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1 Altered Achilles tendon function during walking in people with diabetic neuropathy:
2 implications for metabolic energy saving

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41 **ABSTRACT**

42 The Achilles tendon (AT) has the capacity to store and release elastic energy during
43 walking, contributing to metabolic energy savings. In diabetes patients, it is
44 hypothesised that a stiffer Achilles tendon may reduce the capacity for energy saving
45 through this mechanism, thereby contributing to an increased metabolic cost of walking
46 (CoW). The aim of this study was to investigate the effects of diabetes and diabetic
47 peripheral neuropathy (DPN) on the Achilles tendon and plantarflexor muscle-tendon
48 unit behaviour during walking. Twenty three non-diabetic controls (Ctrl); 20 diabetic
49 patients without peripheral neuropathy (DM) and 13 patients with moderate/severe
50 DPN, underwent gait analysis using a motion analysis system, force plates and
51 ultrasound measurements of the gastrocnemius muscle, using a muscle model to
52 determine Achilles tendon and muscle-tendon length changes. During walking, the DM
53 and particularly the DPN group displayed significantly less Achilles tendon elongation
54 (Ctrl: 1.81; DM 1.66; DPN: 1.54 cm), higher tendon stiffness (Ctrl: 210; DM: 231; DPN:
55 240 N/mm) and higher tendon hysteresis (Ctrl: 18; DM: 21; DPN: 24 %) compared to
56 controls. The muscle fascicles of the gastrocnemius underwent very small length
57 changes in all groups during walking (~0.43cm), with the smallest length changes in the
58 DPN group. Achilles tendon forces were significantly lower in the diabetes groups
59 compared to controls (Ctrl: 2666; DM: 2609; DPN: 2150 N). The results strongly point
60 towards the reduced energy saving capacity of the Achilles tendon during walking in
61 diabetes patients as an important factor contributing to the increased metabolic CoW in
62 these patients.

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

64

65 **New & Noteworthy**

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

72

73 **INTRODUCTION**

74 Diabetes mellitus (DM) is a very prevalent global chronic disease in older adults and is
75 associated with a number of complications including cardiovascular disease, peripheral
76 arterial disease, retinopathy and poor wound healing (16, 14). One of the most common
77 complications of diabetes is diabetic peripheral neuropathy (DPN), with the incidence
78 reported to range between 13 and 68% (44, 6). Diabetes and DPN impact negatively on
79 gait and mobility with implications for quality of life. Diabetes and DPN cause muscle
80 weakness and affect sensory perception altering walking strategy and causing
81 impairments to balance control (13, 30, 20, 5).

82 The muscle-tendon complex is central to all movement tasks, with skeletal muscle
83 generating force, which is transmitted to the skeleton via viscoelastic tendons. In
84 addition to their force transmitting role, tendons also play an important role in energy
85 saving during walking by storing (during stretching) and returning (upon recoil) elastic
86 energy (37, 38, 39, 2). In particular, the Achilles tendon is a long tendon that is

87 important for storing and releasing elastic energy during walking and as such, plays an
88 important role in metabolic energy saving, as it actually 'spares' the muscle from
89 performing a large part of the work (3).

90 Both muscles and tendons are highly malleable tissues, which can modify their
91 properties in response to the habitual level of physiological loading and also the
92 metabolic environment (36, 1, 17). Animal studies show that diabetes causes non-
93 enzymatic glycation of soft tissues, including tendons (34). This non-enzymatic glycation
94 causes increased cross-linking, increasing the stiffness and modulus of the tendon (35,
95 33). Stiffening of the tendon reduces the degree to which it can be stretched, affecting
96 its potential for storing (and subsequently releasing) elastic strain energy during walking
97 and also limiting the ankle joint range of motion (11, 19, 29). In humans, calcification
98 and fascicle disruption have been observed in the diabetic human Achilles tendon (4).
99 Tendons exhibit relatively low mechanical hysteresis, which is defined as the energy
100 lost upon recoil of the tendon (27). In addition to tendon stiffness, the hysteresis of the
101 tendon could also be affected by diabetes. Hysteresis has been shown to increase in
102 humans with ageing (37). An increase in hysteresis would also reduce metabolic energy
103 saving by the Achilles tendon during walking.

104 In dynamometry tests, Couppé et al. (10) found Achilles tendon stiffness and skin
105 connective tissue cross-linking were greater in diabetes patients compared with
106 controls. Cronin et al. (11) found that Achilles tendon length changes during walking at
107 self-selected speed were attenuated in diabetes patients and that this was inversely
108 correlated with diabetes duration.

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

119

120 **MATERIALS AND METHODS**

121 **Participants**

122 Fifty-six participants were involved in this study. Participants were allocated into one of
123 three groups based upon defined criteria: patients with diabetes and moderate-severe
124 peripheral neuropathy (DPN, n=13), patients with diabetes but no neuropathy (DM,
125 n=20) and healthy controls without diabetes or peripheral neuropathy (Ctrl, n=23). Major
126 exclusion criteria included: disorders of the vestibular system, severe vascular disease,
127 neurological, rheumatic disease, cerebral injury, unstable ischemic heart,
128 musculoskeletal injury, foot or lower limb amputation (amputation of the hallux;
129 amputation of more than two lesser toes on one foot; amputation of part of/whole foot)
130 and open foot ulcer and recent surgery affecting gait. Participant characteristics are
131 displayed in Table 1.

132 **Diagnosis of Diabetic Peripheral Neuropathy**

133 The presence and severity of peripheral neuropathy was assessed by using the
134 modified Neuropathy Disability Score (mNDS) and the vibration perception threshold
135 (VPT). The mNDS is a composite score taken from tests measuring the participant's
136 ability to discriminate temperature, detect pain, vibration and the Achilles tendon reflex
137 (6). The VPT is an assessment performed using the probe of a neurothesiometer on the
138 apex of the hallux and increasing the level of vibration until detected by the participant.
139 A random blood glucose test was performed in the Ctrl group to confirm the absence of
140 diabetes (<7 mmol/l) and the above neuropathy tests were conducted to confirm the
141 absence of neuropathy in the Ctrl group resulting from any aetiology.

142

143 **Gait analysis**

144 Gait analysis was performed for the purpose of assessing the contribution of the
145 plantarflexor muscle-tendon complex and the capacity for elastic energy storage and
146 release via the Achilles tendon. To investigate whether the changes are dependent on
147 the walking speed we asked participants to walk along a 10-metre walkway in the gait
148 laboratory at their self-selected speed, as well as at a standardized speed of 1.0 m/s.
149 Walking at the standardized speed was controlled by measuring the velocity of a marker
150 attached to the sacrum after each trial from the motion analysis data and providing
151 immediate feedback for participants as to whether they needed to walk more quickly or
152 more slowly on the next trial to achieve the required speed (1.0 m/s). Kinematic data
153 were collected at 100 Hz using a 10-camera Vicon motion capture system (Vicon,
154 Oxford, UK) and a full-body modified Plug-In-Gait marker set consisting of 54 markers.

155 Where possible motion analysis markers were placed directly onto the skin; to minimise
156 movement artefacts resulting from loose clothing, all participants wore tight-fitting shorts
157 and t-shirts. Ground reaction forces were measured at 1000 Hz from three force
158 platforms (Kistler, Zurich, Switzerland) embedded into the walkway and synchronised
159 with the kinematic data. We have used standard procedures and systems for the
160 calculation of joint moments that are used routinely and have been widely accepted by
161 the biomechanics community (43, 9). Walking trials were repeated until three 'clean' foot
162 contacts with the force platforms were made with right limb, for both speed conditions.
163 During walking, an ultrasonographic imaging device (Aloka SSD-5000, Tokyo, Japan)
164 operating at 25 Hz was used to measure gastrocnemius medialis (MG) muscle fascicle
165 length changes *in vivo*. For these measurements, a linear 7.5 MHz probe with 60 mm
166 field of view was secured around the right lower leg in the mid-sagittal plane of the MG
167 muscle with a custom-built fixation device (Fig. 1). The ultrasound scanning was
168 synchronized with recordings of the kinematic and kinetic data. We have previously
169 shown a high reliability for this technique in measuring fascicle lengths, with an intra-
170 class correlation coefficient of 0.8 (42). All participants wore specialist diabetic shoes
171 (MedSurg, Darco, Raisting, Germany) with a neutral foot-bed, ensuring the diabetic
172 patients walked with safe, appropriate footwear whilst controlling for the effects of
173 footwear on the measured variables by standardising across all groups (Fig. 1).

174

175 **Dynamometry measurements: Measurement of Maximal Plantarflexion Strength**

176 Isometric plantarflexor maximal voluntary contraction (MVC) joint moment (maximum
177 strength) was recorded with participants laying prone with the knee in full extension.

178 The axis of rotation of the ankle, defined as the line connecting the two malleoli, was
179 carefully aligned with the axis of rotation of the dynamometer and the right foot secured
180 to the foot adapter of an isokinetic dynamometer (Cybex NORM, Cybex International,
181 New York, NY, USA). Straps were used around the ankle and also the hips to prevent
182 extraneous movements during maximal plantarflexions. Prior to testing subjects became
183 familiarised with the procedures involved. Participants were instructed to perform
184 maximal isometric plantarflexion contractions at joint angles of 0, 5 and 10 degrees of
185 dorsiflexion, where zero degrees was neutral ankle position: the footplate of the
186 dynamometer perpendicular to the longitudinal axis of the tibia. The subjects were
187 verbally encouraged to produce their maximum effort. Contractions were performed in a
188 randomized order. Two contractions were performed at each ankle angle by allowing a
189 1-min rest interval between bouts and the highest value was considered as the MVC at
190 each ankle angle. Results were subsequently normalised to body mass.

191

192 **Data processing**

193 The purpose of the data analysis was to quantify the Achilles tendon and plantarflexor
194 muscle-tendon complex characteristics during walking. The MG muscle was assessed
195 as representative of the plantarflexor muscle group (41, 44) and measured from every
196 frame of the ultrasound recordings throughout the entire stance phase. On each
197 ultrasound frame, three lines were defined automatically using a custom-script written in
198 MATLAB software (12): one line tracked the superficial aponeurosis, a second line was
199 matched with the deep aponeurosis, and a third line defined the fascicular path of the
200 fascicle movement. From these three lines, fascicle length and pennation angle were

201 calculated on each frame of ultrasound data. Muscle fascicle length was defined as the
202 distance between the superficial and deep aponeurosis parallel to the lines of
203 collagenous tissue. Pennation angle (α) was defined as the angle between the
204 collagenous tissue and the deep aponeurosis, since this deep pennation angle is the
205 one through which force is transmitted along the tendon. The equations by ~~Menegalde~~
206 ~~et al.~~ Grieve et al. (10) were used to calculate the MG muscle-tendon complex (MTC)
207 length change (muscle plus free tendon and aponeurosis in both distal and proximal
208 ends) using the fascicle length changes and the ankle and knee joint displacements
209 measured during walking over the stance phase. The length of the tendon (including
210 both the free tendon and aponeurosis) was found by subtracting muscle fascicle length
211 projected in the direction of the line of force application from the muscle–tendon
212 complex (MTC) length for each time instant. Thus:

$$213 \quad l^t = l^{MTC} - l^m \cos \alpha$$

214 where l^t is the length of the tendon, l^{MTC} is the length of the MTC, l^m is the
215 ultrasound-measured muscle fascicle length, and α is the ultrasound-measured
216 pennation angle.
217

218 Real-time ultrasound scanning was used to determine MG muscle fascicle length
219 changes, while musculotendon complex (MTC) length changes were estimated from
220 ankle and knee joint kinematics. Muscle fascicle and tendon properties were assumed
221 to be consistent along the length of the MTC. The muscle fascicles were also assumed
222 to be parallel to one another. The validity and reliability of the ultrasound measurements
223 *in vivo* during walking have been critically assessed in other studies on the same and
224 similar populations, reporting ICC values between 0.78 and 0.94 (21, 28, 31, 41).

225 **Achilles tendon force calculation and magnetic resonance imaging scanning**

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

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

246

247 **Stiffness and hysteresis during walking**

248 The Achilles tendon stiffness was calculated from the measurements taken during
249 walking as the slope of the loading force-elongation curve by dividing the estimated
250 tendon force (N) by the tendon's elongation (mm) over a force region between 500 and
251 1,500 N. This force region (500-1,500 N) was selected because it allowed comparison
252 between groups over a common force region and enabled the use of measured data
253 points on the force-elongation curve without the need to extrapolate. Mechanical
254 hysteresis is a measure of the energy dissipated upon tendon recoil and converted to
255 heat, an important feature of the mechanical properties of tendon. Mechanical
256 hysteresis was defined as the area between the loading (L) and unloading (UnL) curves
257 and expressed as a percentage:

$$258 \qquad \text{Mechanical hysteresis} = (L - UnL) / L \cdot 100$$

259

260 **Statistics**

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

266

267 **RESULTS**

268

269 **Participant characteristics**

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

272

273 **Peripheral neuropathy assessments**

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

278

279 **Lower limb kinetics and kinematics during walking**

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

285

286 **Plantarflexor muscle-tendon unit behaviour during walking**

287 There were significant differences in the tendon length change between the groups at
288 self-selected walking speed (Ctrl: 1.81 cm; DM 1.66 cm; DPN: 1.54 cm; $P<0.01$) as well
289 as 1.0 m/s (Ctrl: 1.67 cm; DM 1.51 cm; DPN: 1.47 cm; $P<0.01$), where the DPN group
290 expressed smaller tendon length changes. During walking, the DM and particularly the
291 DPN groups displayed significantly higher tendon stiffness (Ctrl: 210; DM: 231; DPN:
292 240 N/mm: $P<0.01$) and higher tendon hysteresis (Ctrl: 18; DM: 21; DPN: 24%: $P<0.01$)

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

303

304 **DISCUSSION**

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

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

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

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

338 The results indicate that the MTC length changes during walking are dependent upon
339 the changes in ankle and knee joint angles (Fig. 3 & 4). Although the magnitude of the
340 between-group differences were relatively small (~2 deg at the ankle and ~4 deg at the
341 knee), a significantly smaller ankle and knee joint range of motion during walking was
342 found in the DPN group compared to the controls (Fig. 4). This resulted in significantly
343 smaller MTC length changes during walking in the diabetes and particularly in the DPN
344 group compared to controls (Fig. 3; Table 1). The present findings of reduced tendon
345 elongations are in line with previous work by Cronin et al. (11) showing that the Achilles
346 tendon length changes during walking are attenuated in long-term diabetic patients, but
347 without reference to a diabetic peripheral neuropathy group.

348 During walking the muscle fascicles of the gastrocnemius underwent very little length
349 change compared to the Achilles tendon and the MTC (Fig. 3) and they could be

350 considered as acting near-isometrically. Indeed, near-isometric behaviour of
351 plantarflexor muscle fascicles has been previously reported in healthy young
352 populations Fukunaga (18), Lichtwark (25), Ishikawa (23), Roberts (39), which functions
353 to allow the Achilles tendon to absorb the length changes of the MTC, thereby
354 facilitating elastic energy storage within the tendon. Although the muscle fascicles were
355 found to actually shorten very little during the propulsive phase of gait in any group (Fig.
356 3), the reduced elastic energy contribution from the Achilles during walking in people
357 with diabetes and particularly in those with DPN indicates that the plantarflexor muscles
358 would need to contribute a greater proportion of the work, thereby increasing the
359 metabolic CoW. Although we did not find a greater length change of the gastrocnemius
360 muscle fascicles for the diabetes groups in the present study, it could be speculated that
361 the uni-articular soleus muscle undergoes greater shortening in the diabetes groups,
362 contributing to the higher muscular contribution and increased CoW. Despite the near-
363 isometric behaviour of muscle fascicles during walking, pennation angles underwent
364 changes in the region of between 22-32 deg, reflecting elongation of the Achilles tendon
365 and aponeurosis, with smaller pennation angle changes seen in the DPN group (Table
366 2).

367 The tendon stiffness data measured during walking in the present study are comparable
368 with a number of previous *in vivo* human studies of the Achilles tendon measured using
369 a dynamometry approach and reporting values ranging between 149 and 207 N/mm
370 (31, 21, 25, 28). The increased tendon stiffness likely results from increased collagen
371 cross-linking due to diabetes and DPN (33, 34), but a thicker tendon with a larger cross-
372 sectional area may also play a role if present (21). Also, values for tendon hysteresis

373 from the present study measured during walking are comparable to dynamometry-
374 based methods reported previously in the literature for the Achilles tendon in the range
375 between 5 and 26 % (31, 25, 28, 15, 24). It should be noted, that whilst previous studies
376 have derived tendon stiffness and hysteresis values from static dynamometry
377 measurements, the present study is unique in determining these tendon properties
378 during walking. It should be acknowledged as a limitation, however, that tendon length
379 changes can result from both tendon loading and also joint rotations. Therefore,
380 measurements of tendon elongation in the previous and present studies reflect not only
381 'true' elongations resulting from tensile forces, but also elongation due to joint rotations.
382 Whilst this is more easily 'corrected' for with the dynamometry-based approach, the
383 complexity of the unique approach followed in the present study mean that joint
384 rotations are more challenging to account for. Nevertheless, the magnitudes of
385 between-group differences in joint rotations were relatively small and therefore unlikely
386 to impact on the present findings (Fig. 4; Table 1).

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

395 force, but it should be acknowledged that there are other smaller plantarflexor muscles
396 contributing the remaining 17% of the force that do not act through the Achilles tendon.
397 The present study has shown reduced Achilles tendon elongation, increased stiffness
398 and hysteresis during walking in people with diabetes and particularly those with DPN,
399 compared to controls. The implications of these findings are a reduced storage and
400 release of elastic energy from the Achilles tendon of diabetes and DPN patients during
401 walking, presumably requiring a greater contribution to the work from plantarflexor
402 muscles. The results strongly point towards the reduced energy saving capacity of the
403 Achilles tendon in diabetes and DPN patients as an important factor contributing to the
404 increased metabolic CoW in these patients.

405

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410

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414

415 **COMPETING INTERESTS**

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

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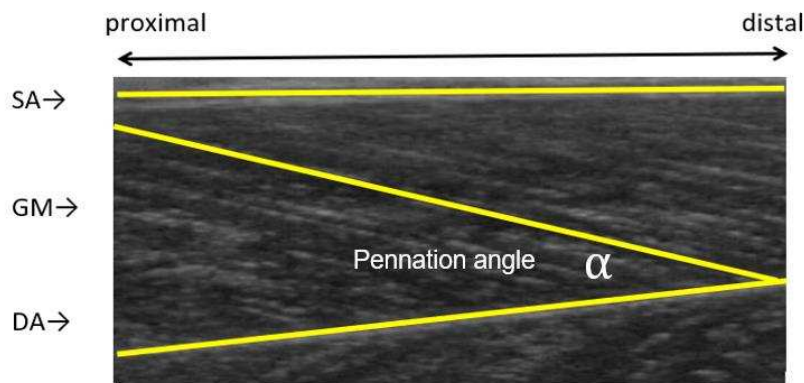
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585 Figure 1. A linear 7.5 MHz probe (A) with 60 mm field of view used for scanning the
 586 gastrocnemius muscle. A custom-built fixation device made of Velcro straps and a
 587 plastic cast moulded to fit the general contour of the calf (B) was used to secure the
 588 probe around the left lower leg, in the mid-sagittal plane of the gastrocnemius muscle
 589 with extra strapping added to further minimise any probe movement (C).
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591
 592 Figure 2. Typical sonograph of the GM muscle. The fascicular trajectory between the
 593 two aponeurosis, as well as the pennation angle (α) are highlighted in white. SA,
 594 superficial aponeurosis; MG, gastrocnemius medialis muscle; DA, deep aponeurosis.
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602 *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

603
 23

604 Healthy controls (Ctrl, n=23), diabetic patients with no neuropathy (DM, n=20) and
605 diabetic patients with moderate/severe neuropathy (DPN, n=13). Significant differences
606 from the Ctrl group are denoted by ** ($P < 0.01$). BMI = body mass index, mNDS =
607 modified neuropathy disability score, VPT = vibration perception threshold. Values are
608 means (standard deviations).
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610 Table 2. *Achilles and plantarflexor muscle-tendon parameters during walking.*

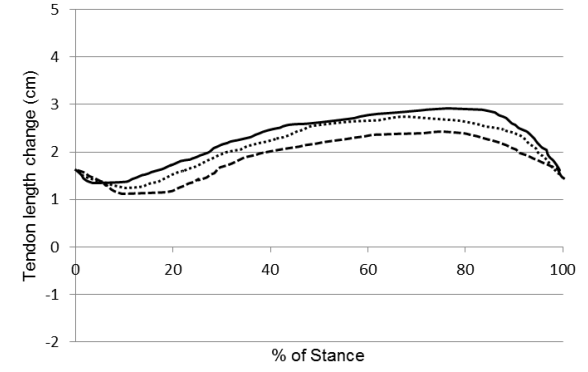
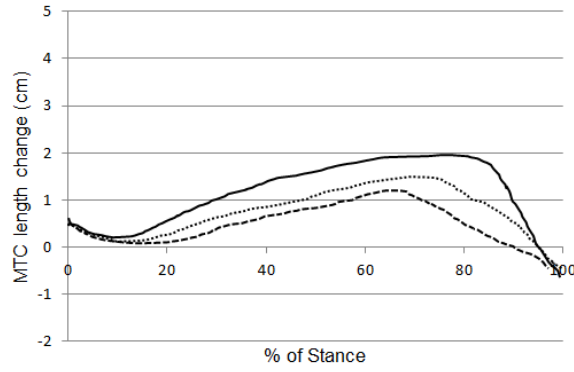
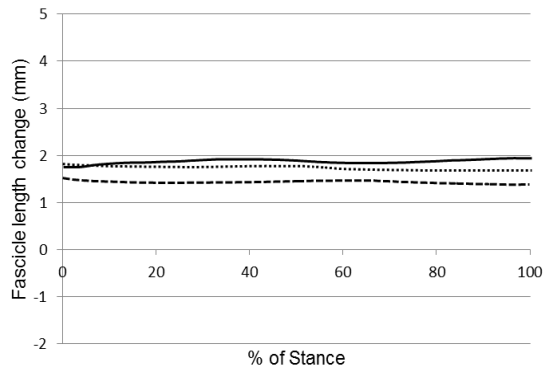
	Ctrl		DM		DPN	
	Self-selected	1 m/s	Self-selected	1 m/s	Self-selected	1 m/s
Walking speed (m/s)	1.43 (0.29)	1.03 (0.17)	1.33 (0.36)	1.04 (0.21)	1.30 (0.34)	0.98 (0.20)
Stiffness (N/mm)	210 (41)	186 (34)	231 (46)**	194 (39)**	240 (49)**	202 (37)**

611

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)**

612 Achilles and plantarflexor muscle-tendon parameters during walking for healthy controls (Ctrl; n=23), diabetic
613 patients with no neuropathy (DM; n=20) and diabetic patients with moderate/severe neuropathy (DPN; n=13).
614 Values are group means and SD; Significant differences from the Ctrl group are denoted by *(P<0.05) or
615 **(P<0.01). MTC – muscle-tendon complex; RoM – range of motion.
616

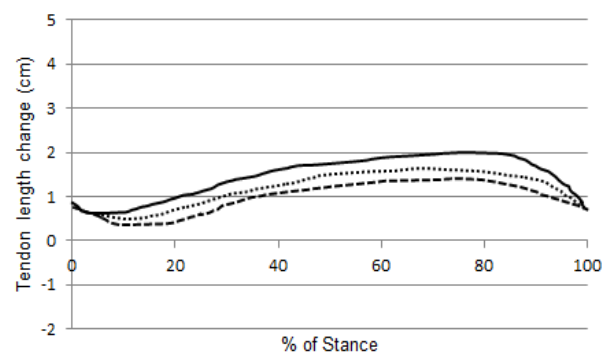
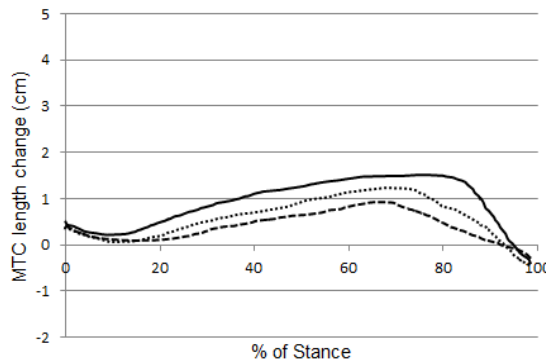
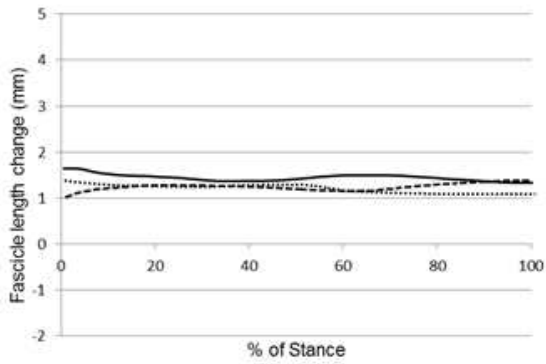
617 Self-selected walking speed



618

619 **1.0 m/s**

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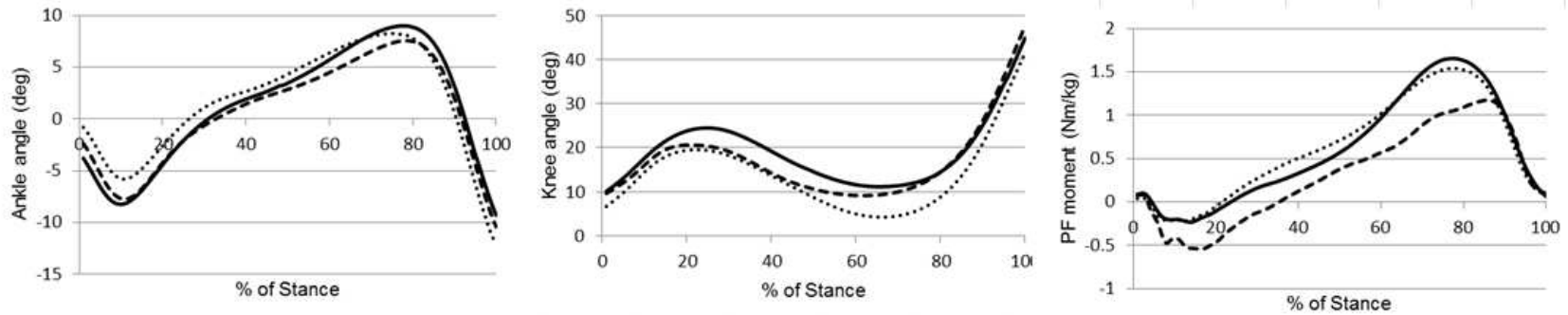


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622 Figure 3. Muscle fascicle length, MTC length and tendon length changes, respectively while walking at self-selected speed
 623 and 1.0 m/s. Values are means. Line graphs: Ctrl - solid line (n=23), DM - dotted line (n=20), DPN - dashed line (n=13).

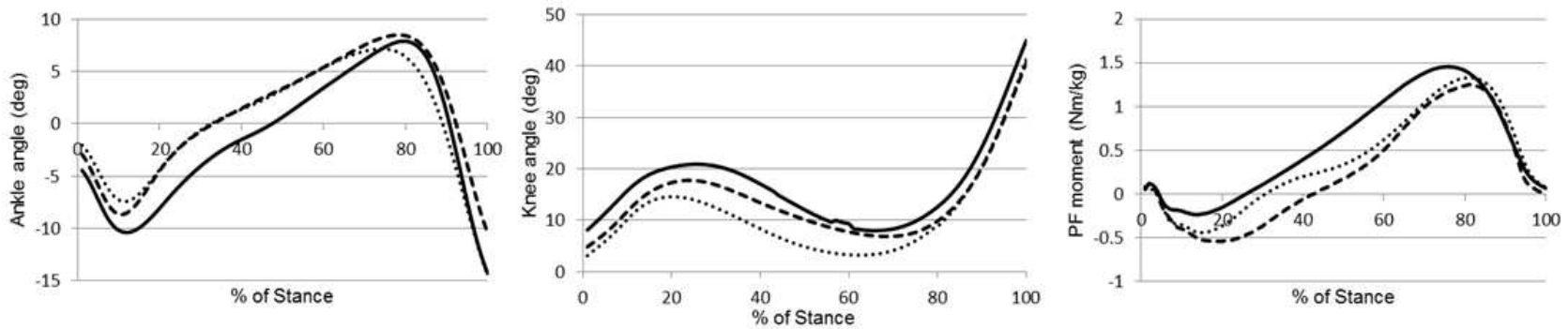
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625 **Self-selected walking speed**



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627 **1.0 m/s**



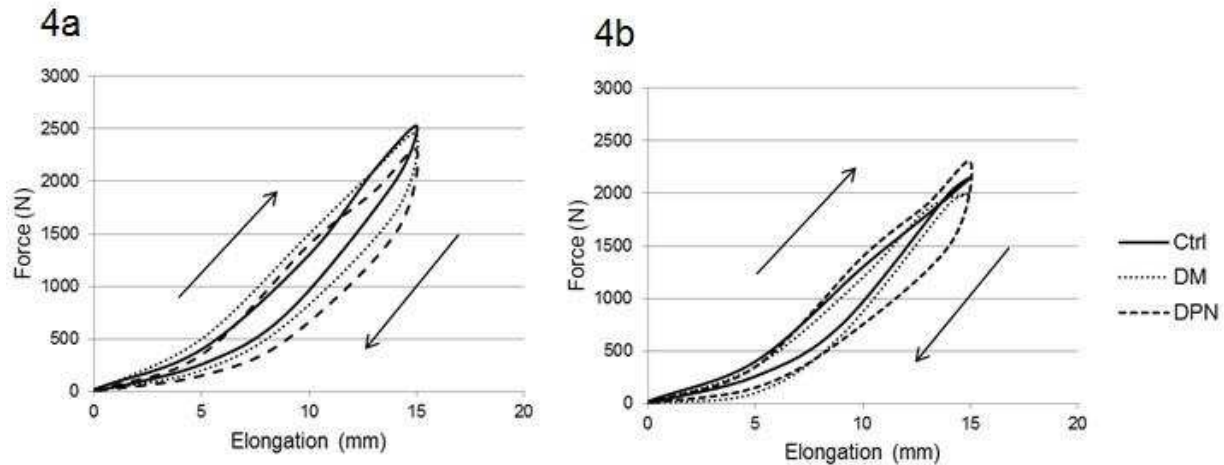
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629 Figure 4. From left to right: ankle and knee range of motion (RoM) and ankle joint moment (AJM) during stance phase while
630 walking at self-selected walking speed and 1.0 m/s for healthy controls (Ctrl), diabetic patients with no neuropathy (DM),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

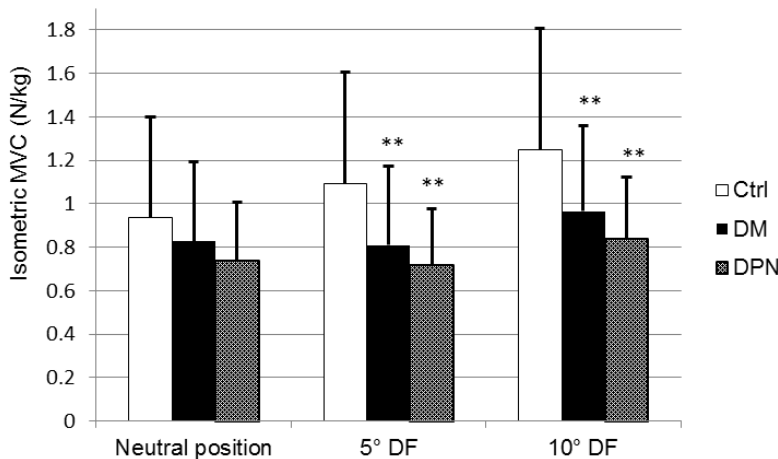
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 637 Figure 5. Achilles tendon force-elongation curves while walking at self-selected speed
 638 (4a) and at 1 m/s (4b) for healthy controls (Ctrl), diabetic patients with no neuropathy
 639 (DM), and diabetic patients with moderate/severe neuropathy (DPN). Values are
 640 means. Line graphs: Ctrl - solid line (n=23), DM - dotted line (n=20), DPN - dashed line
 641 (n=13).
 642



643
 644 Figure 6. Isometric plantarflexion maximal voluntary contraction (MVC) strength for
 645 healthy controls (Ctrl, n=23), diabetic patients with no neuropathy (DM, n=20) and
 646 diabetic patients with moderate/severe neuropathy (DPN, n=13). Values are means and
 647 SD. Significant differences from the Ctrl group are denoted by ** (P<0.01).