, it is noteworthy that this is the first investigation assessing HbA_{1c} changes following a PA programme in DN subjects so it cannot be ruled out that DN subjects are less sensitive to HbA_{1c} changes compared to individuals with diabetes and without neuropathy. Further studies are warranted to determine whether PA programmes trigger comparable effects on HbA_{1c} levels in diabetic patients with and without neuropathy. Beside the effect of PA on diabetes related outcome measures such as sensory neuropathy, motor neuropathy and HbA_{1c} levels, which are discussed above, it is also important to determine the influence of the intervention on risk factors associated with cardiovascular diseases.The next section discusses the

results on the effect of the physical activity programme on traditional cardiovascular risk factors.

7.2.1.4 Traditional cardiovascular risk factors

Results from the present investigation demonstrated that 16 weeks of strengthening and mobility exercises improved some of the abnormalities associated with the metabolic syndrome: namely body composition and hypertension in DN subjects. Thus the intervention triggered beneficial effects on obesity in the form of body fat percentage and systolic blood pressure. However, cholesterol levels in the form of TC, HDL and LDL did not change following the intervention. Results on body composition are discussed first, followed by the results on blood pressure and cholesterol levels.

It is well known that obesity is a common cause of type 2 diabetes and that weight loss should play an important role in the management of this condition. In addition, obesity has been linked with other metabolic disorders associated with type 2 diabetes such as dyslipidemias and hypertension (Krause et al., 1998; Narkiewicz, 2006) as well as with microcirculatory problems (Caballero, 2003). The current intervention, in agreement with previous reports on subjects with type 2 diabetes (Castaneda et al., 2002; Church et al., 2010; Dunstan et al., 2006; Ibañez et al., 2005) showed that resistance training can change body composition in DN subjects by reducing body fat percentage. As to the reasons linked to these losses of fat mass, most investigations have demonstrated an increase in resting metabolic rate in older individuals in response to strength training (Hurley & Roth, 2000) This is mainly explained (60-70% of the inter-individual variability) by training-induced gains in fat-free mass (Tataranni & Ravussin, 1995). Unfortunately, muscle mass was not measured in the present study and it is unknown whether resistance exercises may produce similar adaptations in diabetic subjects with neuropathic complications. However, the fact that only changes in body fat percentage and not body mass reached significant levels suggests an increase in lean mass following the 16 weeks intervention.

In addition to changes in body composition, the present study also demonstrated that 16 weeks of strength training can lower systolic pressure in DN patients. Moreover, the magnitude of the changes (-7 mmHg) agrees with previous investigations with comparable resistance training programmes and population groups (age and baseline

blood pressure) (Castaneda et al., 2002; Cohen et al., 2008; Dunstan et al., 2002). In contrast, other investigations did not find changes in blood pressure in response to strengthening programmes (Church et al., 2010; Dunstan et al., 2006; Sigal et al., 2007). It is well known that changes in blood pressure due to physical activity programmes are dependent on baseline blood pressure levels. Thus, higher baseline blood pressure has been associated with larger training-induced changes (Lesniak & Dubbert, 2001). Most subjects with type 2 diabetes take antihypertensive medications, which explain 1) why most of the investigations assessing the effect of a PA programme on patients with type 2 diabetes have reported baseline blood pressure values within normal range and 2) why some investigations failed to find changes in blood pressure secondary to resistance training interventions. Studies carried out on diabetic patients that found changes in systolic blood pressure following a resistance training programme also reported higher baseline systolic blood pressure (>142 mmHg) (Castaneda et al., 2002; Dunstan et al., 2002). The studies that did not find changes in systolic blood pressure reported lower baseline blood pressure values (<137 mmHg) (Church et al., 2010; Dunstan et al., 2006; Sigal et al., 2007) . Baseline systolic blood pressure values in the present investigation were 142± 11.25 mmHg, which agree with the idea that strengthening exercise are efficient to reduce systolic blood pressure in diabetic subjects with high baseline blood pressure levels. This justification may also explain why the present intervention did not produce significant changes in diastolic blood pressure, which was within normal values in the DN individuals in the EXE group (baseline 80.90± 7.43).The possible mechanisms responsible for the decrease in systolic blood pressure in the present investigation are discussed next.

Exercise-induced changes in the endothelial function, due to an increased NO release, is potentially an important exercise-related hypotensive mechanism (O´Sullivan & Bell, 2000). Previous investigations have demonstrated that resistance training can improve endothelium function (assessed by measuring vasodilatory responses to iontophoretic application of acetylcholine) in individuals with type 2 diabetes (Cohen et al., 2008). Results from the present investigation show a tendency toward greater exercise-induced vasodilatation in the EXE group compared to the CON group following the intervention, which is likely to be due to changes in the endothelial function. However this did not reach significant levels, suggesting that other mechanisms in addition to endothelial function are likely to explain these changes in BP.

Obesity has been consistently associated with hypertension (Narkiewicz, 2006). Based on population studies, risk estimates indicate that at least two-thirds of prevalence of hypertension can be directly attributed to obesity (Krause et al., 1998). The precise mechanism linking obesity to hypertension is not fully understood. However, it is thought that obesity may lead to hypertension by activating the rennin-angiotensin-aldosterone system (Engeli & Sharma, 2000), which is a hormone system that regulates blood pressure and water balance. It is therefore possible that the presumed changes in body composition in the current study following the exercise programme may have led to changes in systolic blood pressure. In support of this idea, several investigations have reported weight reduction to be an efficient therapy to reduce blood pressure in hypertensive subjects (Goldstein, 1992). However, it is not known for certain whether changes in body composition are the cause for the changes in systolic blood pressure in the present investigation.

In addition to this, adaptations in the ANS have also been proposed as a possible mechanism leading to lower blood pressure values. It is believed that an attenuation of sympathetic drive (responsible for increasing HR) and enhancement of the vagal tone (responsible for lowering HR) secondary to physical training may lead to positive for health changes in BP (Lesniak et al., 2001). Physical activity has been shown to modify the sympathico-vagal regulation of the HR in various populations including, healthy individuals (Pichot et al., 2002), cardiac patients (Tygesen et al., 2001), type 2 diabetes patients (Loimaala et al., 2003) as well as diabetes patients with different degrees of autonomic neuropathy (Howorka et al., 1997). In the present study, resting HR was significantly reduced after the training programme, which might suggest adaptations in the ANS. However, the vast majority of studies investigating the effect of exercise training on the ANS have used aerobic exercises, whereas it is unknown whether resistance training could trigger comparable adaptations. Another explanation of the reduced resting HR secondary to the exercise programme could be that the intervention increased stroke volume (volume of blood pumped by the heart with each beat) due to changes in the left ventricle (Tortora & Derrickson, 2006). However, it is very unlikely that a strength training programme can increase stroke volume, unlike aerobic training. Resistance training augments the thickness of the cardiac wall, which results in a more forceful contraction (which does not necessarily influence the volume of blood ejected from the heart in each contraction). Whereas aerobic training enhances the elasticity of the cardiac walls leading to greater expansion and therefore increased stroke volume

(McArdle et al., 2010). It is therefore likely that adaptations in the ANS secondary to the exercise programme may have led to BP changes in the present study. However, further studies are needed in DN subjects to demonstrate whether resistance training can alter ANS function in this population.

Overall, results from the present investigation demonstrated that strength training can lower systolic blood pressure in DN subjects. Although, the exact mechanism/s behind this change in the present study is/are unknown it is possible that changes in body composition and in the ANS may have contributed to lower BP. The fact that diastolic blood pressure, which was within normal levels at baseline, did not change following the intervention, suggests that changes in blood pressure due to PA programmes are dependent on baseline blood pressure levels.

Contrary to the beneficial effect of the exercise programme on body fat percentage and blood pressure, cholesterol levels in the form of TC levels, LDL and HDL were unchanged in the present study. In support of the results from the present study a meta-analysis carried out by Thomas et al. (2006) demonstrated that PA interventions do not appear to produce substantial changes in cholesterol levels in individuals with type 2 diabetes. It is noteworthy that the majority of the participants in the present study were taking anti-cholesterol drugs, which explains why all the variables were within normal range (HDL, LDL and TC). Normal baseline values may also explain that our participants were not sensitive to changes in the lipoprotein profiles.

Summary of general health

Overall, the present investigation demonstrated for the first time that 16 weeks of a PA programme based on strengthening and mobility exercises triggered positive effects on different aspects of health in DN subjects including sensory neuropathy, blood pressure and body composition. On the other hand, the present intervention did not appear to produce substantial changes in strength levels, glucose control and cholesterol levels. The most striking finding among all is that sensory neuropathy was improved following the exercise programme. Although the exact mechanism/s behind this finding is/are still not clear it is possible that exercise-induced improvements in endothelial and/or mitochondrial function could be responsible for this unexpected finding. Unfortunately,

changes in sensory neuropathy were not followed by changes in motor neuropathy. Thus, the present investigation found that 16 weeks of strengthening exercises did not improve strength levels in patients with DN. Although the reasons why DN subjects in the present investigation did not gain strength levels are unknown, it is likely that muscular atrophy secondary to motor neuropathy may have resulted in reduced neural and muscular adaptations to the exercise programme. However, further investigations with larger sample sizes are warranted before drawing any final conclusion about the muscular responses to resistance training in patients with DN.

In addition to health related outcome measures associated with DN the present study also investigated the effect of PA on HbA_{1c} and cardiovascular risk factors in DN subjects. Contrary to previous investigations with subjects with type 2 diabetes the present study found that twice weekly 16 weeks of strengthening and foot mobility exercises did not produce changes in HbA_{1c} level in subjects with DN. It is likely that factors such as low baseline HbA_{1c} levels, changes in hypoglycaemic medications or the frequency of the gym-based sessions may explain the results from the present investigation. However, it cannot be ruled out that DN subjects are less sensitive to HbA_{1c} changes via a PA programme compared to individuals with type 2 diabetes. Further studies are required to determine whether PA programmes produce comparable effects on HbA_{1c} levels in type 2 diabetic individuals with and without peripheral neuropathy. In addition to this, results from the present investigation demonstrated that strength training can reduce obesity and blood pressure in DN subjects.
The alterations in general health discussed are associated with multiple health problems in subjects with DN including neuropathic complications or cardiovascular diseases. Another major problem linked to DN subjects is foot ulceration. It is therefore essential to investigate the effect of PA, not only on neuropathy, glucose levels and traditional cardiovascular risk factors but also on the factors associated with the increased risk of foot ulcers in DN subjects. It seems clear that a combination of gait biomechanics and microcirculatory changes are responsible for the increased risk of foot ulcerations observed in subjects with DN. Results from the effect of the intervention on both gait biomechanics and microcirculation will be discussed.

7.2.2 Gait biomechanics

Changes in gait characteristics are considered a common mechanism by which tissue damage may occur in diabetes patients with peripheral neuropathy (Frykberg et al., 1998; Guldemond et al., 2006). Moreover, it has been hypothesized that therapies aiming to improve joint mobility and muscle weakness in DN subjects could improve gait in this population and consequently reduce foot pressures during walking (Herriott et al., 2004; Akashi et al., 2008). In contrast to this idea, the present study demonstrated for the first time that 16 weeks of strengthening and foot mobility exercises did not produce substantial changes in the gait characteristics of DN subjects including all the gait parameters, except the velocity of the COP at the heel, foot pressures and muscle activation patterns. It should also be noted that the intervention programme did not adversely affect gait in the DN subject, which suggests that strengthening exercises do not increase the risk of foot problems in this population. However, these results should be interpreted with caution since the sample size in the present study was smaller than targeted by the power calculation. This may have affected the power of the study and therefore compromised the interpretation of the results. Discussion of results on the effect of the PA intervention on gait biomechanics is discussed as follows: 1) gait parameters in the form of spatial-temporal and COP parameters; 2) foot pressures and; 3) muscular activity patterns.

Studies on different clinical populations that suffer from muscular weakness including elderly people (Rubenstein et al., 2000), have shown that interventions that improve neuromuscular performance are efficient to modify spatial-temporal gait characteristics (i.e. walking speed or step length). In agreement with this, Brandon et al. (2003) reported that improvements in lower extremity strength levels following 6 months of training were positively associated with mobility performance in subjects with type 2 diabetes (i.e. walking speed). Interestingly, similar results have been obtained in diabetic patients with neuropathic complications. For instance, Allet et al. (2010) found that a resistance based exercise programme improved walking speed in DN patients when comparing pre- and post-intervention data. Contrary to this, the present intervention based on strengthening and foot mobility exercises did not appear to modify spatial-temporal characteristics in DN subjects with moderate neuropathy. It is noteworthy that all the studies presented above reported significant strength gains following the exercise programme, unlike the current study. This suggests that changes

in strength levels are most likely responsible for changes in spatial-temporal characteristics in populations with muscular weakness.

It is generally believed that sensory neuropathy is associated with instability in DN subjects resulting in slower and safer gait in this population (Simoneau et al., 1994; Simmons et al., 1997b). However, in the present study improvements in sensory neuropathy did not result in changes in any spatial-temporal characteristics. It has been hypothesized that instability in DN subjects may be the result of a loss of peripheral sensory receptor function in the lower legs and cannot be attributed exclusively to loss of plantar cutaneous sensation (van Deursen et al., 1999). It is noteworthy that in the cross-sectional study, plantar cutaneous sensation was only correlated to step times whilst it was not significantly correlated to gait velocity, cadence or step length. This suggests that in the present investigation cutaneous sensation was not likely to be the main factor responsible for the alterations in spatial-temporal characteristics observed in the DN group. This may partly explain why improvements in VPT did not influence spatial-temporal in the current investigation. In line with the results from the current study, Eils et al. (2004) and Hohne et al. (2008) investigated changes in gait characteristics following a reduction in the plantar cutaneous sensation in healthy individuals. They found no differences in spatial-temporal characteristics between the control and the reduced sensation conditions. This finding further supports the idea that cutaneous sensation does not appear the main factor responsible for changes in spatial-temporal characteristics. Another reason for this lack of association between sensory neuropathy and spatial-temporal characteristics could be that the DN patients in this study had been living with significant losses of peripheral sensation for many years. Therefore, the gait patterns of these patients reflect not only the effects of the neuropathy itself, but also of any locomotor control strategies these subjects had developed over the years to compensate for their sensory loss. It is therefore likely that changes in gait characteristics in relation to this intervention (if any) may happen beyond the duration of this study.

In line with the results discussed above in relation to spatial-temporal characteristics, the present intervention did not seem to bring about changes in the foot-floor interaction variables, except for the velocity of the COP at the heel. It was hypothesized that strength gains in the dorsi-flexor and plantar-flexor muscles and increase ROM of the ankle joints might augment the distance travelled by the COP during the stance phase,

which has been proposed to potentially reduce stress on the foot plantar surface (Giacomozzi et al., 2002). However, the fact that no significant strength gains were observed in the current study following the intervention may explain the lack of changes in the foot-floor interaction variables. An important limitation of this investigation is that, due to technical difficulties, ROM data could not be analyzed. Therefore, it is impossible to determine whether changes in ROM did not alter loading patterns or whether floor-foot interaction changes were not observed due to lack of alterations in ankle mobility as well as the lack of strength changes.

Interestingly, the present study found a reduction in the velocity of the COP at the heel following the exercise programme. It is generally believed that weakness of the dorsi-flexor muscles causes the foot to reach the foot flat stage quicker and in a less controlled manner, which may lead to increased pressures and longer contact times of the metatarsal heads with the ground in this population compared to healthy individuals (Abboud et al., 2000; Bevans, 1992). Therefore, it is possible to speculate that if changes in DF strength following a resistance training programme can be elicited more effectively this may result in a slower and more controlled drop of the forefoot to the ground after the heel strike. Results from the present study did not show significant changes in strength levels in any muscle group following the intervention. In addition to this, there was a tendency toward higher pressures at the heel (PP and PTI) in the EXE group despite no changes in gait velocity, which can theoretically be associated to changes in the DF muscles. Further research into effective strengthening of DF is however needed before conclusions can be drawn. Furthermore, the present study failed to demonstrate that changes in the foot drop patterns may result in changes in the loading patterns of the metatarsal region. Thus pressures at the metatarsal region, both PP and PTI, were unchanged following the exercise programme despite a smother foot drop in the EXE group, as demonstrated by the reduced velocity of the COP at the heel. This finding suggests that interventions to improve heel strike motor control (i.e. strengthening dorsi-flexor muscles) in DN subjects are likely not to alter foot pressures on the forefoot regions in contrast to previously hypothesized (Abboud et al., 2000, Bevans, 1992).

EMG data also suggests that the 16 weeks of strengthening exercises did not substantially influence muscular activity patterns in DN subjects, which agrees with the lack of changes in gait parameters and foot pressures presented above. However, it is

noteworthy that there is a trend toward higher TA activity in the EXE group over time during the loading phase of the GC compared to the CON group. It is therefore possible that the above-mentioned changes in the velocity of the COP at the heel could be due to a higher TA activity during the loading phase over time in the EXE group and not necessary due to strength gains secondary to the resistance training programme. Eils et al. (2004) and Nurse & Nigg (2001) investigated the effect of changes in plantar foot sensation via ice immersion on EMG activity patterns in healthy individuals. Both studies found a significant reduction in TA activity for the period immediately after the heel strike when the iced condition was compared to the normal condition. The central nervous system relies on sensory input from muscle and cutaneous receptors in the lower extremities to generate effective motor patterns for human posture and locomotion (Gandevia & Burke, 1992). Feedback originating from these receptors provides a constant source of information regarding loading, joint kinematics, and pressure distribution on the plantar surface of the foot (van Deursen, 1998a, Nurse & Nigg, 2001). Since, the present intervention appeared to significantly improve sensation, it is possible that the trend toward changes in TA activity are secondary to improvements in sensory neuropathy. However, the fact that changes in TA activity only reached tendency levels ($p<0.1$) suggests that this interpretation should be taken with caution. Since neuromuscular control strategies develop over the years, it is possible that changes in muscular activity patterns in relation to improvements in sensation (if any) may become more evident beyond duration of this study. However, further studies are required to draw any conclusion on the effect of improvements in cutaneous sensation neuropathy in EMG activation patterns in DN subjects.

Overall, the present investigation found that a physical activity programme based on strengthening and foot mobility exercises did not lead to substantial changes in gait characteristics in DN subjects. Previous investigations on different clinical populations that suffer from muscular weakness (Rubenstein et al., 2000) demonstrated that increases in strength levels can modify spatial-temporal characteristics. The fact that the present investigation did not manage to change strength levels is likely to explain the lack of changes in spatial-temporal characteristics. Interestingly, improvements in sensory neuropathy secondary to the intervention did not appear to change gait characteristics substantially. It is believed that sensory neuropathy is the single most important factor explaining gait alterations in DN subjects (Frykberg et al., 1998; Payne et al., 2002). However, gait parameters, foot pressures and muscular activity patterns

during gait in the present investigation were mostly unchanged despite improvements in sensory neuropathy. The DN in the present study had been living with significant losses of sensation for many years. Therefore, it is likely that improvements in sensation may take a long time before any adaptation in gait occurs (if any). This could partly explain the lack of association between changes in sensory neuropathy and gait in the present study. On the other hand, the present study demonstrated that a PA intervention did not negatively affect gait in DN subjects, which suggests that strengthening exercises do not increase the risk of foot problems in this population. Beside gait characteristics, microcirculation is also known to play an important role in the development of foot ulcers in DN subjects. Therefore, it is important to investigate whether PA can influence microcirculation in DN subjects. Results from the effect of the intervention on microcirculatory parameters are discussed next.

7.2.3 Microcirculation

This study has investigated for the first time the effect of an exercise training programme on: 1) resting microcirculation; 2) microcirculatory responses to an exercise bout in individuals with type 2 diabetic neuropathy; and 3) recovery of the microcirculation from an exercise bout. Thus, the present investigation found that 16 weeks of strengthening and foot mobility exercises: 1) did not change microcirculation during resting conditions in the form of BF and mVO_2; 2) showed a trend toward higher vasodilatory responses to the exercise bout following the exercise programme whilst did not change mVO_2 responses to the exercise bout; 3) improved significantly mVO_2 recovery from the exercise bout whilst did not change BF recovery. Results from the effect of intervention on the microcirculation are discussed in the following order: 1) during resting conditions; 2) in response to the exercise bout; and 3) recovery from the exercise bout.

7.2.3.1 Microcirculation at rest: BF and mVO_2

Results from the present investigation show that 16 weeks of resistance and foot mobility exercises did not influence resting microcirculation in the form of capillary blood flow and oxygen consumption. In line with the results from the present study, previous investigations with type 2 diabetic patients did not find changes in baseline

blood flow following either an aerobic exercise programme (Colberg et al., 2005) or a resistance training programme (Colberg et al., 2006). Regarding changes in baseline mVO_2 values following an exercise programme, it appears this is the first investigation reporting mVO_2 values before and after a physical activity intervention in patients with type 2 diabetes.

7.2.3.2 Microcirculatory responses to the exercise bout

The present investigation found a trend toward greater vasodilatory responses in the exercise group following the intervention. Furthermore, 16 weeks of strength training did not seem to influence the amount of oxygen consumed by the muscle in response to an exercise bout.

With respect to changes in blood flow responses to an exercise bout, this study showed that exercise-induced vasodilatation was enhanced after 16 weeks of resistance training in DN subjects. The beneficial effects of an exercise programme on vascular function probably relates to increasing flow and shear stress on the endothelium, which may stimulate the release of NO and, in turn, improve endothelial function (Higashi & Yoshizumi, 2004; Goto et al., 2003). However, in the present study changes in exercise-induced vasodilatory capacity did not reach significance and were limited to a trend ($p<0.1$). Cohen et al. (2008) demonstrated that a 14-months strength training programme improved vascular responses in 16 adults with type 2 diabetes. Interestingly, Cohen and colleagues (2008) measured vascular function at 2 months and 14 months (end of the exercise programme), and they found that improvements in both endothelial function and smooth muscle responsiveness were significant after 14 months but not at 2 months. It should be noted that the exercise programme and the population age (mean age 60 years) in their study were very similar to the present investigation, which allows the comparison between both studies. Results from Cohen´s study suggest that the duration of the intervention may explain why Cohen et al. (2008) found significant changes in the vascular function in their study while the results in the present investigation only showed a trend.

In agreement with this interpretation, Colberg et al. (2006), who carried out a interventional study for only 8 weeks, found that a resistance training programme of this length was not sufficient to improve the responsiveness of cutaneous perfusion to

local heating in healthy as well as in subjects with type 2 diabetes. The same results were observed by the same investigator when assessing changes in cutaneous perfusion to local heating following 10 weeks of aerobic training in individuals with type 2 diabetes (Colberg et al., 2005). Overall, these results suggest that there may be a correlation between the duration of the exercise programme and vasodilatory changes in type 2 diabetic patients, and that an intervention longer than 16 weeks may be necessary for significant exercise-induced changes in the microcirculation. It should be noted there are methodological differences between the studies presented above and the present investigation, which should be taken into consideration. Firstly, the present study assessed vasodilatory responses in the skeletal muscle while Colberg' studies (Colberg et al., 2005; Colberg et al., 2006) and Cohen et al. (2008) measured cutaneous microcirculation. Secondly, the current study investigated vasodilatory responses to an exercise bout while Colberg and Cohen et al. (2008) examined vasodilatory responses to local heating and iontophoresis of acetylcholine and sodium nitroprussie, respectively.

Although the duration of the intervention appears to be important to find vascular changes, Maiorana et al. (2001) found an increased vascular response to infusion of acetylcholine in subjects with type 2 diabetes following an 8-week training programme. Moreover, the magnitude of improvements was similar to the changes reported by Cohen et al. (2008) after 14 months. There are a few methodological differences between Maiorana's and both the present and Cohen's study. Firstly, Maiorana and colleagues (2001) used a combined aerobic and resistance exercise training compared to the other studies in which only strengthening exercises were used. A combination of aerobic strengthening exercise is known to reduce HbA_{1c} more than either aerobic or anaerobic training alone (Church et al. 2010; Dunstan et al., 2008; Sigal et al., 2007) and HbA_{1c} has been linked to vascular function. It is believed that oxidative stress, which is aggravated by hyperglycaemia, diminishes NO availability affecting the endothelium-dependent function (Schramm et al., 2006; Taddei et al., 1998). This could be a possible mechanism by which a combination of different types of exercises may trigger greater changes in the endothelial function than aerobic or resistance exercises alone. However, more investigations are required to identify whether a combination of exercises may trigger further changes in the vasculature compared to either type of exercises alone. Secondly, Maiorana's participants were significantly younger (mean 52 years of age) than in Cohen et al. (2008) or in the present study, and

age is known to diminish physiological adaptations (ACSM, 2000). Thirdly, Maiorana et al. (2001) measured vascular responses on the macrovasculature. Hamdy et al. (2003) provided evidence that there may be differential effects of exercise on the macro- and microcirculation. Thus, they found that flow-mediated dilation of the brachial artery (measured after 5 minutes of arterial occlusion with a high-resolution vascular ultrasound) was significantly enhanced following a PA intervention while the microvascular reactivity after iontophoresis of acetylcholine (measured with laser Doppler) was unchanged. These results suggest that the microcirculation may be less sensitive to exercise-induced changes than the macrocirculation, which may explain the greater changes in vascular responses reported by Maiorana et al. (2001).

Overall, evidence presented above on subjects with type 2 diabetes suggests that strength training is likely to influence microcirculation in DN subjects and that the length of the intervention in the present study may not have been sufficient for significant changes to be found. This could explain why in the current study improvements in the vasodilatory capacity in response to an exercise bout did not reach significant levels and were limited to a trend ($p<0.1$). This could also be influenced by the fact sample size in the present investigation did not reach the numbers determined by the power calculation. Thus, further investigations with larger numbers are warranted to draw any final conclusions on the effect of strengthening exercises on DN subjects.

Results from the present study also show that a 16-week resistance programme did not promote any changes in the amount of oxygen consumed by the muscle following an exercise bout. This is not a surprising finding since the physical activity programme was based on strengthening exercise. Thus, it is well established that aerobic exercises are more efficient to improve muscular oxidative capacity by increasing the number of mitochondria, the proportion of type II muscle fibres or oxidative enzymes (McArdle et al., 2010).

7.2.3.3 Microcirculatory recovery from the exercise bout

This study investigated for the first time whether a resistance programme may bring about changes not only in the post-exercise microcirculatory responses but also in the recovery phase in DN subjects. Results from the present investigations show that 16

weeks of strengthening and foot mobility exercises did not influence BF recovery whilst improved significantly mVO_2 recovery. The fact the current intervention did not have a significant effect on BF recovery is consistent with the lack of substantial post-exercise changes in the microcirculation (BF and mVO_2). On the other hand, the observation that mVO_2 returned to baseline values significantly quicker in the EXE group compared to the CON after the exercise programme is quite important and was an unanticipated finding. Although the exact mechanism behind this finding is unknown, it appears that the intervention resulted in changes not in the capacity of the muscle to take up oxygen (i.e. changes in the muscle fibre type), but in the efficiency of its usage. It is therefore possible that exercise-induced mitochondrial changes may explain the results observed in the present study. This option is discussed next.

Physical activity programmes have been proven to improve not only overall oxygen transport (circulation) or oxygen uptake capacity, but also the capacity of the skeletal muscle to produce energy (ATP) through the aerobic system (oxidative phosphorylation) (McArdle et al., 2010). Starritt et al. (1999) assessed the in vitro mitochondrial ATP production rate in 7 untrained healthy volunteers, who participated in a 10-day cycle exercise training programme. Resting muscle samples were obtained from VL before and after 5 and 10 days of training. They indicated that mitochondrial ATP production rate measured by a bioluminescence technique can rapidly increase in response to endurance training. Similarly, in vivo studies have used phosphorus magnetic resonance spectroscopy (P-MRS) to show higher ATP production rate in a variety of muscles of trained compared to untrained healthy humans (Larsen et al., 2009) and in response to endurance training of specific muscles (Forbes et al,. 2008). Evidence presented above demonstrates that exercise training can influence mitochondrial function in healthy individuals and consequently produce energy more efficiently. Furthermore, early investigations have reported beneficial changes in the muscle mitochondria function in subjects type 2 diabetes following a physical activity programme (determined via skeletal muscle biopsies) (Hey-Mogensen et al., 2010; Toledo et al., 2007). These findings suggest that changes in the mitochondrial function secondary to physical activity training may lead to a more efficient use of oxygen in the skeletal muscle of type 2 diabetic patients (higher ATP production rate). This could explain the quicker mVO_2 recovery observed in the EXE group after the intervention.

It is noteworthy that all the studies presented above used aerobic exercises for the training programme. Aerobic exercises are well known to trigger physiological adaptation in the aerobic system (McArdle et al., 2010) whereas the current intervention was based on strengthening exercises. It is generally believed that aerobic fitness is reduced considerably in diabetic patients compared to healthy individuals (Ozdirenç et al., 2003; Regensteiner et al., 1998). Therefore, it is possible that a physical activity programme that is not specifically designed to enhance the aerobic system, may reach the minimal threshold stimulus to improve aerobic fitness in this population. In the present study every training session began with a 5-10 minutes warm up period at an increasing intensity on an aerobic machine such as bicycle or cross-trainer. It is therefore possible that the duration and intensity of these exercise bouts were sufficient to trigger physiological adaptations in the aerobic system in the DN subjects who participated in the present study. Consistent with this idea, Colberg et al. (2006) demonstrated improvements in maximal oxygen capacity (VO_{2max}) in a group of patients with type 2 diabetes after a 8-weeks resistance training programme. It is also possible that the intervention may have had an effect on the participant´s daily activities. This could result in increased daily physical activity as part of lifestyle changes and consequently higher fitness levels. It is noteworthy that the intervention resulted in significant improvements in vitality as measured with the QOL questionnaire. Therefore it is possible that if subjects showed an improvement in vitality levels this may increase their capacity to lead to a more active life style, which may in turn lead to changes in aerobic capacity. A limitation of the present study is that aerobic capacity was not determined, and therefore it is not known whether improvements in mVO_2 recovery are secondary to alterations in the aerobic capacity or other mechanisms may be responsible for these changes. Nevertheless, results from the present study show for the first time that strength training improves exercise-induced reoxygenation in DN subjects, likely due to changes in the mitochondria.

Overall, results from the present study demonstrated for the first time that 16 weeks of strength training can influence microcirculatory in DN subjects. The most striking and unexpected finding was that a strength training programme had a significant effect on the mVO_2 recovery. Since the exercise programme did not promote changes in the amount of oxygen consumed by the muscle following the exercise bout, it appears that the intervention resulted in changes not in the capacity of the muscle to take up oxygen but in the efficiency of its usage. Changes in the mitochondrial function secondary to

the exercise programme are likely to explain the results in the current study. Furthermore, the present study showed that exercise-induced vasodilatation was enhanced after 16 weeks of resistance training. However, this change did not reach significance and was limited to a trend. It is possible that the length of the intervention or issues related to the power of the study may partly explain why this change did not reach significant levels. Although gait and microcirculation represent critical outcomes in DN patients due to their link to foot problems, health-related quality of life outcomes are also important. The effect of the PA programme on self-reported QOL will be discussed next.

7.2.4 QOL

QOL represents an important goal for health care professionals and it has been associated with adverse outcomes in people with type 2 diabetes, including poor response to therapy, disease progression and even mortality (Ali et al., 2010; Landman et al., 2010). In addition, QOL is known to be significantly reduced in patients with neuropathic complications (Price & Harding, 2000), as shown by the data presented in the cross-sectional part of the present study. However, the question remains whether QOL is a modifiable risk factor or just a marker of disease burden in this population. Harkness et al. (2010) carried out a meta-analysis to indentify psychosocial interventions that improve both physical and mental health in diabetic patients. They concluded that although there are efficient treatments to improve both diabetes and mental health, they did not identify types of interventions that consistently provide benefits for both simultaneously. The present investigation demonstrated for the first time that a physical activity programme can bring about changes in multiple health-related aspects including QOL in DN subjects.

Interestingly, 16 weeks of resistance training appeared to modify mental and not physical aspects of QOL. Thus, the current study found significant improvements in various mental related dimensions of the SF-36 QOL questionnaire, which included vitality, mental health and overall mental health. However, it could be explained by the fact that strength training and consequently gait characteristics were not changed through the exercise programme. In support of this idea, Ruhland & Shields (1997) demonstrated an inverse correlation between both walking speed and lower-extremity muscle function and scores on the physical function scale of the SF-36 following a

home exercise programme in persons with chronic peripheral neuropathies (no DN subjects were included in the study). Therefore, early evidence suggests that changes in muscle function are required for changes in physical aspects of the QOL to appear.

QOL has been previously associated with blood glucose in patients with type I diabetes (Wikblad et al., 1996) and painful neuropathy (Galer et al., 2000). In line with this evidence the cross-sectional study (part 1 of the main study) showed that HbA_{1c} was correlated to mental health in DN subjects. Since HbA_{1c} was not significantly changed by the exercise programme, it is unlikely that improvements in blood glucose levels can explain changes in mental health. On the other hand, data from the present study suggests that changes in mental health (vitality, mental health and overall mental health) may be related to the exercise programme itself and not to other mediators. It has been proposed that self-esteem and positive feeling may mediate the effect that physical activity has on the mental aspect of QOL (Drewnowski et al., 2001). In addition to this, it is well established that group dynamics can also influence levels of enjoyment (Turner et al., 1997). According to Rejeski & Mihalko (2001) repeated enjoyment with an activity may be related to cognitive judgments about one's overall QOL. Thus, it is very likely that the social aspect of sharing the exercise programme with a group of people may have increased the enjoyment of the session and consequently the mental aspect of QOL.

QOL has been correlated to the severity of the diabetic complications (Price & Harding, 2000). In line with this, Currie et al. (2006) demonstrated that the severity of diabetic peripheral neuropathy symptoms was predictive of decreased quality of life. Since the present investigation improved sensory neuropathy in our DN subjects, it is possible that changes in the severity of sensory loss could also have influenced QOL.

The present investigation demonstrated for the first time that a PA programme can be an efficient tool to enhance not only physical health but also mental health in DN subjects. However, further investigations comparing the effect of group based versus a home based PA intervention are required to distinguish whether changes in the mental aspect of QOL are related to the exercise itself, to the social environment around the exercise programme or to exercise-related changes (i.e. sensory neuropathy).

7.2.5 Summary of the discussion for the intervention study

The cross-sectional study demonstrated that the DN subjects in the present study, in agreement with the literature, suffered from multiple pathologies linked to DN including general health, gait abnormalities, microcirculation and QOL. The aim of this longitudinal study was to investigate the effect of 16 weeks of strengthening and foot mobility exercises on identified pathologies associated to diabetic peripheral neuropathy. Therefore, results of the present study demonstrated that 16 weeks of resistance and foot mobility exercises can trigger beneficial changes in some aspects of general health and microcirculation as well as in QOL. The most striking finding of all is that this intervention study documents for the first time the beneficial effects of a 16-week resistance training programme on sensory neuropathy on patients with peripheral neuropathy. On the other hand, the exercise programme did not seem to have a substantial effect on gait characteristics. Importantly, it should be noted that, no adverse effects related to the intervention were reported in any of the volunteers who participated in the physical activity programme.

Regarding to general health linked to DN, the exercise programme improved sensory neuropathy and cardiovascular risk factors such as blood pressure and obesity. On the other hand the intervention did not appear to influence HbA_{1c} levels, strength and cholesterol levels. Unexpectedly, 16 weeks of resistance training seemed to improve sensory neuropathy in DN patients. Although the underlying mechanisms are not clear it appears that vascular changes secondary to the exercise programme may be responsible for this unexpected finding. On the one hand, it is possible that exercise-induced changes in the endothelial function could be a mechanism through with endoneurial blood flow may be restored and in turn might lead to improved sensory nerves. However, it should be stated that the exercise programme only found a trend toward improved vasodilatory responses following the exercise programme. On the other hand, it is also possible that changes in the mitochondrial function could explain the beneficial changes in the sensory nerves observed in the current study. In support of this interpretation, the present study found a more efficient use of oxygen in the EXE group following the intervention, which could be due to changes in the mitochondria. Unfortunately, changes in the sensory neuropathy were not followed by changes in motor neuropathy. Thus, the present investigation found that 16 weeks of strengthening exercises did not improve strength levels in patients with moderate severity of DN.

Although the reasons why DN in the present study did not gain strength levels are unknown, it is likely that muscular atrophy secondary to motor neuropathy may have resulted in reduced neural and muscular adaptations to the exercise programme. Hence, the present study suggests that interventions to improve strength levels in DN subjects may be more efficient at an earlier stage of the disease.

In addition to health related outcome measures associated with DN the present study also investigated the effect of PA on HbA_{1c} levels and cardiovascular risk factors in DN subjects. Contrary to previous investigations on individuals with type 2 diabetes, the present study found that 16 weeks of twice weekly strengthening and mobility exercise did not produce significant changes in HbA_{1c} levels in DN subjects. It is likely that factors like low baseline HbA_{1c} levels, changes in hypoglycaemic medications or the frequency of the intervention may explain the results from the present investigation. However, since this is the first investigation assessing HbA_{1c} changes following a PA programme in DN subjects it cannot be ruled out that DN subjects are less sensitive to HbA_{1c} changes compared to individuals with type 2 diabetes. In line with previous studies with subjects with type 2 diabetes the present investigation found that a PA programme lowered blood pressure and body fat percentage whilst it did not influence cholesterol levels.

The alterations in general health in DN discussed are well known to be associated with multiple health problems in this population such as neuropathic complications or cardiovascular diseases. Furthermore, another major health problem linked to DN subjects is foot ulceration. Changes in gait biomechanics and microcirculation are responsible for the increased risk of foot problems in DN subjects. Thus, the principal aim of the present investigation was to determine whether a PA programme based on strengthening and mobility exercises can influence gait characteristics and/ or microcirculation in DN subjects. On the one hand, the intervention in the present study did not produce substantial changes in gait characteristics in DN subjects. It was hypothesized in the cross-sectional study that strength gains in the lower limb may result in some changes in gait biomechanics. The fact that the present investigation did not manage to change strength levels is likely to explain the lack of changes in gait characteristics. Interestingly, improvements in sensory neuropathy secondary to the intervention did not appear to change gait characteristics substantially. It is believed that sensory neuropathy is the single most important factor explaining gait alterations in

DN subjects (Frykberg et al., 1998). However, gait parameters, foot pressures and muscular activity patterns during gait in the present investigation were mostly unchanged despite improvements in sensory neuropathy. The DN in the present study had been living with significant losses of sensation for many years. Therefore, it is likely that improvements in sensation may take a long time before any adaptation in gait occurs (if any). This could partly explain the lack of association between changes in sensory neuropathy and gait in the present study. On the other hand, the exercise programme influenced some aspects of microcirculation. Thus, the intervention had a significant effect on the mVO_2 recovery. Since the exercise programme did not promote changes in the amount of oxygen consumed by the muscle following the exercise bout, it appears that the intervention resulted in changes not in the capacity of the muscle to uptake oxygen but in the efficiency of its usage. Changes in the mitochondrial function secondary to the exercise programme are likely to explain the results found in the present study. Furthermore, the present study showed that exercise-induced vasodilatation was enhanced by 16 weeks of resistance training. It is believed that the beneficial effects of an exercise programme on vascular function probably related to increased flow and shear stress on the endothelium, which may stimulate the release of NO and, in turn, improve endothelial function. However, these changes did not reach significance and were limited to a trend. It is possible that the length of the intervention or issues related to the power of the study may partly explain why this change did not reach significant levels.

In addition to the health problems associated with DN, QOL also represents an important goal for health care professionals. The present study demonstrated for the first time that a PA programme can be an efficient tool to enhance not only physical health but also mental health in DN subjects. Thus, the current study found significant improvements in various dimensions of the SF-36 questionnaire including vitality, mental health and overall mental health.

Overall, the present investigation demonstrated that 16 weeks of strengthening and joint mobility exercises influenced some of the health problems associated with DN. It is therefore possible to speculate that if changes in cardiovascular risk factors, microcirculation and QOL were elicited this may have resulted in a better cardiovascular health, less risk of foot problems and better mental health in the DN after the exercise programme. Furthermore, the fact that sensory neuropathy was

improved following the exercise is a very important finding in itself, since up to date there is not a treatment available to reverse neuropathy. However, it is noteworthy that sample size in the present investigation was small so further studies are warranted before drawing any final conclusions about the effect of a physical activity programme based on strengthening exercises and joint mobility exercises on DN patients.

7.3 Clinical implications of the study

The present study investigated, for the first time, the effect of a PA programme on the primary pathologies linked to DN. Several clinical implications emerge from the results of this study. The vast majority of previous studies investigating the effect of a PA programme on subjects with diabetes have been interested in health problems related to type 2 diabetes such as glucose control or cardiovascular risk factors. This investigation demonstrated that exercise-induced changes in health related outcome measures in DN subjects go beyond type 2 diabetes (results from part 2 of the main study). Thus, the present study demonstrated for the first time that 16 weeks of strengthening exercises triggered positive changes in sensory neuropathy in DN subjects. Furthermore, the fact that a PA programme can improve sensory neuropathy may potentially suggest a number of clinical implications. For instance, sensory neuropathy is believed to be the main factor responsible for the high risk of ulcers in DN subjects (Veves et al., 1992). This is related, on the one hand, to the mechanism that a minor trauma in the presence of loss of sensation can remain unattended which can be the start of an ulcer (Dinh & Veves, 2005; Laing, 1998). On the other hand, neuropathy has been linked to microcirculatory problems secondary to changes in the nerve-axon reflex (flare response) (Dinh & Veve, 2004; Schramm et al., 2006). It has been suggested that the impaired flare response observed in patients with DN may be related to both impaired C-nociceptive fibre function and impaired ability of the microvasculature to response to vasomodulators (i.e. NO) secreted by these fibres (Vinik et al., 2001). It is therefore possible to speculate that if an intervention can elicit changes in sensory neuropathy, it may reduce the risk of foot ulceration in DN subjects. Furthermore, loss of sensation is associated with higher number of falls (Dingwell & Cavanagh, 2001), reduced QOL (Currie et al., 2006) and gait abnormalities (Courtemanche et al., 1996). It should be noted that in the present study changes in sensory neuropathy were not associated with changes in gait biomechanics. However, gait patterns in DN subjects reflect not only the effects of the neuropathy itself, but also of any motor control strategies these subjects had developed over the years to compensate for their sensory loss. It is therefore likely that changes in gait characteristics in relation to this intervention (if any) may develop slowly beyond the duration of this study.

Moreover, results from the present investigation suggest, not only that exercise can influence outcome measures related to DN, but also that adaptations to exercise

programmes differ between subjects with type 2 diabetes and DN individuals. Contrary to numerous previous investigations on subjects with type 2 diabetes (Cauza et al., 2005; Dunstan et al., 2006), the present investigation found that 16 weeks of resistance training did not elicit changes in strength levels in DN subjects with moderate neuropathy. This finding demonstrates that DN subjects may be less sensitive to musculo-skeletal adaptations compared to diabetic subjects without neuropathy. It is well documented that gains in muscle strength following a resistance training programme occur from neural and muscular adaptations. Thus, neural factors account for the majority of the strength gains over the first 5 weeks of workouts. Thereafter, muscle fibre adaptations become progressively more important to strength improvement (McArdle et al., 2010). Since the exercise programme lasted 16 weeks, it seems that DN diminishes both neural and muscular adaptations to strengthening exercises. It is possible that muscular atrophy secondary to motor neuropathy (Brash et al., 1999) is partly responsible for these reduced neural and muscular adaptations. Allet et al. (2010), who undertook the only previous study to assess changes in strength levels in DN subjects following an exercise programme, reported only moderate strength gains in DN subjects with mild neuropathy (VPT<4 volts). These results suggest that the improvements in strength levels in DN subjects are likely to occur more readily at an early stage of the disease. Muscle weakness is known to be an important contributing factor for the changes in gait characteristics observed in DN subjects (Mueller et al., 1994). In support of this, the cross-sectional study found that muscular strength was responsible (at least in part) for changes in COP parameters and loading patterns especially under the metatarsal region in the DN group. In addition to this, motor neuropathy is well known to limit physical function (Resnick et al., 2000) and has been associated with poor QOL (Currie et al., 2006). It therefore appears critical to implement strengthening exercise at an early stage of DN before muscular function is impaired to a level which will be more difficult to influence by strength training. However, more studies are needed to confirm that muscular weakness, despite possible improvements in nerve function, cannot be improved in DN subjects.

Overall, these findings highlight the importance of: 1) evaluating PA interventions on the whole range of health problems associated with DN, and not only on glucose control and cardiovascular risk factors and 2) investigating the effect of PA on DN subjects to challenge the assumption that similar adaptations may occur in DN compared to individuals with type 2 diabetes without neuropathic complications.

The aim of this intervention study was to evaluate whether 16 weeks of strengthening and foot mobility exercises could influence health in DN. In line with previous investigations with subjects with type 2 diabetes the present study found that PA can influence outcome measures linked with cardiovascular diseases. Subsequently, it can be speculated that improvements in blood pressure and obesity secondary to the intervention may have decreased the risk of cardiovascular events in the DN subjects who participated in the training programme. A novel attempt of this investigation was to evaluate whether the risk of foot ulcers can be altered through a resistance training programme. Thus, results from the present study demonstrated that although foot pressures were unchanged, microcirculatory responses to a stress condition were improved following the exercise programme. It is therefore possible to speculate that if an intervention can enhance the ability of the microcirculation to respond to a stress condition, this may improve the body's ability to respond to trauma in the foot region more effectively, and therefore the risk of ulcers may be reduced. Furthermore, the present study demonstrated for the first time that certain aspects of QOL can be reversed in DN individuals via a PA programme. Interestingly it appears that aspects of mental health showed greater improvement following the intervention compared to aspects of physical health. Overall the present investigation demonstrated that some of the outcome measures associated with cardiovascular diseases, foot ulcers and mental health in DN subjects are modifiable by PA interventions.

Since DN subjects are at risk to develop foot ulcers (Boulton et al., 1994) and there is little evidence on the effect of PA on this population, it is important to mention that the present intervention did not cause any adverse events in the feet in any of the subjects who participated in the present study. The amount of weight bearing activities among individuals with diabetes is thought to influence the amount of mechanical trauma accumulated by plantar tissue (Cavanagh et al., 1996). This suggests that an adaptation of the exercises used is necessary to avoid foot complications (Kanade et al., 2006). In the present study, to reduce the risk of foot complications related to the exercise programme the amount of weight bearing exercises was reduced to a minimum and only non-impact exercises were chosen. In addition to this, all participants were encouraged to closely examine their feet after each training session to prevent sores and were required to use proper footwear. This study suggests that a well controlled PA programme based on non-impact exercises can be safe in DN subjects. In addition, no

hypoglycaemic episodes were reported by any subject in the study. To reduce the risk of hypoglycaemic episodes subjects were advised before each session to check their sugar levels. If glucose levels were below 100 $mg \cdot dL^{-1}$ the participants were provided with a carbohydrate snack (Flood & Constance, 2002). Again this suggests that physical exercises can be done safely when glucose levels are monitored prior the session.

The present investigation has provided a substantial contribution to the knowledge of rehabilitative exercise in the context of DN. Thus, the present investigation demonstrated for the first time that PA interventions can influence a variety of health related outcome measures in patients with DN, including both outcome measures related to type 2 diabetes such as blood pressure and obesity, and outcome measures related to neuropathy such as sensory neuropathy and microcirculation. Although this early evidence highlights the therapeutic role PA interventions may have on subjects with DN, it should be noted that a number of limitations arose from the present study.

7.4 Limitations of the study

A number of limitations of this study have to be highlighted. For part 1 of the main study, groups were not successfully matched for body mass, which could have biased some of the outcome measures investigated in the present study. However, to minimize this source of bias the effect of body mass on the outcome measures was explored prior to the main analysis. Thereafter, when body mass was identified as a confounding variable ANCOVA was used to control for the effect of body mass in the analysis.

One of the aims of the present study was to investigate gait characteristics in DN subjects. Thus, the cross-sectional study compared gait characteristics between healthy and DN subjects whilst the intervention study investigated the effect of an intervention on gait characteristics. Range of motion is well known to affect gait characteristics in DN subjects including COP parameters and foot pressures (Salsich et al., 2005; D'Ambrogi et al., 2003). For this reason the present investigation intended to measure foot and knee mobility during gait. However, due to technical difficulties this data could not be presented in the present study. This can be seen as a limitation of the present study. Furthermore, the intervention programme, which was based on strengthening and mobility exercises, was intended to influence foot mobility during dynamic conditions. However, it is unknown whether the exercise programme

influenced joint mobility during walking in present study. Nevertheless, it is possible to speculate that if gait characteristics in the form of gait parameters and foot pressures did not change, ROM is not likely to have undergone major changes either.

Another aim of the present study was to investigate microcirculation in subjects with DN due to its association with foot problems. However, it should be stated that microcirculation measurements in the present investigation were obtained from the MGast muscle. Thus, evidence obtained from this muscle cannot necessary be generalized to the foot area, which is where the vast majority of foot ulcers occur in subjects with DN. Future studies should investigate the effect of a PA programme on the foot microcirculation, which may provide insight into how the foot responds to stress conditions.

Another limitation of the study was the sample size. For part 2 of the main study the power calculation determined a minimum of 22 participants per group. However due to difficulties during recruitment, groups in the intervention study were composed of 21 and 20 in the EXE and CON group respectively. These sample sizes were used for the analysis of the majority of outcome measures. However, some data sets were lost for EMG and microcirculation data, which resulted in a further reduction of the sample size for those measurements. Thus, analysis of EMG data was carried out with 19 sets of data for each group whilst analysis of microcirculation data was carried out with 17 sets of data per group. Therefore, the sample size in the present study is viewed as a limitation and future research should include larger number of participants.

Randomized control trials (RCT) are considered to be the preferable study design due to increases in the internal validity. Also the selection and testing bias related to pre-testing subjects with the knowledge of group allocation is minimised (Craig et al., 2008). However, the main problem of RCTs is that they are not easy to implement, for instance, when a substantial proportion of patients refuse the allocation group (Rose & Baker, 1978). In addition to this, a high number of drop outs can compromise the external validity of the study (Bratcher et al., 1970). Previous studies have reported high rates of drop-out in PA interventions with diabetic populations (Thomas et al., 2006). To reduce the number of drop outs in the present study, a study design which allowed participants the opportunity to choose in which group they wanted to be included was used. Randomisation based on patient preferences is considered an acceptable

randomization method when patients have strong preferences for one intervention group or the other (Campbell, et al., 2000; Craig, et al., 2008). Furthermore, in this study the investigator was not blinded with respect to the intervention. However, these are viewed as a limitation and future research should: 1) carry out RCTs; and 2) blind the investigator with respect to the intervention.

Strength gains are related to the specificity of the training exercises (McArdle et al., 2010). Thus, an isometrically trained muscle shows greatest strength improvement when measured isometrically. The same principle applies to muscles trained during dynamic conditions, where the greatest improvements in strength will be observed when the muscle is measured dynamically. In the present investigation the training programme was based on dynamic exercises whereas strength measurements were carried out isometrically. This is viewed as a limitation of the present investigation. It is possible that this limitation in the methodology of the present study could have affected the sensitivity of the measurements to identify strength changes following the exercise programme. Thus, it is important that future studies measure strength levels taking into account the specificity of strength-training responses.

7.5 Future research

In relation to the cross-sectional study, future research should focus on investigating activity patterns during walking in DN patients. The majority of studies have investigated kinetic or kinematic data, whereas only a handful of studies have assessed EMG patterns in DN subjects. Furthermore, preliminary studies number 2 and 3 have demonstrated that EMD values differ between DN and healthy individuals, especially in the plantar-flexor and dorsi-flexor muscles, which highlights the importance of calculating individual EMD values when processing EMG activity. Therefore, studies are warranted to investigate muscular activity patterns in DN subjects whilst taking into account individual EMD values.

Results from the present investigation suggest that microcirculatory alterations in DN subjects are linked to: 1) vasodilatory capacity and 2) oxygen uptake capacity. Whereas changes in the vasodilatory capacity have been widely investigated, the exact

mechanism/s behind the reduced skeletal muscle oxygen uptake capacity in DN subjects remains unclear. Therefore, future studies should attempt to clarify this issue.

The present investigation is the first study that has demonstrated that 16 weeks of resistance training can influence some of the pathologies linked to DN. However, future studies with larger populations and using different type PA interventions (i.e. type of exercise, intensity, duration, etc.) are needed at this stage (what Campbell et al. (2000) referred to as "exploratory phase"), before any final conclusions can be drawn about the overall effect of PA interventions on the primary health problems associated with DN (what Campbell et al. (2000) referred to as "definitive randomised controlled trial"). Furthermore, due to the exploratory nature of the study, it is impossible to know for certain the mechanisms and/or reasons behind some of the exercise-induced adaptations observed in the present investigation with DN patients. For this reason future studies are warranted to explore these mechanisms in more detail. A number of future studies are proposed below to clarify the role of PA to influence factors such as sensory neuropathy, motor neuropathy, HbA_{1c}, gait, microcirculation and QOL.

The most striking finding of the intervention study was that 16 weeks of strengthening exercise influenced sensory neuropathy in DN subjects. Sensory neuropathy in the current study was quantified by VPT whilst conduction velocity measurements of the sensory nerves, which are considered the gold standard to assess nerve function, were not conducted. Future studies should assess sensory nerve function directly to understand better the effect of PA on sensory neuropathy. Furthermore, other forms of physical activity should be investigated to establish which intervention is more efficient to preserve sensory neuropathy in this population.

Results from the present study suggest that muscular weakness is more difficult to preserve in patients with more severe DN. There is only one previous investigation assessing the effect of an exercise programme on muscular strength in DN subjects and they found moderate strength gain in subjects with mild neuropathy (Allet et al. 2010). It appears that changes in muscular function are dependent on the degree of neuropathy. However, more studies are needed: 1) to draw any final conclusion about the adaptability of the muscle to strengthening exercises; and 2) to understand the reasons why the muscles of DN subjects may be less sensitive to exercise-induced adaptations

in the form of neural and muscular adaptations. Furthermore, it would be interesting to assess

The main target of rehabilitation programmes in patients with diabetes is to improve glucose control. Previous studies demonstrated that strength training can lower HbA_{1c} levels in subjects with type 2 diabetes (Castaneda, et al. 2002; Cohen et al., 2008; Dunstan et al., 2002). This is the first study investigating the effect of an exercise programme on HbA_{1c} in DN subjects and contrary to previous investigations on patients with type 2 diabetes the present intervention did not elicit changes in this outcome measure. It is likely that factors such as low baseline HbA_{1c} levels, changes in hypoglycaemic medications or the frequency of the intervention may explain the results from the present investigation. However, it cannot be ruled out that DN subjects are less sensitive to HbA_{1c} changes compared to individuals with diabetes and no neuropathic complications. Further studies are therefore warranted to determine whether PA programmes result in comparable effects on HbA_{1c} levels in both subjects with type 2 diabetes and DN individuals.

Muscular weakness is considered an important factor explaining changes in gait biomechanics in DN subjects. One of the objectives of the present investigation was to determine whether strength gains could influence gait characteristics in DN subjects. The fact that the intervention did not trigger changes in strength levels may partly explain why gait characteristics were not altered by the exercise programme. Future research should focus on investigating whether strength gains in subjects with an early DN (who may still be more sensitive to muscular adaptations) can modify gait in this population. Furthermore, the present investigation found that improvements in sensory neuropathy did not influence gait characteristics in DN subjects. It has been speculated in the present study that changes in gait biomechanics secondary to improvements in sensory neuropathy may occur (if any) beyond the length of this study. Therefore, if improvements in sensory neuropathy are confirmed by future investigations, it would be interesting to extend the duration of the study to allow enough time for the locomotor gait strategies to change.

The present study found a trend toward improvements in the vasodilatory capacity in the EXE group following the exercise programme. A few studies investigating subjects with type 2 diabetes demonstrated that PA can improve the vasodilatory capacity via

improvements in the endothelium (Cohen et al., 2008; Maiorana et al., 2001). It has been hypothesized earlier in the discussion that the length of the intervention in the present study may have been insufficient for significant changes to be found. In addition, the sample size in the present investigation did not reach the numbers determined by the power calculation. Future studies with a larger group of participants are warranted to further understand the effect of PA programmes in general, and strength training in particular, on the vasodilatory capacity in DN subjects. Furthermore, the present study demonstrated for the first time that a PA programme based on strengthening exercise can improve the mVO_2 recovery. However, future studies are needed to confirm this novel finding. In the present study it was speculated that changes in the mVO2 recovery may be related to changes in aerobic fitness secondary to the exercise programme. Future studies assessing aerobic fitness and microcirculatory function are warranted to determine whether changes in the ability of the muscle to recover from an exercise bout are secondary to changes in aerobic capacity, or whether other mechanisms may trigger this adaptation.

The present investigation demonstrated for the first time that a PA programme can be an efficient tool to enhance not only physical health but also mental health in DN subjects. However, further investigations comparing the effect of group based versus a home based PA intervention are required to distinguish whether changes in the mental aspect of QOL are related to the exercise itself, to the social environment the exercise programme is undertaken in or to exercise-related changes (i.e. sensory neuropathy).

In summary, more studies are warranted to broaden the understanding of the effect of PA programmes on the primary pathologies associated with DN named cardiovascular risk factors, glucose control, sensory neuropathy, motor neuropathy, gait alterations, microcirculation and QOL .

CHAPTER 8

8 Conclusions

It is well established that DN is a complex condition that affects different aspects of health. Pathologies associated with DN include metabolic abnormalities, alterations in traditional cardiovascular risk factors, gait alterations, microcirculatory impairments and poor QOL. The question remains whether a rehabilitative exercise programme can influence these primary pathologies which have been linked to DN. Thus, this investigation was composed of two different studies: a cross-sectional study to investigate differences between DN and healthy individuals in the primary pathologies associated with DN; and an intervention study to investigate to the effect of a PA programme on identified pathologies linked to peripheral neuropathy in subjects with DN.

The aim of the cross-sectional study was two fold. Firstly, it aimed to compare the findings of this study to previously published investigations. The present study confirmed that DN produced alterations in outcome measures related to cardiovascular diseases, gait characteristics, microcirculation and QOL. Secondly, the cross-sectional study aimed to provide some additional information on: 1) gait characteristics by investigation muscular activity patterns; and 2) microcirculation by assessing for the first time exercise-induced microcirculatory responses in the form of blood flow and oxygen consumption in patients with DN. This study investigated gait characteristics not only from a kinetic and kinematic perspective, which are the most evaluated outcome measures in this population but also from an EMG perspective. Only a handful of studies have previously investigated EMG patterns in DN subjects and none of them took into consideration EMD when processing EMG data. Results from the present study provide early evidence that foot loading patterns are associated with EMG activity. This suggests that muscular function should be considered as a factor partly responsible for high foot plantar pressures, especially under the metatarsal region, which are often observed in DN subjects. In addition to that, EMG data from the cross-sectional study provided some insight about motor control in DN subjects. It appears that the DN subjects in this study adopted an anticipatory strategy to efficiently produce force at the right moment in time (demonstrated by an early muscular activation and same peak activity). With regard to the microcirculation, the present study investigated,

for the first time, exercise-induced microcirculatory responses in DN subjects. The present study not only found that oxygen delivery was reduced in the DN group, in agreement with previous studies on individuals with type 2 diabetes, but also the ability of the muscle to uptake oxygen. Overall, the cross-sectional study demonstrated that DN is a very complex condition affecting different aspects of health such as general health, gait characteristics, microcirculation and self reported QOL. It can be speculated that if those outcome measures can be modified, health in DN subjects can be enhanced.

The aim of the intervention study was to evaluate for the first time whether 16 weeks of a PA programme based on strengthening and foot mobility exercises can influence different aspects of health that are altered in DN subjects. The most remarkable finding was that the exercise programme improved sensory neuropathy. This is an important finding since sensory neuropathy is linked to a variety of health problems associated with DN such as foot ulcers or poor QOL. Furthermore, it shows that PA activity can influence health beyond type 2 diabetes related health problems. Another interesting finding from this study was that high intensity resistance training did to seem not improve strength levels in patients with moderate DN as expected, despite improvements in neural health as demonstrated by the changes in sensory neuropathy. This finding not only suggests that exercise can influence outcome measures related to DN, but also that adaptations to exercise programmes may differ between type 2 and DN individuals. This highlights the importance of investigating the effect of PA on DN subjects and not to assume that similar adaptations may occur in DN subjects compared to individuals with type 2 diabetes.

Furthermore, this study represents an accumulation of evidence that PA programmes can influence some of the health related outcome measures, which are altered in DN subjects. Thus, 16 weeks exercise training improved the cardiovascular risk profile by decreased blood pressure and body fat percentage in DN subjects. Furthermore, the intervention improved exercise-induced responses of the microcirculation. Additionally, the PA programme improved the exercise-induced vasodilatory capacity in the exercise group. However, this change did not reach significance and was limited to a trend ($p<0.1$). On the other hand, the exercise programme did significantly improve mVO_2 recovery (reoxygenation) from an exercise bout in the EXE group compared to the CON group over time. Although there were no changes in the capacity of the muscle to take up oxygen following the intervention, changes in the efficiency of its use were

observed. The fact that a PA programme can influence microcirculation in DN patients is a very important finding since impairments in the microcirculation are associated with major health problems such as reduced exercise capacity or foot ulceration. Moreover, the exercise programme influenced certain aspects of self reported QOL. Interestingly it appears that aspects of mental health showed greater improvement following the intervention compared to aspects of physical health. Overall, the intervention study demonstrated that some of the health related outcome measures that affect DN subjects are modifiable via a PA programme. This is only the first of many steps required to fully understand the potential of PA interventions in influencing health in DN subjects. Thus, more evidence needs to be conducted at this "exploratory phase" (Campbell et al., 2000) to evaluate different components of a PA intervention (i.e. type of exercise, intensity, duration, etc.). This will broaden the knowledge base around the effect of different types of stimuli on the different outcome measures related to DN.

The present study on the primary pathologies associated with DN has provided a substantial contribution into the research of rehabilitative programmes based on physical activity in patients with DN. Results from the present investigation highlighted the value of PA as a therapeutic tool in subjects with DN to modify different aspects of health in this population including sensory neuropathy. Furthermore, the present investigation demonstrated the importance of investigating the effect of PA programmes on DN subjects and not to assume that similar adaptations may occur in DN subjects compared to individuals with type 2 diabetes without neuropathic complications.

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APPENDICES

Appendix 1. Literature search

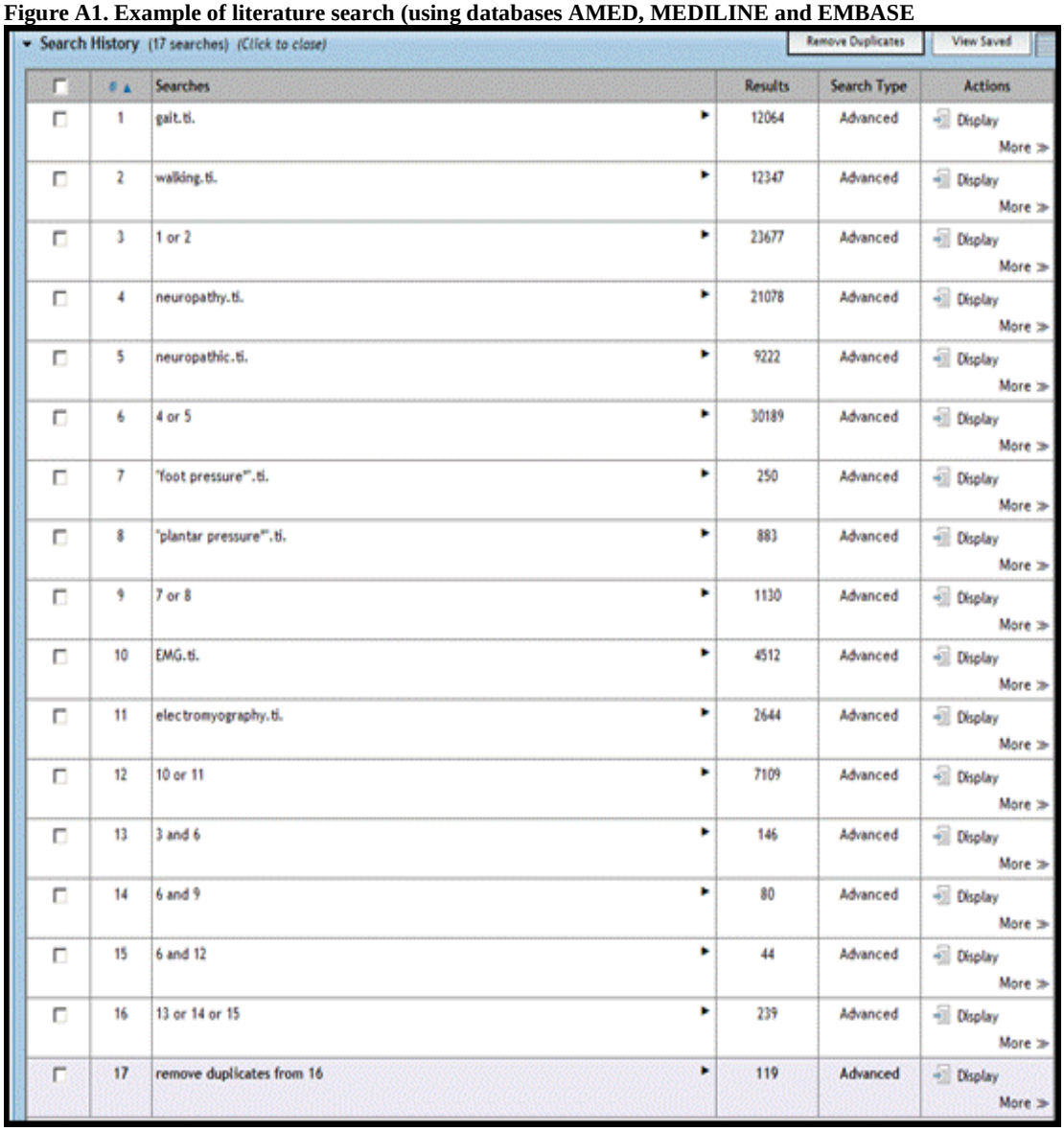
Table A1. Keywords included for literature search on identified pathologies related to DN

	Cardiovascular diseases	**Gait**	**Microcirculation**	**QOL**	
	Cardiovascular disease/s Metabolic syndrome Type 2 diabetes	Gait/Walking EMG/ Electromyography Foot Pressures/ plantar pressures Neuropathy/ Neuropathic	Microcirculation Blood flow Vasodilatation Endothelium/ endothelial Neuropathy/ Neuropathic	Quality of life Neuropathic/ neuropathy	
Results	64	119	86	66	335
Relevant after reviewing	21	26	39	18	104

Table A2. Keywords included for the literature search on the effect of PA on identified pathologies related to DN

	Cardiovascular diseases	**Gait**	**Microcirculation**	**QOL**	
	Cardiovascular disease/s Metabolic syndrome Type 2 diabetes Physical Activity/ exercise training	Gait/Walking EMG/ Electromyography Foot Pressures/ plantar pressures Neuropathy/ Neuropathic Type 2 diabetes Physical Activity/ exercise training	Microcirculation Blood flow Vasodilatation Endothelium/ endothelial Type 2 diabetes Neuropathy/ Neuropathic Physical Activity/ exercise training	Quality of life Neuropathic/ Neuropathy Type 2 diabetes Physical Activity/ exercise training	
Results	42	61	18	10	132
Relevant after reviewing	23	8	10	8	49

Figure A1. Example of literature search (using databases AMED, MEDILINE and EMBASE



Search History (17 searches) (Click to close) — Remove Duplicates — View Saved

	#	Searches	Results	Search Type	Actions
☐	1	gait.ti.	12064	Advanced	Display More ≫
☐	2	walking.ti.	12347	Advanced	Display More ≫
☐	3	1 or 2	23677	Advanced	Display More ≫
☐	4	neuropathy.ti.	21078	Advanced	Display More ≫
☐	5	neuropathic.ti.	9222	Advanced	Display More ≫
☐	6	4 or 5	30189	Advanced	Display More ≫
☐	7	"foot pressure"".ti.	250	Advanced	Display More ≫
☐	8	"plantar pressure"".ti.	883	Advanced	Display More ≫
☐	9	7 or 8	1130	Advanced	Display More ≫
☐	10	EMG.ti.	4512	Advanced	Display More ≫
☐	11	electromyography.ti.	2644	Advanced	Display More ≫
☐	12	10 or 11	7109	Advanced	Display More ≫
☐	13	3 and 6	146	Advanced	Display More ≫
☐	14	6 and 9	80	Advanced	Display More ≫
☐	15	6 and 12	44	Advanced	Display More ≫
☐	16	13 or 14 or 15	239	Advanced	Display More ≫
☐	17	remove duplicates from 16	119	Advanced	Display More ≫

Appendix 2: Home exercises

HOME EXERCISES

Warm up:

Start each session by moving each body segment. You can do it from the top to the bottom so we do not miss any.

Note: Please do all the exercises **very slowly** and trying to reach the "**highest amplitude for each movement".**

1. Head
    a. Standing position (remember in standing position we keep the knees a little bit bent so the back is in a better position):
        i. Move the head forward and backwards (5 times).
        ii. Move the head from side to side (5 times).
2. Shoulders
    a. Standing position.
        i. Move the shoulders in circles (5 moving forward and 5 backwards)
3. Arms
    a. Standing position
        i. Move right arm in circles. Remember to follow the hand with your eyes. (5 times moving forward and 5 backwards.
        ii. Repeat the same movement with the left arm.
4. Trunk
    a. Standing position
        i. Rotate your trunk to the right and to the left During the last part of the rotation we perform a reaching movement with both arms. (5 rotations to each side)
5. *Ankles*
    a. Sitting position.
        i. Make the alphabet with your feet. Both of them together. You can use a chair underneath the calf muscles to help hold your legs while doing it. The ankles should be kept on the air.

Resistant band exercisers (it is important you find difficult to make 10 reps with that tension). Remember you can always make the exercise more difficult by increasing the tension on the band;

1. Shoulders
    a. Seated position:
        i. Hold the band with your arms straight and both hands just a few inches from each other at the lower part of your back. From that

position move the hands away from each other. Keep always the arms straight (10 repetitions).

ii. Repeat the same movement with the arms higher up in your back. Arms still straight all the way through (10 reps).

2. Chest
    a. Seated position

        1. Place the band around your back and hold it with your fists (Palm facing down) by the side of the trunk (elbows bent and backwards). Move your arms to and extended position. Do not chance the position of the fist during the whole movement. (10 reps).

3. Back
    a. Seated position
        1. Step on the band with both feet and hold the band with you arms a little bit in flexion aside the body. The fist should face the floor. Bend your body forward. Bend your arms with the fist always facing the floor.

4. Biceps
    a. Seated position.

        1. Step on the band with both feet and hold the band with you arms straight aside the body. Without moving the shoulders bend your arms simultaneously (10 reps).

5. Triceps
    a. Seated position

        i. Pick up your band with the right arm bent aside the trunk and elbow pointing forward. The left arm holds the other side of the band by the lower back. Keeping the elbow and shoulder still straighten the arm. (10 reps).

6. Calf Muscles
    a. Seating position

        1. Pass the band around the forefoot of the right foot and hold it with both hands. Make the band tight and move the forefoot down (like pressing the pedal in the car). (15 reps)

        2. Repeat with the left foot

7. Tibiales
    a. Seating position
        1. Attach the band on the leg of a table, place the band around your forefoot. Place yourself at a distance enough to make the band tight. Pull your toes up. (15 reps)

2. Repeat with the left foot

b. Ankle muscles.
   i. Sitting position

      1. Place the band around the forefoot of your right foot, step on the band with the left foot and with the heel of the right foot touching the floor move the forefoot out to the side. (15 reps)

      2. Do the same with the left foot.

      3. Place the band around the forefoot of your right foot, tie the band out to the side with the leg of a table and with the heel of the right foot touching the floor move the forefoot inwards.

      4. Do the same with the left foot.

      5. *Pass the band around the forefoot of the right foot and hold it with both hands. Write the alphabet on the air with your foot. Think of the toes as the tip of a pen. (do it once)*

      6. *Do the same with the left foot.*

Cool down

b. Sitting position.
   i. Make the alphabet with your feet. Both of them together. You can use a chair underneath the calf muscles to help hold your legs while doing it. The ankles should be kept on the air.

Appendix 3: Invitation letter

Dear_____,

The Department of Physiotherapy is looking at the effects of a physical activity programme in people with diabetes and loss of sensation. Such a programme could really benefit participants, so the Podiatry Department (University Hospital of Wales) has agreed to collaborate and contact people with diabetes that may be suitable for this research study. We would like to offer you the opportunity to participate in a free physical activity programme carried out by the Physiotherapy Department specifically designed for patients with diabetes and loss of sensation.

The study will investigate:

- the effect of a physical activity program (over 16 weeks) in preventing the risk of ulcers in people with diabetes and loss of sensation
- the effect of this program on general health (i.e. Cholesterol levels, glucose levels, weight loss, etc)

The benefits to patients could be:

- Ulcer prevention
- Improved glucose control
- Improved blood flow (healthier arteries).
- Reduce risk of cardiovascular problems by improving cholesterol levels, reducing body weight and improving general fitness.

A possible side-effect could be changes in glucose levels due to physical activity. More details can be found in the enclosed information sheet (risk section, page 3).

Before you decide it is important for you to understand why the research is being completed and what it will involve. Please take a moment to read the information sheet (enclosed) in which a clear explanation of the study is provided. You are under no obligation to take part, as participation is entirely voluntary.

To make it easer for you to respond, the Physiotherapy department will contact you within the next two weeks to ask whether you are interested in participating in this study. In the meantime if you wish to ask any questions, please feel free to contact either the Podiatry Department (Dr Jane Lewis) or the researcher conducting the study at the Department of Physiotherapy.

Yours sincerely,

Dr Jane Lewis
Podiatrist
Work: [redacted]
Email address: [redacted]

Alejandro Meana-Esteban
MSc Sport and Health Sciences
Work: [redacted]
Mobile: [redacted]

Appendix 4: Information sheet

School of Healthcare Studies
Department of Physiotherapy
Director Dr R W M van Deursen MCSP MSc PhD ILTM
Adran Ffisiotherapi
Cyfarwyddwr Dr R W M van Deursen MCSP MSc PhD ILTM

DATE: 11/02/2008
VERSION Nº 3.1

Cardiff University
Tŷ Dewi Sant
Heath Park
Cardiff CF14 4XN
Tel *Ffôn* +44(0)29 2074 2267
Fax *Ffacs* +44(0)29 2074 2267
E-mail *E-bost* Physiotherapy@cf.ac.uk
Prifysgol Caerdydd
Tŷ Dewi Sant
Mynydd Bychan
Caerdydd CF14 4XN

INFORMATION SHEET

(Participants with diabetic neuropathy)

"The effect of non-impact resistance training on patients with diabetic neuropathy: *Acute responses and lon-term adaptation*".

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish.

(Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study). Please, ask us if there is anything that is not clear or if you would like more information.

PART 1

What is the reason for the study?
Physical activity has been recognized to have an important role in health promotion and prevention of diseases. It is generally accepted that exercise reduces the risk of cardiovascular diseases (i.e. stroke or heart attack) and mortality in both healthy and clinical population**.** However, physical activity recommendations for people with peripheral neuropathy are based on evidences obtained from studies with non-neuropathic people with diabetes whereas non attempt has been made to demonstrate the positive effect of an exercise program on people with peripheral neuropathy.

What is the purpose of the study?
The aim of this study is to determine whether and to what extent an exercise program using muscle strengthening exercises have a positive effect on glycaemic control, cholesterol levels, circulation, daily activity levels and quality of life in people with diabetes and peripheral neuropathy. A more detailed explanation of the exercise program and the type of exercises you will be asked to do can be found in the section "what will happen to me if I take part (next page)".

This study will be run by Dr Robert van Deursen, Professor Patricia Price and Alejandro Meana-Esteban (PhD student) in collaboration with Prof Keith Harding.

Why was I chosen to take part in this study?
You have been invited to participate in the study because you have been diagnosed with diabetes peripheral neuropathy.

Do I have to take part?
It is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving any reason. This would not affect the standard of care you receive.

What will happen to me if I take part?

If you agree to participate in the study, you will be asked:

1. To undergo an exercise program for 16 weeks. The program will aim to improve strength levels of your legs as well as other parts of your body (arms and trunk) by exercising against a fixed resistance which will be adjusted throughout the program.

   The exercise program will be split between controlled and home-based sessions. All the controlled sessions will take place in the outpatients physiotherapy gym at the University Hospital of Wales (UHW). Training sessions throughout the study will be conducted 4 times per week (twice in the gym and twice at home). The gym based sessions will last for 60 minutes whereas the home based exercise will last for 30 minutes.
   The exercise program has been designed and will be carried out by a qualified practitioner (Alejandro Meana-Esteban, MSc in Health and Sport Science).

   Note: Not all the participants with diabetes will be asked to undergo the exercise training since by chance (randomly) you will be distributed into either Exercise or Control groups (Non-exercisers) (Please, go to Chart 1 in the last page of this document for further clarification regarding the study groups).

2. To visit the Research Centre of Clinical Kinesiology (RCCK), situated in Heath Campus, twice during the study. The first visit will take place before the physical activity program starts and the second will take place after the exercise program finishes.

   During each visit to the RCCK each participant will be tested: glucose levels, cholesterol levels, blood pressure, height, weight, *fat %*, strength levels, circulation both at rest and when exercising and walking characteristics.

   During each visit, you will be asked to two main tasks:

   1. Walk on a 9 meters walk-way to analyze your walking characteristics.
   2. Produce movements with your right leg against a fixed resistance to determine your strength levels. When performing this task a cable will be secured with a plaster on the skin of your leg (calf muscle), which will be connected to a piece of equipment to give us information regarding your circulation when exercising.

   You will also be asked to complete two questionnaires. One questionnaire will contain questions regarding to your daily physical activities, whereas the second will contain health related questions.

Each visit to the Research Centre of Clinical Kinesiology will last approximately 120 minutes.

For further information on the structure of the research can be found in Chart 1 (last page of this information sheet).

How will blood samples be taken?
A lancet will be used to analyze blood for glucose and cholesterol levels. A lancet is a fine, sharp pointed needle used for pricking the skin. You can choose whether to prick yourself on the fingertip or to be pricked by a qualified person.

Expenses
Travel and parking expenses will be available for all participants in the study. Heath campus has got car parking facilities in which you can park your car.

What are the possible benefits of taking part?
We cannot promise the study will help you directly but the information we gather from this study will help improve our understanding of the effects of diabetic neuropathy on the lower legs and may provide recommendations for treatment of people with diabetic neuropathy.

Is there any risk associated with the study?

- The most common risk associated with physical activity in people with diabetic neuropathy is the development of foot ulcers. However the risk of foot ulceration will be minimized in this study by choosing non-impact activities.

- When exercising people with diabetes face some metabolic risks due to the exercise-induced fluctuations in their blood glucose levels. However, those risks will be minimized in this study by controlling the sugar levels both before and after exercising.

- Due to the type of exercises (strength training) that will be carried out in the present study, you may experience post-training muscular soreness. The principal symptom secondary to this condition is pain when exercising the fatigued muscles. However, the risk associated with this condition is trivial and no further complications related to muscle soreness are expected. In order to reduce the discomfort associated to muscle soreness, your *exercise* programme will start with low loads of training and will gradually increase both its volume and intensity. This will reduce the potential muscle fatigue provoked by the exercise intervention. However, if you experience discomfort associated with muscle soreness, your following training session will be adapted to minimize the risk of further soreness in the muscles affected. If the discomfort persists training will be stopped temporarily to allow the fatigued muscles to fully recover.

On top of that you will undergo an ECG (heart scan) to make sure that you do not suffer from any *overt* cardiac condition that may be worsened by the exercise program. For the ECG to be done you will be asked to lie on a therapeutic bed for 10 minutes whilst signals from your heart will be recorded on a computer using 12 leads secured with a plaster on the skin around your chest.

In case of concern please feel free to contact us at RCCK on the telephone number: 02920687739.

What happens if I am excluded from the study after undergoing the ECG at rest?
If the electrocardiogram shows clinically relevant abnormalities you will be immediately referred to *your GP*.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in part 2.

What happens when the research study stops?
After your second visit to the Research Centre of Clinical Kinesiology your participation in the study will finish.

Confidentiality- Will my taking part in this study be kept confidential?
Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2

What will happen if I don't want to carry on with the study?
If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

What If there is a problem?

In the event that something goes wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Cardiff University but you may have to pay your legal costs. The study is indemnified by Cardiff University, there is a limit of indemnity of £5,000,000.

If a participant, who has given informed consent, loses capacity to consent during the study, the participant would be withdrawn from the study and the data already collected with consent would be retained and used in the study.

Confidentiality- Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential, and any information about you which leaves RCCK will have your name and address removed so that you cannot be recognised.

GP notification
With permission, your GP will be informed of your participation in the trial.

What happens to the results of the research study?
The results of this study may be presented at conferences and published in scientific journals. A summary of the results will be sent to you after completion of the study.

Who is organising the study?
The study is being organized by the Department of Physiotherapy, School of Health Sciences, Cardiff University.

Who has reviewed the study?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Research and Development Committee and the South East Wales Research Ethics Committee (Panel D).

Contact for further information
In case you would like to discuss any part of the project in greater detail then please do not hesitate to contact Alejandro Meana-Esteban. The details are displayed below:

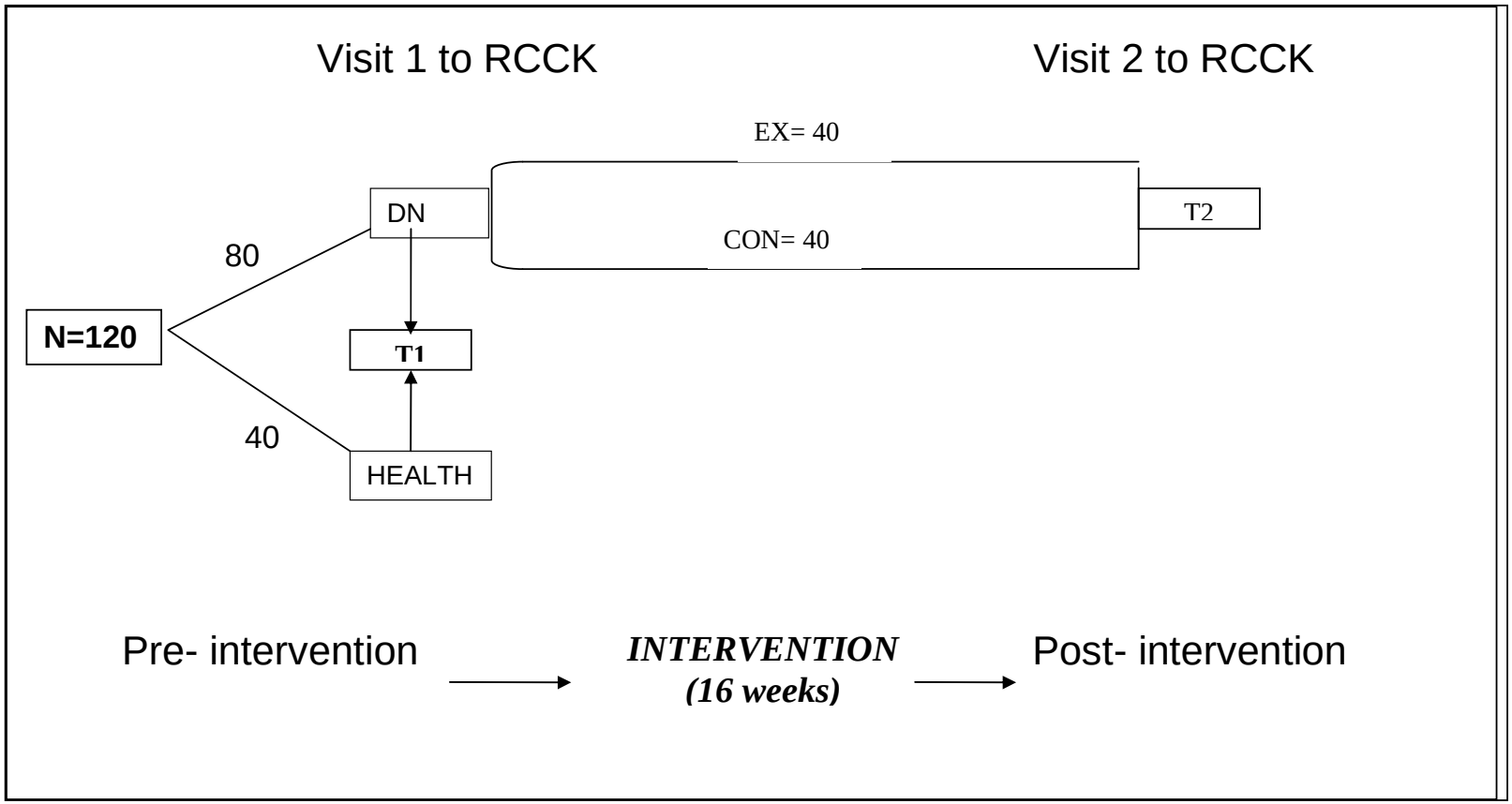
Research Centre for Clinical Kinesiology
Department of Physiotherapy
Ty Dewi Sant
Cardiff University
Cardiff CF14 4XN

Telf: [redacted]
Email: [redacted]

Thank you very much for your attention,

Alejandro Meana-Esteban
MSc Health and Sport Sciences

Chart 1. Sample size distribution. *Population groups:* Neuropathic group (DN) and healthy group (HEALTH); *Intervention groups:* exercise group (EX) and control group (CON); *Tests*: Test 1 (pre-intervention measurements) and T2 (Post-intervention measurements).



School of Healthcare Studies
Department of Physiotherapy
Director Dr R W M van Deursen MCSP MSc PhD ILTM
Adran Ffisiotherapi
Cyfarwyddwr Dr R W M van Deursen MCSP MSc PhD ILTM

Cardiff University
Tŷ Dewi Sant
Heath Park
Cardiff CF14 4XN
Tel *Ffôn* +44(0)29 2074 2267
Fax *Ffacs* +44(0)29 2074 2267
E-mail *E-bost* Physiotherapy@cf.ac.uk
Prifysgol Caerdydd
Tŷ Dewi Sant
Mynydd Bychan
Caerdydd CF14 4XN

DATE: 11/02/2008
VERSION Nº 3.2

INFORMATION SHEET

(Healthy group)

"The effect of non-impact resistance training on patients with diabetic neuropathy: *Acute responses and long-term adaptation*".

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish.

(Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study). Please, ask us if there is anything that is not clear or if you would like more information.

PART 1

What is the reason for the study?

Physical activity has been recognised to have an important role in health promotion and prevention of diseases. It is generally accepted that exercise reduces the risk of cardiovascular diseases (i.e. stroke or heart attack) and mortality in both healthy and clinical population**.** However, physical activity recommendations for people with peripheral neuropathy are based on evidence obtained from studies with non-neuropathic people with diabetes whereas no attempt has been made to demonstrate the positive effect of an exercise programme on people with peripheral neuropathy.

What is the purpose of the study?

The aim of this study is to determine whether and to what extent an exercise programme using muscle strengthening exercises have a positive effect on glycemic control, cholesterol levels, circulation, daily activity levels and quality of life in people with diabetes and peripheral neuropathy.

This study will be run by Dr Robert van Deursen, Professor Patricia Price and Alejandro Meana-Esteban (PhD student) in collaboration with Prof Keith Harding.

Why was I chosen to take part in this study?
You are a healthy subject with no symptoms of diabetes and you will be part of the control group. It is important to create a control group with non-diabetic subjects in order to quantify the effect of diabetes neuropathy in that clinical population.

Do I have to take part?
It is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving any reason. This would not affect the standard of care you receive.

What will happen to me if I take part?
If you agree to participate in the study, you will be asked:

1. To visit the Research Centre of Clinical Kinesiology (RCCK), situated in Heath Campus, on one occasion.

   During each visit to the RCCK each participant will be tested: blood pressure, height, weight, fat %, strength levels, circulation both at rest and when exercising and walking characteristics.

   During each visit, you will be asked to perform two main tasks:

   1. Walk on a 9 meters walk-way to analyze your walking characteristics.
   2. Produce movements with your right leg against a fixed resistance to determine your strength levels. When performing this task a cable will be secured with a plaster on the skin of your leg (calf muscle), which will be connected to a piece of equipment to give us information regarding your circulation when exercising.

   You will also be asked to complete two questionnaires. One questionnaire will contain questions regarding to your daily physical activities, whereas the second will contain health related questions.

   Each visit to the Research Centre of Clinical Kinesiology will last approximately 120 minutes.

For further information on the structure of the research can be found in Chart 1 (last page of this information sheet).

Expenses
Travel and parking expenses will be available for all participants in the study. Heath campus has got car parking facilities in which you can park your car.

What are the possible benefits of taking part?
We can not promise the study will help you directly but the information we gather from this study will help improve our understanding of the effects of diabetic neuropathy on the lower legs and may provide recommendations for treatment of people with diabetic neuropathy.

Is there any risk associated with the study?
No, there are no risks for you to participate in this study.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in part 2.

What happens when the research study stops?
After your visit to the Research Centre of Clinical Kinesiology your participation in the study will finish.

Confidentiality- Will my taking part in this study be kept confidential?
Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2

What will happen if I don't want to carry on with the study?
If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

What If there is a problem?
In the event that something goes wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Cardiff University but you may have to pay your legal costs. The study is indemnified by Cardiff University, there is a limit of indemnity of £5,000,000.

Confidentiality- Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential, and any information about you which leaves RCCK will have your name and address removed so that you cannot be recognised.

What happens to the results of the research study?
The results of this study may be presented at conferences and published in scientific journals. A summary of the results will be sent to you after completion of the study.

Who is organising the study?
The study is being organized by the Department of Physiotherapy, School of Health Sciences, Cardiff University.

Who has reviewed the study?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Research and Development Committee and the South East Wales Research Ethics Committee (Panel D).

Contact for further information
In case you would like to discuss any part of the project in greater detail then please do not hesitate to contact Alejandro Meana-Esteban. The details are displayed below:

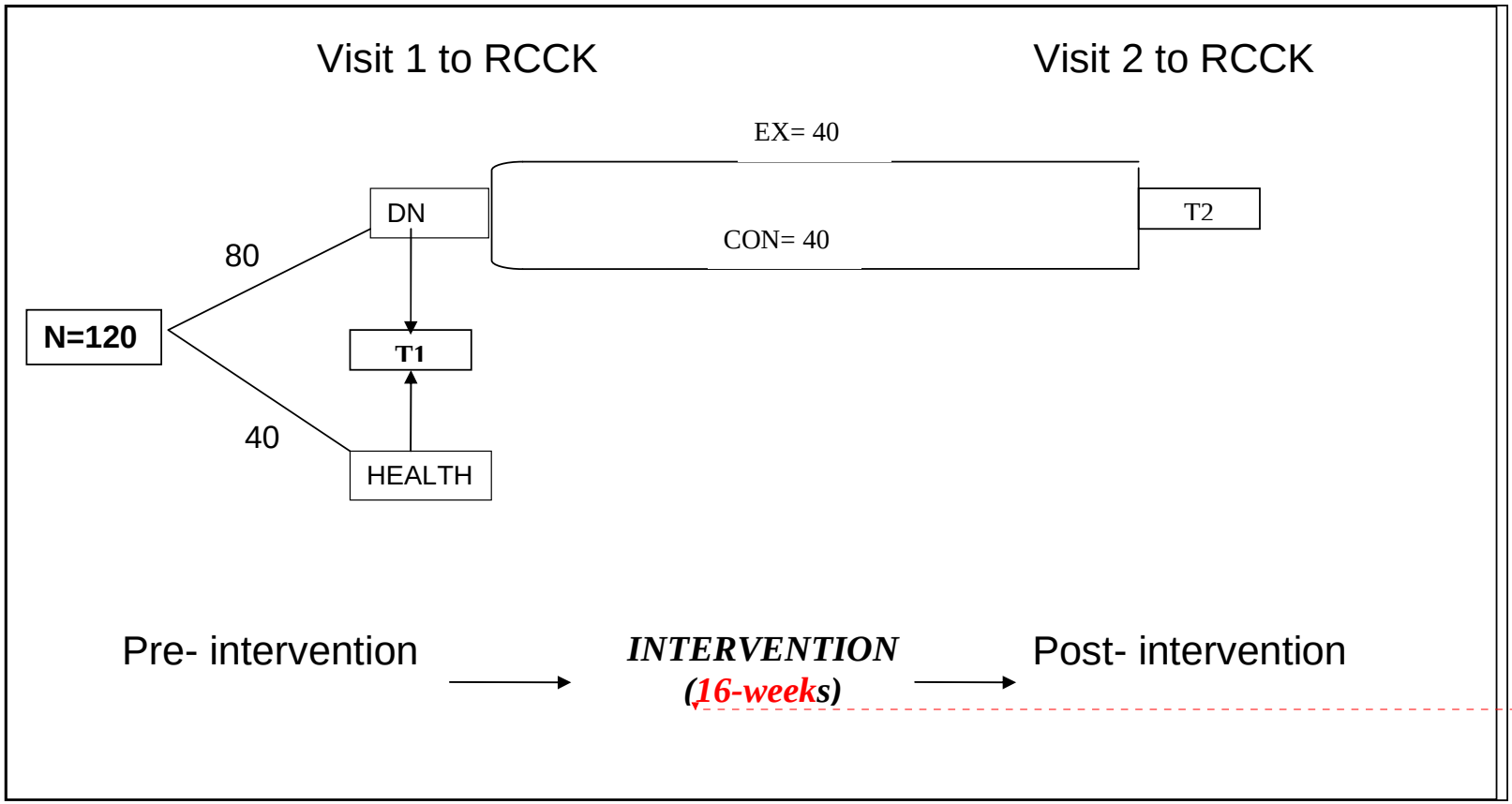
Research Centre for Clinical Kinesiology
Department of Physiotherapy
Ty Dewi Sant
Cardiff University
Cardiff CF14 4XN

Tel: [redacted]
Email: [redacted]

Thank you very much for your attention,

Alejandro Meana-Esteban
MSc Health and Sport Sciences

Chart 1. Sample size distribution. *Population groups:* Neuropathic group (DN) and healthy group (HEALTH); *Intervention groups:* exercise group (EX) and control group (CON); *Tests*: Test 1 (pre-intervention measurements) and T2 (Post-intervention measurements).



Deleted: *16 week*

Appendix 5: Phone interview

QUESTIONNAIRE

NAME: _____
DATE OF BIRTH: ____ /____ / ____
CONTACT NUMBER: _____

1. Which type of diabetes do you have:
a) Type I ☐
b) Type II ☐

2. Are you currently taking insulin:
a) Yes ☐
b) No ☐

COMPLICATIONS SECONDARY TO DIABETES

3. Do you suffer from loss of sensation in your feet – (this may be slight or significant)
a) Yes ☐
b) No (Skip to Question 5) ☐

4. Is the loss of sensation in your feet painful?
a) Yes ☐
b) No ☐

5. Do you suffer from any foot deformities:
a) Yes (Major deformity) ☐
b) No (Minor deformity eg: claw toes) ☐

6. Do you have any current foot ulcers?
a) Yes ☐
b) No ☐

7. Do you suffer from retinopathy (severe vision loss)?
a) Yes ☐
b) No ☐
c) No but I suffer from other diabetes related eye problems eg. Glaucoma ☐

8. Do you suffer from any form of kidney disease or are you on dialysis?
a) Yes ☐
b) No ☐

9. Do you have any history of kidney failure?
a) Yes ☐
b) No ☐

10. Do you have any history of heart problems eg: heart attack or angina?
a) Yes ☐
Please specify: _____

b) No ☐

P.T.O

11. Are you aware of any abnormalities or complications with your heart?

a) Yes ☐

Please specify: _____

b) No ☐

12. Are you on any medication?

a) Yes ☐

Please list names: _____ _____

_____ _____ _____

_____ _____ _____

_____ _____ _____

b) No ☐

13. Do you suffer from any <u>severe</u> lung disease?

a) Yes ☐

b) No ☐

14. Do you have any history of poor circulation in the lower limb?

a) Yes ☐

b) No ☐

15. Are you capable of walking independently to perform your daily activities without a walking aid?

a) Yes ☐

b) No ☐

16. Do you suffer from pain in the calves after walking 100 yards?

a) Yes ☐

b) No ☐

17. During the last 7 days, how many days did you do moderate physical activity, e.g. walking to the shops, carrying light loads, or cycling at a regular pace.

a) Number of days ☐

b) Average amount of time per activity _____

18. During the last 7 days, how many days did you do heavy physical activity, e.g. DIY, carrying heavy loads, or cycling.

a) Number of days ☐

b) Average amount of time per activity _____

THANK YOU FOR COMPLETING THE QUESTIONNAIRE
PLEASE RETURN THE QUESTIONNAIRE IN THE ENVELOPE PROVIDED

Appendix 6: Confirmation letter

Dear _____ _____ ,

Thank you for agreeing to participate in our research study. We look forward to meeting you on:

Date: **Wednesday 16th December 2009**
Time: **9.30 am**
Location: **RCCK Lab, Basement floor, Ty Dewi Sant, Cardiff University Heath Park, Cardiff, CF14 4XN.**
(Please see map attached)

If you are travelling by car, you may park in the University Hospital of Wales Multi-Storey car park (expenses will be reimbursed). Please report to the car park office on arrival and give them the reference number **"VP765"** to be able to park for £3 total.

We would request for you to bring (if possible) the following items when you attend:

1 x T-shirt

1 x Pair of shorts

Should this however pose a problem, please do not worry as these may be supplied on the day. We would kindly request that you would refrain from coffee on the day you come to the lab and from any unusual physical exacerbation 24 hours prior to attending the lab.

I will contact you the day before your appointment to confirm there are no problems. Should you wish to rearrange this appointment or have any further queries, please do not hesitate to contact us on: [redacted]

Yours Sincerely

ALEJANDRO MEANA-ESTEBAN

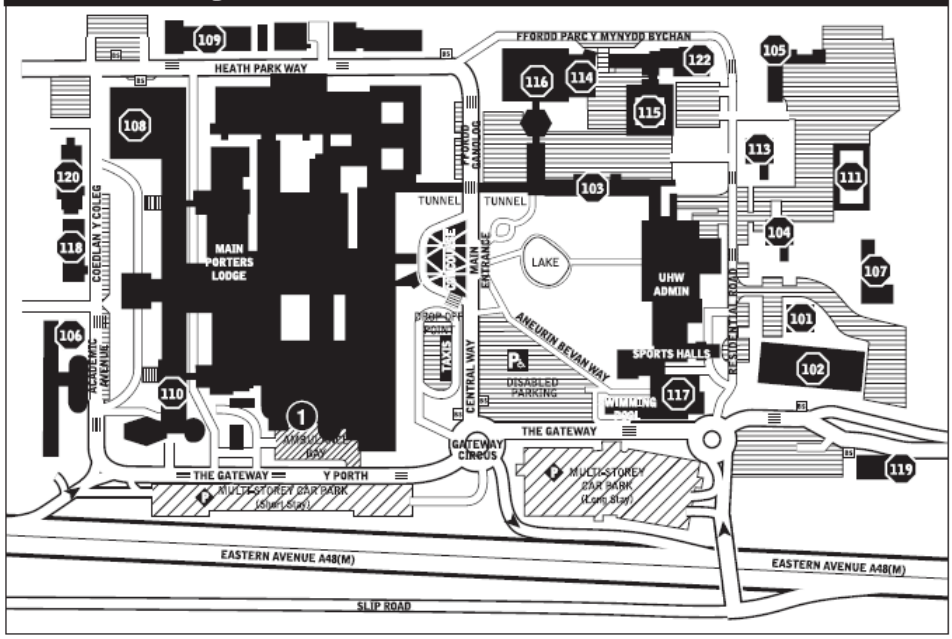
Heath Park Campus

Tel Switchboard: **029 2074 7747**

The University shares the Heath Park Campus with the University Hospital of Wales (UHW).

Brecknock House	101	Healthcare Studies	116	Nursing & Midwifery	116	Tŷ Maeth	119
Cardiff Medicentre	102	Henry Wellcome Research Building	108	Pembroke House	113	Wales Heart Research Institute	120
Cardigan House	103	Institute of Medical Genetics	109	Postgraduate Medical and Dental Education	122	Student Support Centre	103
Carmarthen House	104	Medical School	110	Radnor House	115		
Denbigh House	105	Monmouth House	111	Sports & Social Club	117		
Dental School/Hospital	106	Neuadd Meirionnydd	122	Tenovus Building	118		
Glamorgan House	107	New Lecture Theatre complex	114	Tŷ Dewi Sant Building	116		

Heath Park Campus



* You are looking for building number 116 on map The is a reception when coming from Concourse. You ask for the lab on the basement. If there is any problem do not worry, I will go to the reception to pick you up

Appendix 7: Informed consent sheet

School of Healthcare Studies
Dean *Deon* M J Booy BA MA TDipCOT ILTM
Department of Physiotherapy
Director Dr R W M van Deursen MCSP MSc PhD ILTM
Adran Ffisiotherapi
Cyfarwyddwr Dr R W M van Deursen MCSP MSc PhD ILTM

DATE: 11/02/2008
VERSION Nº 3

Cardiff University
Tŷ Dewi Sant
Heath Park
Cardiff CF14 4XN
Tel *Ffôn* +44(0)29 2074 2267
Fax *Ffacs* +44(0)29 2074 2267
E-mail *E-bost* Physiotherapy@cf.ac.uk
Prifysgol Caerdydd
Tŷ Dewi Sant
Mynydd Bychan
Caerdydd CF14 4XN

CONSENT FORM

Title of Project*:* "The effect of low impact resistance exercises on patients with diabetes neuropathy: *Acute responses and long-term adaptation"***.**

Name of Researchers: Dr Robert van Deursen
Alejandro Meana-Esteban

Please initial box

1. I confirm that I have read and understand the information sheet dated 11/02/2008 (Version 3) for the above study. I have had the opportunity to consider the Information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that photography and/or video-recording is used in this study. ☐
4. I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from Cardiff University, from regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
5. I agree my GP being informed of my participation in the study. ☐
6. I agree to take part in the above study. ☐

Name of Patient	Signature	Date
Witness	Signature	Date
Name of person taking consent	Signature	Date

Appendix 8: ECG sheet

School of Healthcare Studies
Department of Physiotherapy
Director Dr R W M van Deursen MCSP MSc PhD ILTM
Adran Ffisiotherapi
Cyfarwyddwr Dr R W M van Deursen MCSP MSc PhD ILTM

Cardiff University
Tŷ Dewi Sant
Heath Park
Cardiff CF14 4XN
Tel *Ffôn* +44(0)29 2074 2267
Fax *Ffacs* +44(0)29 2074 2267
E-mail *E-bost* Physiotherapy@cf.ac.uk
Prifysgol Caerdydd
Tŷ Dewi Sant
Mynydd Bychan
Caerdydd CF14 4XN

ECG REPORT

Research Project: The effect of non-impact resistance training on patients with diabetic neuropathy: *Acute responses and long-term adaptation.*

Subject code (as written on the ECG sheet):

Are there any abnormalities in this subjects' ECG

that would exclude him/her from the study? ***Yes*** ***No***

If yes, could you briefly explain the abnormalities observed in the ECG to inform his/her GP?

Date ***Signature***

Dr. Sharmila Khot, MB BS, DA, MD, FRCA, FFPMRCA(Consultant in Anaesthesia and Pain Medicine)

This sheet (and any attachments) is confidential and may contain personal views. If you have received or found it in error, do not use, copy or disclose the information in any way. Please notify Alejandro Meana-Esteban (contact number: [redacted]) immediately of this error.

Appendix 9: Ethical approval

Canolfan Gwasanaethau Busne
Business Services Centre

South East Wales Research Ethics Committee - Panel D

Telephone: 02920 376822/6823
Facsimile: 02920 376835

19 February 2008

Dr R Van Deursen
Director of Physiotherapy
Cardiff University
Research Centre For Clinical Kinaesiology;
Department Of Physiotherapy
School of Healthcare Studies,
Heath Park, Cardiff University
CF14 4XN

Dear Dr Van Deursen

Full title of study: **The effect of non-impact resistance exercise on patients with diabetic neuropathy: Acute responses and long-term adaptattions**
REC reference number: **08/WSE04/8**

Thank you for your letter of 4th December 2008, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair, Dr DEB Powell.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). There is no requirement for [other] Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Canolfan Gwasanaethau Busnes
Ty Churchill
17 Ffordd Churchill
Caerdydd, CF10 2TW
Ffôn: 029 20 376820 WHTN: 1809
Ffacs: 029 20 376826

Business Services Centre
Churchill House
17 Churchill Way
Cardiff, CF10 2TW
Telephone: 029 20 376820 WHTN: 1809
Fax: 029 20 376826

rhan o Addysgu Bwrdd Iechyd Lleol Powys / part of Powys Teaching Local Health Board

Approved documents

The final list of documents reviewed and approved by the Committee is as follows

Document	*Version*	*Date*
Application	5.5	04 December 2007
Investigator CV	A Meana-Esteban	01 December 2007
Investigator CV	R van Deursen	
Protocol	3	11 February 2008
Covering Letter	R van Deursen	04 December 2007
Letter from Sponsor	Cardiff University	05 December 2007
Compensation Arrangements	UMAL	01 August 2007
Questionnaire: SF 36 Health Survey		
Questionnaire: Modified Baecke Questionnaire for older adults		
Questionnaire: Telephone Interview	2.2 - Healthy Group	11 February 2008
Questionnaire: Telephone Interview	2.1 - Patients	11 February 2008
Letter of invitation to participant	1	25 November 2007
GP/Consultant Information Sheets	No Version	
Participant Information Sheet	3.1 - Patients	11 February 2008
Participant Information Sheet	3.2 - Healthy Group	11 February 2008
Participant Consent Form	3	11 February 2008
Response to Request for Further Information		
Flow Chart	No Version	

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://www.rdforum.nhs.uk/rdform.htm.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

Here you will find links to the following

a) Providing feedback. You are invited to give your view of the service that you have received from the National Research Ethics Service on the application procedure. If you wish to make your views known please use the feedback form available on the website.
b) Progress Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
c) Safety Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
d) Amendments. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
e) End of Study/Project. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nationalres.org.uk .

08/WSE04/8	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

pp.

Dr D E B Powell
Chair

Email: jagit.sidhu@bsc.wales.nhs.uk

Enclosures: *Standard approval conditions - SL-AC1*
Dr RWM van Deursen

Copy to: