Reduced lower limb muscle strength and volume in patients with Type 2 diabetes in relation to neuropathy, intramuscular fat and vitamin D levels

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Abstract

Introduction: Patients with diabetes may develop muscle weakness and atrophy of the lower limbs and are at increased risk of falling. The underlying basis of these abnormalities has not been fully explained.

Aims: To objectively quantify muscle strength and size in patients with type 2 diabetes mellitus (T2DM) in relation to the severity of neuropathy, intramuscular non-contractile tissue (IMNCT) and vitamin D deficiency.

Methods: 20 patients with T2DM and 20 healthy control subjects were matched by age, gender and BMI. They underwent assessment of strength and size of knee extensor, flexor and ankle plantar and dorsiflexor muscles in relation to the severity of diabetic sensori-motor neuropathy (DSPN), amount of intramuscular non-contractile tissue and serum 25OHD levels.

Results: Patients with T2DM had significantly reduced knee extensor (P= 0.003) strength and muscle volume of both knee extensors (P= 0.045) and flexors (P= 0.019). Ankle plantar flexor strength (P=0.001) was also significantly reduced but without a reduction in ankle plantar flexor (P= 0.23) and dorsiflexor (P=0.45) muscle volumes. IMNCT was significantly increased in the ankle plantar (P= 0.006) and dorsiflexors (P= 0.005) of patients with T2DM compared to controls. Patients with DSPN had significantly lower knee extensor strength compared to those without DSPN (P= 0.02), but there was no difference in knee extensor volume (P=0.38) and ankle plantar flexor strength or volume (P= 0.21 and P= 0.96 respectively). There was no significant difference for the strength and volume of knee extensors (P=0.32 and P= 0.18) or ankle plantar flexors (P=0.58 and P=0.12) between T2DM patients with a 25 hydroxyvitamin D (25OHD) <25nmol/L vs >25 nmol/L.

Conclusions: Patients with T2DM have a significant reduction in proximal and distal leg muscle strength and a proximal but not distal reduction in muscle volume, possibly due to greater intramuscular fat accumulation in distal muscles.

Proximal but not distal muscle strength was related to the severity of peripheral neuropathy, but not with IMNCT or the level of 25OHD.

Key words: Diabetes, muscle strength, muscle size, intramuscular fat, peripheral neuropathy, vitamin D.

Introduction

Although diabetic polyneuropathy manifests primarily in the form of sensory and autonomic dysfunction, an increasing body of evidence shows that ankle and knee motor dysfunction may also be a major manifestation (1-3). Motor dysfunction presents as muscle weakness, a reduction in muscle mass, limitation of joint flexibility and range of motion, ultimately impacting upon gait and whole body movements (4-6).

Whilst weakness and atrophy of the distal muscles and decreased ankle mobility and strength have been demonstrated in several previous studies and related to the severity of neuropathy (7-9) underlying mechanisms have not been explored. Previous studies have not performed a comprehensive assessment of muscle strength in relation to morphology and internal composition. Thus patients with diabetes and obesity have an increased amount of intramuscular non-contractile tissue (IMNCT) which is highly correlated to insulin resistance and a reduction of muscle strength in the calf and thigh muscles (1, 2, 10).

Variations in muscle volume (11) may contribute to the alterations in strength, and as many patients with diabetes have obesity they may have larger muscle size, but the presence of diabetic neuropathy may result in muscle atrophy (7). Thus, previous studies have shown atrophy of the ankle plantar and dorsiflexor muscles as well as knee extensors in patients with diabetic neuropathy compared to patients without neuropathy and control subjects (2, 4, 6, 8). However, the effect on more proximal leg muscles (knee extensors and flexors), i.e. those muscles which confer a major effect on postural stability and gait performance is not established. Indeed maximal isometric muscle strength is related directly to muscle cross sectional area (11-13).

It has been suggested that a decline of muscle strength and muscle size, with increased intramuscular fat infiltration and a reduction in physical performance in healthy elderly subjects may be related to vitamin D deficiency (14, 15). Motor dysfunction can occur in those with mild and particularly severe vitamin D

deficiency (14, 16). Furthermore, 93% of patients complain of non-specific musculoskeletal pain, which may be attributed to vitamin D deficiency (17). The degree of vitamin D deficiency is currently categorised according to circulating levels of 25-hydroxy vitamin D (25OHD) such that adequate is defined as: >75 nmol/L (>30ng/ml); insufficient: 75-50 nmol/L (20-30 ng/ml); deficient: 25-50nmol/L (10-20ng/ml) and severely deficient: <25 nmol/L (<10ng/ml) (18). The underlying basis of vitamin D deficiency related muscle symptoms and dysfunction is likely to be complex, but proximal myopathy is a major manifestation of those with severe vitamin D deficiency (17). Vitamin D receptors decline in elderly subjects (17, 19, 20) and vitamin D deficiency is associated with atrophy of skeletal muscle fibers (Type II) and a decline in muscle strength, with an increased risk of falls (17, 21). We have previously shown a high prevalence of vitamin D deficiency in patients with diabetes (22) and vitamin D levels are inversely correlated with obesity, diabetes and high triglycerides (23). Although previous studies have investigated specific aspects of motor function in patients with Type 2 diabetes, there has not been a comprehensive assessment of skeletal muscle strength, morphology and internal composition in relation to neuropathy, IMNCT and 25OHD.

The purpose of this study was to investigate muscle strength deficits in distal and proximal extensors and flexors in the lower limb of patients with Type 2 diabetes and relate this to muscle size, the severity of peripheral neuropathy, IMNCT and vitamin D deficiency.

Methods

20 patients with Type 2 diabetes and 20 control subjects without diabetes underwent assessment at the muscle function laboratory at Manchester Metropolitan University. Subjects with severe musculoskeletal problems, neurological, orthopaedic or surgical problems, severe foot deformities, foot ulcers, amputations and pregnant women were excluded from the study. The study was approved by the UK National Health Service (NHS) ethics committee, local Research Ethics Committees at the University of Manchester and the Manchester Metropolitan University and written informed consent was obtained from all subjects prior to participation. This research adhered to the tenets of the declaration of Helsinki.

Assessment of Neuropathy

All patients with diabetes underwent assessment of body mass index (BMI), blood pressure, HbA1c, lipid profile [total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides], albumin creatinine excretion ratio , estimated glomerular filtration rate and 25 (OH)D. Symptoms of DPN were assessed using the Neuropathy Symptom Profile. Neurological deficits were evaluated using the simplified neuropathy disability score (NDS), which is comprised of vibration perception, pin-prick, temperature sensation and presence or absence of ankle reflexes. Vibration perception threshold (VPT) was tested using a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilfrod, Nottingham, UK). Cold (CT), warm (WT) thresholds, cold induced pain (CIP) and warm induced pain (WIP) were established on the dorsolateral aspect of the foot using the TSA-II NeuroSensory Analyser (Medoc Ltd., Ramat-Yishai, Israel). . Electro-diagnostic studies were undertaken using a Dantec “Keypoint” system (Dantec Dynamics Ltd, Bristol, UK) equipped with a DISA temperature regulator to keep limb temperature constantly between 32-35°C. Sural sensory nerve amplitude (SNAP), sural sensory nerve conduction velocity (SNCV) and peroneal motor nerve conduction velocity (PMNCV) and amplitude (PMNA) were assessed by a consultant neurophysiologist. (Diabetic sensorimotor polyneuropathy) DSPN

was defined according to the Toronto criteria (24). Control subjects only underwent an assessment of VPT and NDS.

All study subjects were scanned with a laser IVCCM [Heidelberg Retinal Tomograph III Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany)]. All images were captured using the "section" mode in the Heidelberg Explorer of the HRT III RCM and ~6 high clarity images/subject were analysed from the central sub basal nerve plexus. Four parameters were established to assess corneal nerve fiber damage; Corneal nerve fiber density (CNFD): the total number of nerve fibers (no./mm^2); Corneal nerve branch density (CNBD): the total number of nerve branches (no./mm^2); Corneal nerve fiber length (CNFL): the total length of all nerve fibers (mm/mm^2) within the area of cornea and Corneal nerve fiber tortuosity (CNFT): the degree of non-linearity of the nerve fibers. These parameters were quantified using semi-automated, purpose-written, proprietary software (CCMetrics®, M. A. Dabbah, Imaging Science Biomedical Engineering, University of Manchester, Manchester, UK).

Intraepidermal nerve fiber density (IENFD) was quantified in skin biopsies from the dorsum of the foot using our established techniques (25).

Isokinetic dynamometer (Cybex)

Maximal isometric muscle strength for knee extensors and ankle plantar flexors was assessed using an isokinetic dynamometer (Cybex Norm, Ronkonkoma, NY). The dynamometer measured joint torque (Nm) at the knee and ankle, which reflects the net forces acting around the respective joints and the internal tendon moment arm lengths. Since this measure (joint torque) primarily reflects the force produced by the major muscle groups acting around these joints (knee and ankle extensors, respectively) and for the purpose of optimising clinical understanding, we use the term 'muscle strength' in the manuscript to refer to the measurement of joint torque.

Tests were performed at three different angles for both the knee and ankle joints on the dominant leg. To test knee extensor joint torque, participants were seated

and secured on the chair of the dynamometer with their knees flexed at 90 degrees (0 degrees = full knee extension) and their hip angle at 85 degrees (0 degrees = supine position). Three maximal voluntary isometric contractions of the knee extensors were performed at 3 knee joint angles tested in a randomised order: 85, 70, and 55 degrees of knee flexion, with a two minute rest interval between contractions and the highest value was recorded.

To test ankle plantar flexor joint torque participants were positioned prone on the dynamometer with the knee in full extension and the ankle secured into the footplate of the dynamometer. Maximum voluntary isometric plantar flexor joint torque was assessed at three different joint angles performed in a random order: 0 degrees (neutral position, i.e., right angle between footplate and tibia), -5 degree in dorsiflexion, and -10 in dorsiflexion and also three maximal isometric contractions were performed and the highest value was recorded (Nm). Each maximum isometric contraction for both knee and ankle was held for around 3-4 seconds, with a 60 second rest interval between contractions within each angle and 2 minutes between contractions at different angles.

A range of joint angles were tested to ensure that we encompassed the joint angle where torque peaked (i.e., the optimum angle) for each participant and that we therefore took into account any slight variations in the muscle's force-length relationship between groups.

Magnetic Resonance Imaging (MRI)

A 0.25 Tesla MRI peripheral scanner (G-Scan, Esaote, Italy) was employed to scan the upper and lower regions of the leg with a T1 Gradient Echo scanning sequence with the following parameters: field of view= 200x200mm; matrix= 256x192 pixels; slice thickness= 10mm, inter-slice gap = 1mm; time to echo=16 ms; time to repetition = 685 ms and flip angle = 90 degrees. Serial axial plane images were obtained of the upper and lower leg, from which the cross-sectional areas (CSA) of specific muscles were analysed. Major exclusion criteria for MRI scanning included: women who were or could be pregnant; Ferromagnetic

foreign bodies; Cardiac pacemakers/cardioverter defibrillators; cochlear implants, Intrauterine devices and implanted drug infusion pumps.

Muscle volume calculation

Serial CSAs of the Knee extensors (vastus medialis, vastus intermedius, vastus lateralis and rectus femoris) knee flexors (semimembranosus, biceps femoris and semitendinosus); ankle plantar flexors (soleus, medial and lateral heads of gastrocnemius muscles) and ankle dorsiflexors (tibialis anterior and extensor digitorum longus) were analysed using digitizing software (OsiriX, Pixmeo, Geneva, Switzerland).

The CSA of each muscle was manually analyzed from the serial axial plane scans. To establish how many CSAs were required to be analysed for each specific muscle to provide a representative and accurate muscle volume calculation, three subjects were randomly selected and all of the available CSAs analyzed consecutively. The muscle volume calculated using all available slices was then compared against calculations using measurements from every second and third slice. As a result of the analysis, for the soleus, ankle dorsiflexors, vastus medialis, vastus intermedius, vastus lateralis, semimembranosus, biceps femoris and semitendinosus every third slice was used and for medial and lateral heads of gastrocnemius and rectus femoris every slice was used in the calculation of muscle volume. The sum of all CSAs for each muscle was calculated ($\sum$CSA cm^2) and multiplied by the distance between each muscle section d (m) in order to derive the muscle volume (cm^3) using the following equation:

Muscle volume (cm^3) = ($\sum$CSA cm^2* d)

Intramuscular non-contractile tissue

The density of different tissues is reflected by a different MRI signal intensity. Connective tissues yield low signal intensity values, while fat tissue produces very high signal intensity values, with the signal intensity of skeletal muscle

falling between these two tissues. By measuring the frequency distribution of the signal intensity from a given area of the MRI image, it is possible to determine shifts in signal intensity indicating changes in tissue composition. OsiriX software was used to measure the signal intensity for all muscles, from the region outlined as their CSA. The signal intensity was quantified in each of the muscles studied and a frequency distribution of the signal intensity in that CSA obtained. The signal intensity value with the highest frequency from the selected muscle CSA was recorded when this signal intensity value composed over 10% of the pixel number from the total number of pixels within that specific muscle CSA. Three different levels along the patient's leg were chosen (proximal, mid and distal) according to the different anatomical structure of the muscle. The sum of the three signal intensity values from each muscle was selected and used for further analysis.

Statistical Analysis

An independent samples Student's *t*-test was used to test between-group differences in the measured variables. A Pearson's correlation coefficient was performed to test the relationship between muscle strength and other parameters. Data are presented as mean ± standard deviation (SD) unless otherwise stated.

We have performed a power analysis before the study (a-priori power calculation) using the parameter ankle joint strength (torque) based upon the results of previous studies (7, 26) using the following equation: n = 2 □ ((Zα + Zβ)□σ) / Δ)2. The power analysis indicated that we needed 14 subjects in each of the two groups to detect a difference of 22 Nm between the groups (~20% difference between groups), with an alpha level of 0.05 and a beta level of 0.9 (i.e., power of 90%).To account for drop-out and potential data problems we recruited 20 into each group.

Results

Study Subjects

20 healthy controls (13 males & 7 females) and 20 patients with Type 2 diabetes (8 patients with T2DM had DSPN and 12 had no DSPN), (15 males & 5 females) were assessed. Age, height and BMI were matched in patients with T2DM compared to control subjects (Table1). Patients with T2DM had reasonable control of their glycemia and lipids and evidence of mild neuropathy based on neurological examination, quantitative sensory testing, neurophysiology, corneal confocal microscopy and skin biopsy (Table 1).

Muscle strength and volume

The knee and ankle muscle strength (Nm/kg) was significantly lower in patients with T2DM compared to controls at three different angles. Knee strength at 55º (1.3 ± 0.4 vs 2.1 ± 0.7; P=0.002), 70º (1.3 ± 0.5 vs 2.0 ± 0.8; P= 0.002) and 85º (1.3 ± 0.4 vs 1.8 ± 0.6; P=0.009) was significantly reduced in patients with T2DM compared to control subjects. Ankle strength at 0º (0.6 ± 0.2 vs 0.9 ±0.3; P=0.000), -5º (0.7 ± 0.2 vs 1.04 ± 0.3; P=0.001) and -10º (0.7 ± 0.2 vs 1.1 ± 0.36; P=0.001) was significantly reduced in patients with T2DM compared to control subjects. Accordingly the average knee extensor (1.3±0.5 vs 1.9±0.7; P=0.003) and ankle plantar flexor (0.6±0.2 vs 1.05±0.3; P= 0.001) strength was significantly lower in patients with T2DM compared to controls (Table 2).

Muscle volume

Muscle volume for the knee extensors (P=0.04) and flexors (P=0.01) was significantly lower in patients with T2DM compared to controls (Table 2). There was no significant reduction in ankle plantar (P=0.23) and dorsiflexor (P=0.45) muscle volume in patients with T2DM compared to control subjects (Table 2).

Intramuscular non-contractile tissue

The IMNCT was significantly increased in the soleus (P= 0.006); dorsiflexor (P= 0.005) and lateral gastrocnemius (P= 0.05) muscles in patients with T2DM

compared to controls (Table 2, Fig.1). There were no significant differences in IMNCT in the knee extensors or knee flexors between groups (Table 2).

DSPN v No DSPN

Patients with DSPN (n=8) had significantly lower knee extensor strength (Nm/kg) compared to patients without DSPN (n=12) (1.0±0.4 vs 1.5±0.4; P= 0.028). There was no significant difference in ankle plantar flexor strength between diabetic patients with and without DSPN (0.59±0.31 vs 0.76±0.19; P= 0.21).

Low vs normal vitamin D

There was no significant difference in muscle strength (1.2 ± 0.1 vs 1.3 ± 0.5; P=0.32) and volume (932.1 ± 427.3 vs 1122.7 ± 175.1; P=0.18) of the knee extensors and ankle plantar flexor strength (0.6 ± 0.2 vs 0.7 ± 0.2; P= 0.58) and volume (582.6 ± 306.9 vs 768.1 ± 160.5; P= 0.12) between diabetic patients with 25OHD levels <25nmol/L compared to patients with 25OHD levels >25 nmol/L.

Correlations

There was a significant correlation between knee extensor strength and knee extensor muscle volume (r= 0.57; P= 0.007) in patients with T2DM. There was no significant correlation between ankle plantar flexor strength and ankle plantar flexor volume (r= 0.23, P= 0.297) or between knee and ankle muscle strength with IMNCT (r ranging from -0.34 to -0.02 at ankle and from -0.33 to 0.37 at knee), severity of DSPN (r= -0.36 at ankle and -0.48 at knee) or 25OHD levels (r= -0.12 at ankle and 0.13 at knee) among patients with Type 2 diabetes.

Discussion

We have shown that patients with Type 2 diabetes mellitus have reduced proximal and distal lower limb muscle strength compared to age-matched controls, which is in agreement with other studies (2, 6, 7, 27). For the knee extensors, this was associated with muscle atrophy as reflected by the significantly reduced knee extensor muscle volume in patients with T2DM. In contrast, although the plantar flexor muscle strength was reduced in patients with T2DM, there was no measurable muscle atrophy. This may be attributed to the increase in intramuscular fat, which may 'mask' muscle atrophy. Essentially, since the muscle is infiltrated by increased levels of intramuscular fat, its CSA and volume appear 'artificially' larger than the actual active contractile area. The knee extensors demonstrated both a reduction in muscle strength and volume in patients with T2DM. The increase in intramuscular fat in the lower leg as opposed to the proximal knee extensors and flexors of patients with T2DM may well be related to peripheral neuropathy affecting the distal muscles. The exact mechanism that explains the association between an increase in intramuscular fat and peripheral neuropathy in patients with T2DM is not fully understood. An accumulation of IMNCT particularly in the thigh may further contribute to a reduction in muscle blood flow and insulin diffusion capacity, increasing the local concentration of fatty acids, resulting in insulin resistance of the skeletal muscle in patients with Type 2 DM (28). Aging is also associated with increased IMNCT within muscles of the lower limb in patients with Type 2 DM (29).

We have found marked strength deficits not only in the ankle plantar flexors, which has been shown previously and related to DSPN, but also in proximal knee extensors, which may also be partly attributed to DSPN. Of clinical relevance, the knee extensors are a major antigravity muscle group responsible for propelling and controlling the body during gait and therefore this abnormality may partially explain the recent observation that balance is impaired in patients with diabetic neuropathy (30). Reduced ankle and knee muscle strength in patients with diabetes and particularly those with neuropathy may contribute substantially to

the impairment in gait, increased incidence of falls and severe injuries with hospitalisation. Indeed, resistance training exercises can improve muscle strength, walking speed and reduce the risk of falls (6, 31-33).

Accumulation of IMNCT within skeletal muscle may result in insulin resistance, but has also been shown to correlate with reduced calf and thigh muscle strength in patients with DSPN (1). Magnetic resonance imaging allows accurate quantification of muscle CSA and volume and also allows one to differentiate muscle from fat, connective tissue and bone (34). In the present study knee extensor and flexor muscle volume was significantly smaller and there was also a trend for a smaller distal plantar flexor muscle volume in patients with T2DM. Muscle volume is of course associated with muscle strength and power production (14), in agreement with others (2, 6, 35). In addition to the impairment in lower extremity muscle function alterations in the cartilage, ligaments and tendons may also contribute to instability (36). Thus diabetes also increases the thickness of the Achilles tendon and plantar facia resulting in decreased flexibility of the ankle joint and limited dorsiflexion during walking (36). We have also found knee extensor muscle strength to be reduced significantly in patients with DSPN compared to those without DSPN. Previous studies have found that the severity of neuropathy contributes to an impairment of physical mobility (1). Thus the reduction in physical activity may result in a reduction in the use of the major antigravity muscles (37-39), particularly knee extensors during walking and this is reflected in the reduced strength of the knee extensors in patients with DSPN.

Vitamin D deficiency causes musculoskeletal dysfunction and has been associated with a reduction in muscle strength, size, bone density and increased IMNCT (14, 27). Muscle weakness and atrophy are prominent in patients with diabetes (1, 2, 6, 7) and have been attributed to vitamin D deficiency (22, 23). To our knowledge this is the first study that has systematically examined differences in muscle function and structure in relation to vitamin D deficiency in patients with type 2TDM. Whilst we show that all diabetic patients have insufficient levels of

25(OH)D, a low level of 25OHD (<25 nmol/L) was not related to a reduction in lower limb muscle strength or size (15).

The statistical power for the majority of key variables (muscle strength, size and IMNCT) in this study was 0.83-1, which is very high considering an optimal recommendation is 0.8 (40). Some other variables did fall below this optimal 0.8 threshold and for some of these variables statistical power could have been limiting. Considering that we had such high power for the majority of key variables, the lower power for certain variables may also reflect the fact that no true differences exist between groups in these other variables and would not have been found even with a much larger sample.

Conclusion

This is a small but detailed study, which was adequately powered for the majority of variables examined. Potential confounders such as differences in BMI and ethnicity between groups may have an impact on our findings. However, patients with T2DM show both proximal (knee extensor) and distal (ankle plantar flexor) muscle weakness. Proximal muscle weakness was related to a reduction in muscle volume but distal muscle weakness was not. The latter finding may be as a consequence of greater infiltration of distal intramuscular fat. The reduction in muscle strength was related to DSPN but not to low levels of vitamin D.

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Conflicts of Interest

The authors declare that there are no conflicts of interest associated with this manuscript.

Authors Contributions

M.A. researched data, performs statistical analysis and wrote the manuscript.

N.R. reviewed and revised the manuscript.

F.B. reviewed and revised the manuscript.

A.J.M.B. reviewed and revised the manuscript.

M.J. reviewed and revised the manuscript.

R.A.M. Designed the study, reviewed and revised the manuscript and is principal investigator of the study.

R.A.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Figure

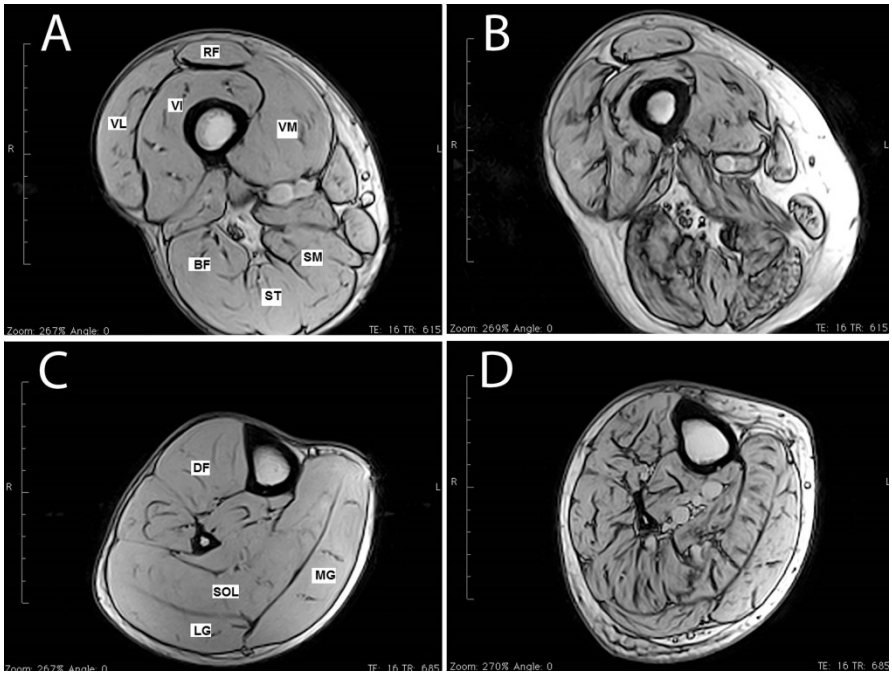

Figure 1. Representative lower limb MRI images from a healthy 69 year old control group volunteer (A and C) and a 67 year old patient with DPN (B and D). Images are from the mid-thigh level (A and B) and mid-tibia level (C and D). Note substantial increase in IMNCT (dark areas inside the muscle cross-sections are connective tissue) in images from patient with diabetes. Letters in A (VM: vastus medialis; VI: vastus intermedius; VL: vastus lateralis; FR: rectus femoris; BF: biceps femoris; ST: semitendinosus; SM: semimembranosus) and C (DF: Dorsiflexors; SOL: soleus; LG: lateral gastrocnemius MG: medial gastrocnemius) are abbreviations of muscle names in Latin. The length of the scale-bar along the left side of each image is 10 cm.

Tables

Table1. Clinical characteristics of the participants.

Parameter	Control	T2DM	P-value
No (male/female)	20(13/7)	20(15/5)	
DSPN (with/without)	NA	8/12	NA
Age (years)	61.5±6.0	63.1±10.8	0.56
Height (m)	1.69±0.09	1.67±0.09	0.53
Body mass (kg)	78.1±11.5	82.6±18.2	0.34
BMI (kg/m^2)	27.2±3.9	29.4±4.1	0.09
Ethnicity (Asian/European)	(9/11)	(2/18)	
Duration of DM (years)	NA	14.9 ± 9.9	
25OHD (nmol/L)	78.9±48.8	72.6±43.5	0.66
25OHD/BMI	2.9±1.9	2.5±1.6	0.48
25OHD/BSA	1.91±0.17	1.95±0.25	0.54
HbA1c (%)	NA	7.34±1.52	NA
Cholesterol (mmol/l)	NA	4.01±0.73	NA
HDL (mmol/l)	NA	1.37±0.887	NA
LDL (mmol/l)	NA	1.76±0.62	NA
Triglycerides (mmol/l)	NA	1.80±1.82	NA
NDS (0-10)	0.4±1.0	3.1±2.6	0.000
VPT(Hz)	6.4±3.0	14.7±11.0	0.003
CT (Cº)	NA	26.1±3.3	NA
WT(ºC)	NA	41.6±4.9	NA
SNCV (m/s)	NA	10.4±11.5	NA
SNAP (µV)	NA	5.2±10.7	NA
PMNCV (m/s)	NA	40.0±11.2	NA
PMNAP (mV)	NA	4.7±4.1	NA
CNFD (no/mm^2)	NA	28.0±9.2	NA
CNBD (no/mm^2)	NA	95.9±41.4	NA
CNFL (mm/mm^2)	NA	23.6±8.57	NA
CNFT (TC)	NA	20.50±4.2	NA
IENFD (no/mm)	NA	7.8±5.4	NA

DM: Diabetes mellitus; BMI: Body mass index; BSA: Body surface area; VD3: Vitamin D3; HbA1c: Glycated Haemoglobin; HDL: High density lipoprotein; LDL: Low density lipoprotein; NDS: Neuropathy disability score; VPT: Vibration perception threshold; CT: Cold threshold; WT: Warm threshold; SNVC: Sural sensory nerve conduction velocity; SNAP: Sural sensory nerve amplitude; PMNCV: Peroneal motor nerve conduction velocity; PMNAP: Peroneal motor nerve amplitude; CNFD: Corneal nerve fiber density; CNBD: Corneal nerve branch density; CNFL: Corneal nerve fiber length; CNFT: Corneal nerve fiber tortuosity; IENFD: Intraepidermal nerve fiber density.

Table 2. Muscle strength, volume and IMNCT (MRI signal intensity values) in patients with T2DM and controls, with a-priori statistical power analysis, statistical difference and the percentage difference between groups.

Variables	Control	T2DM	Statistical power	P-value	% difference
Muscle strength (Nm/kg)					
Knee extensors	1.9±0.7	1.3±0.5	0.99	**0.003**	-32
Ankle plantar flexors	1.0±0.3	0.6±0.2	1	**0.001**	-34
Muscle volume (cm^3)					
MV SOL	418.7±114.8	420.2±132.4	0.06	0.97	0
MV MG	184.8±52.8	170.3±68.5	0.28	0.46	-7
MV LG	106.3±35.8	92.4±36.7	0.53	0.24	-13
SUM Ankle PF	709.9±186.5	617.9±291.2	0.53	0.23	-12
Ankle DF	218.2±49.9	205.3±55.3	0.29	0.45	-5
MV VM	342.3±98.8	328.9±80.2	0.16	0.64	-3
MV VI	342.3±106.4	293.1±62.8	0.83	0.08	-14
MV VL	368.5±106.7	330.6±94.9	0.51	0.24	-10
MV RF	148.5±47.8	117.5±48.7	0.89	0.05	-20
Sum Knee extensors	1201.6±323.2	968.4±395.6	**0.83**	**0.04**	-19
MV SM	227.7±58.4	194.1±56.7	0.83	0.07	-14
MV BF	288.4±77.3	246.4±68.3	0.83	0.08	-14
MV ST	152.2±51.4	131.5±40.5	0.64	0.16	-13
Sum Knee flexors	668.5±172.9	517.5±219.6	**0.96**	**0.01**	-22
IMNCT (Pixel Intensity)					
Soleus	3453.3±356.0	3736.0±240.0	**1**	**0.006**	8
MG	2751.8±325.3	2901.7±464.9	0.52	0.25	5
LG	2039.6±282.5	2231.4±314.9	**0.89**	**0.05**	9
DF	3748.7±321.8	4103.6±414.2	**1**	**0.005**	9
VM	3628.4±138.3	3636.2±251.5	0.07	0.90	0
VI	3553.8±155.2	3594.0±205.9	0.26	0.49	1

VL	3670.4±239.7	3668.1±349.6	0.05	0.98	-0
RF	3303.1±206.1	3437.6±306.1	0.76	0.11	4
SM	3571.3±342.5	3543.6±353.9	0.10	0.80	-0
BF	3411.3±214.5	3457.9±276.9	0.21	0.56	1
ST	3513.2±360.7	3715.4±427.9	0.74	0.12	5

An independent sample *t*-test was used to represent the statistical differences in muscle strength, volume and intramuscular non-contractile tissue (IMNCT) in controls vs patients with diabetes (DM). SOL: soleus; DF: Dorsiflexors; PF: Plantar flexors; MG: medial gastrocnemius; LG: lateral gastrocnemius; VM: vastus medialis; VI: vastus intermedius; VL: vastus lateralis; RF: rectus femoris; SM: semimembranosus; BF: biceps femoris; ST: semitendinosus.