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Running title: Elastic energy storage in diabetic neuropathy

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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 Achilles tendon may reduce the capacity for energy saving through this mechanism, thereby contributing to an increased metabolic cost of walking (CoW). The aim of this study was to investigate the effects of diabetes and diabetic peripheral neuropathy (DPN) on the Achilles tendon and plantarflexor muscle-tendon unit behaviour during walking. Twenty three 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 of the gastrocnemius muscle, using a muscle model to determine Achilles tendon and muscle-tendon length changes. During walking, the DM and particularly the DPN group displayed significantly less Achilles tendon elongation (Ctrl: 1.81; DM 1.66; DPN: 1.54 cm), higher tendon stiffness (Ctrl: 210; DM: 231; DPN: 240 N/mm) and higher tendon hysteresis (Ctrl: 18; DM: 21; DPN: 24 %) compared to controls. The muscle fascicles of the gastrocnemius underwent very small length changes in all groups during walking (~0.43cm), with the smallest length changes in the DPN group. Achilles tendon forces were significantly 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 during walking in diabetes patients as an important factor contributing to the increased metabolic CoW in these patients.

Keywords: elastic energy storage, tendon stiffness, lower limb, biomechanics, diabetes.

New & Noteworthy

From measurements taken during walking we observed that the Achilles tendon in people with diabetes and particularly people with diabetic peripheral neuropathy was stiffer, elongated less and was subject to lower forces compared to controls without diabetes. These altered properties of the Achilles tendon in people with diabetes reduce the tendon's energy saving capacity and contribute towards the higher metabolic energy cost of walking in these patients.

INTRODUCTION

Diabetes mellitus (DM) is a very prevalent global chronic disease in older adults and is associated with a number of complications including cardiovascular disease, peripheral arterial disease, retinopathy and poor wound healing (16, 14). One of the most common complications of diabetes is diabetic peripheral neuropathy (DPN), with the incidence reported to range between 13 and 68% (44, 6). Diabetes and DPN impact negatively on gait and mobility with implications for quality of life. Diabetes and DPN cause muscle weakness and affect sensory perception altering walking strategy and causing impairments to balance control (13, 30, 20, 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 also play an important role in energy saving during walking by storing (during stretching) and returning (upon recoil) elastic energy (37, 38, 39, 2). In particular, the Achilles tendon is a long tendon that is

important for storing and releasing elastic energy during walking and as such, plays an important role in metabolic energy saving, 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 habitual level of physiological loading and also the metabolic environment (36, 1, 17). Animal studies show that diabetes causes non-enzymatic glycation of soft tissues, including tendons (34). This non-enzymatic glycation causes increased cross-linking, increasing the stiffness and modulus of the tendon (35, 33). 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 ankle joint range of motion (11, 19, 29). In humans, calcification and fascicle disruption have been observed in the diabetic human Achilles tendon (4). Tendons exhibit relatively low mechanical hysteresis, which is defined as the energy lost upon recoil of the tendon (27). In addition to tendon stiffness, the hysteresis of the tendon could also be affected by diabetes. Hysteresis has been shown to increase in humans with ageing (37). An increase in hysteresis would also reduce metabolic energy saving by the Achilles tendon during walking.

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. Cronin et al. (11) found that Achilles tendon length changes during walking at self-selected speed were attenuated in diabetes patients and that this was inversely correlated with diabetes duration.

The impact of changes in Achilles tendon and plantarflexor muscle function induced by diabetes and diabetic neuropathy remain unknown during walking. The aim of this study was to investigate the effects of diabetes and diabetic peripheral neuropathy on plantar flexor muscle-tendon behaviour during walking at self-selected and controlled speeds. We hypothesized that the Achilles tendon would function in a manner that reduced its energy contribution during walking in diabetes patients and particularly in those with diabetic neuropathy compared to controls. As a result, a greater contribution would be required from the plantarflexor muscles for walking, requiring more energy and contributing to the higher cost of walking (CoW) that we have recently reported in people with diabetes (32).

MATERIALS AND METHODS

Participants

Fifty-six participants were involved in this study. Participants were 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. Participant characteristics are displayed in Table 1.

Diagnosis of Diabetic Peripheral Neuropathy

The presence and severity of 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 were 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. To investigate whether the changes are dependent on the walking speed we asked participants to walk along a 10-metre walkway in the gait laboratory at their self-selected speed, as well as at a 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) and 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. We have used standard procedures and systems for the calculation of joint moments that are used routinely and have been widely accepted by the biomechanics community (43, 9). Walking trials were repeated until three 'clean' foot contacts with the force platforms were made with right 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 (MG) muscle fascicle length changes *in vivo*. For these measurements, a linear 7.5 MHz probe with 60 mm field of view was secured around the right lower leg in the mid-sagittal plane of the MG muscle with a custom-built fixation device (Fig. 1). The ultrasound scanning was synchronized with recordings of the kinematic and kinetic data. We have previously shown a high reliability for this technique in measuring fascicle lengths, with an intra-class correlation coefficient of 0.8 (42). 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 controlling for the effects of footwear on the measured variables by standardising across all groups (Fig. 1).

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 to produce their maximum effort. 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 each ankle angle. Results were subsequently normalised to body mass.

Data processing

The purpose of the data analysis was to quantify the Achilles tendon and plantarflexor muscle-tendon complex characteristics during walking. The MG muscle was assessed as representative of the plantarflexor muscle group (41, 44) and measured from every frame of the ultrasound recordings throughout 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. Muscle fascicle length was defined as the distance between the superficial and deep aponeurosis parallel to the lines of collagenous tissue. Pennation angle (α) was defined as the angle between the collagenous tissue and the deep aponeurosis, since this deep pennation angle is the one through which force is transmitted along the tendon. The equations by ~~Menegaldo et al.~~ Grieve et al. (10) were used to calculate the MG 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.

Real-time ultrasound scanning was used to determine MG muscle fascicle length changes, while musculotendon complex (MTC) length changes were estimated from ankle and knee joint kinematics. 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 (21, 28, 31, 41).

Achilles tendon force calculation and magnetic resonance imaging scanning

Achilles tendon forces were calculated during walking throughout the stance phase by dividing the net plantarflexion joint moments (Nm) by the Achilles tendon internal moment arm length measured using a 0.25T magnetic resonance imaging (MRI) scanner (E-Scan, Esaote Biomedica, Genoa, Italy). The MRI scanning was performed with the participant in the upright standing position (i.e., full weight-bearing MRI) to mimic as closely as possible the conditions experienced on the ankle joint and Achilles tendon during walking. To calculate the Achilles tendon moment arm we used the Reauleaux method for identification of the ankle joint centre of a rotation, with the principle of a segment (the talus) rotating about a stationary (tibia) segment (40, 26). The centre of rotation was first defined using MRI images taken at 10 degrees of plantarflexion and 10 degrees of dorsiflexion, after which the distance between the Achilles tendon action line and the centre of rotation was measured on an MRI scan performed at the neutral ankle position.

The plantarflexion joint moments were derived from the kinematic and kinetic data using Visual 3D software (C-motion Inc., MD, USA). Elongation of the Achilles tendon was calculated as described in the above section. The Achilles tendon force and elongation were normalised to 100 points to represent the entire stance phase. Therefore, the Achilles tendon force-elongation curve was derived, as shown in Fig. 5, where the loading phase (arrow pointing up) represents 10-70% of the stance phase and the unloading phase (arrow pointing down) the final 30%, as described in Table 2.

Stiffness and hysteresis during walking

The Achilles tendon stiffness was calculated from the measurements taken during walking as the slope of the loading force-elongation curve by dividing the estimated tendon force (N) by the tendon's elongation (mm) over a force region between 500 and 1,500 N. This force region (500-1,500 N) was selected because it allowed comparison between groups over a common force region and enabled the use of measured data points on the force-elongation curve without the need to extrapolate. Mechanical hysteresis is a measure of the energy dissipated upon tendon recoil and converted to heat, an important feature of the mechanical properties of tendon. 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 (Ctrl; DM; DPN). 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 $p<0.05$.

RESULTS

Participant characteristics

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

Peripheral 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 diabetes 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 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. During walking, the DM and particularly the DPN groups displayed significantly higher tendon stiffness (Ctrl: 210; DM: 231; DPN: 240 N/mm: $P<0.01$) and higher tendon hysteresis (Ctrl: 18; DM: 21; DPN: 24%: $P<0.01$)

compared to controls. There were no differences in the fascicle lengths during standing between the groups ($P>0.05$). Average fascicle length change data during the stance phase show that the DPN group was significantly lower ($P<0.01$) than the Ctrl group for both self-selected speed and 1.0 m/s during two different phases, 10-70% and 70-100% of the stance (Table 2), while the DM group was different from the Ctrl group only at 1.0 m/s (Table 2). Significant differences 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). Significant differences in the pennation angle changes were found between DPN and the Ctrl as well as the DM and the Ctrl groups for both speeds during loading and unloading phases (Table 2).

DISCUSSION

This study has shown for the first time that there is reduced Achilles tendon elongation during the loading phase of walking (10-70% stance) and reduced tendon recoil during the subsequent propulsive phase (70-100% stance) in people with diabetes and to the greatest extent in those with DPN compared to controls (Table 2; Fig. 3). Further novelty is in uncovering the mechanism of this during walking, by showing that people with diabetes and particularly those with DPN demonstrated a higher stiffness and hysteresis of the Achilles tendon compared to the Ctrl group (Fig. 4; Table 5). Taken together the present findings strongly indicate a reduced elastic energy contribution from the Achilles tendon during walking in people with diabetes and to a greater extent in those with DPN, with implications for increasing the metabolic CoW in patients with diabetes and DPN as we have recently shown (32).

The increased tendon stiffness observed in the diabetes groups shows that for the same application of force, the Achilles tendon is less extensible during walking, which means that less energy can be stored. The increased stiffness is further compounded by the fact that less force is applied on the Achilles tendon in the DM and particularly the DPN groups (Fig 5; Table 2). The lower tendon forces applied during walking in diabetic patients is the result of lower joint moments being developed, which reflect a natural strategy to lower the demands of walking (7, 8, 22). 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 of the present study (Fig. 6). The maximum plantarflexor strength deficits were most marked as the ankle moved further into dorsiflexion (Fig. 6), which is closely

aligned with the position of the ankle during walking when the Achilles tendon is undergoing elongation (Fig. 3 & 4). Hence, lower moments developed while the ankle is in dorsiflexion during walking means lower forces applied to elongate and store energy within the Achilles tendon.

Once energy is stored in the Achilles tendon, the majority is returned upon tendon recoil, but some is lost due to internal damping, known as hysteresis. It was found that Achilles tendon hysteresis was significantly higher in people with diabetes, and to the greatest extent in those with DPN compared to controls. This further compounds the effect of reduced energy stored in the tendon upon loading resulting from increased tendon stiffness, since a lower proportion of the energy stored will be returned upon recoil.

The results indicate that the MTC length changes during walking are dependent upon the changes in ankle and knee joint angles (Fig. 3 & 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 found 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. 3; Table 1). The present findings of reduced tendon elongations are in line with previous work by Cronin et al. (11) showing that the Achilles tendon length changes during walking are attenuated in long-term diabetic patients, but without reference to a diabetic peripheral neuropathy group.

During walking the muscle fascicles of the gastrocnemius underwent very little length change compared to the Achilles tendon and the MTC (Fig. 3) 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 Fukunaga (18), Lichtwark (25), Ishikawa (23), Roberts (39), 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 found to actually shorten very little during the propulsive phase of gait in any group (Fig. 3), 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. Although we did not find a greater length change of the gastrocnemius muscle fascicles for the diabetes groups in the present study, it could be speculated that the uni-articular soleus muscle undergoes greater shortening in the diabetes groups, contributing to the higher muscular contribution and increased CoW. Despite the near-isometric behaviour of muscle fascicles during walking, pennation angles underwent changes in the region of between 22-32 deg, reflecting elongation of the Achilles tendon and aponeurosis, with smaller pennation angle changes seen in the DPN group (Table 2).

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 (31, 21, 25, 28). The increased tendon stiffness likely results from increased collagen cross-linking due to diabetes and DPN (33, 34), but a thicker tendon with a larger cross-sectional area may also play a role if present (21). Also, values for tendon hysteresis

from the present study measured during walking are comparable to dynamometry-based methods reported previously in the literature for the Achilles tendon in the range between 5 and 26 % (31, 25, 28, 15, 24). 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 as a limitation, however, 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).

We calculated ankle joint moments using the inverse dynamics technique, which provides the net joint moment. In calculating the net joint moment, this technique takes into account agonist and antagonistic moments acting around the joint, but cannot distinguish differences in for example, the level of antagonist muscle coactivation between groups. Using this standard approach to calculate Achilles tendon forces, an assumption is made that that the force generated by all of the plantarflexor muscles acts through the Achilles tendon. Based on data of muscle physiological cross-sectional area (17), the soleus and gastrocnemius muscles will contribute 83% of the plantarflexion

force, but it should be acknowledged that there are other smaller plantarflexor muscles contributing the remaining 17% of the force that do not act through the Achilles tendon.

The present study has shown reduced Achilles tendon elongation, increased stiffness and hysteresis during walking in people with diabetes and particularly those with DPN, compared to controls. The implications of these findings are a reduced storage and release of elastic energy from the Achilles tendon of diabetes and DPN 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 and DPN patients as an important factor contributing to the increased metabolic CoW in these patients.

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COMPETING INTERESTS

None of the authors had any financial or personal conflict of interest with regard to this study.

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Figure 1. A linear 7.5 MHz probe (A) with 60 mm field of view used for scanning the gastrocnemius muscle. A custom-built fixation device made of Velcro straps and a plastic cast moulded 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 with extra strapping added to further minimise any probe movement (C).

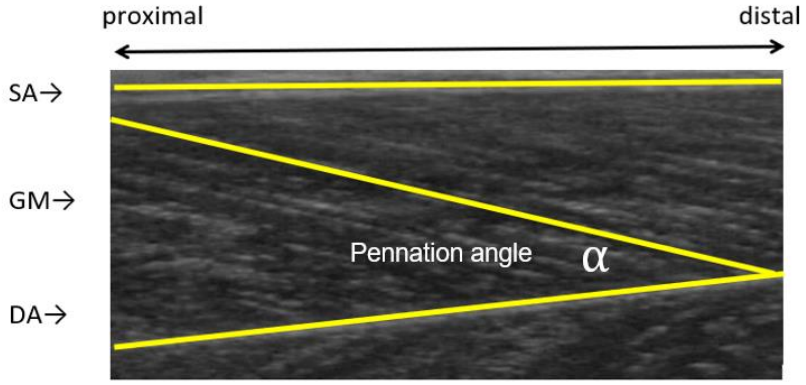

Figure 2. Typical sonograph of the GM muscle. The fascicular trajectory between the two aponeurosis, as well as the pennation angle (α) are highlighted in white. SA, superficial aponeurosis; MG, gastrocnemius medialis muscle; DA, deep aponeurosis.

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).

610 Table 2. *Achilles and plantarflexor muscle-tendon parameters during walking.*

	Ctrl		**DM**		**DPN**	
	Self-selected	**1 m/s**	Self-selected	**1 m/s**	Self-selected	**1 m/s**
Walking speed (m/s)	1.43 (0.29)	1.03 (0.17)	1.33 (0.36)	1.04 (0.21)	1.30 (0.34)	0.98 (0.20)
Stiffness (N/mm)	210 (41)	186 (34)	231 (46)**	194 (39)**	240 (49)**	202 (37)**

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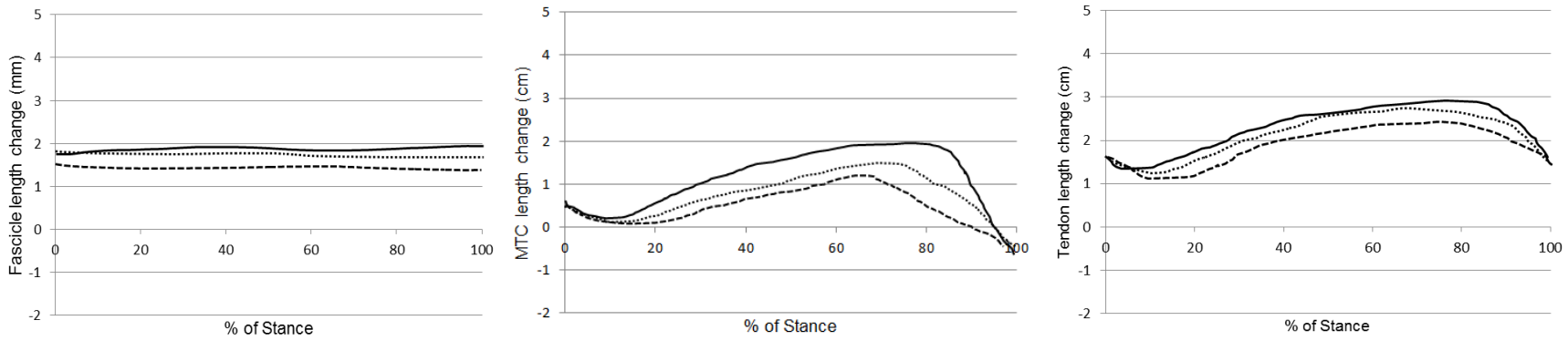
Hysteresis (%)	18 (3)	17 (3)	21 (5)**	19 (4)*	24 (6)**	21 (5)**
Standing fascicle length (cm)	5.15 (1.5)		5.08 (1.4)		5.19 (1.3)	
Tendon length change (cm)	1.81 (1.0)	1.67 (0.7)	1.66 (0.5)*	1.51 (0.6)*	1.54 (0.8)**	1.47 (0.6)**
Fascicle length change (cm) 10-70 % of stance (loading)	0.58 (0.08)	0.53 (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)**
Pennation angle change (deg) 10-70% stance (loading)	26.8 (6.3)	24.9 (3.4)	25.7 (8.9)**	24.7 (5.0)**	25.1 (9.2)*	22.4 (8.0)*
Pennation angle change (deg) 70-100% stance (unloading)	31.9 (9.9)	30.7 (7.2)	29.6 (6.1)**	29.2 (6.9)**	28.8 (8.9)*	22.8 (7.7)**

Achilles and plantarflexor 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

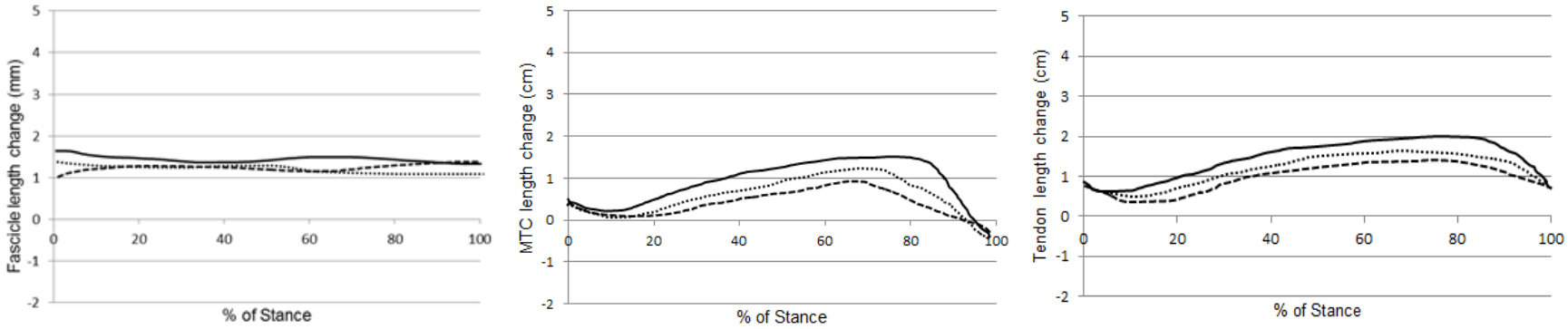
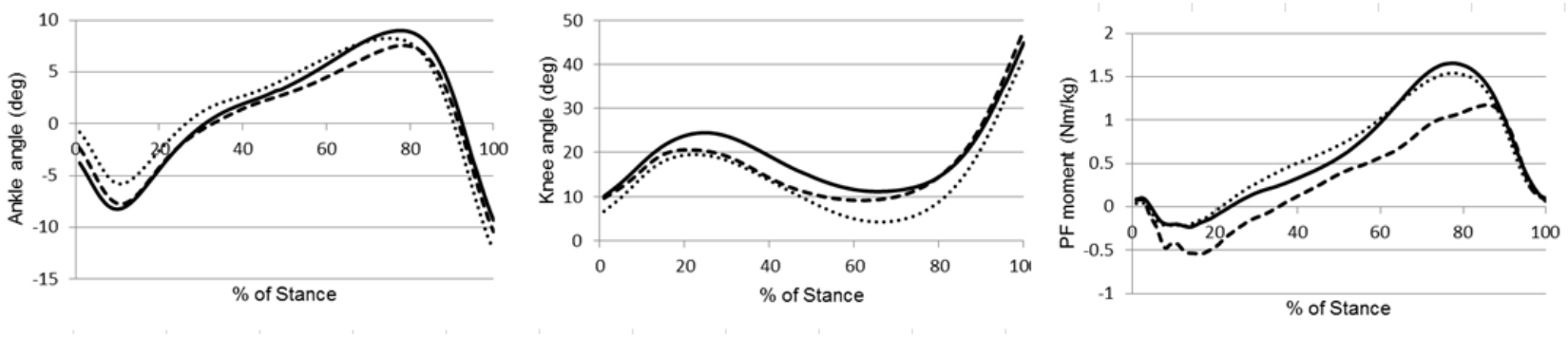


Figure 3. Muscle fascicle length, MTC length and tendon length changes, respectively while walking at self-selected speed and 1.0 m/s. Values are means. Line graphs: Ctrl - solid line (n=23), DM - dotted line (n=20), DPN - dashed line (n=13).

Self-selected walking speed



1.0 m/s

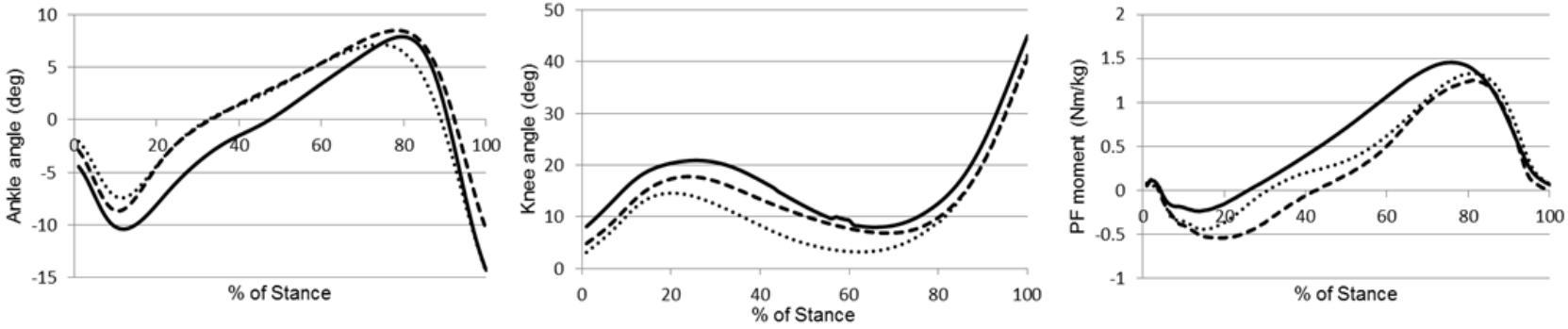


Figure 4. From left to right: ankle and knee range of motion (RoM) and ankle joint moment (AJM) during stance phase while walking at self-selected walking speed and 1.0 m/s for healthy controls (Ctrl), diabetic patients with no neuropathy (DM), and diabetic patients with moderate/severe neuropathy (DPN).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

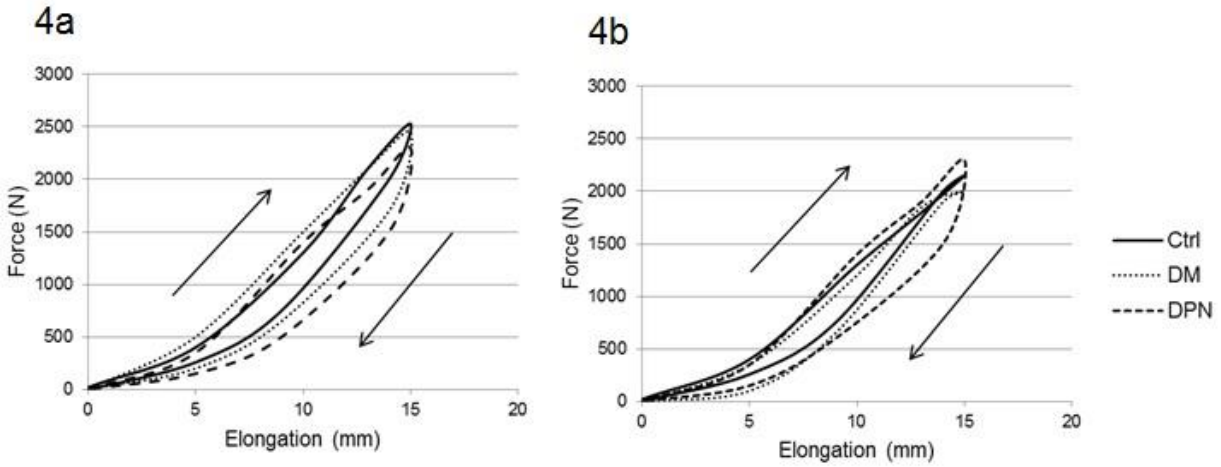


Figure 5. Achilles tendon force-elongation curves while walking at self-selected speed (4a) and at 1 m/s (4b) for healthy controls (Ctrl), diabetic patients with no neuropathy (DM), and diabetic patients with moderate/severe neuropathy (DPN). Values are means. Line graphs: Ctrl - solid line (n=23), DM - dotted line (n=20), DPN - dashed line (n=13).

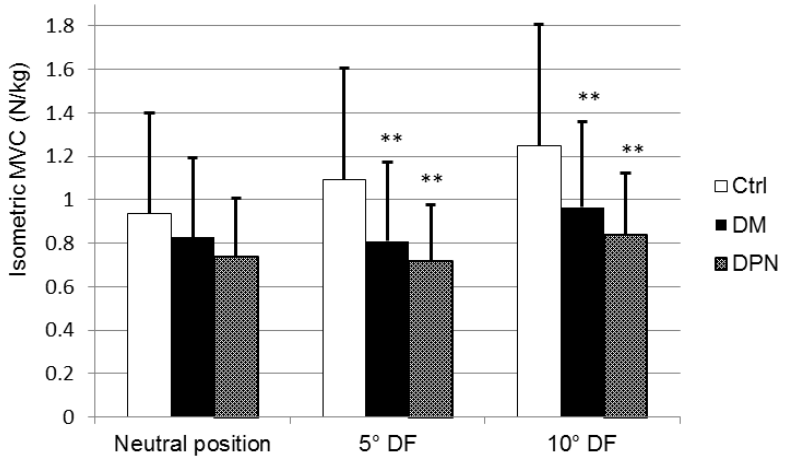


Figure 6. Isometric plantarflexion maximal voluntary contraction (MVC) strength for healthy controls (Ctrl, n=23), diabetic patients with no neuropathy (DM, n=20) and diabetic patients with moderate/severe neuropathy (DPN, n=13). Values are means and SD. Significant differences from the Ctrl group are denoted by ** (P<0.01).