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Biomechanics and the metabolic cost of
walking in people with diabetes

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Dissemination of study findings

1 Published articles

Petrovic M, Deschamps K, Verschueren SM, Bowling FL, Maganaris CN, Boulton AJM, Reeves ND. Is the metabolic cost of walking higher in people with diabetes? *J Appl Physiol* 120: 55-62, 2016.

2 Conferences presentations

Petrovic M, Brown SJ, Bowling FL, Deschamps K, Verschueren SM, Maganaris CN, Boulton AJM, Reeves ND. The role of the external lever arm of the foot in walking efficiency in people with diabetes. Diabetic Foot Study Group (DFSG). Bratislava, Slovakia. [Oral presentation] 2014.

Petrovic M, Bowling FL, Deschamps K, Verschueren SM, Maganaris CN, Boulton AJM, Reeves ND. Does the energy cost of walking explain why patients with DPN walk more slowly? European Association for the Study of Diabetes (EASD). Stockholm, Sweden. [Poster presentation] 2015.

Thesis abstract

Diabetes mellitus is a serious worldwide disease characterised by pathological metabolism of sugars. Diabetic peripheral neuropathy (DPN) is a common complication of diabetes involving dysfunction of peripheral nerves. Diabetes is known to alter a number of biomechanical aspects of gait, but it remains unknown as to whether these alterations could impact upon the metabolic cost of walking (CoW). The aim of this thesis was to investigate the CoW in people with diabetes and examine biomechanical factors that could contribute to explaining any potential differences. Data were generated from three groups: patients with DPN (n=14), patients with diabetes but without peripheral neuropathy (DM, n=22), and controls without diabetes (Ctrl, n=31). Gait assessment was performed using a Vicon motion analysis system and Kistler force plates while participants walked at a range of matched speeds (between 0.6 and 1.6 m/s). Oxygen consumption was measured continuously whilst participants walked on a motor-driven treadmill at the range of matched walking speeds. Ultrasonographic imaging data from the plantarflexor muscle-tendon complex (MTC) were collected in vivo during walking to determine MTC properties. Magnetic resonance imaging of the ankle joint in the standing position was used to quantify the internal leverage around the ankle. Isometric plantarflexor maximal voluntary contraction strength was measured using a dynamometer. The CoW was significantly higher in the DPN group across a range of matched walking speeds and also in the DM group at selected speeds, compared to Ctrl. Despite the higher CoW in patients with diabetes, concentric lower limb joint work was significantly lower in DM and DPN groups compared to Ctrl. A greater value for the effective mechanical advantage (EMA) at the ankle joint was found in the DPN and DM groups compared to Ctrl, meaning that the ankle plantarflexor muscles developed relatively lower forces to generate a given joint moment compared to Ctrl. The increased EMA was mainly caused by a smaller external moment arm of the ground reaction force in the DPN and DM groups compared to Ctrl. The DPN group reduced the joint moment at the ankle during walking by applying the ground reaction force more proximally on the foot, or at an angle directed more towards the ankle, thereby reducing the external moment arm and increasing the EMA around the ankle. The DPN group demonstrated significantly less Achilles tendon elongation during walking, higher stiffness and higher hysteresis compared to Ctrl. These properties mean that the Achilles tendon would store and release less energy in the DPN group during walking, requiring more work from the plantarflexor muscles. Vertical displacement of the centre of mass during walking was not different between groups and is therefore unlikely to be a factor in itself that contributes towards the increased CoW in people with diabetic neuropathy. A higher cumulative joint work resulting from an increased cadence may contribute to the higher CoW in patients with diabetes, along with a reduced elastic energy contribution from the Achilles tendon.

1. INTRODUCTION

1.1 Introduction to Diabetes mellitus

Diabetes mellitus (DM) is a serious worldwide endocrinological disease characterised by pathological metabolism of sugars. The term diabetes mellitus describes a group of chronic metabolic disorders characterised by hyperglycemia resulting either from a deficiency of insulin production, or decreased ability to transduce the insulin signal (insulin resistance), or both (7). Diabetes is a global epidemic with significant morbidity, very common in older people and is often undiagnosed (67). Diabetes is associated with a range of serious complications that result in reduced quality of life and premature mortality. The prevalence of diabetes is increasing at an alarming rate and the condition presents lifelong health problems (27). There are four main types of diabetes:

Type 1 is caused by an autoimmune reaction where the body's defence system attacks the insulin-producing beta cells in the pancreas. Type 1 diabetes is developed if the body cannot produce any insulin. Usually appears before the age of 40 years. It is the least common of the two main types of diabetes and accounts for around 10% of all people with diabetes (35).

Type 2 diabetes develops when the body can still produce insulin, but this is either not sufficient, or the insulin has little or no intended effect (known as insulin resistance). This type of diabetes usually appears in people over the age of 40 years, though in South Asian and African-Caribbean people, it often appears much earlier, appearing typically after the age of 25 years. Type 2 diabetes is the more common of the two main types and accounts for around 90% of people with diabetes (34).

Type 2 diabetes typically affects patients who are overweight and display lower level of physical activity, but evidence suggests that stressful experience might affect diabetes, both its onset and its exacerbation (79). Diabetes is a very common condition among older adults. Secondary diabetes develops in serious health failures such as pancreatic damage, hepatic cirrhosis, endocrinological disease/therapy, or anti-viral/anti-psychotic therapy.

Gestational diabetes is another form of diabetes, affecting pregnant women without a previous history of diabetes, but who develop a high blood glucose level during pregnancy. Gestational diabetes has a tendency to occur around the 24th week of pregnancy and occurs when insulin receptors do not function properly. This is likely due to pregnancy-related factors such as the presence of human placental lactogen that interferes with susceptible insulin receptors. This in turn causes inappropriately elevated blood sugar levels.

The main complications associated with diabetes are cardiovascular diseases, nephropathy, retinopathy and peripheral neuropathy (46) and this will be discussed further in one of the following sections.

1.2 Symptoms of DM Type 1 and Type 2.

There are a variety of symptoms present in diabetes: increased thirst, urinating frequently, particularly at night, increased hunger (especially after eating), feeling very tired, dry mouth, non-healing skin infections, unexplained weight loss and loss of muscle bulk, blurred vision, fatigue, cramps, Itchiness around the genitals and recurrent infections including thrush Infections. The three classic symptoms of diabetes are thirst, polyuria (excessive urination) and weight loss. As glucose is lost

in the urine it draws fluid and other small molecules with it, causing excessive urination, which in turn causes dehydration and thirst. Weight is lost because of rapid breakdown of fat and protein reserves to compensate for the loss of glucose and metabolic inefficiency due to lack of insulin action. The breakdown of protein primarily occurs from skeletal muscles, explaining the reduction in body mass through muscle atrophy. In most cases the presence of diabetes is associated with several of these symptoms occurring together.

1.3 Risk factors and prevalence

Type 1 diabetes is not currently preventable, while Type 2 is more complex and is a combination of genes and life habits (lifestyle) and prevention is certainly possible. Simple lifestyle measures have been shown to be effective in preventing or delaying the onset of type 2 diabetes (153).

The main risk factors for developing diabetes are:

1. Genetics (family history)
2. Obesity
3. Sedentary way of life and lack of exercise (hypokinesia)
4. Stress
5. Poor nutrition
6. High blood pressure, heart attack, stroke, coronary diseases
7. Ethnicity.

The International Diabetes Federation (IDF) estimates that in 2014 the five countries with the highest numbers of people with diabetes were India, China, the United

States, Russia and Brazil, also low and middle income countries face the greatest burden of diabetes. Li et al. (77) shows that the “top three” countries with the absolute numbers of people with diabetes are India, China, and the USA. Large increases in prevalence are also expected, mostly in countries such as Bangladesh, Brazil, Indonesia, Japan, and Pakistan. Among other factors, ethnicity can be one of the factors that can decrease or increase risk of developing diabetes. In some cases that can be explained by socio-economic factors, studies have shown that even with equal access prevalence of diabetes differs between people of different ethnicity. The prevalence of adult Type 2 diabetes is about three to five times greater in African-Caribbean and South Asian people (Table 1), respectively, compared with the white European population. Certain gender differences are present in both type 1 and 2. Diabetes prevalence among the countries is ranging from 2.4% to 37.5% (138, 160, 20). The world health organisation estimates that by 2025 as many as 200–300 million people worldwide will have developed type 2 diabetes (74). Approximately 15% of adult people in developed countries have diabetes. In the United Kingdom, 3.2 million people (6% of the total United Kingdom population) have been diagnosed with diabetes (67). By 2025, it is predicted that there will be more than four million people with diabetes in the United Kingdom (8). An additional worldwide problem is undiscovered/undiagnosed diabetes and it is estimated that there are up to half a million people in the United Kingdom who have diabetes but have not yet been diagnosed.

Table 1. *Countries with the highest prevalence of diabetes (67).*

Country	% prevalence
Tokelau	37.5
Federated States of Micronesia	35.0
Marshall Islands	34.9
Cook Islands	25.7
Saudi Arabia	24.0
Nauru	23.3
Kuwait	23.1
Qatar	22.9

1.4 Financial costs

In the United Kingdom, it is currently estimated that 10% of the total NHS budget is spent on diabetes. This works out at around £9 billion a year (based on 2007/2008 budget for the NHS of approximately £90.7 billion), or £173 million a week, or £25 million a day (35). Patients with diabetes require at least two to three times the health-care resources to patients without diabetes, and approximately 15% of health care budgets are spent on diabetes related care. In 2012, the cost of diabetes to the United States of America healthcare system was \$245 billion, of which \$69 billion was due to reduced productivity, and \$176 billion was spent on direct medical costs. Hex et al. (58) presented the current and the future costs of Type 1 and Type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs. In their study they estimated total costs screening, testing, treatment, management and complications, in 2011 to be £10 billion and estimated cost for 2035 to increase to £17 billion.

1.5 Complications of Diabetes mellitus

The main complications associated with diabetes are cardiovascular diseases, nephropathy, stroke, peripheral arterial disease, retinopathy, skin problems, poor wound healing and peripheral neuropathy (43, 46). The prevalence of diabetes is increasing because of the ageing population, obesity and sedentary way of life. The chronic hyperglycemia is associated with long-term damage, failure or dysfunction of many tissues and organs (65). Diabetes can cause peripheral nerve dysfunction, which might be one pathway through which diabetes leads to decreased physical function, particularly in the lower limbs (97).

Other complications of diabetes might include foot deformity (146), deterioration in the function of large afferent nerve fibres, slow eye movements (57), motor and vestibular impairment (121).

Neuropathies, muscle weakness and balance impairments, either together or individually, can lead to gait abnormalities including improper pressure distribution on the foot, a longer stance phase and shorter steps than observed in people without diabetes (21, 57, 95, 123, 91). These gait and balance impairments in diabetes are not simply a matter of academic interest; people with diabetes are fifteen times more likely to report experiencing a fall-related injury during standing and walking when compared to people without diabetes (118, 139, 36, 118). Although there are many complications related to diabetes, this introduction will focus mainly on diabetic peripheral neuropathy (DPN), as one of the most significant complications that affects gait in people with diabetes.

1.5.1 Diabetic Peripheral Neuropathy

Diabetic peripheral neuropathy is a long-term complication and dysfunction of

peripheral nerves. The main cause is neurovascular alterations to the nerve fibres and blood vessels supplying the nerve endings, resulting in reduced or absent nerve conduction (35). The European association for the study of diabetes defines DPN as “the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes” (17). The incidence of DPN ranges from 13 to 68% in diabetes populations (145, 97, 17). Nerves become damaged and people affected by neuropathy are 15 times more likely to experience an injury during walking (38, 25). DPN affects the sensory, motor and autonomic components of the nervous system, manifesting as a loss of protective sensation, intrinsic foot muscle dysfunction and anhidrosis (diminished sweating response) of the foot. Both age and duration of diabetes are independent risk factors for DPN. Moreover, neuropathic patients walk slower, have longer double support time (two feet in contact with the ground), shortened stride lengths, decreased ground reaction forces and decreased ankle moments and powers compared to matched controls (21, 57, 116, 96, 73, 37), while the impact of diabetes on the cost of walking (CoW) remains unknown. Symptoms of DPN may include sensory deficits in light touch and proprioception and motor deficits in terms of reduced strength in the ankle muscles. The sensory and motor deficits may subsequently result in balance disorders and an increased risk of falling (21), which may lead to injuries and hospitalization in this group of patients (91).

1.5.2 Diabetic Peripheral Neuropathy and gait impairment

Patients with diabetic peripheral neuropathy exhibit decreased stability both, while standing and during walking (16, 48, 134, 19). Several authors (128, 73) have found

gait pattern deviations in diabetic peripheral neuropathy and diabetes mellitus patients. For instance, important deviations were revealed in hip, knee, ankle joints and trunk movement patterns over the entire stance phase of gait in both DPN and DM subjects (128). Fernando et al. (41) presented that DPN participants walked slower than healthy control subjects (50, 126, 129) and two studies reported slower walking speeds in the DPN group compared to the DM patients (128). DPN-related changes in the lower limbs may lead to functional gait variations; predominantly related to reduced range of movement of joints, reduced active muscle power and changes in gait mechanics (9). Gait related changes and developing foot ulcers are very often consequences of neuropathy (143). DPN can have catastrophic consequences for patients, as this leads to foot ulceration and increased risk of limb amputation, significant healthcare costs, reduced quality of life and reduced mobility (41, 17, 135). Handsaker (57) showed that training may favourably alter muscle activations and increase the speed of ankle and knee strength development in people with DPN, potentially reducing the risk of falling and improving safety during the everyday task of stair walking. Therefore, understanding the impact of DPN on the biomechanical aspects of human locomotion is clinically important (45). Boulton (17) recommends all diabetic patients, regardless of their type of diabetes, duration of diabetes, or age, require careful clinical examination of the lower extremities and feet at least once a year.

1.6 Glycation and Diabetes mellitus

The hallmark of diabetes mellitus, whether Type I or type II, is hyperglycemia. Clinical complications associated with diabetes are most likely the consequence of hyperglycemia via both altered metabolic pathways and non-enzymatic glycation of

proteins. The nonenzymatic glycation of proteins is accelerated in diabetes due to elevated blood glucose concentration. The Amadori product of nonenzymatic glycation will further cross-link with other proteins to form advanced glycosylation end products (158). Advanced glycation end products (AGEs) represent a heterogeneous group of chemical products resulting from a non-enzymatic reaction between reducing sugars and proteins, lipids, nucleic acids, or a combination of these (99). The glycation process (glucose fixation) affects circulating proteins (serum albumin, lipoprotein, insulin, hemoglobin), whereas the formation of AGEs implicates reactive intermediates such as methylglyoxal. Glycation involves a series of reactions in which proteins bind non-enzymatically to reducing sugars such as glucose (3, 51). AGEs have been identified in the kidney, nerve, arteries and heart of diabetic animals and humans (3, 135, 44, 98) Thus, the accumulation of these compounds has been implicated in the etiology of the diabetic complications of nephropathy, retinopathy, cataract, neuropathy and accelerated vascular disease (137, 44, 98). Hyperglycemia is still considered the principal cause of diabetes complications (104). The recognition and binding of AGEs to RAGE contribute to the microvascular and macro vascular complications of diabetes. It has been shown in animal models of diabetes (111, 112, 113) that non-enzymatic glycation affect other tissues such as tendon. This causes increased cross-linking, increasing the stiffness and modulus of the tendon.

1.7 Gait characteristics in people with DM and DPN

Walking represents the most convenient and the most usual way of transport for humans. Walking is one of the most practiced of all motor skills (72). Also walking is a popular, convenient and a relatively safe form of exercise (60) that holds great

promise for weight management (56, 69). Weight management is most effective when individuals can accurately determine how much energy they expend during exercise, which, in the case of walking, is dependent on speed (22). In the work of Sasaki & Neptune (101) it is suggested that previous studies indicated that the two primary energy saving mechanisms in walking are the passive exchange of potential and kinetic energy (25) and elastic energy utilization (59). Assuming that walking can be modelled as an inverted-pendulum, the maximum theoretical efficiency of the energetic exchange between kinetic and potential energy (i.e., energy recovery) is only as high as 65% and varies depending on walking speed (26) and stride frequency (93). During human gait, the storage and return of elastic energy in compliant structures is an important energy saving mechanism that will reduce the necessary muscle fibre work and be an important determinant of the preferred gait mode (i.e., walk or run) at a given speed.

The main question this thesis addresses the impact of diabetes and diabetic peripheral neuropathy on the CoW. The further questions investigate possible mechanisms accounting for any differences in the CoW, including vertical displacement of the centre of mass (CoM) and the cadence.

The purpose of the studies comprising this thesis are to investigate the metabolic cost of walking in people with diabetes and further elucidate biomechanical factors that alter gait characteristics and that may impact upon the metabolic cost of walking. Treadmill walking has frequently been used to assess in older adults due to the ability to control walking speed closely (28, 42, 80, 84, 151). It is hypothesized that walking at slower speeds (as is the case in diabetes patients) may be mechanically less efficient (100). There are many possible factors, which can explain oxygen

uptake and the CoW, among them are shorter strides, longer double support time, higher values of ground reaction forces, presence of neuropathies and a stiffer Achilles tendon.

It is particularly interesting to investigate, from a biomechanical point of view, what is the impact of diabetic peripheral neuropathy and diabetes without neuropathy on walking characteristics.

Walking patterns of diabetic patients are explained in many studies:

A review of the main aspects of gait in people with diabetes and diabetic neuropathy follows:

1.7.1 Spatio-temporal gait parameters

The gait speed of diabetes and control patients was described in many studies. The self-selected walking speed in diabetic patients has been observed to range from 0.7 to 1.24 m/s and was significantly lower than that of controls, which ranged from 0.9 to 1.47 m/s (41, 21, 91, 128, 110). Petrovsky et al. (105) described a significantly higher walking speed in controls compared to groups with either type 1 or 2 diabetes. Additionally, they assessed slower reaction times in patients with diabetes and a much slower gait while turning than compared to control subjects. They demonstrated that subjects with type 2 diabetes used an average of two steps to turn, whereas control subjects on average used one step. The subjects with type 2 diabetes took 1.66 s to execute this free pivot, whereas the control subjects took, on average, 0.78 s. Stride length findings in diabetes patients has been observed to range from 1.04 to 1.38 m and was significantly lower than that of for the Ctrl group, which ranged from 1.14 to 1.54 m (127, 129, 95, 87, 30, 37, 91). Step length differences between the DPN and the Ctrl groups were well documented in many

studies (29, 91, 11, 95, 5, 157), with constant findings of shorter steps in the DPN groups. Step-cycle length was described in several studies (36, 37, 38, 95, 91, 57). Values ranged from 1.38 to 1.54 m for controls, and from 1.08 to 1.38 m in diabetic patients. Cadence (steps/min) at self-selected walking speed has been described in many studies (95, 157, 49, 110, 87, 76, 123, 159, 4). All mentioned studies showed that the DPN group had a higher cadence compared to the Ctrl group and as the main responsible factors were suggested as slower self-selected speed, shorter steps and shorter strides.

Table 1. Self-selected walking speed reported from the literature in three different participant groups (Ctrl – controls, DM – Diabetes mellitus (without neuropathy), DPN – Diabetic Peripheral Neuropathy).

	Ctrl	DM	DPN
	Self-selected walking speed (m/s)		
Sawacha et al. (2009a)	1.27	1.10	1.10
Sawacha et al. (2009b)	1.00	-	1.00
Savelberg et al. (2012b)	1.22	1.30	1.40
Allet et al. (2009)	1.49	1.27	1.19
Dingwell et al. (2000)	1.47	-	1.24
Brown et al. (2014)	1.39	1.28	1.22
Menz et al. (2004)	1.21	-	0.98
Jor`dan et al. (2014)	1.14	-	1.05
Raspovic (2013)	1.3	1.4	1.2
Savelberg et al. (2012a)	1.18	1.06	1.02
Mueller et al. (1995)	1.26	1.09	-
Martinelli et al. (2013)	1.03	-	0.89
Salsich & Mueller (2000)	1.29	-	1.12
de Mettelinge et al. (2013)	1.38	-	1.04
Kwon et al. (2013)	0.9		0.7

Gait cycle time has been investigated in various studies. Gait cycle time in diabetic patients was in range between 1.15 to 1.26 s, with the time ranging from 1.00 to 1.22 s for controls (118, 119, 120, 121, 95, 96, 38, 30, 36, 37, 38). Richardson et al. (118) showed that environmental factors have a significant effect on all spatio-temporal

gait parameters in diabetic subjects. In a challenging environment in which either walking surface conditions or lighting intensity was manipulated, a decrease in step length and walking speed and an increase in step width, step-width variability, step width to step length ratio and step-time variability were observed. Furthermore, the controls did not decrease their step length or increase step width in a challenging environment, unlike patients with diabetes. Menz et al. (95) found comparable results, reporting that the walking speed of patients with DPN was 19% slower while walking on a level surface and 25% slower on an irregular surface than among healthy controls.

1.7.2 Kinematics of gait

Petrovsky et al. (105, 106, 107) compared diabetic patients with and without neuropathy to healthy controls and showed that subjects with diabetes walked significantly slower than control subjects and with a wider stance (base of support), both for walking in a linear path and when making turns. The accelerometers measured side-to-side and forward–backward directions. The coefficient of variation was higher at the head than the shoulders and higher for the hip than the shoulders for both controls and diabetics. However, the coefficient of variation for movement was much larger in diabetic patients. Menz et al. (91) found smaller magnitude accelerations in patients with diabetes compared to controls and recorded more erratic acceleration signals in diabetic patients, particularly at the head. Dingwell et al. (36) used a tri-axial accelerometer on the upper body to measure the standard deviation of accelerations and reported no difference between diabetic patients with neuropathy and healthy controls. Consistently smaller ranges of motion (RoM) at the ankle, knee and hip in the DPN group have been reported from a range of studies

and likely underlies the shorter step length reported in diabetes patients. Ankle RoM in diabetic patients was reported to have a range of 21 - 23.6 degrees, and 24.4 - 26.7 degrees for controls. Values of the knee RoM were reported to have a range of 25.5 - 54.3 degrees for the DPN group and 30.7 - 57.7 degrees for the Ctrl group. Hip RoM for the DPN group was reported to have a range of 38.9 - 43.2 degrees for the DPN group and 40.5 - 44.7 for the Ctrl group (1, 50, 87, 95, 110, 123). The reported values for the range of joint movement (i.e., the minimum to maximum joint movement) were higher at the hip for diabetic patients compared to controls, but lower at the ankle with more variation in range reported at the knee in diabetic patients.

1.7.3 Kinetics of gait

The ground reaction forces (GRF) have been investigated both in diabetes and controls patients. It was anticipated that DPN patients would exhibit higher GRF due to neurological deficit and reduced proprioception, causing a relatively higher impact with the ground. Constantly higher values of the GRFs have been reported in the diabetes patients compared to controls (129, 142, 125, 159) with the values at initial contact in the range of 82.5 and 104 N/BW% for the diabetes patients and 80-91.2 N/BW% for the control group. Sacco et al. (123) were the only group who differentiated the two peaks of the GRF, the first at heel strike and another at the moment of propulsion. For the first peak, they agreed with Katoulis et al. (73) and Uccioli et al. (142) who did not find a difference in the mean GRFs between controls and patients with or without neuropathy. Concerning the second peak, however, they found a significant difference between vertical forces of controls and the values of the diabetic group. In addition to patients with and without neuropathy, Katoulis et

al. (73) evaluated a group of diabetes patients with previous ulcers. They described a decrease in the maximum value of the vertical component of the GRF for these patients compared to healthy controls, and diabetic patients without neuropathy ($p < 0.03$). Meier et al. (89) investigated the anterior posterior (A/P) and medio-lateral (M/L) forces during stopping tasks. The participants were instructed to walk at their self-selected walking speed and to stop in front of the marked stopping line on the walkway. They found a slower A/P speed of the centre of mass (CoM), and larger A/P and M/L centre of pressure overshoots than in controls. Furthermore, they described decreased shock absorption at heel strike and Uccioli et al. (142) described significantly reduced peak forces recorded mainly during heel strike and push-off for patients with diabetes compared to a control group. During walking, movement is caused by moments of force generated by muscles around ankle, knee and hip joints. Joint moments are a measure of the rotational force acting around a joint, allowing an indication of the magnitude of the muscle force and they have been well investigated both in diabetic and non-diabetic populations. Similar findings have been reported across a range of studies (21, 128, 76, 95, 96, 159) where patients with diabetes are reported as developing lower ankle joint moments during walking. The knee joint extension moment tended to be lower in DPN participants. In a study that did not control gait velocity, Kwon et al. (76) found similar results comparing subjects with and without DPN, but in addition they reported decreased maximal plantarflexion moments and knee joint extension moments Mueller et al. (95) found reduced maximal plantarflexion moments in diabetic polyneuropathy. Katoulis et al. (73) described lower knee joint moments in the DPN group compared to controls. They also notice that gait alterations in people with diabetes and neuropathy could

facilitate foot injuries, thus contributing to frequent foot ulceration. As the main cause for lower moments all authors suggested that this alteration might be caused by a decline in plantarflexor strength and slower self-selected walking speed in people with neuropathy. External and internal moment arms are important measurements contributing to the calculation of the effective mechanical advantage around a joint. The external moment arm (ExtMA) length around the ankle during walking is defined as the perpendicular distance between the resultant GRF vector in sagittal plane and the ankle joint centre of rotation. A smaller ExtMA at the ankle means that either the resultant GRF is applied closer to the ankle joint centre or the angle of application is more towards the ankle, making the ExtMA smaller, thereby minimising the ankle joint moment (assuming a similar GRF). The effective mechanical advantage around the ankle is given by the ratio of the internal (Achilles tendon moment arm) to the ExtMA, with lower values reflecting a relatively greater contribution from the plantarflexor muscles towards the joint moment required to overcome the external resistance applied (15). Indeed, it has been shown in a healthy population how a marked increase in the ExtMA around the knee and therefore a marked reduction in the effective mechanical advantage at the knee, likely accounts for the marked increase in the cost of transport when going from walking to running (14). To the best of our knowledge there are no previous studies reporting the effective mechanical advantage in diabetes patients during walking, but it has been measured for different human and animal populations (10, 14, 54, 130, 136).

1.7.4 Muscle activity during gait

Neuropathic damage of the nerves affects motor control of the lower limbs, as DPN affects proprioception of lower limb position. Patients with DPN display a decreased

nerve conduction velocity, and impaired contractile properties as a result of non-enzymatic glycation, which in tandem results in a slower muscle response (65, 90, 124). During locomotion, patients with DPN exhibit delayed peak muscle activations, despite an earlier activation of muscles, which may lead to decreased motor control during walking (57). Handsaker et al. (57) observed the delayed activation during stair ascent of the knee extensors and plantarflexors in patients with diabetic neuropathy. These delayed activations may explain a slower speed of strength generation in the knee and ankle extensors of the DPN group. During stair descent, changes are observed in the plantarflexors with a significantly earlier activation, a longer time to peak activation, and longer duration of activation. Persons suffering from diabetes used nearly twice the muscular activity compared to controls to initiate and maintain gait at a lower walking speed. The earlier activation of the plantarflexors in patients with DPN is expected to be an anticipatory mechanism, preparing the ankle joint to stabilize before contact with the step actually occurs (Kwon et al., 2003; Sacco et al., 2000). It has been well documented significantly earlier activation of the gastrocnemius medialis, lateralis, soleus, tibialis anterior, vastus lateralis, and biceps femoris muscles in DPN patients, in addition to a prolonged cessation times of tibialis anterior and vastus medialis muscles (41, 2, 4, 50, 76, 124, 129).

1.7.5 Centre of mass vertical displacement during gait

Large vertical centre of mass displacement (CoM) results in increasing in the metabolic cost of walking, because of greater mechanical work performed at the ankle, knee and hip joints (52). It has been suggested by Chwala et al. (29) that the main element of biomechanical cost of walking is energy used to control the

displacements of the CoM. Kinematic methods are predominantly used to determine the vertical displacements of the CoM during a gait cycle (40). Race walking events can be used as an example of how the CoM influences the metabolic CoW, where the speeds must be significantly higher, slightly above the threshold speed, but then the gait becomes more “costly”. To minimise the CoW individuals movement should be optimised towards minimal vertical oscillations of the centre of mass combined with a smooth passage from the heel-strike to toe-off phase involving only small changes in kinetic energy.

1.8 Tendon properties in people with Diabetes mellitus

Muscles attach to the skeleton via tendons. Tendons are force transmitters enabling skeletal movement. They are structural links between muscles and bones (114, 161, 24) and they transfer forces to the skeleton and the environment. Tendons are mechanically responsible for transmitting muscle forces to bone, and in doing so, permit locomotion and enhance joint stability (70). Tendons are spring-like structures and they respond by increasing their mechanical stiffness in response to chronic loading and decreasing with chronic unloading (102). Tendons consist of a collagenous matrix and demonstrate viscoelastic properties. This property enables forces to transmit (75), store and return elastic strain energy during locomotion and other movements (55, 47). The muscle-tendon complex has a very important role in gait mechanics of humans. The tendon is not an inert structure; both muscles and tendons are highly malleable tissues (32) and just as skeletal muscle displays plasticity to changes in the level of physiological loading. The function of tendons can be classified into two categories: tensile force transmission, storage and release

of elastic strain energy during locomotion (81). Tendons of both animals (23, 154, 155) and humans have been shown to respond to loading levels higher than those experienced habitually (running in animals and resistive training in humans), by increasing their tensile stiffness (115). Animal studies show that diabetes affects non-enzymatic glycation of soft tissues, such as tendon (113). This causes increased cross-linking, increasing the stiffness and modulus of the tendon (111, 112). Furthermore, tendon tensile stiffness is increased, limiting the range of joint motion at the ankle and knee (31, 87). In humans, calcification and fascicle disruption have been observed in the diabetic human Achilles tendon (13). Stiffening of the tendon will reduce the degree to which it can be stretched, affecting its potential for storing elastic strain energy during walking. The Achilles tendon is particularly important for storing and releasing elastic energy during walking (6) and can lead to the significant metabolic energy savings, as it actually 'spares' the muscle from performing a large part of the work. Since tendons exhibit low mechanical hysteresis, most of the elastic energy stored during stretching is returned on recoil (82). Thickness and stiffness of plantar fascia (the flat band of tissue (ligament) that connects calcaneus to toes) and Achilles tendon are increased in type I and type II diabetic subjects, mainly in those with peripheral neuropathy (1). In patients with diabetes however, it is hypothesised that this energy saving mechanism of the Achilles tendon will play a far less-significant role during walking compared to healthy matched-controls. As a result, it is hypothesised that the plantarflexor muscles will need to contribute a relatively greater proportion of the energy required, thereby increasing the energy cost of walking for diabetic patients compared to matched-controls. Cronin et al. (31) found the Achilles tendon length changes to be attenuated in the DM patients and that they

were inversely correlated with diabetes duration, as was the ankle range of motion. Also, they found that tendon length changes were independent of walking speed and age in the diabetic group.

1.9 The Metabolic Cost of walking

A parameter that characterizes locomotion is the metabolic cost of walking (CoW) and is defined as the energetic cost needed to travel a given of distance. Of particular interests are several studies that examined the CoW in different cohorts and different conditions. Houdijk et al. (61) found that the effort for balance control can elicit a meaningful metabolic energy demand. The same authors also found that the increased mechanical work for the step-to-step transition from prosthetic to intact limb contributes to the increased metabolic energy cost of amputee walking. Van Engelen et al. (114) proved that tibiotalar arthrodesis leads to higher metabolic energy cost during walking. Energy expenditure of stroke patients has been investigated during postural control tasks (63), as well as the treadmill and overground walking. The main conclusions are that impaired balance control should not be overlooked as a contributing factor to the increased energy cost of walking in patients with stroke, and improving or assisting balance control should be considered to reduce the energy cost of hemiplegic. They have provided further evidence that active control of medio-lateral stability during walking imposes a metabolic demand even in young healthy people. IJmker et al. (66) demonstrated that the effect of lateral stabilization on energy cost is independent of walking speed, suggesting that medio-lateral stability is not influenced by walking speed in young healthy persons. The CoW is an important factor that could contribute towards dictating a slower self-

selected walking speed in diabetes patients. As walking speed increases, joint moments and work are expected to increase (39, 152), increasing the CoW. The slower self-selected speed may therefore reflect the most efficient strategy for diabetes patients as previously shown in other populations (92, 86, 12, 162). The CoW is known to be higher in healthy elderly people compared to young adults, which likely reflects energetic inefficiencies in older people (92). Knowing some key 'inefficiencies' are present in people with diabetes, it might be expected that the CoW would be higher in people with diabetes, but so far this remains unknown. A higher CoW in people with diabetes may underpin the lower physical activity levels and lower habitual walking distances in this population and may also contribute towards a negative spiral where there is a greater perception of difficulty for walking, which causes less engagement in physical activity (Maluf et al., 2003; Morrato et al., 2003; Tudor-Locke, 2002, 2004), leading to poorer metabolic control and worsening of the diabetic condition. To allow intervention to break this negative cycle, it is therefore important to understand the factors that contribute to increasing the CoW in diabetes.

1.10 Thesis aim

The purpose of this thesis was to investigate the energy cost of walking in people with diabetes and examine biomechanical factors that could contribute to explaining any potential differences.

1.11 Thesis outline

This thesis will take the form of six chapters, focusing around the presentation of four experimental chapters. The first experimental chapter reports the metabolic cost of walking (CoW) in people with diabetes and diabetic peripheral neuropathy across a

range of matched walking speeds and gives an insight of the muscle concentric work. Experimental chapter 2 investigates the external moment arm and the effective mechanical advantage around the ankle during walking across a range of matched walking speeds in people with diabetes and diabetic peripheral neuropathy. Experimental chapter 3 examines muscle-tendon behaviour during walking for its potential role in the CoW in people with diabetes and diabetic peripheral neuropathy. The final experimental chapter 4 investigates the vertical displacement of CoM during walking across a range of matched walking speeds in people with diabetes and diabetic peripheral neuropathy. The last chapter summarises all findings, brings conclusions, limitations and possible future work.

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2. Experimental chapter one - Is the metabolic cost of walking higher in people with diabetes?

2.1 ABSTRACT

People with diabetes walk slower and display biomechanical gait alterations compared to controls, but it remains unknown whether the metabolic cost of walking (CoW) is elevated. Thirty-one non-diabetic controls (Ctrl); 22 diabetic patients without peripheral neuropathy (DM) and 14 patients with moderate/severe DPN, underwent gait analysis using a motion analysis system and force plates and treadmill walking using gas analyser to measure oxygen uptake. The aim of this study was to investigate the CoW and the lower limb concentric joint work as a major determinant of the CoW, in patients with diabetes and diabetic peripheral neuropathy (DPN). The CoW was significantly higher particularly in the DPN group compared to controls and also in the DM group (at selected speeds only) compared to controls,

across a range of matched walking speeds. Despite the higher CoW in patients with diabetes, concentric lower limb joint work was significantly lower in DM and DPN groups compared to controls. The higher CoW is likely due to energetic inefficiencies associated with diabetes and DPN reflecting physiological and biomechanical characteristics. The lower concentric joint work in patients with diabetes might be a consequence of kinematic gait alterations and may represent a natural strategy aimed at minimizing the CoW.

2.2 INTRODUCTION

Diabetes mellitus (DM) is a disease with a global reach, the prevalence of which is increasing at an alarming rate, with type 2 diabetes being particularly common among older adults. The prevalence of diabetes in most developed countries ranges between 2.1% (Iceland) and 10.5% Brazil (70, 82, 13). The world health organisation estimates that by 2025 as many as 200–300 million people worldwide will have developed type 2 diabetes (69).

Diabetic peripheral neuropathy (DPN) is one of the most common complications associated with diabetes occurring in 30–50% of patients and causing dysfunction of peripheral nerves (17, 22). Diabetic neuropathy affects sensory, motor and autonomic components of the nervous system. In terms of complications arising from diabetic neuropathy and impacting upon gait, a loss of sensory perception and impaired muscle function are major factors.

Diabetes patients have consistently been shown to display a slower self-selected walking speed, and take shorter strides compared to age-matched controls (19, 46, 28). Diabetic patients also generate lower knee and ankle joint moments compared

to controls during walking (56, 52, 14). It could be suggested that diabetic patients walk more slowly at least in part to keep the joint moment demands of gait lower, which may therefore explain their lower walking speed. However, lower joint moments during gait in diabetic patients have also been shown to be independent of walking speed (14).

The cost of walking (CoW) is another important factor that could contribute towards dictating a slower self-selected walking speed in diabetes patients. As walking speed increases, joint moments and work are expected to increase (24, 79), increasing the CoW. The slower self-selected speed may therefore reflect the most efficient strategy for diabetes patients as previously shown in other populations (53, 6, 49, 84).

The CoW is known to be higher in healthy elderly people compared to young adults, which likely reflects energetic inefficiencies in older people (53). Despite previous studies describing gait alterations in people with diabetes, the CoW and its relation to walking speed remains unknown in this clinical population. Lower limb concentric joint work is closely related to the CoW, with higher joint work being linked to a higher CoW (24, 79). Knee and ankle concentric joint work has recently been shown to be lower in people with diabetes during walking at a self-selected speed compared to controls (14), which might suggest a lower CoW as a result. However, there are also a number of energetic inefficiencies present in patients with diabetes that might increase the CoW for any given speed. For example, the effects of non-enzymatic glycation has been shown to stiffen tendons in animal models of diabetes (30, 58, 61, 62, 63). A stiffer Achilles tendon may reduce the amount of elastic energy stored in the tendon during walking (based upon the assumption of lower forces and

therefore smaller elongations resulting from the lower joint moments developed in diabetic patients compared to controls). Reduced elastic energy storage in the Achilles tendon would increase the amount of energy required from ankle muscles, thereby increasing the CoW. Other factors that could contribute to energetic inefficiencies during walking in diabetic patients include altered leverage around the foot due to diabetic foot deformities and increased antagonist muscle co-activation (80, 19, 33).

The aim of this study was therefore to investigate the CoW (and the lower limb joint work as a major determinant of the CoW) in patients with diabetes and diabetic neuropathy compared to controls at a range of matched walking speeds. I hypothesised that due to the above-mentioned inefficiencies in diabetes patients, they would display a higher CoW when walking at the same speed compared to controls and that this would be more marked in diabetes patients with DPN compared to those without.

2.3 MATERIALS AND METHODS

Participants

After receiving ethical approval from all relevant bodies, a total of sixty seven participants gave written informed consent to participate in this study. All procedures in this study complied with the declaration of Helsinki. All participants were aged over 40 years and were allocated into one of three groups: healthy controls without diabetes or peripheral neuropathy (Ctrl, n=31, 19 men), patients with diabetes but no neuropathy (DM, n=22, 12 men) and patients with diabetes and moderate-severe peripheral neuropathy (DPN, n=14, 14 men). All participants were assessed to confirm they satisfied the inclusion criteria for each group. Exclusion criteria for participation in the study were vascular disease, unstable ischemic heart, neurological, rheumatic disease, cerebral injury, disorders of the vestibular system, musculoskeletal injury, recent surgery affecting gait, foot or lower limb amputation (amputation of the hallux; amputation of more than two lesser toes on one foot; amputation of part of/whole foot) and open foot ulcer. Information about duration and type of diabetes, smoking habits and use of current medication was obtained via questionnaire. The majority of the DM and the DPN patients reported taking insulin,

cholesterol-lowering medication and diabetes medication, while from the whole sample (including controls) only 2 people reported smoking. Participant characteristics are displayed in Table 3.

Assessment of peripheral neuropathy

A clinical evaluation was undertaken to quantify neuropathy in diabetic patients and to confirm the absence of neuropathy in healthy controls. Peripheral neuropathy was assessed by using the modified Neuropathy Disability Score (mNDS) and the vibration perception threshold (VPT). The mNDS is a combined score taken from tests measuring the patient's ability to detect temperature, pain, vibration and the Achilles tendon reflex (10). The VPT was assessed by placing the probe of the biothesiometer on the apex of the hallux and increasing the level of vibration until detected by the participant. A random blood glucose test was performed in the Ctrl group to confirm the absence of diabetes and the above neuropathy tests conducted to confirm the absence of neuropathy in the Ctrl group resulting from any aetiology.

Modified Plug-in-gait based marker set

A full-body Plug- In-Gait marker set (Vicon®, 2002) was selected due to the minimal number of markers required for a full-body model. The plug-in-gait model uses joint calibration markers for both segment definition and tracking, thereby minimising the need for additional tracking markers. Whilst the model employed for the studies within this thesis include additional tracking markers, the intention of these markers is to provide additional redundancy in the model rather than to replace the calibration markers. The plug-in-gait model has previously been shown to have good

repeatability, and good comparability to other models including a six-degrees of freedom approach for sagittal plane motion. Some markers have fewer placement constraints still; these are tracking markers only and are not used for defining the body segment; rather during the model's calibration their position is calculated relative to the segment in order to define it's position during gait, these markers then provide more reliable tracking of the segments during the trials.



Figure 1. Marker placements in anterior, posterior and lateral views.

Table 1. Upper body marker placements (head, arms and torso).

Marker	Landmark/Placement
1. Head 1	Headband: left front
2. Head 2	Headband: right front
3. Head 3	Headband: left back
4. Head 4	Headband: right back
5. Right Shoulder	Acromio-clavicular joint (right)
6. Left Shoulder	Acromio-clavicular joint (right)
7. Cervical 7	7 th cervical vertebra
8. Thorax 10	10 th Thoracic vertebra
9. Sternum	Xiphoid process
10. Scapula	Inferior angle
11. Clavicula	Incisura jugularis
12. Left Upper Arm	On upper arm between Elbow and Shoulder (Left)
13. Left Elbow	Lateral epicondyle (Left)
14. Left Wrist Medialis	Styloid process of Radius (Left)
15. Left Wrist Lateralis	Styloid process of Ulna (Left)
16. Left Finger	On the hand just proximal to the 2 nd metacarpal head (Left)
17. Right Upper Arm	On upper arm between Elbow and Shoulder (Right)
18. Right Elbow	Lateral epicondyle (Right)
19. Right Wrist Medialis	Styloid process of Radius (Right)
20. Right Wrist Lateralis	Styloid process of Ulna (Right)
21. Right Finger	On the hand just proximal to the 2 nd metacarpal head (Right)

Table 2. Lower body marker placements (pelvis and legs).

22. Left SISA	Left anterior superior iliac spine
23. Right SISA	Right anterior superior iliac spine
24. Sacrum 1	Left posterior superior iliac spine
25. Sacrum 2	Right posterior superior iliac spine
26. Sacrum 3	Placed midway between Sacrum 1 and Sacrum 2 markers and slightly inferior
27. Left Upper Thigh	On the lateral side of the left thigh, approximately halfway between hip centre and knee centre. It should be aligned anteroposteriorly with the knee and hip extension/flexion axes
28. Right Upper Thigh	On the lateral side of the right thigh, approximately halfway between hip centre and knee centre. It should be aligned anteroposteriorly with the knee and hip extension/flexion axes
29. Left Knee Lateral	Lateral femoral epicondyle (Left leg) – lateral side of knee flexion/extension axis

30. Right Knee Lateral	Lateral femoral epicondyle (Right leg) – lateral side of knee flexion/extension axis
31. Left Knee Medial	Medial femoral epicondyle (Left leg) medial mirror of Left Knee Lateral marker so that the line connecting the two markers approximates the flexion/extension joint axis.
32. Right Knee Medial	Medial femoral epicondyle (Right leg) medial mirror of Right Knee Lateral marker so that the line connecting the two markers approximates the flexion/extension joint axis.
33. Left Shank 1	Top 1/3 of shank, in the same plane as the knee and ankle flexion/extension axes (Left leg)
34. Left Shank 2	Bottom 1/3 of shank, in the same plane as the knee and ankle flexion/extension axes (Left leg)
35. Left Shank 3	Placed midway between Left Shank 1 and Left Shank 2 markers and slightly inferior
36. Right Shank 1	Top 1/3 of shank, in the same plane as the knee and ankle flexion/extension axes (Right leg)
37. Right Shank 2	Bottom 1/3 of shank, in the same plane as the knee and ankle flexion/extension axes (Right leg)
38. Right Shank 3	Placed midway between Right Shank 1 and Right Shank 2 markers and slightly inferior
39. LANKMED	Medial malleolus (Left ankle)
40. RANKMED	Medial malleolus (Right ankle)
41. LANKLAT	Lateral malleolus (Left ankle)
42. RANKLAT	Lateral malleolus (Right ankle)
43. LHEEL	On the left calcaneus
44. RHEEL	On the right calcaneus
45. Left B1	Base of the first metatarsal bone (Left foot)
46. Left H1	Head of the fifth metatarsal bone (Left foot)
47. Left B5	Base of the first metatarsal bone (Left foot)
48. Left H5	Head of the fifth metatarsal bone (Left foot)
49. Right B1	Base of the first metatarsal bone (Right foot)
50. Right H1	Head of the fifth metatarsal bone (Right foot)
51. Right B5	Base of the first metatarsal bone (Right foot)
52. Right H5	Head of the fifth metatarsal bone (Right foot)
53. Left Toe	Tip of left toe
54. Right Toe	Tip of right toe

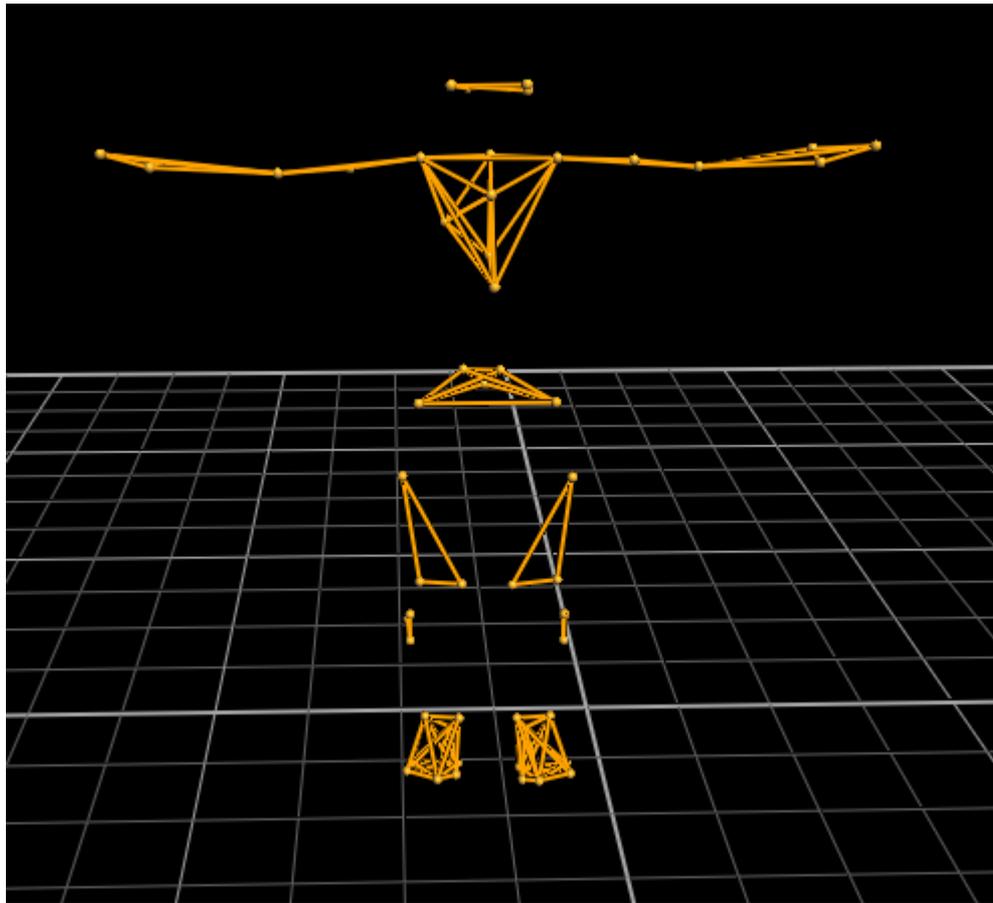


Figure 2. Fully labelled standing calibration file in Nexus (Vicon, Oxford, UK).

Gait analysis

Participants were asked to walk along a 10-metre walkway in the gait laboratory. Participants were instructed to walk the length of the walkway at a series of different walking speeds performed in a specific order (0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 m/s). Walking speed was controlled by measuring the velocity of a marker attached to the sacrum after each trial from the motion analysis data and providing immediate feedback for participants as to whether they needed to walk more quickly or more slowly on the next trial to achieve the required speed, allowing +/- 5% deviation from the required speed. Participant's starting position was altered by the experimenters to ensure a 'clean' (i.e., no overlap outside the force platform) foot-strike on one or two of the force platforms per walking trial without alteration to their natural gait. Walking trials were repeated until at least

three 'clean' foot contacts with the force platforms were made per limb, per speed condition. Kinematics were collected at 100 Hz using a full-body modified Plug-In-Gait marker set with 54 markers and a 10-camera Vicon motion capture system (Vicon, Oxford, UK) positioned around the 10-meter walkway. Kinetics were simultaneously collected at 1000 Hz from three force platforms (Kistler, Zurich, Switzerland) embedded into the middle of the walkway. Where possible markers were placed directly onto the skin; to minimise movement artefacts resulting from loose clothing all participants wore tight-fitting shorts and tops. All participants wore specialist diabetic shoes (MedSurg, Darco, Raisting, Germany) with a neutral foot-bed, ensuring the diabetic patients walked with safe, appropriate footwear whilst minimising the effect of footwear by standardising across all participants.

Oxygen uptake measurements and metabolic calculations

Prior to testing, all participants completed walking familiarisation sessions for a minimum of 6 minutes on the treadmill to become accustomed to the task of treadmill walking and enable a natural walking style to be achieved. Measurements of expired air were acquired whilst participants walked on a motor-driven treadmill (Woodway Ergo ELG 70, Weil am Rhein, Germany) set at six different walking velocities (0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 m/s). The treadmill was inclined by 1% from horizontal for the purpose of increasing the similarity of oxygen uptake demands with level ground walking as previously shown (34, 38). Participants wore a facemask, which passed expired air into an automated analyser (Cortex Metalyser 3B, Biophysik, Leipzig, Germany). The analyser, calibrated prior to each testing session, provided breath-by-breath data sent via telemetry to a computer. Oxygen consumption (VO_2) was measured continuously

using this online system. The net VO_2 during walking was determined as:

$$\text{Net } VO_2 = \text{gross } VO_2 - \text{resting } VO_2^*$$

**resting VO_2 was measured during quiet standing on the treadmill prior to walking.*

Net VO_2 was expressed relative to body mass for all participants. The cost of walking was calculated using the mean rate of oxygen consumption for VO_2 data collected between the 3rd and 4th minute of each stage.

Net VO_2 was converted to joules using an energetic equivalent and calculated using the specific respiratory exchange ratio (RER) value from each participant as (29): $VO_2 \cdot (4.94 \cdot \text{RER} + 16.04)$. The CoW was calculated by dividing VO_2 by the walking speed and multiplying this value for the energy equivalent. Using the RER and calculating the energetic equivalent in this way takes into account possible differences between groups due to the contribution of the anaerobic energy system. Nine participants (Ctrl=3, DM=1, DPN=5) were unable to walk for a sufficient period of time at 1.6 m/s to derive adequate VO_2 measurements at this specific speed.

Gait biomechanical analysis

Temporal–spatial parameters (walking speed, stance time) were calculated from the gait analysis testing session described above using Visual 3D software (C-motion Inc., MD, USA), using the process of inverse dynamics to calculate joint powers. Power curves during stance were calculated to assess concentric (positive) periods of power during the stance phase to calculate concentric joint work done, defined as the positive power-time integral (14). Concentric joint work done was then subsequently normalised to body mass. Work done (ankle, knee, and hip) was calculated taking into account data from both legs, across at least

three trials (data from at least six stance phases).

Statistics

A one-way analysis of variance (ANOVA) was performed for all variables to assess between group differences. If the ANOVA was significant, a Fisher's least significant difference (LSD) post-hoc test was used to test for differences between the diabetes groups (DM and DPN) and the control group. All values presented are means and standard deviation. All statistical tests were performed on SPSS statistical package (SPSS v21, Chicago, Illinois) with significance set at $p < 0.05$.

2.4 RESULTS

Participant characteristics

There were significant differences between the groups in age, body mass and

BMI, which were significantly greater in the DPN group (Table 3, $p < 0.01$).

Neuropathy assessments

As expected, the DPN group displayed significantly higher values for the VPT and the mNDS compared to the Ctrl group (Table 3). The VPT and mNDS for the DM group were not significantly different from the Ctrl, underlining that this diabetic patient group had no neuropathy (Table 3).

Temporal–spatial gait parameters

The DPN group displayed significantly longer single limb stance times and shorter step lengths in all given speeds compared to Ctrl group (Table 4).

Total joint work during walking at different speeds

Total concentric work showed a very consistent pattern across all speeds with the Ctrl group displaying the highest values, followed by lower values in the DM group and the lowest values observed in the DPN group (Fig. 3). Compared to the Ctrl group, significantly lower joint work was observed at all speeds for the DPN group and all but 1.4 m/s for the DM group.

Ankle, knee and hip joint work during walking

Ankle concentric joint work was lower for the DPN group compared to the Ctrl group, reaching significance at gait velocities of 0.8; 1.2; 1.4 and 1.6 m/s (Fig. 3). Knee concentric joint work was significantly lower in the DPN group compared to Ctrl at gait velocities of 0.6; 0.8; 1.0; 1.2 and 1.6 m/s. In the DM group, knee concentric joint work was significantly lower compared to Ctrl at the gait velocity of 0.6 m/s. Hip concentric joint work was lower for the DPN group compared to

Ctrl reaching significance at velocities of 0.6; 0.8 and 1.6 m/s.

Cost of walking at different speeds

There were significant differences in the CoW between the groups across the matched speeds tested, with the general pattern of a higher CoW in the DPN group, followed by the DM group and the lowest CoW in the Ctrl group (Table 4; Fig. 4). Significant differences in the CoW were mainly found between the DPN and Ctrl groups (at 0.6; 0.8; 1.0; 1.2 and 1.6 m/s), with some significant differences also present between DM and Ctrl groups at the higher gait velocities (1.4 and 1.6 m/s).

Table 3. Participant characteristics and results from neuropathy assessments.

Variable	Group		
	Ctrl	DM	DPN
Age (yr)	56 (10)	51 (9)**	66 (14)**
Body mass (kg)	76 (10)	80.5 (12)	91.5 (18)**
Height (m)	1.72 (0.12)	1.71 (0.09)	1.73 (0.11)
BMI (kg/m ²)	26 (3)	28 (4)	31 (4)**
NDS (Score/10)	1 (1)	2 (1)	7 (2)**
VPT (Volts)	6.1 (3.4)	8.2 (3.4)	27.4 (9.1)**
Diabetes duration (years)	-	14 (12)	14 (11)
Type 1 diabetes	-	7	4
Type 2 diabetes	-	15	10

Healthy controls (Ctrl, n=31), diabetic patients with no neuropathy (DM, n=22) and diabetic patients with moderate/severe neuropathy (DPN, n=14). Significant differences from the Ctrl group are denoted by *(P<0.05) or ** (P<0.01). BMI = body mass index, NDS = neuropathy disability score, VPT = vibration perception threshold. Values are means (standard deviations).

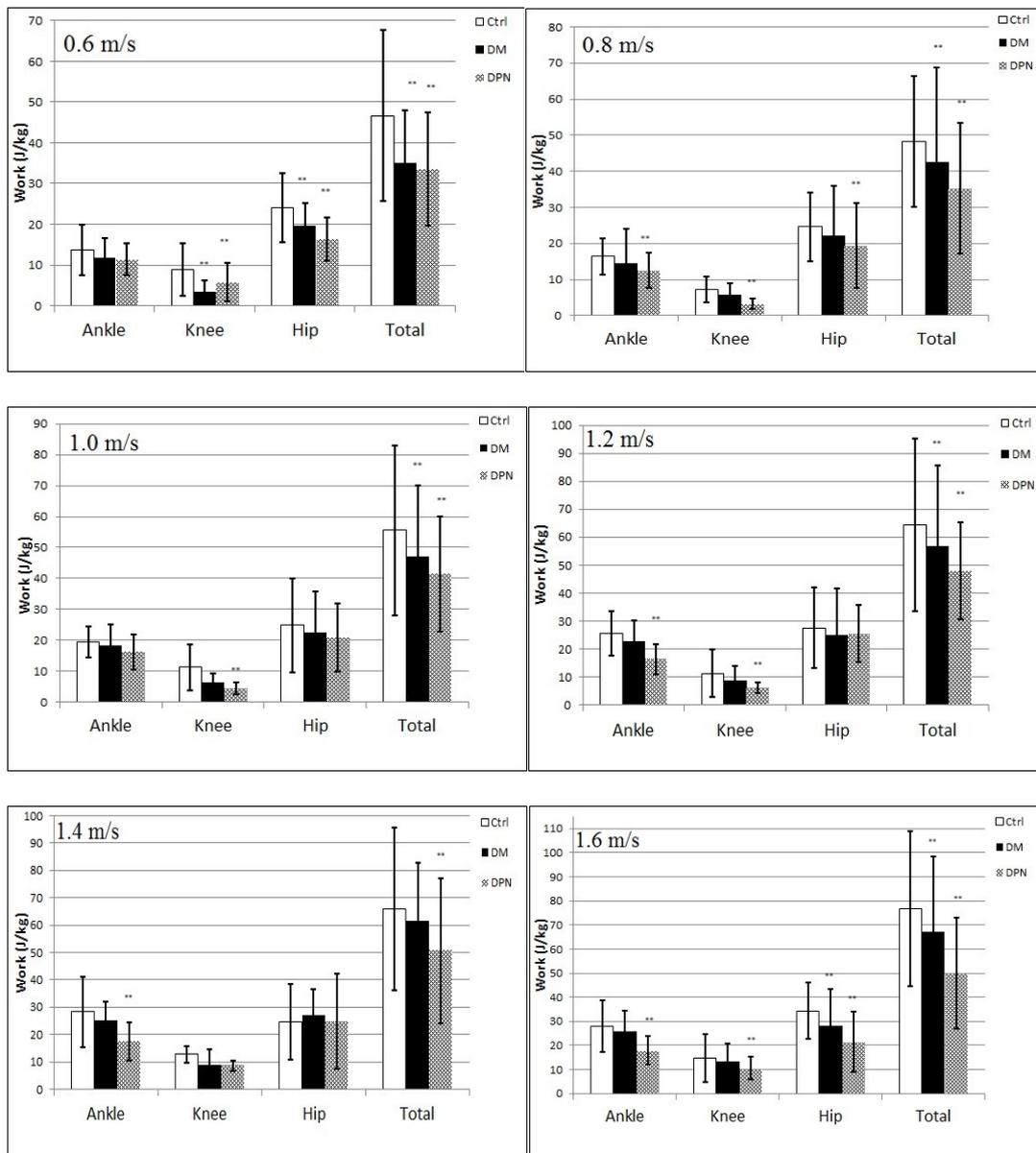


Figure 3. Lower limb ankle, knee, hip and total concentric joint work across walking speeds from 0.6 to 1.6 m/s for healthy controls (Ctrl, n=31), diabetic patients with no neuropathy (DM, n=22) and diabetic patients with moderate/severe neuropathy (DPN, n=14). Values are group means and SD, **denotes significantly ($P < 0.01$) different from the control group.

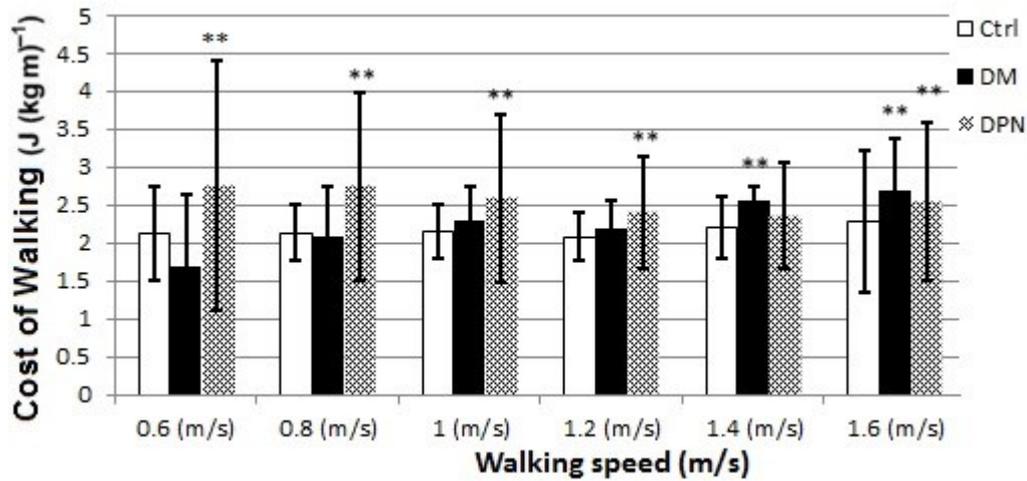


Figure 4. The cost of walking (CoW) plotted across walking speeds from 0.6 to 1.6 m/s for healthy controls (Ctrl, n=31), diabetic patients with no neuropathy (DM, n=22) and diabetic patients with moderate/severe neuropathy (DPN, n=14). Nine participants (Ctrl=3, DM=1, DPN=5) were unable to walk for long enough to calculate the CoW at 1.6 m/s. Values are group means and SD, **denotes significantly ($P < 0.01$) different from the control group.

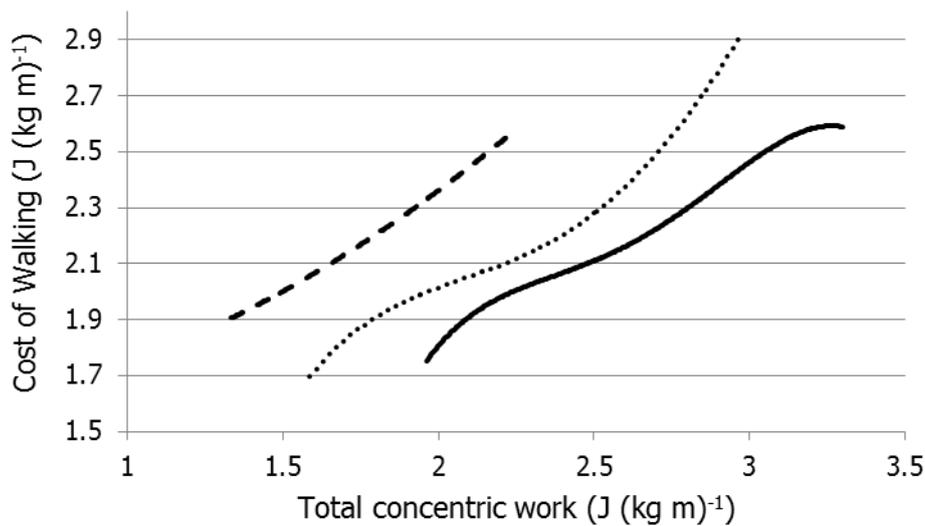


Figure 5. Mean data for the cost of walking (CoW) plotted against total concentric work during walking at walking speeds from 0.6 to 1.6 m/s for healthy controls (Ctrl, n=31), diabetic patients with no neuropathy (DM, n=22) and diabetic patients with moderate/severe neuropathy (DPN, n=14). The curves were fitted with a cubic function to yield R^2 values over 0.98. Line graphs: Ctrl - solid line, DM - dotted line, DPN - dashed line.

Table 4. Temporal-spatial gait parameters and net oxygen uptake.

Variable	Group		
	Ctrl	DM	DPN
0.6 m/s			
Actual speed (m/s)	0.57 (0.24)	0.59 (0.16)	0.61(0.11)
Step length (m)	0.59 (0.20)	0.57 (0.24)	0.51 (0.09)**
Single limb stance time (sec)	0.902 (0.20)	0.841(0.23)	0.958 (0.05)**
Net VO ₂ (ml/min kg)	3.81 (1.11)	3.05 (1.69)	4.93 (2.95)**
RER	0.89 (0.05)	0.93 (0.08)	0.96 (0.09)
0.8 m/s			
Actual speed (m/s)	0.82 (0.27)	0.78 (0.21)	0.77 (0.19)
Step length (m)	0.63 (0.21)	0.57 (0.21)	0.53 (0.05)**
Single limb stance time (sec)	0.801 (0.15)	0.842 (0.21)	0.960 (0.05)**
Net VO ₂ (ml/min kg)	5.11 (0.89)	5.00 (1.55)	6.56 (2.94)**
RER	0.86 (0.09)	0.87 (0.11)	0.97 (0.07)
1.0 m/s			
Actual speed (m/s)	1.02 (0.17)	1.04 (0.28)	0.97 (0.13)
Step length (m)	0.69 (0.15)	0.67 (0.05)	0.64 (0.04)*
Single limb stance time (sec)	0.713 (0.13)	0.741 (0.05)	0.884 (0.05)*
Net VO ₂ (ml/min kg)	6.44 (1.08)	6.89 (1.32)	7.75 (3.29)**
RER	0.84 (0.04)	0.91 (0.06)	0.93 (0.03)
1.2 m/s			
Actual speed (m/s)	1.18 (0.16)	1.22 (0.15)	1.22 (0.23)
Step length (m)	0.76 (0.11)	0.75 (0.17)	0.69 (0.07)*
Single limb stance time (sec)	0.579 (0.31)	0.617 (0.05)	0.682 (0.06)*
Net VO ₂ (ml/min kg)	7.46 (1.15)	7.89 (1.29)	8.62 (2.65)**
RER	0.87 (0.08)	0.91 (0.04)	0.91 (0.07)
1.4 m/s			
Actual speed (m/s)	1.45 (0.19)	1.44 (0.12)	1.46 (0.19)
Step length (m)	0.79 (0.12)	0.77 (0.17)	0.71 (0.11)*
Single limb stance time (sec)	0.555 (0.15)	0.579 (0.21)	0.621 (0.14)*
Net VO ₂ (ml/min kg)	9.22 (1.69)	10.73 (0.80)**	9.87 (2.89)
RER	0.90 (0.07)	0.89 (0.05)	0.93 (0.06)
1.6 m/s			
Actual speed (m/s)	1.62 (0.27)	1.57 (0.17)	1.59 (0.12)
Step length (m)	0.81 (0.11)	0.80 (0.04)	0.74 (0.02)*
Single limb stance time (sec)	0.499 (0.15)	0.498 (0.11)	0.525 (0.01)*
Net VO ₂ (ml/min kg)	10.97 (4.45)	12.84 (3.35)**	12.19 (4.99)**
RER	0.89 (0.04)	0.90 (0.07)	0.98 (0.06)

Healthy controls (Ctrl, n=31), diabetic patients with no neuropathy (DM, n=22) and diabetic patients with moderate/severe neuropathy (DPN, n=14). Significant differences from the Ctrl group are denoted by *(P<0.05) or **(P<0.01). Values are means (standard deviations). Gait parameters were collected on the laboratory walkway.

2.5 DISCUSSION

This study has shown for the first time that when walking speed is matched, patients with diabetic neuropathy have a higher CoW compared to controls (Fig. 4). Despite a higher CoW, patients with diabetic neuropathy showed significantly reduced concentric lower limb joint work compared to controls at these matched speeds. The finding of lower joint work in patients with diabetic neuropathy is surprising considering that under 'normal' conditions lower concentric work is clearly linked to a lower CoW (67, 57), but I suggest possible reasons for this below.

The finding of a higher CoW in patients with diabetic neuropathy when walking speed was matched likely reflects energetic inefficiencies resulting from a number of physiological and biomechanical factors. Firstly, animal models of diabetes have shown that tendons are stiffer due to the effects of non-enzymatic glycation. In human diabetic patients, this likely applies to the long Achilles tendon, which plays a major role in energy saving during walking under 'normal' circumstances (3). Stiffening of the Achilles tendon with diabetes and especially diabetic neuropathy (presumably due to longer exposure with poor glycaemic control), would reduce the extensibility of the tendon. Based upon the lower joint moments developed in patients with diabetic neuropathy during gait (46, 52, 56, 81), it would be expected that the force on the Achilles tendon would be lower compared to controls. The stiffer Achilles tendon of patients with diabetic neuropathy would be expected to elongate less compared to controls, storing less elastic energy and requiring more energy to be generated by the plantarflexor muscles (assuming similar hysteresis compared to controls), thereby contributing to a higher CoW in diabetes patients.

Higher levels of muscle co-activation during walking have been reported in

diabetic patients compared to controls (1, 32). Considering that locomotion should reflect a fine balance between activation and de-activation of agonist and antagonist muscles during specific phases of the gait cycle, an increase in the level of muscle co-activation will increase metabolic energy cost and could therefore be another factor contributing to increase the CoW at a given speed in patients with DPN. Foot deformities are common in diabetic patients (29, 78) and even subtle changes in foot structure would alter the application of force to the ground during walking (43, 51). Changes in the application of force to the ground during walking (and running) will alter the mechanical leverage around the ankle joint, i.e., the external moment arm. This has been shown both in humans and animals (7, 8, 9, 5, 41, 42, 68, 12) and therefore such changes may increase the CoW in patients with DPN. Another contributing factor to the higher CoW in the DPN group is the increased step frequency (the DPN group had a shorter step length for a given speed, therefore requiring a higher step frequency) and greater body mass compared to the DM and the Ctrl groups. These two factors (increased step frequency and greater body mass) would increase the internal work required for moving the lower limbs and may contribute to a higher CoW in people with diabetes and particularly those with DPN (54).

A higher CoW was clearly evident in patients with diabetes (DM group) and particularly in those with diabetic neuropathy (DPN group) across the matched walking speeds. In this study I examined a range of different walking speeds (from 0.6 until 1.6 m/s) and observed that the differences in the CoW between groups were most evident at the lower gait velocities (0.6-1.2 m/s; Fig. 4). At the higher walking speeds, the pattern changes slightly with the CoW still remaining higher in patients with diabetes and diabetic neuropathy compared to controls, but with the differences being less evident than at the slower walking speeds. This may

be explained by patients with diabetic neuropathy moving closer towards their maximal oxygen uptake when walking at velocities of 1.4 m/s and above. It is well known that diabetes patients engage in less physical activity (48, 55, 72, 73) and are therefore likely less fit i.e., have a lower maximal oxygen uptake compared to non-diabetic controls (40, 65, 66). It is also a possibility that diabetes patients might have reached the lactate threshold earlier than controls (i.e., at lower walking speeds), which could have influenced the VO_2 kinetics and the time to reach a relatively constant VO_2 . Specifically, with heavy exercise above the lactate threshold the VO_2 slow component (i.e., the gradual rise in VO_2 with constant workload) may be more pronounced (77) and there is a risk that diabetes patients may have reached their lactate threshold earlier than controls, thereby influencing our estimate for the CoW differently between diabetes and control participants. Although I did not measure the lactate threshold or the maximal oxygen uptake in our participants, previous studies have shown that the lactate threshold occurs in other populations at a VO_2 between 50 and 55 ml/kg/min, or at running speeds of between 3.75 and 4.73 m/s (64, 26, 83, 2). These VO_2 values (50-55 ml/kg/min) and running speeds (3.75-4.73 m/s) are considerably higher compared to those measured in our study (VO_2 values of up to 13 ml/kg/min and walking speeds of up to 1.6 m/s; Table 4), and despite these previous reports being in healthy populations, it may suggest that all participants in the present study were well below their lactate threshold. Future work could be conducted to compare the CoW between these groups at relative exercise intensities, taking into account individual lactate thresholds.

The CoW data in the present study are comparable with a number of previous studies conducted in similar populations reporting values ranging between 1.1 and 5 J (kg m)⁻¹ (76, 25, 75, 35, 15, 36, 6, 16, 18, 20, 53, 59). In the DPN group

the CoW showed a U-shaped relationship with walking speed as previously reported in other populations (53), but this relationship was not as clearly evident in the DM and Ctrl groups (Fig. 4). All three groups showed the same consistent pattern of increasing net VO_2 with increasing walking speed. Slight differences in the RER values between groups likely explain the lack of a consistent U-shaped relationship between the CoW and walking speed across all three groups. The DPN group displayed particularly high standard deviations for the CoW (Fig. 4) and VO_2 data (Table 4). This high within-group variance is a consistent characteristic reported in previous studies with DPN patients for other gait variables, but here I also highlight the within-group variance associated with VO_2 and CoW parameters in DPN patients.

Across the matched walking speeds in the present study, there was a consistent pattern of lower total concentric joint work being developed by the DM group and particularly the DPN group compared to controls (Fig. 3). A slower walking speed is a consistent finding of previous studies in diabetic patients (5, 52, 60, 28). Whilst most other studies have examined only self-selected walking speed (45, 21), the present study is the first to examine a range of different functionally relevant matched walking speeds (between 0.6 and 1.6 m/s) in the diabetic patient population. Since lower limb joint work is known to be closely linked to the CoW, joint work was examined in the present study to provide insight to the mechanism(s) for group differences in the CoW. I found a consistent pattern of lower joint work in the DM group and particularly in the DPN group compared to the Ctrl group for the hip, knee and ankle joints across walking speeds (Fig. 3). Theoretically, the same lower limb joint work was associated with a higher CoW in diabetic patients and particularly in patients with DPN, which can be observed by projecting vertically from any point on the x-axis on Fig. 5.

It was surprising that diabetic patients were actually able to match the same walking speed as controls despite generating significantly reduced lower limb joint work. This interesting aspect might be explained by a number of kinematic alterations to gait made by diabetic patients with implications for joint kinetics. This may represent an 'altered gait strategy' in people with diabetes to enable them to meet the task demands in the face of compromised musculoskeletal properties and already elevated CoW due to energetic inefficiencies. Diabetic patients display a reduced lower limb range of motion during walking compared to controls. This is achieved at least in part via shorter steps taken by diabetic patients during walking (Table 4). It is known that DM and DPN patients are able to lower joint moments and walk with shorter steps and this translates to less flexed joints, which in general means that the moment arms of the ground reaction force are smaller compared to the situation with more flexed joints. Smaller moment arms will lower the joint moments and since joint work is derived from the product of joint moments and joint angular speed (joint power), this kinematic strategy likely contributes towards reducing the joint work done during walking. Concentric contractions are associated with a relatively high metabolic load, whereas in contrast, this is much lower for isometric and eccentric contractions (27, 23). Despite these strategies to lower the joint moments, patients with DPN have a higher CoW presumably due to metabolic inefficiencies discussed above. If patients with DPN did not employ these 'altered gait strategies' presumably the CoW would be even higher.

There are some limitations in the present study that should be acknowledged. Firstly, several participants were not able to complete walking on the treadmill at the highest speed (1.6 m/s). Secondly, body mass was significantly different between groups, however, this should not affect the two main parameters of the

CoW and joint work, since both parameters were normalised for body mass. Also, the higher body mass in patients with DPN is a well-known characteristic of this population described in the literature (45, 39, 37). Although only a mean of 10 years difference, patients in the DPN group were significantly older than controls (66 to 56 years, respectively), which might be a confounding factor for some of the variables examined. I did not measure blood lactate to confirm that all participants were working below their lactate threshold. This is a consideration since the VO_2 slow component is much more pronounced during exercise above the lactate threshold compared to below as discussed above. Although the intensity of the exercise during walking in the present study was unlikely sufficient for participants to exceed their lactate threshold based on comparison with previous studies (64, 26, 83, 2), it remains a note of caution since it would affect our interpretation of the CoW data if there were between-group differences in the onset of the lactate threshold occurring within the range of walking speeds examined.

I have shown that the CoW is higher in patients with diabetes and particularly in those with diabetic neuropathy compared to controls when walking speed is matched. This higher CoW is likely due to energetic inefficiencies in diabetic patients reflecting physiological and biomechanical characteristics and occurs despite the development of lower concentric joint work in patients with diabetes and diabetic neuropathy.

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3. Experimental chapter two - Altered leverage around the ankle in people with diabetes: a natural strategy to modify the muscular contribution during walking?

3.1 ABSTRACT

Diabetes patients display a number of gait alterations compared to controls including a higher cost of walking. The aim of this study was to investigate the external moment arm (ExtMA) and effective mechanical advantage (EMA) at the ankle in patients with diabetes and diabetic peripheral neuropathy compared to controls as a potential mechanism contributing to the increased cost of walking. Thirty one non-diabetic controls (Ctrl); 22 diabetes patients without peripheral neuropathy (DM) and 14 patients with moderate/severe diabetic peripheral neuropathy (DPN), underwent gait analysis using a motion analysis system and force plates. Internal moment arms were determined using magnetic resonance imaging during weight-bearing and external moment arms were calculated using gait analysis. A greater value ($P < 0.01$) for the EMA at the ankle joint was found in the DPN (0.488) and DM (0.46) groups compared to Ctrl (0.448). This means that the ankle plantarflexor muscles develop relatively lower forces to generate a given joint moment compared to controls. The increased EMA was mainly caused by a smaller external moment arm in the DPN (9.63cm; $P < 0.01$) and DM (10.31cm) groups compared to Ctrl (10.42cm). Here, I uncover a new mechanism through which patients with diabetes and particularly those with DPN reduce the joint moment at the ankle during walking – by applying the ground reaction force more proximally on the foot or at an angle directed more towards the ankle, increasing the EMA around the ankle and thereby reducing the ankle joint moment.

3.2 INTRODUCTION

Diabetes presents a global health challenge and the prevalence is increasing rapidly, ranging between 2.4% to 24% across various international countries (44, 48, 7). The world health organisation estimates that by 2025 as many as 200-300

million people worldwide will have developed type 2 diabetes (19). One of the major complications of diabetes is diabetic peripheral neuropathy (DPN), which occurs in 30–50% of patients with diabetes, causing dysfunction of peripheral nerves (10, 13). Diabetic peripheral neuropathy affects not only sensory but also motor nerves, having implications for movement dysfunction (35, 18, 9). People with diabetes walk less and at a slower speed and engage in lower levels of physical activity compared to match controls (27, 30, 45, 46). Other gait characteristics in diabetic patients include taking shorter strides, spending relatively longer in double support (two feet in contact with the ground) and generating lower knee and ankle joint moments compared to matched controls (31, 11, 22, 9, 12, 14, 24, 29, 31). I have recently shown a higher cost of walking (CoW) across a range of matched walking speeds in patients with diabetes and especially in those with DPN compared to controls. This higher CoW in people with diabetes may underpin the lower physical activity levels and lower habitual walking distances in this population and may contribute towards a negative spiral where there is a greater perception of difficulty for walking, which causes less engagement in physical activity, leading to poorer metabolic control and worsening of the diabetic condition. To allow intervention to break this negative cycle, it is therefore important to understand the factors that contribute to increasing the CoW in diabetes.

One potential factor that might contribute to increasing the CoW is a greater external moment arm (ExtMA) of the resultant ground reaction force (GRF) around the ankle, since this will increase the relative contribution from the plantarflexor muscles. The effective mechanical advantage (EMA) around the ankle is given by the ratio of the internal (Achilles tendon moment arm) to the ExtMA, with lower values reflecting a relatively greater contribution from the

plantarflexor muscles towards the joint moment required to overcome the external resistance applied (5). Indeed, it has been shown in a healthy population how a marked increase in the ExtMA around the knee and therefore a marked reduction in the EMA at the knee, likely accounts for the marked increase in the cost of transport when transitioning from walking to running (4). Although running is not an issue of investigation here in diabetes patients, this clearly illustrates the concept of how differences in external leverage around joints can impact upon on the energy cost of locomotion.

In diabetes patients the ExtMA at the knee would not be increased since they take shorter strides and have less flexed joints compared to controls (31, 11, 22). However, many diabetes patients have some level of foot deformity such as a high arch, or toe deformities (16, 47), which may result in applying force to the ground more distal on the foot, increasing the ExtMA around the ankle, decreasing the EMA and thereby increasing the relative contribution from the plantarflexor muscles. The EMA around the ankle in diabetic patients could be also affected by altered use of the lower limb and foot caused by sensory deficits and plantarflexor muscle weakness. A relative increase in the contribution from ankle plantarflexor muscles during walking may partly explain the increased CoW in diabetes patients and especially those with DPN.

The aim of this study was to establish whether there are differences in the ExtMA and EMA at the ankle in patients with diabetes and DPN compared to controls at a range of matched walking speeds, as a potential mechanism contributing to the increased CoW recently observed in diabetes patients (32). I hypothesized that the ExtMA will be higher and the EMA will be lower in diabetes patients compared to controls.

3.3 MATERIALS AND METHODS

Participants

After receiving ethical approval for the study from all relevant bodies, a total of sixty seven participants were recruited, who gave their written informed consent to participate. Participants were aged over 40 and allocated into one of three

groups based upon defined criteria: patients with diabetes and moderate-severe peripheral neuropathy (DPN, n=14, 12 men), patients with diabetes but no neuropathy (DM, n=22, 12 men) and healthy controls without diabetes or peripheral neuropathy (Ctrl, n=31, 19 men).

All participants were assessed to confirm they met the inclusion criteria. Major exclusion criteria included: severe vascular disease, unstable ischemic heart, neurological, rheumatic disease, cerebral injury, disorders of the vestibular system, musculoskeletal injury, recent surgery affecting gait, foot or lower limb amputation (amputation of the hallux; amputation of more than two lesser toes on one foot; amputation of part of/whole foot) and open foot ulcer. A questionnaire was used to obtain the following information: duration and type of diabetes, smoking habits and use of current medication. The majority of the DM and the DPN patients reported taking insulin, cholesterol-lowering medication and diabetes medication, while from the whole sample (including controls) only 2 people reported smoking. Participant characteristics are displayed in Table 1.

Clinical assessment of peripheral neuropathy

A clinical evaluation was undertaken to quantify neuropathy in diabetes patients and to confirm the absence of neuropathy in healthy controls. Peripheral neuropathy was assessed by using the modified Neuropathy Disability Score (mNDS) and the vibration perception threshold (VPT). The mNDS is a composite score taken from tests measuring the patient's ability to detect temperature perception, pain, vibration and the Achilles tendon reflex (6). The VPT is an assessment performed using a biothesiometer placed on the apex of the hallux and increasing the level of vibration until detected by the participant. A random blood glucose test was performed in the Ctrl group to confirm the absence of

diabetes and the above neuropathy tests conducted to confirm the absence of neuropathy in the Ctrl group resulting from any aetiology.

Gait analysis

Kinematic data were collected at 100 Hz using a full-body modified Plug-In-Gait marker set with 54 markers and a 10-camera Vicon motion capture system (Vicon, Oxford, UK) positioned around the 10-meter walkway. Ground reaction forces were measured at 1000 Hz synchronously with motion capturing using three force platforms (Kistler, Zurich, Switzerland) embedded into the walkway. Where possible markers were placed directly onto the skin; to minimise movement artefacts resulting from loose clothing all participants wore tight-fitting shorts and t-shirts. Participants were instructed to walk the length of the walkway at different walking speeds performed in a specific order (0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 m/s). Walking speed was controlled by measuring the velocity of a marker attached to the sacrum after each trial from the motion analysis data and providing immediate feedback for participants as to whether they needed to walk more quickly or more slowly on the next trial to achieve the required speed. Walking trials were repeated until at least three 'clean' foot contacts with the force platforms were made per limb, per speed condition. All participants wore specialist diabetic shoes (MedSurg, Darco, Raisting, Germany) with a neutral foot-bed, ensuring the diabetes patients walked with safe, appropriate footwear whilst minimising the effect of footwear by standardising across all participant groups.

MRI scanning and analysis

Magnetic resonance imaging (MRI) was used to quantify the internal Achilles tendon (AT) moment arm length at the ankle as previously described (26). The internal moment arm was defined as the perpendicular distance from the centre of rotation on the talus to the AT line of action (26). Internal moment arm lengths were determined with participants standing upright (i.e., full weight-bearing) in a 0.25T MRI scanner (E-Scan, Esaote Biomedica, Genoa, Italy). Weight-bearing scans were acquired across the predominant range of ankle joint angles (10 degrees dorsiflexion, neutral position, 10 degrees plantarflexion) experienced during walking, to relate these measurements as closely as possible to the conditions of walking. In the present study rotation of the ankle joint from plantarflexion to dorsiflexion was treated as a single planar mechanism (25, 39, 41, 38). The ankle joint instant centre of rotation was located following the graphical approach described by Reuleaux (37) for ankle angle rotations from -10 to 10 deg. Instant centre of rotation was determined by measuring the rotation of the talus, which was considered to represent the whole rotating foot, relative to the tibia. The AT moment arm was measured on the neutral ankle scan as the perpendicular distance from the centre of rotation on the talus to the line of action on the AT. All images were analysed using a custom-script written in MATLAB software.

Measurement of the external moment arm at the ankle during walking and foot length

Foot length was measured in the standing position as the distance between the end of the big toe and the heel. The external moment arm (ExtMA) length around the ankle during walking was defined as the perpendicular distance between the

resultant GRF vector in sagittal plane and the ankle joint centre of rotation. The ankle joint centre of rotation was defined from the markers positioned on both lateral and medial malleoli. The ExtMA was quantified throughout the stance phase on every motion analysis frame from integration of the kinematic data with the GRF data.

Calculation of the EMA

Consistent with the approach used in previous studies (4, 5, 36), the EMA around the ankle joint was calculated as the ratio of the internal moment arm length to the external moment arm length (Fig. 2).

$$\text{Effective Mechanical Advantage} = \frac{\text{Internal moment arm}}{\text{External moment arm}}$$

The ExtMA values were quantified across the stance phase from the kinematic data as described above. The internal moment arm values were measured using MRI as described above and calculated across the stance phase by using the measured ankle joint angle data and the previously reported ratio of internal moment arm to ankle joint angle determined from MRI (38).

Gait biomechanical analysis

Gait variables were calculated using Visual 3D software (C-motion Inc., MD, USA): external moment arm lengths, joint moments, GRFs and ankle, knee and hip joint angles. Joint moments and GRFs were normalised to body mass. Data for the external moment arms, joint moments and GRFs were collected during

the stance phase, while ankle, knee and hip joint ranges of motion (RoM) were analysed throughout the gait cycle. Means across both legs and three trials were used for all variables presented.

Statistics

A one-way analysis of variance (ANOVA) was performed for all variables to assess between group differences. If the ANOVA was significant, a Fisher's least significant difference (LSD) post-hoc test was used to test for differences between the diabetes groups (DM and DPN) and the control group. An analysis of covariance (ANCOVA) was performed for the external moment arm at peak ankle joint moment using foot length as the covariate. All values presented are means and standard deviation. Significance was set at $p < 0.05$.

3.4 RESULTS

Participant characteristics

Significant differences existed between the groups in age, body mass and BMI, with the DPN group being older and heavier with a greater BMI compared to controls (Table 1, $P < 0.01$).

Diabetic Peripheral Neuropathy

As expected, patients with DPN displayed significantly higher mNDS and VPT than the Ctrl and the DM groups (Table 1). There were no differences ($P > 0.05$) in the mNDS or VPT between the Ctrl and the DM groups, underlining that this diabetes group had no neuropathy.

Temporal–spatial gait parameters

The DPN group displayed significantly longer single limb stance times and shorter step lengths in all given speeds compared to Ctrl group (Table 2).

External moment arm at peak ankle joint moment during walking & gait parameters

The ExtMA length at peak ankle joint moment was significantly smaller ($P < 0.01$) in the DPN group compared to the Ctrl group at walking speeds of 0.6; 1.0 and 1.4 m/s and for the mean across all speeds (Table 2). Significant differences ($P < 0.01$) were also observed in the external moment arm length between the DM and Ctrl groups at a walking speed of 1.4 m/s. The DPN group displayed significantly ($P < 0.01$) longer single limb stance times and shorter step lengths in all given speeds compared to the Ctrl group (Table 2).

Internal moment arm and EMA during walking

There were no differences in the internal moment arm length in the DPN and the DM groups compared to the Ctrl group ($P > 0.05$). The EMA at the ankle was

significantly ($P<0.01$) higher in the DPN group compared to the Ctrl group at walking speeds of 0.6; 1.0; 1.2; 1.4, 1.6 m/s and for the mean across all walking speeds (Table 2). The EMA at the ankle was also significantly ($P<0.01$) higher in the DM group compared to the Ctrl group at walking speeds of 0.6; 0.8, 1.4 m/s and for the mean across all walking speeds (Table 2).

Ground reaction forces during walking

Ground reaction forces were significantly higher ($P<0.01$) in the DPN group compared to the Ctrl at walking speeds of 0.6; 0.8; 1.0; 1.4 and 1.6 m/s and for the mean across all walking speeds (Table 2). Significantly higher ($P<0.01$) GRF values were also found in the DM group compared to the Ctrl group at the walking speed of 1.6 m/s and for the mean across all speeds (Table 2).

Peak ankle joint moments & lower limb kinematics during walking

Peak ankle plantarflexion joint moments were significantly lower ($P<0.01$) in the DPN compared to the Ctrl group for all walking speeds including the mean across all speeds (Table 2), with the exception of values at 1.2 m/s. Peak ankle plantarflexion joint moments were also significantly lower ($P<0.01$) in the DM compared to the Ctrl group at walking speeds of 1.2, 1.4 and 1.6 m/s and for the mean across all speeds (Table 2).

A significantly ($P<0.01$) smaller ankle, knee and hip joint RoM was observed in the DPN group compared to the Ctrl group across all walking speeds (Table 3). Joint RoM was also significantly ($P<0.01$) reduced in the DM group compared to the Ctrl group at the ankle (1.0 and 1.2 m/s), knee (0.8; 1.0; 1.2 m/s and for the mean values) and hip (all speeds except 0.6 m/s). Between group differences (range of motion) for the DPN and Ctrl groups were in the range 11-15% for the

ankle, 4-6% for the knee and 9-11% for the hip across the range of speeds examined. Smaller percentage differences were found when the Ctrl group was compared to the DM group across the range of speeds (1-5% for the ankle, 1-3% for the knee and 4-8% for hip). Smaller ankle RoM in the DPN group was mainly brought about through a significantly reduced peak dorsiflexion angle (Table 4). Smaller knee RoM was the result of significantly ($P<0.01$) reduced peak flexion and knee extension in the DPN group compared to the Ctrl group (Table 4). Despite overall reductions in hip RoM in the DPN compared to Ctrl group (Table 3), the DPN group displayed significantly ($P<0.01$) greater hip flexion (Table 4). The overall reductions in hip RoM were explained by significantly ($P<0.01$) reduced hip extension in the DPN group compared to Ctrl (Table 4).

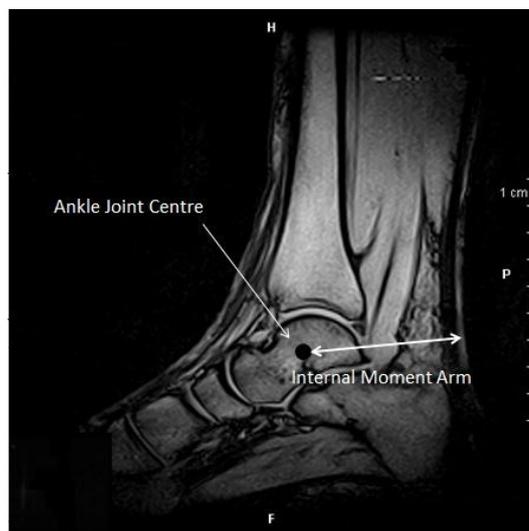


Figure 1. An example sagittal plane MRI scan of the lower limb showing the measurement of the internal moment arm length (indicated by the white arrow).

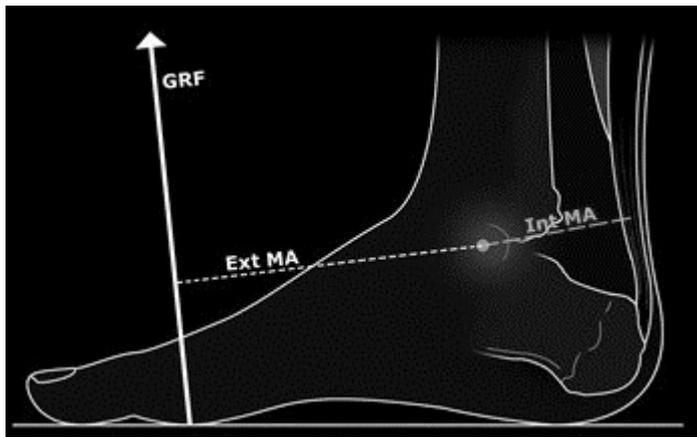


Figure 2. Diagram showing the external moment arm length (Ext MA; black dashed line) as the perpendicular distance between the resultant GRF vector and the joint centre of rotation (●); the internal Achilles tendon moment arm (Int MA; red dashed line) as the perpendicular distance between the tendon's action line and the ankle joint centre (●). The EMA is calculated as: $IntMA/ExtMA$.

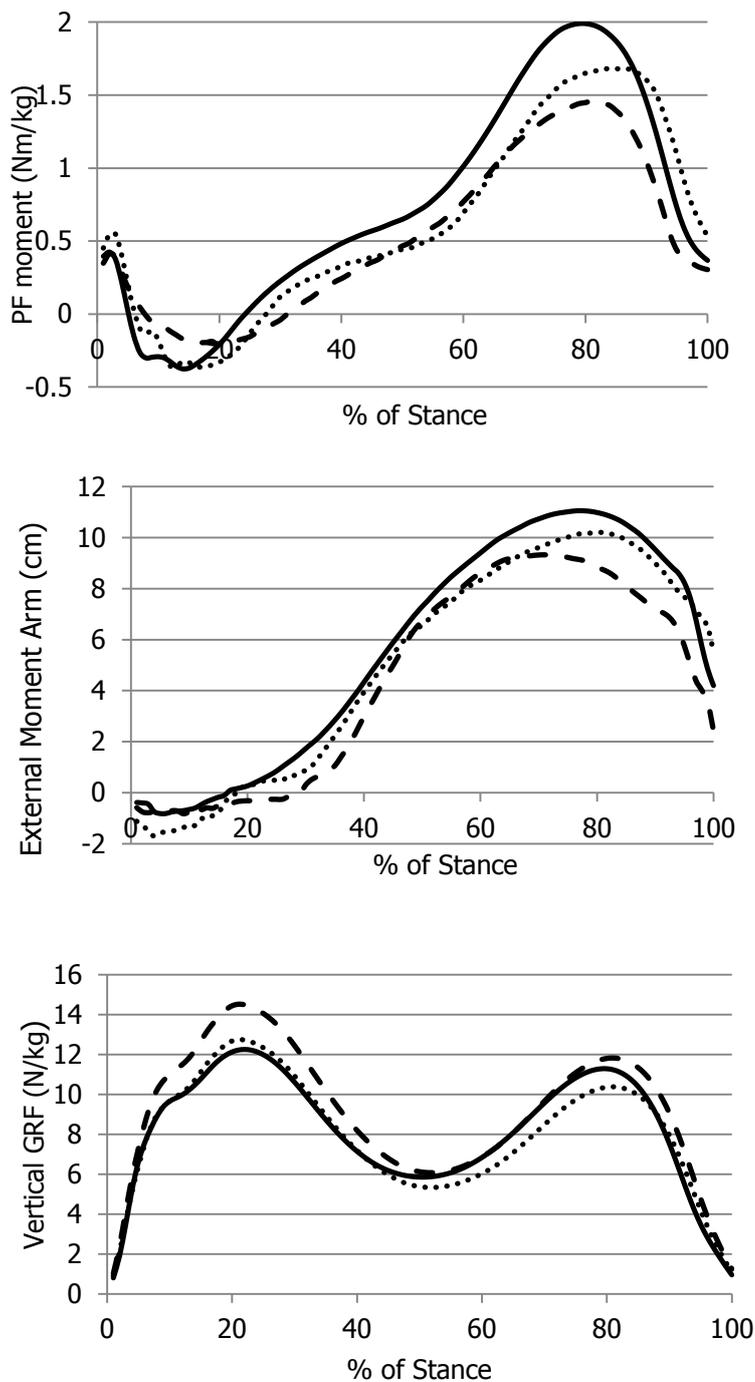


Figure 3. External moment arm (EMA), ankle joint moment (AJM) and ground reaction forces (GRFs) during stance phase while walking at 1.4 m/s for healthy controls (Ctrl), diabetic patients with no neuropathy (DM), and diabetic patients with moderate/severe neuropathy (DPN). Values are means. Line graphs: Ctrl - solid line (n=31), DM - dotted line (n=22), DPN - dashed line (n=14).

Table 1. Participant demographics and diabetes characteristics by study group.

Variable	Group		
	Ctrl	DM	DPN
Age (yr)	56 (10)	51 (9)**	66 (14)**
Body mass (kg)	76 (10)	80.5 (12)	91.5 (18)**
Height (m)	1.72 (0.12)	1.71 (0.09)	1.73 (0.11)
BMI (kg/m²)	26 (3)	28 (4)	31 (4)**
Foot length (cm)	25.43 (1.76)	24.77 (2.1)	25.81 (2.4)
Internal MA (cm)	4.72 (0.27)	4.73 (0.30)	4.98 (0.21)
mNDS (Score/10)	1 (1)	2 (1)	7 (2)**
VPT (Volts)	6.1 (3.4)	8.2 (3.4)	27.4 (9.1)**
Diabetes duration (years)	-	14 (12)	14 (11)
Type 1 diabetes	-	7	4
Type 2 diabetes	-	15	10

Healthy controls (Ctrl, n=31), diabetes patients with no neuropathy (DM, n=22) and diabetes patients with moderate/severe neuropathy (DPN, n=14). Significant differences from the Ctrl group are denoted by ** (P<0.01). BMI = body mass index, mNDS = modified neuropathy disability score, VPT = vibration perception threshold. Values are means (standard deviations).

Table 2. Biomechanical parameters at the ankle joint and temporal-spatial parameters during walking at different matched speeds.

Speed (m/s)	ExtMA @ peak AJM (cm)			Peak AJM (Nm/kg)			Peak GRF (N/kg)			EMA			Single limb stance time (s)			Step length (m)		
	Ctrl	DM	DPN	Ctrl	DM	DPN	Ctrl	DM	DPN	Ctrl	DM	DPN	Ctrl	DM	DPN	Ctrl	DM	DPN
0.6	11.71 (3.74)	11.79 (3.14)	9.54 (4.41)**	1.53 (0.53)	1.47 (0.47)	1.28 (0.45)**	9.91 (2.81)	9.63 (2.70)	11.22 (3.27)**	0.402 (0.09)	0.424 (0.06)**	0.486 (0.08)**	0.90 (0.20)	0.84 (0.23)	0.96 (0.05)**	0.59 (0.20)	0.57 (0.24)	0.51 (0.09)**
0.8	9.81 (4.14)	10.03 (3.47)	10.22 (4.01)	1.69 (0.59)	1.57 (0.50)	1.32 (0.41)**	10.84 (2.87)	10.52 (2.78)	11.91 (3.38)**	0.458 (0.07)	0.473 (0.04)**	0.462 (0.07)	0.80 (0.15)	0.84 (0.21)	0.96 (0.05)**	0.63 (0.21)	0.57 (0.21)	0.53 (0.05)**
1.0	10.25 (3.57)	10.30 (3.68)	9.70 (4.98)**	1.52 (0.53)	1.45 (0.57)	1.40 (0.52)	10.80 (2.74)	11.13 (2.70)	12.74 (3.02)**	0.460 (0.06)	0.465 (0.11)	0.485 (0.09)**	0.71 (0.13)	0.74 (0.05)	0.88 (0.05)*	0.69 (0.15)	0.67 (0.05)	0.64 (0.04)*
1.2	9.51 (3.71)	9.63 (4.03)	9.48 (4.55)	1.74 (0.60)	1.50 (0.58)**	1.56 (0.55)**	12.01 (2.94)	11.78 (2.82)	12.97 (3.17)	0.486 (0.04)	0.488 (0.08)	0.494 (0.05)**	0.58 (0.31)	0.62 (0.05)	0.68 (0.06)*	0.76 (0.11)	0.75 (0.17)	0.69 (0.07)*
1.4	11.02 (4.65)	9.97 (4.81)**	8.46 (5.54)**	1.99 (0.74)	1.68 (0.66)**	1.46 (0.55)**	12.25 (3.08)	12.74 (3.01)	14.52 (3.43)**	0.426 (0.05)	0.461 (0.04)**	0.508 (0.06)**	0.56 (0.15)	0.58 (0.21)	0.62 (0.14)*	0.79 (0.12)	0.77 (0.17)	0.71 (0.11)*
1.6	10.24 (5.40)	10.18 (5.49)	10.43 (6.14)	2.09 (0.81)	1.80 (0.67)**	1.62 (0.60)**	13.13 (3.28)	11.28 (2.86)**	14.16 (3.48)**	0.460 (0.03)	0.451 (0.04)	0.493 (0.09)**	0.50 (0.15)	0.50 (0.11)	0.53 (0.01)*	0.81 (0.11)	0.80 (0.04)	0.74 (0.02)*
Mean	10.42 (4.20)	10.31 (4.10)	9.63 (4.93)**	1.74 (0.64)	1.59 (0.56)**	1.44 (0.51)**	9.82 (2.95)	11.18 (2.81)**	12.92 (3.29)**	0.448 (0.05)	0.460 (0.06)**	0.488 (0.07)**	0.68 (0.18)	0.68 (0.14)	0.77 (0.06)**	0.71 (0.15)	0.68 (0.14)	0.63 (0.06)**

Healthy controls (Ctrl; n=31), diabetes patients with no neuropathy (DM; n=22) and diabetes patients with moderate/severe neuropathy (DPN; n=14). Significant differences from the Ctrl group are denoted by *(P<0.05) or **(P<0.01). Values are means (standard deviations). ExtMA @ peak AJM – external moment arm around the ankle at peak ankle joint moment. Peak AJM – peak ankle joint moment. Peak GRF – peak vertical ground reaction force. EMA - Effective mechanical advantage.

Table 3. Ankle, knee and hip joint ranges of motion (RoM) over the gait cycle at different matched speeds.

Speed (m/s)	RoM Ankle (deg)			RoM Knee (deg)			RoM Hip (deg)		
	Ctrl	DM	DPN	Ctrl	DM	DPN	Ctrl	DM	DPN
0.6	23.1 (8.1)	22.7 (7.8)	20.8 (9.5)**	64.5 (24.4)	62.6 (23.7)	61.2 (27.4)**	44.9 (16.5)	42.4 (12.7)	40.8 (15.8)**
0.8	23.7 (7.4)	23.5 (8.5)	21.1 (7.8)**	66.6 (27.6)	64.8 (28.3)**	62.7 (29.1)**	46.6 (17.9)	43.1 (15.8)**	42.9 (16.0)**
1.0	25.6 (9.0)	24.4 (8.3)*	22.5 (9.4)**	67.7 (25.1)	66.1 (21.2)**	64.9 (24.0)**	46.8 (14.8)	44.8 (16.6)**	42.1 (19.3)**
1.2	26.4 (7.5)	25.2 (8.7)*	23.7 (8.4)**	69.4 (22.9)	67.7 (26.6)**	66.5 (27.3)**	47.9 (16.7)	44.5 (15.7)**	43.2 (18.7)**
1.4	26.8 (10.3)	26.0 (9.1)	23.4 (9.2)**	69.8 (27.8)	69.3 (25.8)	67.3 (30.4)**	49.8 (13.7)	47.3 (14.2)**	45.7 (15.3)**
1.6	27.3 (9.9)	26.9 (8.6)	24.3 (9.0)**	71.0 (28.4)	70.3 (31.2)	68.4 (30.7)**	50.7 (19.4)	48.4 (18.9)**	46.5 (21.4)**
Mean	25.4 (8.7)	24.7 (8.5)	22.4 (8.8)**	68.2 (26.0)	66.8 (26.1)**	65.2 (28.1)**	47.8 (16.5)	45.1 (16.7)**	43.5 (17.8)**

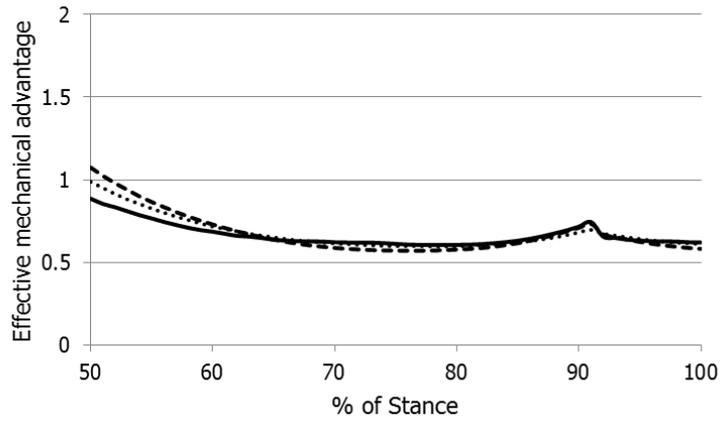
Healthy controls (Ctrl, n=31), diabetic patients with no neuropathy (DM, n=22) and diabetic patients with moderate/severe neuropathy (DPN, n=14). Significant differences from the Ctrl group are denoted by *(P<0.05) or **(P<0.01). Values are means (standard deviations). RoM – range of motion.

Table 4. Peak joint angle values for the ankle, knee and hip during the gait cycle at different matched speeds.

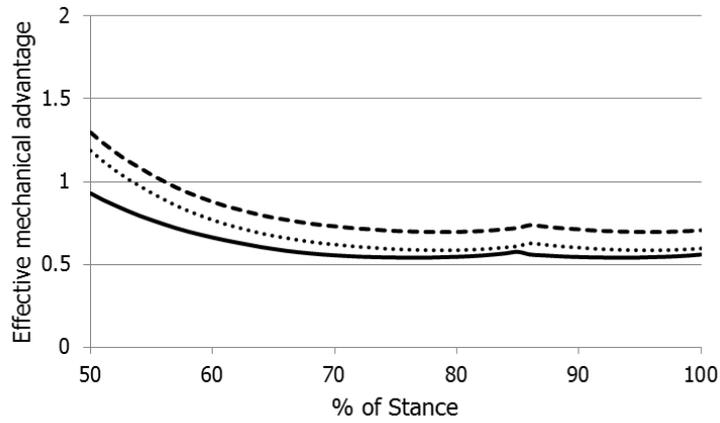
Speed (m/s)	Peak ankle angle						Peak knee angle						Peak hip angle					
	Ctrl		DM		DPN		Ctrl		DM		DPN		Ctrl		DM		DPN	
	DF	PF	DF	PF	DF	PF	Flex	Ext	Flex	Ext	Flex	Ext	Flex	Ext	Flex	Ext	Flex	Ext
0.6	10.3	12.8	10.1	12.6	8.3**	12.5	55.2	9.3	53.0	9.6	51.0**	10.2**	37.7	7.2	36.8	5.6**	38.0	2.8**
0.8	10.4	13.3	10.4	13.1	8.1**	13.0	57.4	9.2	55.3	9.5	52.6**	10.1**	38.7	7.9	37.3	5.8**	38.1	4.8**
1.0	10.4	15.2	9.8	14.6	7.9**	14.6	59.5	8.2	57.4	8.7	56.0	8.9	38.0	8.8	37.5	7.3	38.6	3.5**
1.2	10.5	15.9	10.1	15.1	8.6**	15.1	61.0	8.4	58.9	8.8	57.3**	9.2**	38.1	9.8	38.4	6.1**	39.8**	3.4**
1.4	10.7	16.1	10.6	15.4	8.2**	15.2**	64.3	5.5	62.9	6.4**	59.9**	7.4**	36.2	13.6	39.1**	8.2**	40.1**	5.6**
1.6	10.9	16.4	11.5	15.4	9.0**	15.3**	67.6	3.4	66.1	4.2**	61.9**	6.5**	35.5	15.2	39.3**	9.1**	42.4**	4.1**
Mean	10.5	14.9	10.3	14.4	8.1**	14.3	60.8	7.4	59.0	7.8	56.5**	8.7**	37.4	10.4	38.0	7.1**	39.5**	4.0**

Healthy controls (Ctrl, n=31), diabetic patients with no neuropathy (DM, n=22) and diabetic patients with moderate/severe neuropathy (DPN, n=14). Significant differences from the Ctrl group are denoted by *(P<0.05). Values are means. DF – dorsiflexion, PF – plantarflexion, Flex – flexion, Ext – extension.

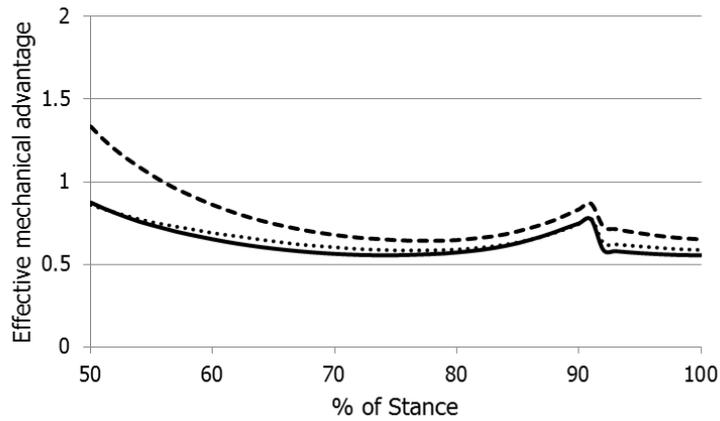
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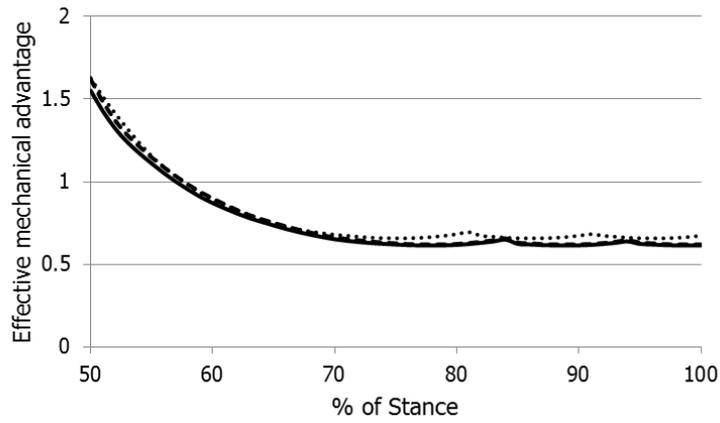
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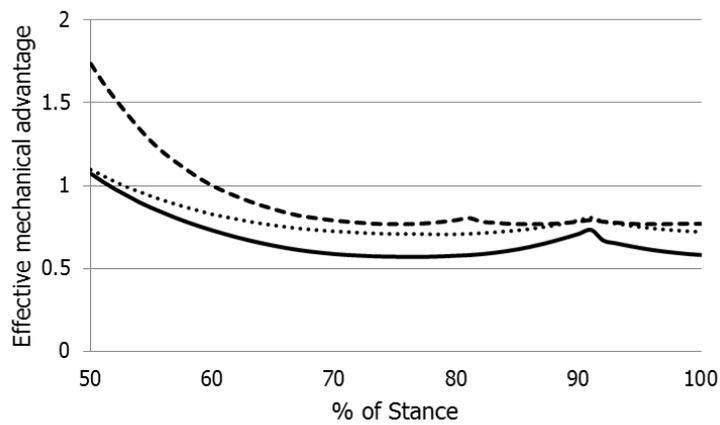
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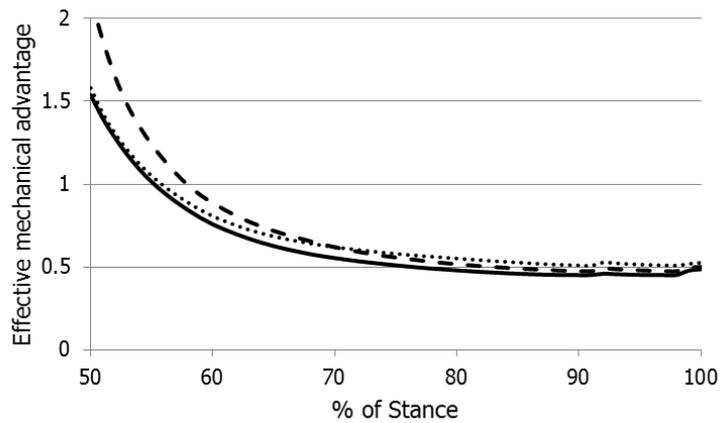


Figure 4. Effective mechanical advantage (EMA) values across the entire stance phase. Line graphs: Ctrl - solid line, DM - dotted line, DPN - dashed line. Values are means.

3.5 DISCUSSION

I have recently shown that patients with diabetes and especially those with DPN have a higher CoW compared to controls (32). In the present study I investigated whether differences between diabetes patients and healthy controls in the ExtMA and EMA around the ankle joint could be potential mechanisms underpinning the above finding. I established that patients with diabetes and especially those with DPN have a smaller ExtMA and a higher mechanical advantage around the ankle joint compared to controls (Fig. 3; Table 2), which is in contrast with our hypothesis that the ExtMA will be higher and the EMA will lower in the diabetes patients.

The smaller ExtMA at the ankle in patients with diabetes and especially those with DPN was evident across all walking speeds (Table 2) and means that either the resultant GRF was applied closer to the ankle joint centre, or the angle of application was more towards the ankle, making the ExtMA smaller, thereby minimising the ankle joint moment. The effects of this can be seen by the reduced ankle joint moment in patients with DPN compared to controls across all matched walking speeds (Table 2).

It has previously been shown that diabetes patients reduce joint moments by taking shorter strides with less flexed joints (2, 23, 29, 31, 33, 9). Our unexpected findings demonstrate a mechanism through which people with diabetes and particularly those with DPN reduce the joint moment at the ankle during walking – by applying the GRF more proximally on the foot or at an angle more towards the ankle, reducing the ExtMA around the ankle and thereby reducing the ankle joint moment (Figs. 2 & 3). No differences in foot length existed to explain the smaller ExtMA found in patients with DPN (Table 1) and further, this parameter (foot length) was also entered as a covariate in the statistical analysis of variance.

Our finding of a smaller ExtMA and greater EMA around the ankle does not appear to explain an increased CoW, as the plantarflexor muscles would need to produce not higher, but smaller contractile forces to rotate the foot and propel the body forward. However, the consequent reduction in the force applied to the Achilles tendon would result in reduced tendon elongation and therefore reduced storage of elastic strain energy. The reduced contribution from elastic strain energy stored in the Achilles tendon could impact upon the CoW, but this requires further investigation.

The joint kinematics from the present study provides insight as to how patients with DPN might have been able to execute this natural strategy of reducing the ExtMA around the ankle and thereby minimising the ankle joint moment. The ankle joint RoM over the gait cycle was reduced in patients with DPN compared to controls as a result of a reduced peak dorsiflexion angle (Tables 3 & 4). This reduced dorsiflexion suggests that patients with DPN were not able to allow the tibia to rotate over the foot to the same extent as controls during the mid-stance phase, further evidenced by the reduced knee flexion (Table 4), thereby applying force to the ground more proximally on the foot and reducing the ExtMA around the ankle as a result. These joint range of motion limitations may also raise the possibility that this may not be a natural strategy of choice, but rather in contrast, patients with DPN may adopt this strategy since they have no other possibilities due to such limitations.

Whilst the total hip joint RoM during walking was reduced in diabetes patients and especially those with DPN compared to controls (Table 3), patients with diabetes and to the greatest extent those with DPN flexed the hip more than controls (Table 4). This kinematic strategy fits very well with the 'hip strategy' previously reported in other studies (31, 34), whereby diabetes patients have

been observed to 'drag' the leg forwards into the swing phase from the hip, rather than 'propelling' the leg off from the ground using the ankle plantarflexors. Whilst greater knee and hip RoM occurs with increasing walking speeds in all groups, a consistently smaller RoM at the knee and hip in the DPN group (Table 3) underlies the shorter step length reported in the present study and is comparable with a number of previous studies conducted in diabetes patients (47, 34, 28, 15, 1, 31, 32).

Through measuring the ExtMA during walking and the internal Achilles tendon moment arm in the weight-bearing condition using MRI, I calculated the EMA around the ankle. The present study found a greater value for the EMA at the ankle joint in diabetes patients and especially in those with DPN compared to controls (Table 2). To the best of our knowledge there are no previous studies reporting the EMA in diabetes patients during walking, but it has been measured for different human and animal populations (4, 40, 42, 17, 3). The internal AT moment arm values in the present study (Table 1) measured using MRI are comparable with those reported from previous human studies conducted in other populations reporting values ranging between 3.7 and 5.3 cm (26, 4, 38, 43). The EMA was calculated throughout the stance phase (Fig. 4), but the most functionally relevant point to report was considered to be from 50% till 100% of the stance phase. Figure 4 shows that for the same joint work there is still a higher CoW in DPN.

Whilst previous studies have consistently reported lower ankle joint moments in diabetes patients during walking, this has typically been at the self-selected speed, which is consistently lower in diabetes patients as they seek to minimise the demands of the task. Here I show that when walking speed is matched, joint moments are consistently lower in the DM and particularly in the DPN group

compared to controls (Table 2). The reduction in the ExtMA and increasing the EMA at the ankle in the diabetes groups is relatively independent of walking speed, whereas ankle joint moments and the vertical GRF increase with increasing walking speed (Table 2). It is also noteworthy that although the peak ankle joint moments were significantly lower in the diabetes groups compared to controls, the vertical GRF was significantly higher especially in the DPN group (Table 2). This higher GRF seems to underline the importance of the strategy in patients with diabetes and particularly those with DPN for reducing the ExtMA around the ankle to lower the ankle joint moment substantially below that of controls. What remains unclear is whether the way in which the DPN group walked represents a natural strategy to lower the demands, or whether they have no other possibility to walk differently because of any foot deformities present and inflexible joints in the lower limb and within the foot.

In terms of study limitations, the mean body mass was significantly different between groups (being higher in the DPN group), however, this should not affect the ankle ExtMA, EMA, or the joint moments since these were normalised for body mass. Furthermore, the higher body mass of patients with DPN is a well-known characteristic of this clinical population described by previous studies (23, 20, 21). Although only a mean of 10 years difference, patients in the DPN group were significantly older than controls (66 to 56 years, respectively), which might be a confounding factor for some of the variables examined, but unlikely to affect the main variables of interest: the ankle ExtMA and EMA.

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4. Experimental chapter three – Achilles tendon properties during walking in patients with diabetes: implications for metabolic energy saving

4.1 ABSTRACT

The Achilles tendon (AT) has the capacity to store and release elastic energy during walking, contributing to metabolic energy savings. In diabetes patients, it is hypothesised that a stiffer tendon may reduce the capacity of the tendon for energy saving, thereby contributing to an increased metabolic cost of walking in this population. The aim of this study was to investigate the effects of diabetes and diabetic peripheral neuropathy (DPN) on plantarflexion muscle-tendon behaviour during walking at self-selected and a controlled (1.0 m/s) walking speed. 23 non-diabetic controls (Ctrl); 20 diabetic patients without peripheral neuropathy (DM) and 13 patients with moderate/severe DPN, underwent gait analysis using a motion analysis system, force plates and ultrasound measurements from the gastrocnemius muscle. The DM and particularly the DPN group displayed significantly lower Achilles tendon elongation, higher stiffness (Ctrl: 210; DM: 231; DPN: 240 N/mm) and higher hysteresis (Ctrl: 18; DM: 21; DPN: 24 %) while walking compared to controls. The muscle fascicles of the gastrocnemius underwent very small length changes. Achilles tendon forces were lower in the diabetes groups compared to controls (Ctrl: 2666; DM: 2609; DPN: 2150 N). The results strongly point towards the reduced energy saving capacity of the Achilles tendon in diabetes patients as an important factor contributing to the increased metabolic CoW in these patients.

4.2 INTRODUCTION

Diabetes mellitus (DM) is a common problem in older adults worldwide and is associated with many complications such as cardiovascular diseases, nephropathy, stroke, peripheral arterial disease, retinopathy, skin problems, poor wound healing and peripheral neuropathy (16, 14). One of the most common complications in diabetes is diabetic peripheral neuropathy (DPN). The incidence of DPN has been reported to range between 13 and 68% in diabetes populations (41); while Boulton (6) reports up to 50% of diabetes patients are affected by neuropathy.

Scientists and clinicians are particularly interested in the impact of diabetes and DPN on gait and mobility since it has a direct impact on person's quality of life. Diabetes has been reported to affect level walking, stair negotiation and cause impairments to balance control during walking and standing (13, 29, 42, 19, 5).

The muscle-tendon complex is central to all movement tasks, with skeletal muscle generating force, which is transmitted to the skeleton via viscoelastic tendons. In addition to their force transmitting role, tendons can also play an important role in energy saving by storing (during stretching) and returning (during recoil) elastic energy (36, 37, 38, 2). In particular, the Achilles tendon is important for storing and releasing elastic energy during walking and can lead to significant metabolic energy savings, as it actually 'spares' the muscle from performing a large part of the work (3).

Both muscles and tendons are highly malleable tissues, which can modify their properties in response to the level of physiological loading and also the metabolic environment (35, 1, 17). Animal studies show that diabetes causes non-enzymatic glycation of soft tissues, such as tendon (33). This non-enzymatic glycation causes increased cross-linking, increasing the stiffness and modulus of

the tendon (32, 34). Stiffening of the tendon reduces the degree to which it can be stretched, affecting its potential for storing (and subsequently releasing) elastic strain energy during walking and also limiting the range of joint motion (11, 18, 27). In humans, calcification and fascicle disruption have been observed in the diabetic human Achilles tendon (AT) (4). Since tendons exhibit relatively low mechanical hysteresis, most of the elastic energy stored during stretching is returned on recoil (25), but there is the capacity for hysteresis to also be affected by diabetes as has been shown to occur in human ageing (36).

Cronin et al. (10) found the Achilles tendon length changes during walking at self-selected speed to be attenuated in diabetes patients and that they were inversely correlated with diabetes duration. In dynamometry tests, Couppé et al. (10) found Achilles tendon stiffness and skin connective tissue cross-linking were greater in diabetes patients compared with controls.

The role of the Achilles tendon during walking and the relative contribution required from the plantarflexors muscles remains unknown in diabetes patients. The aim of this study was to investigate the effects of diabetes and diabetic peripheral neuropathy on muscle and tendon behaviour during walking at self-selected and a controlled speed. I hypothesized that the AT would contribute less to elastic energy during walking due to its increased stiffness in diabetes patients compared to controls and as a result a greater contribution would be required from the plantarflexor muscles, requiring more energy and contributing to explain the higher cost of walking (CoW) in people with diabetes.

4.3 MATERIALS AND METHODS

Participants

After receiving ethical approval for the study from all relevant bodies, a total of sixty seven participants were recruited, who gave their written informed consent to participate. Due to logistical reasons the measurements for this study were acquired in 56 of these participants. All participants were aged over 40 and allocated into one of three groups based upon defined criteria: patients with diabetes and moderate-severe peripheral neuropathy (DPN, n=13), patients with diabetes but no neuropathy (DM, n=20) and healthy controls without diabetes or peripheral neuropathy (Ctrl, n=23). Major exclusion criteria included: disorders of the vestibular system severe vascular disease, neurological, rheumatic disease, cerebral injury, unstable ischemic heart, musculoskeletal injury, foot or lower limb amputation (amputation of the hallux; amputation of more than two lesser toes on one foot; amputation of part of/whole foot) and open foot ulcer and recent surgery affecting gait. Information about the duration and type of diabetes, smoking habits and use of current medication were obtained by using a questionnaire. The vast majority of the DM and the DPN patients reported taking insulin, cholesterol-lowering medication and diabetes medication. Participant characteristics are displayed in Table 1.

Diagnosis of Diabetic Peripheral Neuropathy

The presence and severity of peripheral neuropathy was assessed through clinical evaluation, which was undertaken to quantify neuropathy in diabetes patients and to confirm the absence of neuropathy in controls. Peripheral neuropathy was assessed by using the modified Neuropathy Disability Score (mNDS) and the vibration perception threshold (VPT). The mNDS is a composite

score taken from tests measuring the participant's ability to discriminate temperature, detect pain, vibration and the Achilles tendon reflex (6). The VPT is an assessment performed using the probe of a neurothesiometer on the apex of the hallux and increasing the level of vibration until detected by the participant. A random blood glucose test was performed in the Ctrl group to confirm the absence of diabetes (<7 mmol/l) and the above neuropathy tests conducted to confirm the absence of neuropathy in the Ctrl group resulting from any aetiology.

Gait analysis

Gait analysis was performed for the purpose of assessing the contribution of the plantarflexor muscle-tendon complex and the capacity for elastic energy storage and release via the Achilles tendon. Participants were asked to walk along a 10-metre walkway in the gait laboratory at their self-selected speed, as well as at standardized speed of 1.0 m/s. Walking at the standardized speed was controlled by measuring the velocity of a marker attached to the sacrum after each trial from the motion analysis data and providing immediate feedback for participants as to whether they needed to walk more quickly or more slowly on the next trial to achieve the required speed (1.0 m/s). Kinematic data were collected at 100 Hz using a 10-camera Vicon motion capture system (Vicon, Oxford, UK) a full-body modified Plug-In-Gait marker set consisting of 54 markers. Where possible motion analysis markers were placed directly onto the skin; to minimise movement artefacts resulting from loose clothing all participants wore tight-fitting shorts and t-shirts. Ground reaction forces were measured at 1000 Hz from three force platforms (Kistler, Zurich, Switzerland) embedded into the walkway and synchronised with the kinematic data. Walking trials were repeated until at least three 'clean' foot contacts with the force platforms were made with each limb, for

both speed conditions. During walking, an ultrasonographic imaging device (Aloka SSD-5000, Tokyo, Japan) operating at 25 Hz was used to measure gastrocnemius medialis (GM) muscle fascicle length changes *in vivo*. For these measurements, a linear 7.5 MHz probe with 60 mm field of view was tightly secured around the right lower leg in the mid-sagittal plane of the GM muscle with a custom-built fixation device (Fig. 1). The ultrasound scanning was synchronized with recordings of the kinematic and kinetic data. All participants wore specialist diabetic shoes (MedSurg, Darco, Raisting, Germany) with a neutral foot-bed, ensuring the diabetic patients walked with safe, appropriate footwear whilst minimising the effect of footwear by standardising across all participants (Fig. 1).



Figure 1. A linear 7.5 MHz probe (A) with 60 mm field of view. A custom-built fixation device made of Velcro straps and a plastic cast molded to fit the general contour of the calf (B) was used to secure the probe around the left lower leg, in the mid-sagittal plane of the gastrocnemius muscle (C) (Fukunaga et al., 2001).

Dynamometry measurements: Measurement of Maximal Plantarflexion Strength

Isometric plantarflexor maximal voluntary contraction (MVC) joint moment (maximum strength) was recorded with participants laying prone with the knee in full extension. The axis of rotation of the ankle, defined as the line connecting the two malleoli, was carefully aligned with the axis of rotation of the dynamometer and the right foot secured to the foot adapter of an isokinetic dynamometer (Cybex NORM, Cybex International, New York, NY, USA). Straps were used

around the ankle and also the hips to prevent extraneous movements during maximal plantarflexions. Prior to testing subjects became familiarised with the procedures involved. Participants were instructed to perform maximal isometric plantarflexion contractions at joint angles of 0, 5 and 10 degrees of dorsiflexion, where zero degrees was neutral ankle position: the footplate of the dynamometer perpendicular to the longitudinal axis of the tibia. The subjects were verbally encouraged for additional motivation to perform static contractions with the ankle plantarflexor with a maximum possible effort at all three ankle angles and they were encouraged to hold each contraction for up to 5-6 s. Contractions were performed in a randomized order. Two contractions were performed at each ankle angle by allowing a 1-min rest interval between bouts and the highest value was considered as the MVC at that ankle angle. Results were subsequently normalised to body mass.

Data processing

The purpose of the data analysis was to quantify the plantarflexor muscles and Achilles tendon characteristics during walking. The gastrocnemius medialis (GM) muscle was assessed as a representative of the plantarflexor muscle group (39, 40). The GM muscle fascicle lengths were measured from every frame of the ultrasound recordings during the entire stance phase. On each ultrasound frame, three lines were defined automatically using a custom-script written in MATLAB software (12): one line tracked the superficial aponeurosis, a second line was matched with the deep aponeurosis, and a third line defined the fascicular path of the fascicle movement. From these three lines, fascicle length and pennation angle were calculated on each frame of ultrasound data. The pennation angle was defined as the angle that the fascicle made with the deep aponeurosis.

Muscle fascicle length was defined as the distance between the superficial and deep aponeurosis parallel to the lines of collagenous tissue (Fig. 2). Pennation angle (α) was defined as the angle between the collagenous tissue and the deep aponeurosis. The equations by Menegaldo et al. (28) were used to calculate the GM muscle-tendon complex (MTC) length change (muscle plus free tendon and aponeurosis in both distal and proximal ends) using the fascicle length changes and the ankle and knee joint displacements measured during walking over the stance phase. The length of the tendon (including both the free tendon and aponeurosis) was found by subtracting muscle fascicle length projected in the direction of the line of force application from the muscle–tendon complex (MTC) length for each time instant. Thus:

$$l^t = l^{MTC} - l^m \cos \alpha$$

where l^t is the length of the tendon, l^{MTC} is the length of the MTC, l^m is the ultrasound-measured muscle fascicle length, and α is the ultrasound-measured pennation angle. Muscle fascicle and tendon properties were assumed to be consistent along the length of the MTC. The muscle fascicles were also assumed to be parallel to one another. The validity and reliability of the ultrasound measurements in vivo during walking have been critically assessed in other studies on the same and similar populations, reporting ICC values between 0.78 and 0.94 (11, 26, 30, 39).

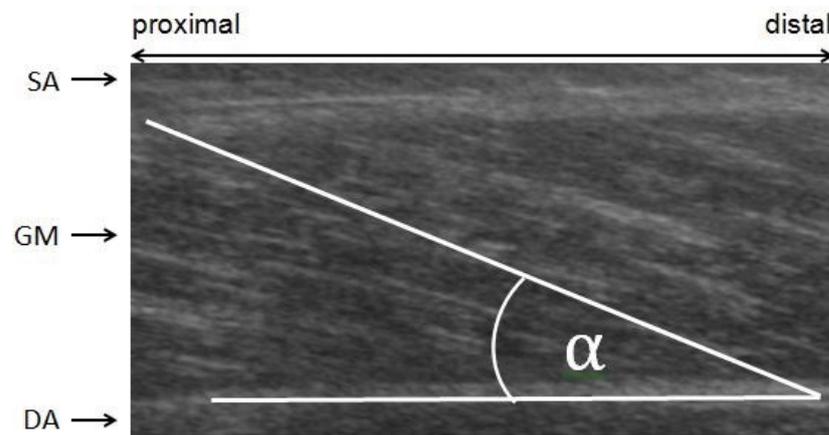


Figure 2. Typical sonograph of the GM muscle. The fascicular trajectory between the two aponeurosis, as well as the pennation angle (α) are highlighted in white. SA, superficial aponeurosis; GM, gastrocnemius medialis muscle; DA, deep aponeurosis.

Achilles tendon force

Achilles tendon (AT) forces were calculated during walking throughout the stance phase by dividing the net plantarflexion joint moments (Nm) by the AT internal moment arm length (m). The plantarflexion joint moments were derived from the kinematic and kinetic data using Visual 3D software (C-motion Inc., MD, USA). MRI scanning and analysis was used to quantify the internal AT moment arm length at the ankle as previously described (24). Elongation of the AT was calculated as described in the above section. The AT force and elongation were normalised to 100 points to represent the entire stance phase. Therefore, the AT force-elongation curve was derived, as shown in Figs. 5 and 6, where the loading phase (arrow pointing up) represents 10-70% of the stance phase and the unloading phase (arrow pointing down) the remaining 30%, as described in Table 2. The area between the loading and unloading curves represents the AT's hysteresis, which is the energy dissipated upon recoil.

Stiffness and hysteresis during walking

The AT stiffness was calculated from the measurements taken during walking as the slope of the loading curve by dividing the estimated tendon force (N) by the tendon's elongation (mm) over a force region between 500 and 1500 N (26). The AT hysteresis was calculated by dividing the difference between the area under the loading and the unloading curves by the area under the loading curve alone. This provides a measure of the energy converted to heat, an important feature of the mechanical properties of tendon. The mechanical hysteresis was defined as the area between the loading (L) and unloading (UnL) curves and expressed as a percentage:

$$\text{Mechanical hysteresis} = (L - UnL) / L \cdot 100$$

Statistics

A one-way analysis of variance (ANOVA) was performed for all variables to assess between group differences. If the ANOVA was significant, a Fisher's least significant difference (LSD) post-hoc test was used to test for differences between the diabetes groups (DM and DPN) and the control group. All values presented are means and standard deviation. Significance was accepted at $p < 0.05$.

4.4 RESULTS

Participant characteristics

Participant characteristics are shown in Table 1. There were no significant differences between the groups in age and BMI (Table 1).

Neuropathy assessments

As expected, the DPN group displayed significantly higher values for the VPT and the mNDS compared to the Ctrl group (Table 1). The VPT and mNDS for the DM group were not significantly different from the Ctrl, underlining that this diabetic patient group had no neuropathy (Table 1).

Lower limb kinetics and kinematics during walking

Peak ankle plantarflexion joint moments were significantly lower ($P < 0.01$) in the DPN and the DM compared to the Ctrl group for both, self-selected and 1.0 m/s walking speeds (Table 2). A significantly ($P < 0.01$) lower ankle and knee joint range of motion (RoM) was observed in the DPN and the DM groups compared to the Ctrl group for self-selected and 1.0 m/s walking speeds (Table 2).

Plantarflexor muscle-tendon unit behaviour during walking

There were no differences in the fascicle length during standing in the DPN and the DM groups compared to the Ctrl group ($P > 0.05$). Average fascicle length change data show that the DPN group was significantly different ($P < 0.01$) than the Ctrl group for both self-selected speed and 1.0 m/s, while the DM group was different than the Ctrl group only at 1.0 m/s. Significant differences ($P < 0.01$) in the MTC length change were found between the DPN and the Ctrl as well as the DM and the Ctrl groups for both walking speeds (Table 2). There were significant

differences in the tendon length change between the groups at self-selected walking speed (Ctrl: 1.81 cm; DM 1.66 cm; DPN: 1.54 cm; $P<0.01$) as well as 1.0 m/s (Ctrl: 1.67 cm; DM 1.51 cm; DPN: 1.47 cm; $P<0.01$), where the DPN group expressed smaller tendon length changes. The DM and particularly the DPN group displayed significantly lower Achilles tendon elongation, higher stiffness (Ctrl: 210; DM: 231; DPN: 240 N/mm: $P<0.01$) and higher hysteresis (Ctrl: 18; DM: 21; DPN: 24 %: $P<0.01$) while walking compared to controls.

Table 1. *Participant characteristics and results from neuropathy assessments.*

Variable	Group		
	Ctrl	DM	DPN
Age (yr)	55 (7)	57 (8)	61 (7)
BMI (kg/m ²)	26 (4)	28 (4)	29 (5)
mNDS (Score/10)	1 (1)	2 (1)	7 (2)**
VPT (Volts)	6.1 (3)	8.2 (4)	27.4 (9)**
Diabetes duration (years)	-	14 (13)	17 (11)
Type 1 diabetes	-	6	4
Type 2 diabetes	-	14	9

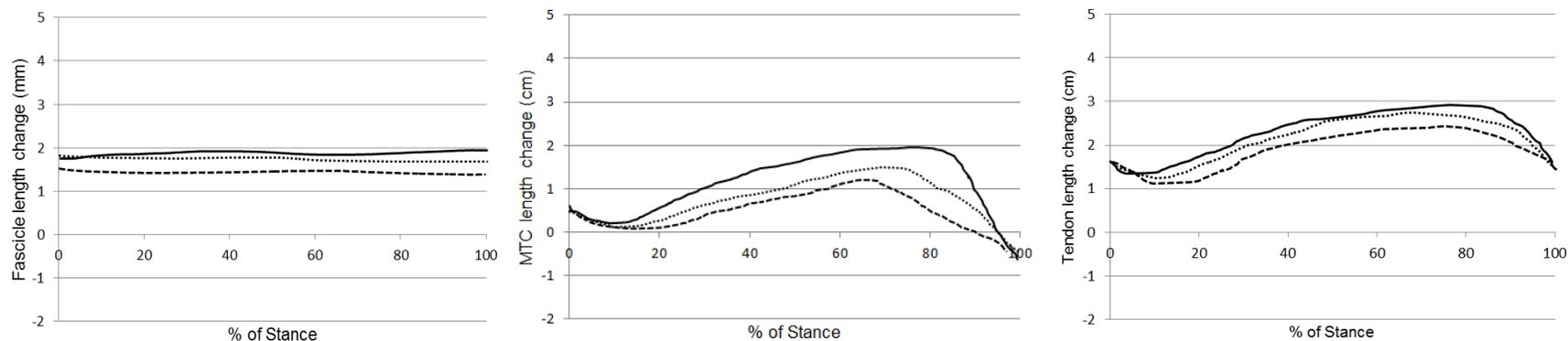
Healthy controls (Ctrl, n=23), diabetic patients with no neuropathy (DM, n=20) and diabetic patients with moderate/severe neuropathy (DPN, n=13). Significant differences from the Ctrl group are denoted by ** ($P<0.01$). BMI = body mass index, mNDS = modified neuropathy disability score, VPT = vibration perception threshold. Values are means (standard deviations).

Table 2. Plantarflexor muscle-tendon and Achilles tendon parameters during walking.

	Ctrl		DM		DPN	
	Self-selected		Self-selected		Self-selected	
Walking speed (m/s)	1.43	1.0	1.33	1.0	1.30	1.0
Stiffness (N/mm)	210 (41)	186 (34)	231 (46)**	194 (39)**	240 (49)**	202 (37)**
Hysteresis (%)	18 (3)	17 (3)	21 (5)**	19 (4)*	24 (6)**	21 (5)**
Standing fascicle length (cm)	5.15 (1.5)		5.08 (1.4)		5.19 (1.3)	
Fascicle length change (cm)	0.57 (0.06)	0.52 (0.16)	0.41 (0.04)	0.37 (0.04)**	0.35 (0.09)**	0.41 (0.12)**
MTC length change (cm)	1.62 (0.3)	1.41 (0.4)	1.13 (0.4)**	0.95 (0.3)**	0.84 (0.3)**	0.63 (0.2)**
Tendon length change (cm)	1.81 (1.0)	1.67 (0.7)	1.66 (0.5)*	1.51 (0.6)*	1.54 (0.8)**	1.47 (0.6)**
Fascicle length change (cm) 10-70 % of stance (loading)	0.58 (0.08)	0.53 (0.19)	0.42 (0.05)**	0.39 (0.06)**	0.38 (0.12)**	0.44 (0.14)**
Fascicle length change (cm) 70-100% of stance (unloading)	0.54 (0.04)	0.50 (0.12)	0.38 (0.04)**	0.33 (0.04)**	0.31 (0.07)**	0.37 (0.11)**
MTC length change (cm) 10-70 % of stance (loading)	1.21 (0.2)	1.11 (0.3)	0.89 (0.3)**	0.81 (0.2)*	0.76 (0.2)**	0.69 (0.1)**
MTC length change (cm) 70-100% of stance (unloading)	1.44 (0.1)	1.20 (0.1)	0.97 (0.1)**	0.84 (0.1)**	0.63 (0.1)**	0.58 (0.1)**
Tendon length change (cm) 10-70 % of stance	1.96 (0.6)	1.71 (0.4)	1.65 (0.3)**	1.26 (0.4)**	1.18 (0.5)**	0.81 (0.4)**
Tendon length change (cm) 70-100% of stance	1.92 (0.4)	1.82 (0.3)	1.63 (0.2)**	1.41 (0.2)**	0.78 (0.3)**	1.15 (0.2)**
Achilles Tendon forces (N)	2666 (242)	2343 (288)	2609 (167)*	2256 (290)**	2150 (177)**	2288 (241)**
Ankle RoM (deg)	26.4 (7.9)	25.1 (8.7)	25.3 (7.1)**	24.2 (8.1)**	25.1 (8.6)**	22.3 (9.5)**
Knee RoM (deg)	69.7 (26.1)	67.8 (24.9)	67.0 (21.5)**	66.0 (21.3)**	64.8 (30.2)**	64.7 (23.5)**

Gastrocnemius muscle fascicle length and other muscle-tendon parameters during walking for healthy controls (Ctrl; n=23), diabetic patients with no neuropathy (DM; n=20) and diabetic patients with moderate/severe neuropathy (DPN; n=13). Values are group means and SD; Significant differences from the Ctrl group are denoted by *(P<0.05) or **(P<0.01). MTC – muscle-tendon complex; RoM – range of motion.

Self-selected walking speed



1.0 m/s

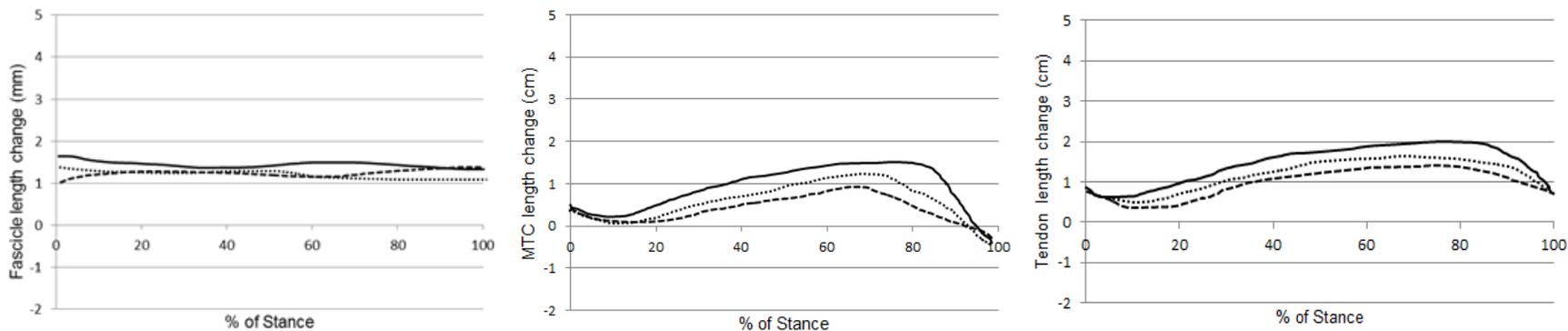


Figure 1. Muscle fascicle length, MTC length and tendon length changes respectively while walking at self-selected speed and 1.0 m/s. Values are means. Line graphs: Ctrl - solid line (n=23), DM - dotted line (n=20), DPN - dashed line (n=13).

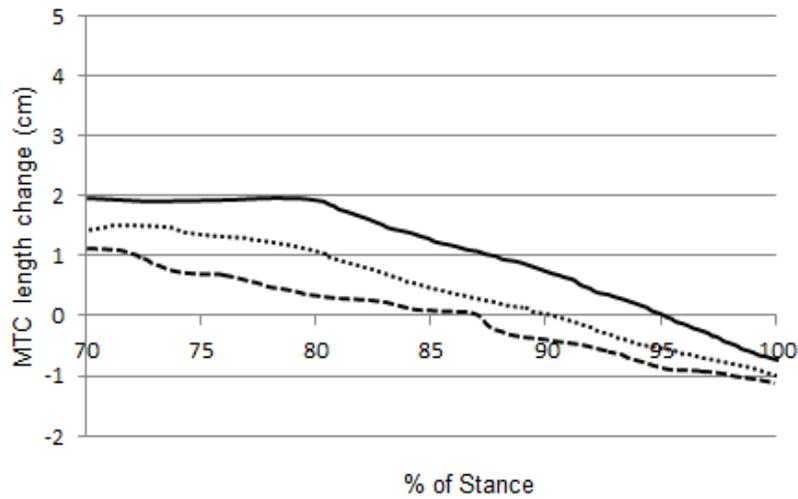


Figure 2. MTC length changes during walking at self-selected walking speed. Values are means. Line graphs: Ctrl - solid line (n=23), DM - dotted line (n=20), DPN - dashed line (n=13).

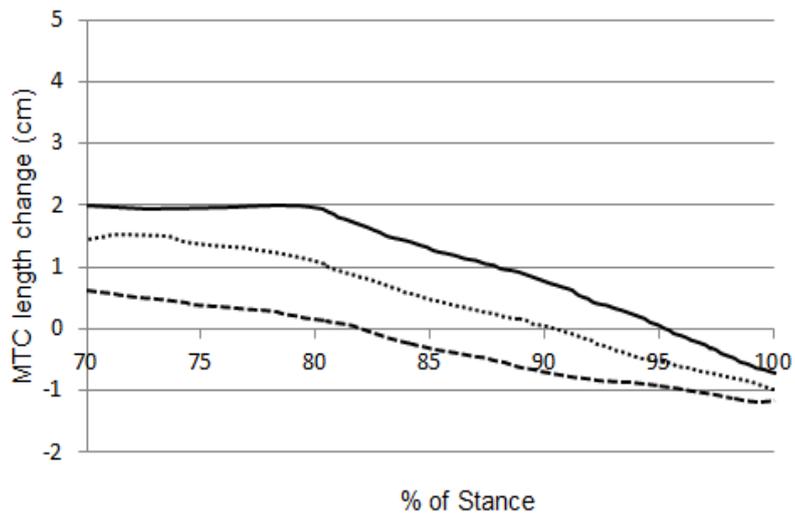
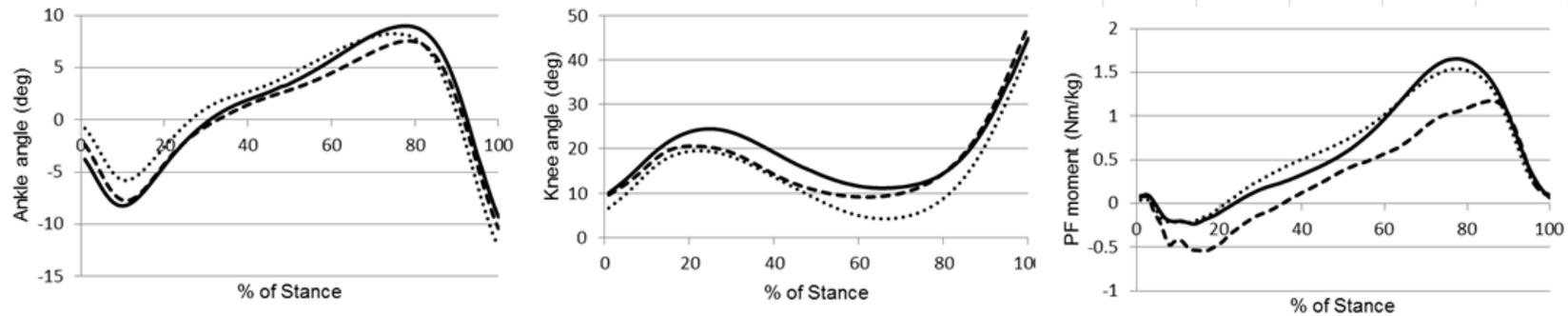


Figure 3. MTC length changes during walking at 1.0 m/s. Values are means. Line graphs: Ctrl - solid line (n=23), DM - dotted line (n=20), DPN - dashed line (n=13).

Self-selected walking speed



1.0 m/s

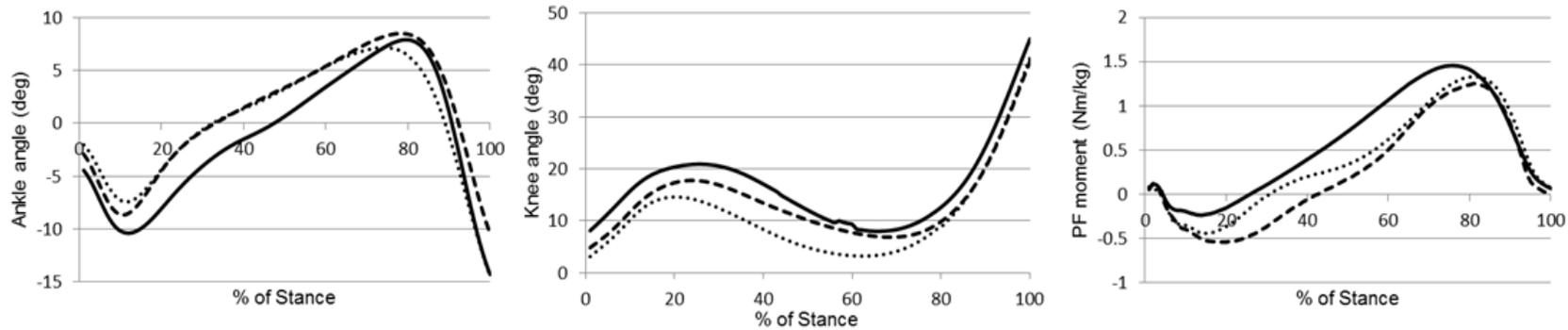


Figure 4. Knee and ankle range of motion and ankle joint moment (AJM) during stance phase while walking at self-selected walking speed and 1.0 m/s for healthy controls (Ctrl), diabetic patients with no neuropathy (DM), and diabetic patients with moderate/severe neuropathy (DPN). Values are means. Line graphs: Ctrl - solid line (n=23), DM - dotted line (n=20), DPN - dashed line (n=13).

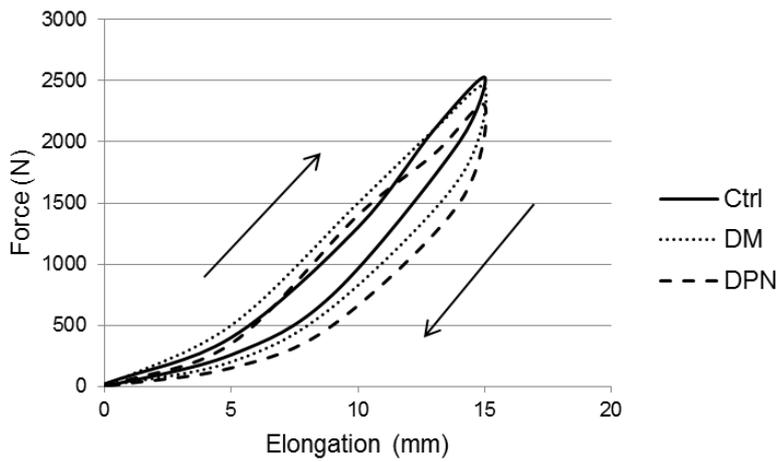


Figure 5. Achilles tendon force-elongation curves while walking at self-selected speed for healthy controls (Ctrl), diabetic patients with no neuropathy (DM), and diabetic patients with moderate/severe neuropathy (DPN). Values are means. Line graphs: Ctrl - solid line (n=23), DM - dotted line (n=20), DPN - dashed line (n=13).

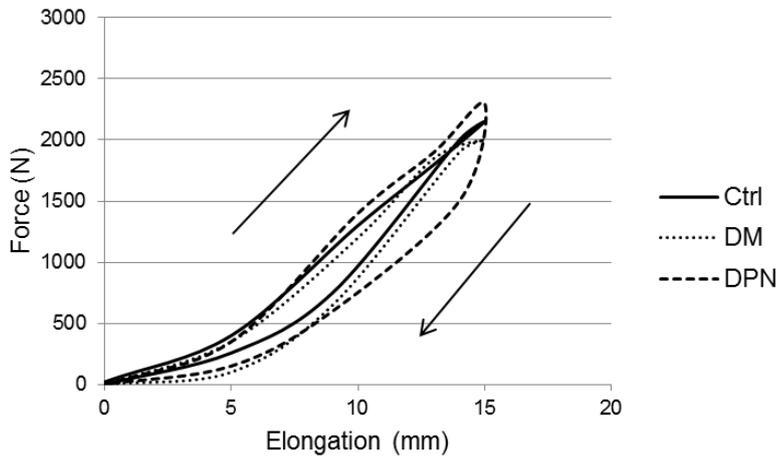


Figure 6. Achilles tendon force-elongation curves while walking at 1.0 m/s for healthy controls (Ctrl), diabetic patients with no neuropathy (DM), and diabetic patients with moderate/severe neuropathy (DPN). Values are means. Line graphs: Ctrl - solid line (n=23), DM - dotted line (n=20), DPN - dashed line (n=13).

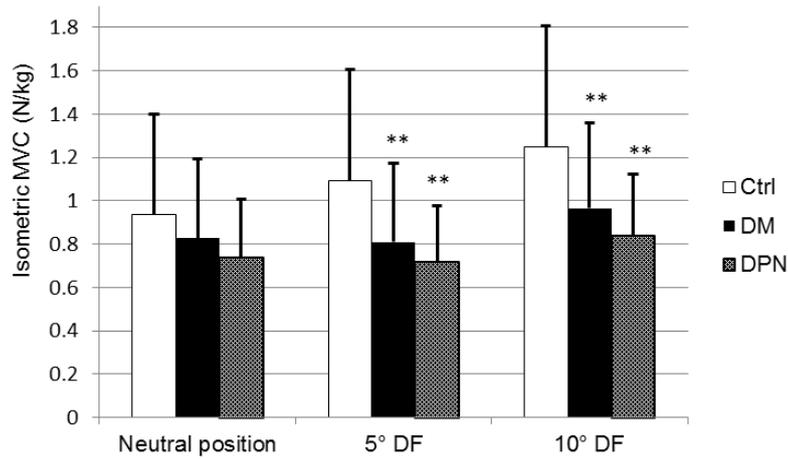


Figure 7. Isometric plantarflexion maximal voluntary contraction (MVC) strength for healthy controls (Ctrl, n=23), diabetic patients with no neuropathy (DM, n=20) and diabetic patients with moderate/severe neuropathy (DPN, n=13). Values are means and SD. Significant differences from the Ctrl group are denoted by ** (P<0.01).

4.5 DISCUSSION

This study has shown reduced Achilles tendon elongation during the loading phase of walking (10-70% stance) and reduced recoil during the subsequent propulsive phase (70-100% stance) in people with diabetes and particularly those with DPN compared to controls (Table 2; Fig. 1). People with diabetes and particularly those with DPN demonstrated a higher stiffness and hysteresis of the Achilles tendon measured during walking compared to the Ctrl group (Figs. 5 & 6; Table 2). Taken together the present findings strongly indicate a reduced elastic energy contribution from the Achilles during walking in people with diabetes and particularly in those with DPN, with implications for increasing the metabolic CoW in patients with diabetes.

The increased stiffness observed in the diabetes groups shows that for the same application of force the AT is less extensible during walking, which means that less energy can be stored, with less therefore available to return. The increased stiffness is further compounded by the fact that less force is applied on the AT in the DM and particularly the DPN groups (Figs. 5 & 6; Table 2). The lower tendon forces applied during walking in diabetic patients are the result of lower joint moments being developed, which reflect a natural strategy to lower the demands of walking (7, 8, 20). This requirement to lower the demands of walking stems from the lower muscular capabilities of diabetes patients, exemplified by the lower maximum plantarflexor strength observed in both diabetes groups (Fig. 7). The maximum plantarflexor strength deficits were most marked as the ankle moved further into dorsiflexion (Fig. 7), which is closely aligned with the position of the ankle during walking when the Achilles tendon is undergoing elongation (Fig. 1 & 4). Hence, lower moments developed in dorsiflexion during walking means lower forces applied to elongate and store energy in the AT.

The results show that the MTC length changes during walking are dependent upon the changes in ankle and knee joint angles (Fig. 1 & 4). Although the magnitude of the between-group differences were relatively small (~2 deg at the ankle and ~4 deg at the knee), a significantly smaller ankle and knee joint range of motion during walking was observed in the DPN group compared to the controls (Fig. 4). This resulted in significantly smaller MTC length changes during walking in the diabetes and particularly in the DPN group compared to controls (Fig. 1; Table 1). The present findings of reduce tendon elongations are in line with previous work by Cronin et al. (11) showing that the AT length changes during walking are attenuated in long-term diabetic patients.

During walking the muscle fascicles of the gastrocnemius underwent very little length change compared to the Achilles tendon and the MTC (Fig. 1) and they could be considered as acting near-isometrically. Indeed, near-isometric behaviour of plantarflexor muscle fascicles has been previously reported in healthy young populations Fuknaga (17), Lichtwark (23), Ishikawa (21), Roberts (37), which functions to allow the Achilles tendon to absorb the length changes of the MTC, thereby facilitating elastic energy storage within the tendon. Although the muscle fascicles were observed to actually shorten very little during the propulsive phase of gait in any group (Fig. 1), the reduced elastic energy contribution from the Achilles during walking in people with diabetes and particularly in those with DPN indicates that the plantarflexor muscles would need to contribute a greater proportion of the work, thereby increasing the metabolic CoW.

The tendon stiffness data measured during walking in the present study are comparable with a number of previous *in vivo* human studies of the Achilles tendon measured using a dynamometry approach and reporting values ranging

between 149 and 207 N/mm (30, 10, 23, 26). Also, values for tendon hysteresis from the present study measured during walking are similar to dynamometry-based methods reported previously in the literature for the Achilles tendon in the range between 5 and 26 % (30, 23, 26, 15, 22). It should be noted, that whilst previous studies have derived tendon stiffness and hysteresis values from static dynamometry measurements, the present study is unique in determining these tendon properties during walking. It should be acknowledged that tendon length changes can result from both tendon loading and also joint rotations. Therefore, measurements of tendon elongation in the previous and present studies reflect not only 'true' elongations resulting from tensile forces, but also elongation due to joint rotations. Whilst this is more easily 'corrected' for with the dynamometry-based approach, the complexity of the unique approach followed in the present study mean that joint rotations are more challenging to account for. Nevertheless, the magnitudes of between-group differences in joint rotations were relatively small and therefore unlikely to impact on the present findings (Fig. 4; Table 1).

Increased tendon stiffness means that the tendon will not elongate as much, and therefore not store as much energy. This reduced length changes is important because plantarflexor muscles have relatively short fibres and long tendons and therefore most of the length changes are achieved by lengthening and shortening of tendon, rather than length changes occurring within the muscle. This pattern of length change can be seen from presented data (most of the length changes occur within the tendon rather than within the muscle fascicles).

Tendon stiffness measurements have traditionally been performed during isometric contractions performed on a dynamometer. Clearly these dynamometry measurements are more constrained as they allow control over many variables. For example, with dynamometry measurements there is no movement of the

ankle and all of the measured elongations can be attributed to the load applied by muscle contraction. Although measurements of tendon stiffness performed during gait offer high ecological validity, control over certain variables is more limited. For example, the ankle joint angle is not fixed, which can lead to some tendon elongations attributed to joint rotation rather than just the load applied to the tendon. Although ankle joint movement occurred during the measurements, the RoM in the ankle and knee were not so different between groups as to mask any differences found in tendon stiffness.

The present study has shown reduced Achilles tendon elongation, increased stiffness and hysteresis during walking in people with diabetes compared to controls. The implications of these findings are a reduced storage and release of elastic energy from the Achilles tendon of diabetes patients during walking, presumably requiring a greater contribution to the work from plantarflexor muscles. The results strongly point towards the reduced energy saving capacity of the Achilles tendon in diabetes patients as an important factor contributing to the increased metabolic CoW in these patients.

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5. Experimental chapter four – Vertical displacement of the centre of mass during walking in people with diabetes: can it explain a higher metabolic cost of walking?

5.1 ABSTRACT

People with diabetes display biomechanical gait alterations compared to controls and have an increased metabolic cost of walking (CoW), but it remains unknown

whether differences in the vertical displacement of centre of mass (CoM) may play a role in this increased CoW. Thirty-one non-diabetic controls (Ctrl); 22 diabetic patients without peripheral neuropathy (DM) and 14 patients with moderate/severe DPN, underwent gait analysis using a motion analysis system and force plates while walking at a range of matched speeds. The aim of this study was to investigate CoM displacement and its relation with step length as a potential explanatory factor in the previously observed increased CoW with diabetes. Vertical displacement of the CoM was measured over the gait cycle. Vertical displacement of the CoM during walking was not different between diabetes patients with and without diabetic neuropathy compared to controls across the range of matched speeds examined and is therefore unlikely to be a factor in itself that contributes towards the increased CoW observed recently in people with diabetic neuropathy. The higher CoW in patients with diabetes may not be explained by the CoM displacement, but rather may be more related to shorter step lengths, increased cadence and the associated increased internal work and higher muscles forces developed by walking with more flexed joints.

5.2 INTRODUCTION

Diabetes is a global epidemic with significant morbidity and particularly common with increasing age (15). Diabetes is associated with a range of serious complications that result in reduced quality of life and premature mortality. Diabetic peripheral neuropathy (DPN) is one of the most severe complications of diabetes, occurring in 30–50% of all diabetic patients (7). DPN-related changes in the lower limbs lead to functional gait adaptations including taking shorter steps, having a higher cadence but slower self-selected and maximum walking speed (6, 11, 23, 30, 28, 19). Other major gait adaptations include reduced range of joint movement (3) and reduced muscle strength and power characteristics (6).

I have recently shown how the metabolic cost of walking (CoW) is higher in people with diabetes and particularly in those with DPN compared to controls (27). During walking mechanical work is done to continuously raise and lower the body centre of mass (CoM), which requires metabolic energy expenditure. The body CoM moves like an inverted pendulum during human walking, with the pendulum action conserving mechanical energy (1). More specifically, by keeping the knee relatively straight during the single leg stance phase of gait giving rise to the arc of the CoM, the leg supports body mass with relatively little muscle force.

Like an inverted pendulum, the CoM rises/decelerates in the first half of the stance phase and then falls/accelerates during the second half of the stance phase (8, 20, 21, 31). Consequently, in the first half of the stance phase, kinetic energy is converted into gravitational potential energy (9), whereas in the second half of the stance phase, the opposite conversion occurs. During walking, the CoM has a sinusoidal pattern in the vertical direction with two peaks occurring. The first vertical peak of the CoM occurs around 30% of the gait cycle during single-limb stance as the CoM is 'vaulted' over the straight stance limb in an inverted pendulum manner, while the second peak occurs around 80% of the gait cycle during the terminal mid-stance phase.

Increasing the CoM displacement in a type of up and down 'bobbing' action leads to an increase in the CoW compared to a normal gait (25, 22). Equally, if gait is manipulated to minimise or eliminate any vertical displacement of the CoM by walking in a 'crouched' style with very flexed limbs, there is an increase in the CoW compared to normal gait (26, 22, 14). Hence, there appears to be an 'optimum' vertical displacement for the CoM in terms of its effect on the metabolic

CoW, where deviations from this optimum seem inefficient in terms of energy cost.

Stride length also seems intrinsically linked to the CoM vertical displacement and the associated CoW. It has been shown that stride lengths lower than the optimal reduced the vertical displacement of the CoM and increased the CoW, while stride lengths greater than the optimal increased the CoM vertical displacement and increased the CoW (14). Since it is known that diabetes patients take shorter steps compared to controls, it might be hypothesised that that this would reduce the vertical displacement of the CoM, thereby increasing the CoW. Since walking speed may be a confounding factor in the relationship between step length and CoM displacement, in the present study I chose to compare the CoM displacement at matched walking speeds between patients with diabetes and controls. Therefore, this study examined the vertical displacement of the CoM while walking at different speeds. I hypothesised that diabetes patients have a reduced vertical CoM displacement that might explain previously reported finding of a greater CoW, with a reduced step length being a potential factor underpinning the suggested CoM behaviour.

5.3 MATERIALS AND METHODS

Participants

After receiving ethical approval from all relevant bodies, sixty seven participants gave written informed consent to participate in this study. All procedures in this study complied with the declaration of Helsinki. All participants were aged over 40 years and were allocated into one of three groups: patients with diabetes and moderate-severe peripheral neuropathy (DPN, n=14, 14 men), patients with diabetes but no peripheral neuropathy (DM, n=22, 12 men) and healthy controls without diabetes or peripheral neuropathy (Ctrl, n=31, 19 men). All participants were assessed to confirm they satisfied the inclusion criteria for each group. Major exclusion criteria for participation in the study included peripheral vascular disease, musculoskeletal injury, recent surgery affecting gait, foot or lower limb

amputation (amputation of the hallux; amputation of more than two lesser toes on one foot; amputation of part of/whole foot) and open foot ulcer. Information about duration and type of diabetes, smoking habits and use of current medication was obtained via questionnaire. The majority of the DM and the DPN patients reported taking insulin, cholesterol-lowering medication and diabetes medication, while from the whole sample (including controls) only 2 people reported smoking.

Assessment of peripheral neuropathy

A clinical evaluation was undertaken to quantify peripheral neuropathy in diabetic patients and to confirm the absence of neuropathy in healthy controls. Peripheral neuropathy was assessed by using the modified Neuropathy Disability Score (mNDS) and the vibration perception threshold (VPT). The mNDS is a combined score taken from tests measuring the patient's ability to detect temperature, pain, vibration and the Achilles tendon reflex (5). The VPT was assessed by placing the probe of the biothesiometer on the apex of the hallux and increasing the level of vibration until detected by the participant. A random blood glucose test was performed in the Ctrl group to confirm the absence of diabetes (<7 mmol/l) and the above neuropathy tests conducted to confirm the absence of neuropathy in the Ctrl group resulting from any aetiology.

Gait analysis

Participants were asked to walk along a 10-metre walkway in the gait laboratory at a series of standardised speeds (0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 m/s), as well as at their maximum walking speed. The standardised walking speeds were controlled by measuring the velocity of a marker attached to the sacrum after each trial from the motion analysis data and providing immediate feedback for

participants as to whether they needed to walk more quickly or more slowly on the next trial to achieve the required standardised speed. Participant's starting position was altered by the experimenters to ensure a 'clean' (i.e., no overlap outside the force platform) foot-strike on one or two of the force platforms per walking trial without alteration to their natural gait. Walking trials were repeated until at least three 'clean' foot contacts with the force platforms were made with each limb, for each speed condition. Kinematics were collected at 100 Hz using a 10-camera Vicon motion capture system (Vicon, Oxford, UK) positioned around the 10-meter walkway, tracking a full-body modified Plug-In-Gait marker set consisting of 54 markers. Kinetics were simultaneously collected at 1000 Hz from three force platforms (Kistler, Zurich, Switzerland) embedded into the middle of the walkway. Where possible markers were placed directly onto the skin; to minimise movement artefacts resulting from loose clothing all participants wore tight-fitting shorts and tops. All participants wore specialist diabetic shoes (MedSurg, Darco, Raisting, Germany) with a neutral foot-bed, ensuring the diabetic patients walked with safe, appropriate footwear whilst minimising the effect of footwear by standardising across all participants.

Centre of mass displacement

Gait variables (stride length, step length and cadence) were calculated from the kinematic data using Visual 3D software. The vertical displacement of the CoM was also measured from the kinematic data using Visual 3D software (C-motion Inc., MD, USA). Motion data collected during gait analysis were processed, and Dempster's segment parameter model was used to calculate mass distribution for each body segment, thereby allowing accurate calculation of the entire body centre of mass. This measurement was calculated as the range of vertical

displacement of the CoM (Figure 1; Figure 2) during the whole gait cycle, using the mean of the three trials from each person.

Statistics

A one-way analysis of variance (ANOVA) was performed for all variables to assess between group differences. If the ANOVA was significant, a Fisher's least significant difference (LSD) post-hoc test was used to test for differences between the diabetes groups (DM and DPN) and the control group. All values presented are means and standard deviation. Significance was set at $p < 0.05$.

5.4 RESULTS

Participant characteristics

There were significant differences between the groups in age, body mass and BMI, which were significantly greater in the DPN group (Table 1, $p < 0.01$).

Neuropathy assessments

As expected, the DPN group displayed significantly higher values for the VPT and the mNDS compared to the Ctrl group (Table 1). The VPT and mNDS for the DM group were not significantly different from the Ctrl, underlining that this diabetic patient group had no neuropathy (Table 1).

Step length and cadence

The DPN group displayed significantly shorter step lengths in all given speeds compared to Ctrl group (Table 2). The DPN group had significantly higher cadence in all given speeds compared to Ctrl group.

Centre of mass displacement at different speeds

There were significant differences in the CoM displacement between the groups only at maximum walking speed, where the DPN group expressed greater vertical displacement of the CoM (Fig. 1).

Table 1. Participant characteristics and results from neuropathy assessments.

Variable	Group		
	Ctrl	DM	DPN
Age (yr)	56 (10)	51 (9)**	66 (14)**
Body mass (kg)	76 (10)	80.5 (12)	91.5 (18)**
Height (m)	1.72 (0.12)	1.71 (0.09)	1.73 (0.11)
BMI (kg/m ²)	26 (3)	28 (4)	31 (4)**
NDS (Score/10)	1 (1)	2 (1)	7 (2)**
VPT (Volts)	6.1 (3.4)	8.2 (3.4)	27.4 (9.1)**
Diabetes duration (years)	-	14 (12)	14 (11)
Type 1 diabetes	-	7	4
Type 2 diabetes	-	15	10

Healthy controls (Ctrl, n=31), diabetic patients with no neuropathy (DM, n=22) and diabetic patients with moderate/severe neuropathy (DPN, n=14). Significant differences from the Ctrl group are denoted by ** (P<0.01). BMI = body mass index, NDS = neuropathy disability score, VPT = vibration perception threshold. Values are means (standard deviations).

Table 2. Temporal-spatial gait parameters.

Variable	Group		
	Ctrl	DM	DPN
0.6 m/s			
Step length (m)	0.59 (0.12)	0.57 (0.12)	0.51 (0.09)**
Cadence (steps/m)	108 (0.61)	108 (0.74)	113 (0.41)**
0.8 m/s			
Step length (m)	0.63 (0.14)	0.57 (0.12)	0.53 (0.15)**
Cadence (steps/m)	112 (0.84)	113 (0.67)	116 (0.68)**
1.0 m/s			
Step length (m)	0.69 (0.15)	0.67 (0.05)	0.64 (0.04)*
Cadence (steps/m)	117 (1.12)	119 (1.08)	122 (1.25)**
1.2 m/s			
Step length (m)	0.76 (0.11)	0.75 (0.17)	0.69 (0.07)*
Cadence (steps/m)	124 (1.16)	125 (1.27)	128 (1.08)**
1.4 m/s			
Step length (m)	0.79 (0.12)	0.77 (0.17)	0.71 (0.11)*
Cadence (steps/m)	127 (1.56)	129 (1.47)	131 (1.49)**
1.6 m/s			
Step length (m)	0.81 (0.11)	0.80 (0.04)	0.74 (0.02)*
Cadence (steps/m)	129 (0.98)	132 (0.48)	135 (0.63)**
Maximum walking speed (m/s)	1.92 (0.11)	1.88 (0.16)**	1.68 (0.22)**
Step length (m)	0.85 (0.07)	0.79 (0.06)*	0.78 (0.12)**
Step length (m)	143 (1.18)	140 (1.27)*	129 (0.98)**

Cadence (steps/m)

Healthy controls (Ctrl, n=31), diabetic patients with no neuropathy (DM, n=22) and diabetic patients with moderate/severe neuropathy (DPN, n=14). Significant differences from the Ctrl group are denoted by * (P<0.05) or ** (P<0.01). Values are means (standard deviations). Gait parameters were collected on the laboratory walkway.

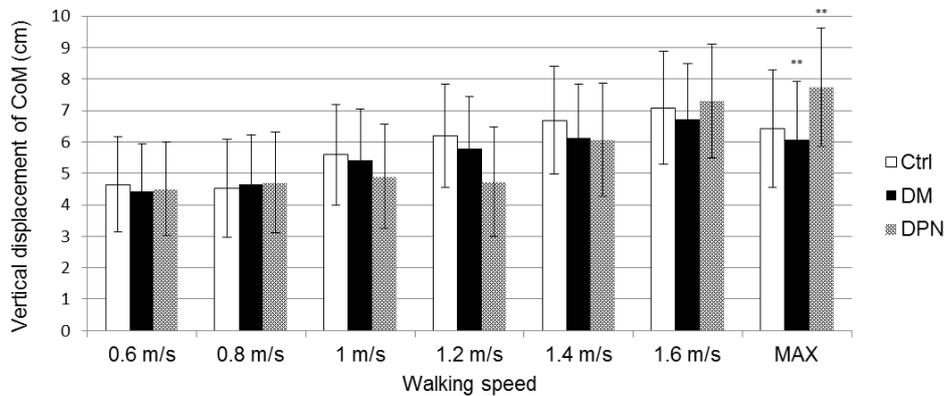


Figure 1. Centre of mass (CoM) vertical (Z) displacement across walking speeds from 0.6 to 1.6 m/s and maximum walking speed for healthy controls (Ctrl; n=31), diabetic patients with no neuropathy (DM; n=22) and diabetic patients with moderate/severe neuropathy (DPN; n=14). Values are group means and SD; ** denotes significantly (P<0.01) different from the control group.

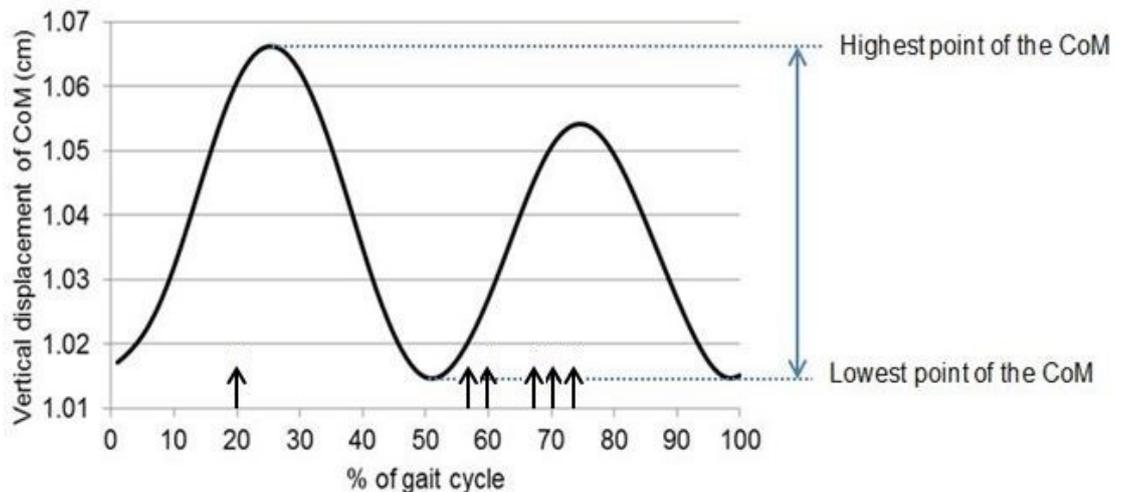


Figure 2A. Example trace from one participant showing the vertical displacement of the CoM over the gait cycle. ↑ start of double support phase, ↑↑ end of single support phase (start of double support phase), ↑↑↑ start of swing phase 70%.

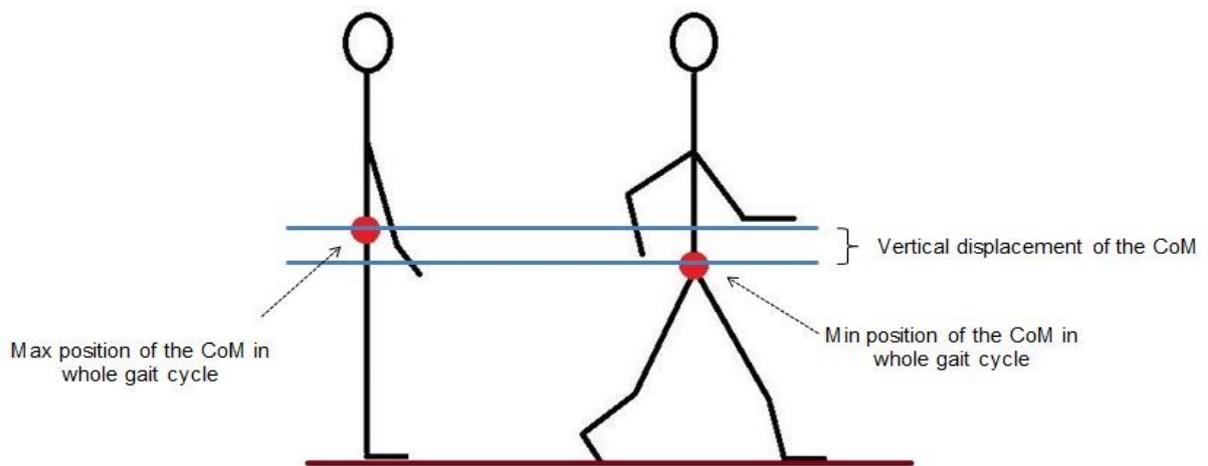


Figure 2B. Schematic illustration of the rise and fall of the CoM during the gait cycle.

5.5 DISCUSSION

This study has shown that the vertical displacement of the CoM during walking is not different between diabetes patients with and without diabetic neuropathy compared to controls across a range of matched speeds (Fig. 1) and is therefore unlikely to be a factor in itself that contributes towards the increased CoW observed recently in people with diabetic neuropathy (27). The exception to this was at maximum walking speed where patients with diabetes and those with diabetic neuropathy showed an increased or a decreased CoM displacement respectively compared to controls (Fig 1).

It has previously been shown that stride lengths shorter and longer than the optimum lead to reduced and increased CoM displacements respectively, increasing the metabolic CoW in both situations (14). In that study subjects increased their metabolic cost when they reduced their vertical CoM movement by taking shorter strides. Subjects also expended more metabolic energy when they walked with greater stride length than their preferred stride length. Previous work (13) has shown that as stride length increases, metabolic energy expenditure and mechanical work performed on the CoM also increase. This is not caused by CoM displacement per se but rather by the additional negative work performed to redirect the CoM velocity during step-to-step transitions and by positive work to restore the energy lost. Although I did find consistently shorter step lengths across matched walking speeds in patients with diabetes and particularly those with diabetic neuropathy, this did not alter the vertical displacement of the CoM (Fig. 1). The lack of effect of stride shortening on the CoM might be due to the fact that people with diabetes and neuropathy have adapted to a different optimal step length, which is consistently shorter compared to controls across the range of speeds examined, or that they have adopted a different step length based on the total metabolic CoW rather than the cost associated with CoM displacement. The exception to this was at maximum walking speed where the CoM displacement was different in both diabetes groups compared to controls (Fig. 1). Both diabetes groups took shorter steps compared to controls, but the DPN group displayed an increased CoM displacement, while the DM group displayed a decreased CoM displacement. This might reflect the fact that asking people with diabetes to walk as fast as they can disturbs the normal regulatory control that they exert over gait and may force them to adopt strategies that are suboptimal in terms of energy efficiency. This is supported by

the observation that all groups adapted to increased walking speeds between 0.6 and 1.6 m/s by increasing cadence, whereas in the transition from walking at 1.6 m/s to maximum walking speed the DPN group were unable to increase cadence any further and instead needed to increase step length, presumably compromising the optimal step length (Table 2).

Consistent with the shorter steps taken by both diabetes groups compared to controls was the higher cadence required to meet the required matched walking speeds by the diabetes patients (Table 2). An increased cadence in the diabetes groups would require greater internal work from the muscles to move the legs during walking (24). Although I have previously found the joint work developed during a single stance phase to be lower in patients with diabetes and even more so in those with diabetic neuropathy, this would be repeated more often over a given distance in diabetes patients because of a higher cadence. Therefore, a higher cadence for any given walking speed could explain the higher CoW previously reported in patients with diabetes and those with diabetic neuropathy (27).

In the absence of differences in the CoM displacement, another possible explanation for the higher CoW previously reported in diabetes patients is that they might be producing greater muscle force without performing as much joint work per stance phase. This would be consistent with previous reports from walking with a 'crouched gait' by excessively flexing the joints (26, 22). Diabetes patients were observed to walk with shorter steps, which is known to be achieved by greater flexion in the lower limb joints. This likely gives rise to higher muscle forces to sustain the more flexed joint positions as previously observed (29) and consequently a higher metabolic CoW. Therefore, the effective mechanical advantage (muscle force moment arm/ground reaction force moment arm) may

be worse in diabetic patients, which would mean that more muscle force would be required to overcome the moment of the ground reaction force – hence higher CoW. This factor may also explain why/how diabetes patients have adopted “optimum” CoM displacement per stride length as a strategy to minimise CoW (meaning unaltered compared to controls). This relates to the Achilles tendon, which plays a major role in energy saving during walking under ‘normal’ circumstances (2). Gordon et al. (14) presented in their study a manipulation of step length above and below the optimal, and found that the CoM vertical displacement increases and decreases over that observed at the self-selected step length. Both increased and decreased vertical displacement of the CoM beyond were associated with a higher energy cost compared to that observed at self-selected step length, suggesting an optimal vertical displacement of the CoM where energy cost is minimised.

In my opinion the main issues determining the CoW are higher cadence and higher cumulative joint work, stiffer Achilles tendon with higher hysteresis, potentially co-activation (it is hypothesized but not measured). There are many factors, not just one, but maybe one of the most important factors is related to stiffer tendons because these tendons might also cause some of the other reported factors such as shorter steps, longer contact time, lower joint moments, smaller RoM and smaller elongation. It is difficult to say for sure that these relations are causative or just correlative; one way to prove this would be to manipulate stride length and cadence, one by one in some systematic way in healthy participants to see what effect they might have. A future study might use some form of regression analysis in terms of defining one potential factor responsible for a higher CoW, but this analysis would need 10 participants for

every variable entered into a regression analysis and would therefore need more participants.

In my opinion stiffer tendons are maybe one of the most important factors in the higher CoW and it can be compared with driving a car with flat tires that will just increase fuel consumption, i.e. CoW.

My idea about improving efficiency of walking is that it will not be achieved just by performing any kind of aerobic exercises while still driving with flat tires (stiffer tendons), but also through appropriate stretching training with the aim of increasing tendon compliance. Increasing flexibility of plantar muscles would also allow them to store and release more energy and as a logical consequence would be longer steps, shorter contact times and lower cadence.

To best of our knowledge this is the first study that has investigated the CoM displacement during walking in a diabetic population. It could be considered as a limitation of the present study that body mass was significantly different between groups. However, the higher body mass of patients with diabetes (especially those with DPN) is a well-known characteristic of this population described in the literature (19, 17, 16) and is unlikely to have directly affected the CoM vertical displacement. If anything it might be expected that increased body mass might reduce the extent to which the CoM is displaced, but this was not found in the present study indicating that group differences body mass did not influence the present results. Although only a mean of 10 years difference, patients in the DPN group were significantly older than controls (66 to 56 years, respectively), which might be a confounding factor for some of the variables examined.

I have shown that there are no differences in the vertical displacement of the CoM in patients with diabetes compared with controls when walking speed is matched, with the exception of the maximum walking speed. The higher CoW in patients

with diabetes may not be explained by the CoM displacement, but rather may be more related to shorter step lengths, increased cadence and the associated increased internal work and higher muscles forces developed by walking with more flexed joints.

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6. Conclusions & future directions

Summary of main findings

The main aim of this thesis was to investigate the cost of walking in people with diabetes mellitus (DM) and diabetic peripheral neuropathy (DPN) and to examine the biomechanical factors that could contribute to explaining any potential differences. The work for this thesis investigated level walking as a part of everyday life activities. Although there is a wide base of research that had been investigating gait patterns in the diabetic population, this is the first body of work that has investigated the energy cost of walking (CoW) in people with diabetes.

In chapter two it was observed that DPN patients have shorter steps, longer single limb stance time, lower concentric work and higher energy cost of walking when walking speed is matched. The lower concentric joint work in patients with diabetes might be a consequence of kinematic gait alterations and may represent a natural strategy aimed at minimizing the CoW. This 'altered gait strategy' in people with diabetes enables them to meet the task demands in the face of compromised musculoskeletal properties and already elevated CoW due to energetic inefficiencies. The main finding of this chapter is that people with diabetes and diabetic peripheral neuropathy have a higher CoW when the walking speeds are matched. This finding is likely due to energetic inefficiencies associated with diabetes and DPN reflecting physiological and biomechanical characteristics as well as the cumulative effect of potentially higher joint work over a given time/distance due to higher cadence. It was surprising that diabetic patients were actually able to match the same walking speed as controls despite generating significantly reduced lower limb joint work. The same lower limb joint work was associated with a higher CoW in diabetic patients and particularly in patients with DPN, which can be observed in Fig. 5. Schenau and Cavanagh (14) concluded that there are no models available which predict reliable measures for positive and negative power from active elements of individual muscles and their efficiencies and that joint work cannot be used as a reliable measure for a prediction of the CoW. Another contributing factor to the higher CoW in the DPN group may be the increased step frequency (the DPN group had a shorter step length for a given speed, therefore requiring a higher step frequency). These two factors (lower limb joint work and higher step frequency) would increase the internal work required for moving the lower limbs and may contribute to a higher CoW in people with diabetes and particularly those with DPN. For human walking,

gross cost of transport is U-shaped. Although theoretically self-selected speed should be on the bottom of the U-shaped curve that is not always the optimum speed of moving which reflects the minimal energy expenditure during the walking.

In the third chapter I have presented that diabetic patients and especially those with DPN displayed increased effective mechanical advantage (EMA) and smaller external moment arm (ExtMA) at the ankle across all walking speeds. The increased EMA was mainly caused by a smaller external moment arm of the ground reaction force in the DPN and DM groups compared to Ctrl. The DPN group reduced the joint moment at the ankle during walking by applying the ground reaction force more proximally on the foot, or at an angle directed more towards the ankle, thereby reducing the external moment arm and increasing the EMA around the ankle.

Another finding of this chapter was the reduced lower limb range of motion (RoM) during walking in diabetic patients compared with controls. This was achieved via shorter steps taken by diabetic patients during walking. It is known that DM and DPN patients are able to lower joint moments and walk with shorter steps, and this translates to less flexed joints, which in general means that the moment arms of the ground reaction force are smaller compared with the situation with more flexed joints. These findings demonstrate a mechanism through which people with diabetes and particularly those with DPN reduce the joint moment at the ankle during walking – by applying the GRF more proximally on the foot or at an angle more towards the ankle, reducing the ExtMA around the ankle and thereby reducing the ankle joint moment. The increased effective mechanical advantage (EMA) was mainly caused by a smaller ExtMA in the DPN and DM groups compared to Ctrl. Whilst the total hip joint RoM during walking was reduced in

diabetes patients and especially those with DPN compared to controls, patients with diabetes and to the greatest extent those with DPN flexed the hip more than controls. This kinematic strategy fits very well with the 'hip strategy' previously reported in other studies (10, 13), whereby diabetes patients have been observed to 'drag' the leg forwards into the swing phase from the hip, rather than 'propelling' the leg off from the ground using the ankle plantarflexors.

In chapter four I have shown the consequent reduction in the force applied to the Achilles tendon (AT) would result in reduced tendon elongation and therefore reduced storage of elastic strain energy in the diabetic population. The reduced contribution from elastic strain energy stored in the Achilles tendon could impact upon the gait mechanics and efficiency. My main findings are a smaller MTC length change, increased stiffness and hysteresis of the Achilles tendon in the DPN group compared to the control group. A stiffer tendon will reduce storage and release of energy from the AT. One of the causes might be non-enzymatic glycation of tissues and it might contribute to explaining the altered lower limb biomechanics, especially the limited ankle and knee RoM in people with diabetes, higher cadence, shorter steps, longer contact time and lower ankle joint moments.

In chapter five I investigated the role of the vertical centre of mass (CoM) displacement during walking and its potential impact on the CoW. The main finding of this chapter shows that it is not the CoM displacement itself that is contributing to increased energy cost, but it is more likely linked to step length and cadence alterations i.e. although diabetic patients were able to generate reduced lower limb joint work (Chapter 2) they displayed increased step frequency which cumulatively might increase metabolic cost of walking. The vertical displacement of the centre of mass seems to be minimised by the DPN

group around the speeds associated with self-selected walking speed i.e., 1 and 1.2 m/s.

Considering findings in all four experimental chapters I suggest that the major factors contributing to the increased energy CoW in patients with diabetes and DPN must include shorter strides and increased cadence for their effect on internal work and also a reduced energy contribution from the Achilles tendon. The work for this thesis was the first to address all of these issues in people with diabetes in order to try to give us a broader picture of the energy CoW in people with diabetes and especially diabetic peripheral neuropathy and to understand the biomechanical factors influencing the CoW.

Another potential factor is the role of the muscle co-activation. Although I have not assessed muscle co-activation in the work for this thesis, if increased it would contribute to a greater energy consumption by the muscles.

The main findings of this thesis are that, when the walking speed is matched, people with diabetes and diabetic peripheral neuropathy walked with shorter strides, longer contact time with the ground, smaller external moment arms, smaller range of motion at the ankle, knee and hip, higher cadence, lower ankle joint moments, having weaker plantarflexors, producing lower positive muscle work, altered muscle-tendon behaviour with less energy stored in their tendons. The data indicates that each group choose to walk at a self-selected speed where the energy expenditure was optimal. Other important findings are significant differences in maximal walking speeds where the DPN group walked slower than the Ctrl group. The maximum walking speed was highest in controls, lower in patients with diabetes and lower still in patients with DPN. The maximum walking speed attainable might be considered as a useful indicator of physical capacities and this could be useful focus for future studies.

The methods used to quantify oxygen uptake during walking are good and certainly well established with good reliability ($ICC > 0.80$) (12, 3, 1, 11, 4).

In terms of the statistical analysis of the results in the present thesis, it could have been argued to perform a two-way analysis of variance (ANOVA) with group (3 levels) and walking speed (6 levels) as the two independent variables to avoid performing repeated tests with a one-way ANOVA. However, the output from the two-way ANOVA would be a main effect for speed, a main effect for group and an interaction effect. The results from the two main effects would not be of interest since they would take an average of the other main independent variable in giving an output for the other. To answer the specific hypotheses set out in the present thesis, multiple comparisons would have needed to be performed using post-hoc tests within the interaction effect. There might be an argument to state that some kind of adjustment for multiple comparisons could have been performed to adjust the alpha level. One such test is the Bonferroni correction, however this is a very conservative method that divides alpha level by number of comparisons and conversely increases the likelihood of making a type 2 error. It should also be considered that the analysis of the results were hypothesis-driven and not simply to find any possible differences from any number of possibilities. As a compromise if I accepted significance only for p-values of below 0.01, very few variables would drop out of significance and further this is in line (although not as conservative) with the principals of tests to adjust for multiple comparison..

Also, the other possibility to sub-divide the controls in similar age groups as the diabetics with and without neuropathy was not considered because it would bring the group numbers down to very small numbers, which would result in a loss of statistical power.

Further findings of interest and future directions

Type 2 diabetes typically affects patients who are overweight and display a lower level of physical activity, but evidence suggests that stressful experience might affect diabetes, both its onset and its exacerbation. A higher CoW in people with diabetes may underpin the lower physical activity levels and lower habitual walking distances in this population and may also contribute towards a negative spiral where there is a greater perception of difficulty for walking, which causes less engagement in physical activity (6, 9, 14, 15, 16), leading to poorer metabolic control and worsening of the diabetic condition. To allow intervention to break this negative cycle, it is therefore important to understand the factors that contribute to increasing the CoW in diabetes.

I found that stride length and cadence seem to be important factors in terms of the cumulative amount of work done for its effects on the CoW and future studies might try to manipulate stride length to see how that influences the CoW when the stride length is matched between Ctrl and DM and DPN patients and to compare the CoW. Possible interventions to reduce the CoW would be to alter tendon stiffness through appropriate stretching training with the aim of increasing tendon compliance. Increasing flexibility of lower limb muscles would also allow them to store and release more energy and as a logical consequence would be longer steps, shorter contact times and lower cadence. Furthermore, a measure of muscle co-activation, through EMG with appropriate normalization, would be useful in further studies and would be able to determine if the efficiency of the muscle is compromised.

In relation to balance it might be interesting to investigate potential differences in the CoW between people with diabetes and poor balance control compared to those with diabetes but with better balance control. We might hypothesise that

instability and the associated corrections needed could increase the CoW. This might be challenging since we know (1) balance is impaired to a greater extent in people with DPN, so apportioning appropriate groups could be a challenge.

Considerations and limitations

A higher level of co-activation from agonist-antagonist muscles is a potential candidate for contributing towards the higher CoW observed in people with diabetes as it is presented previously (5, 7, 8). This important aspect was not assessed in the present work for this thesis, but future work should address this issue by measuring the level of co-activation from lower limb muscles and also relating this level of activation to the maximal activation capacity of the same muscles. In the present thesis, although walking speed was matched between groups, step length was free to vary and as discussed could have been one of the factors contributing to the increased CoW observed in diabetes patients. Future work might try to match step length while evaluating the impact on the CoW in patients with diabetes. However, this approach may also face the confounding effect of walking speed, since matching both step length and walking speed might approach the limits of some patient's capabilities. In terms of the participant characteristics for the work composing this thesis, although only a mean of 10 years difference, patients in the DPN group were significantly older than controls (66 to 56 years, respectively), which might be a confounding factor for some of the variables examined. I did not measure blood lactate, which might have been particularly relevant to Chapter 2 for confirming that all participants were working below their lactate threshold. This is a consideration since the VO_2 slow component is much more pronounced during exercise above the lactate threshold compared to below. Since a number of inefficiencies relating to the

CoW in patients with diabetes may relate to the muscle, future work might investigate the muscular causes of the increased CoW at a cellular level.

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