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## **Distal lower limb strength is reduced in subjects with impaired glucose tolerance and is related to elevated intramuscular fat level and vitamin D deficiency**

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## What's new?

- There are no studies on the structure and function of lower limb muscles in people with impaired glucose tolerance (IGT).
- We believe such a study may provide insights into the early mechanisms of motor dysfunction in people with Type 2 diabetes.
- People with IGT have a significant reduction in distal but not proximal leg muscle strength and no evidence of proximal or distal muscle atrophy.
- Distal weakness was associated with increased distal intramuscular non-contractile tissue, small fibre neuropathy and vitamin D deficiency in subjects with IGT.

## Abstract

**Aim** To quantify muscle strength and size in subjects with impaired glucose tolerance (IGT) in relation to intramuscular non-contractile tissue, the severity of neuropathy and vitamin D level.

**Methods** A total of 20 subjects with impaired glucose tolerance and 20 control subjects underwent assessment of strength and size of knee extensor, flexor and ankle plantar and dorsi-flexor muscles, as well as quantification of intramuscular non-contractile tissue and detailed assessment of neuropathy and serum 25-hydroxy vitamin D levels.

**Results** In subjects with impaired glucose tolerance, proximal knee extensor strength ( $P=0.17$ ) and volume ( $P=0.77$ ), and knee flexor volume ( $P=0.97$ ) did not differ from those in control subjects. Ankle plantar flexor strength was significantly lower ( $P=0.04$ ) in the subjects with impaired glucose tolerance, with no difference in ankle plantar flexor ( $P=0.62$ ) or dorsiflexor volume ( $P=0.06$ ) between groups. Intramuscular non-contractile tissue level

was significantly higher in the ankle plantar flexors and dorsiflexors ( $P=0.03$ ) of subjects with impaired glucose tolerance compared with control subjects, and it correlated with the severity of neuropathy. Ankle plantar flexor muscle strength correlated significantly with corneal nerve fibre density ( $r= 0.53$ ;  $P=0.01$ ), a sensitive measure of small fibre neuropathy, and was significantly lower in subjects with vitamin D deficiency ( $P=0.02$ ).

**Conclusions** People with impaired glucose tolerance have a significant reduction in distal but not proximal leg muscle strength, which is not associated with muscle atrophy, but with increased distal intramuscular non-contractile tissue, small fibre neuropathy and vitamin D deficiency.

## Introduction

Diabetic polyneuropathy has traditionally been considered to manifest itself initially in the form of sensory and autonomic dysfunction, followed by later motor dysfunction [1]. Motor dysfunction presents as weakness, a reduction in muscle mass and limitation of joint range of motion [2]. Weakness and atrophy of the distal muscles has been shown in several previous studies and is related to the severity of diabetic neuropathy [2,3]. Sensorimotor neuropathy in the lower limbs has implications for the control of whole body movement and has been proposed to contribute significantly to increasing the risk of falls during common daily gait tasks [3]

In people with Type 2 diabetes, muscle strength has been related to features of metabolic syndrome [4]. People with diabetes and obesity have an increased amount of intramuscular adipose tissue, which is highly correlated with insulin resistance and a reduction of muscle strength in the calf and thigh muscles. This accumulation of intramuscular non-contractile

tissue (IMNCT) in obese people can paradoxically enlarge the cross-sectional area of the muscles [5], despite reducing muscle area *per se*. Obesity, aging and polyneuropathy are also associated with increased IMNCT [6]. Vitamin D deficiency is related to muscle dysfunction and pain, and in severe deficiency it can lead to marked proximal weakness [7] and reduced physical activity [8].

We believe that a detailed study of the structure and function of lower limb muscles in people with impaired glucose tolerance (IGT) may provide insights into the early mechanisms of motor dysfunction in Type 2 diabetes. It may also identify potential early targets for intervention, which may reverse or limit progression to more overt motor pathology associated with Type 2 diabetes. Previous studies assessing muscle strength and structure in participants with IGT are limited to clinical examination of muscle strength and reflexes, and indeed, not surprisingly, have shown no abnormality [9]. Furthermore, only one study in postmenopausal women with IGT has shown an improvement in muscle mass and function after eccentric training [10], indicating a degree of reversibility. In the present study, we undertook a detailed quantification of lower limb muscle strength and structure in relation to IMNCT and neuropathy. Additionally, we assessed the effect of vitamin D deficiency on more subtle aspects of muscle function.

## Methods

A total of 20 subjects were identified with IGT and 20 subjects with normal glucose tolerance, based on an oral glucose tolerance test. These subjects underwent assessment at the muscle function laboratory at Manchester Metropolitan University and the National Institute for Health Research (NIHR)/Wellcome Trust Clinical Research Facility. Subjects aged 60–80 years old, who were able to walk independently without any assistive device, were included

in the study. Subjects with known musculoskeletal problems, neurological, orthopaedic or surgical problems, severe foot deformities, foot ulcers, amputations and pregnant women were excluded from the study. This study was approved by the UK National Health Service (NHS) ethics committee and the local Research Ethics Committees at the University of Manchester and the Manchester Metropolitan University. Written informed consent was obtained from all subjects prior to participation. This research was conducted in accordance with the declaration of Helsinki.

### **Clinical, metabolic and neuropathy assessment**

The IGT group underwent assessment of blood pressure, HbA<sub>1c</sub>, lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides), albumin–creatinine excretion ratio, estimated GFR and serum 25-hydroxyvitamin D [25(OH)D] levels. The assay for 25(OH)D was an automated platform assay (ImmunoDiagnostic Systems Ltd, Bolden, UK), which is based on chemiluminescence technology. Signs and symptoms of neuropathy were assessed using the neuropathy symptom profile, the Neuropathy Disability Score (NDS) and vibration perception threshold (VPT) using a neurothesiometer (Horwell Scientific Laboratory Supplies, Nottingham, UK). Cold and warm thresholds, cold-induced pain and warm-induced pain 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 and 35°C. Sural sensory nerve amplitude, sural sensory nerve conduction velocity and peroneal motor nerve conduction velocity and amplitude were assessed by a consultant neurophysiologist. The control group only underwent an assessment of NDS and VPT. To

provide a reference comparison we therefore used data from age-matched control subjects who had previously undergone assessment for all comparable metabolic and neuropathy measures in our laboratory.

### **Corneal confocal microscopy**

All the subjects included in the study underwent laser *in vivo* corneal confocal microscopy (Heidelberg Retinal Tomograph III Rostock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany) and four variables were quantified: corneal nerve fibre density [total number of nerve fibres (number/mm<sup>2</sup>)]; corneal nerve branch density [total number of nerve branches (number/mm<sup>2</sup>)]; corneal nerve fibre length [total length of all nerve fibres (mm/mm<sup>2</sup>) within the area of the cornea]; and corneal nerve fibre tortuosity (degree of non-linearity of the nerve fibres). These variables were quantified using semi-automated, purpose-written, proprietary software (CCMetrics<sup>®</sup>, M. A. Dabbah, Imaging Science Biomedical Engineering, University of Manchester, Manchester, UK).

### **Intraepidermal nerve fibre density**

A 3-mm punch skin biopsy was taken from the dorsum of the foot, ~2 cm above the second metatarsal head under local anaesthesia (1% lidocaine) and 50-µm frozen sections were cut and immunostained using anti-human PGP 9.5 antibody (Abcam, Cambridge, UK). Nerve fibres were demonstrated using SG chromogen (Vector, Burlingame, CA, USA) and examined under a Zeiss AxioImager M2 microscope at 400× magnification. Intraepidermal nerve fibre density was quantified according to established criteria and expressed as number/mm [11].

### **Isokinetic dynamometer**

The maximum isometric (static) muscle strength for knee extensors and ankle plantar flexors was assessed using an isokinetic dynamometer (Cybex Norm, Ronkonkoma, NY, USA). The dynamometer measured joint torque (Nm), which reflects the net forces acting around the knee and ankle joints and the anatomical leverage at these joints. The force produced by the major muscle groups acting around these joints is mainly reflected by the measure of joint torque, which we refer to as 'muscle strength' in the present paper. Details of the methodology used are included in Appendix S1.

### **Magnetic resonance imaging**

The thigh and lower leg were scanned using a 0.25-Tesla magnetic resonance imaging (MRI) peripheral scanner (G-Scan, Esaote, Italy). A T1 gradient echo scanning sequence was used with the following parameters: field of view = 200×200mm; matrix = 256×192 pixels; slice thickness = 10 mm, inter-slice gap = 1 mm; time to echo = 16 ms; time to repetition = 685 ms and flip angle = 90°. Serial axial plane images were obtained of the upper and lower leg, from which the cross-sectional areas of the individual muscles were analysed. The following subjects were excluded from MRI scanning: women who were or could be pregnant; subjects in whom ferromagnetic foreign bodies were detected; and those with cardiac pacemakers/cardioverter defibrillators, cochlear implants, intrauterine devices or implanted drug infusion pumps.



### **Muscle volume calculation**

We analysed serial cross sectional areas of the knee extensors (vastus medialis, vastus intermedius, vastus lateralis and rectus femoris), knee flexors (semi-membranosus, biceps femoris and semi-tendinosus), ankle plantar flexors (soleus, medial and lateral heads of the gastrocnemius muscle) and ankle dorsiflexors (tibialis anterior, extensor digitorum longus and extensor hallucis longus; these three dorsiflexors were measured as a group rather than their individual constituents because of difficulties in validly delineating each individual muscle along its entire length) using image analysis software (OsiriX, Pixmeo, Geneva, Switzerland), as detailed in Appendix S1.

### **Intramuscular non-contraction tissue**

The MRI signal intensity reflects the density of different tissues. Connective tissue yields low signal intensity values, while fat tissue produces very high signal intensity values, with the signal intensity value of skeletal muscle falling between these two. Details of the methodology used are provided in Appendix S1.

### **Statistical analysis**

We performed a power analysis before beginning the study (*a priori* power calculation) using the variable ankle joint strength (torque), based on the results of a previous study [12]. The power analysis indicated that we would need 14 subjects in each group to detect a difference of 22 Nm (~20% difference between groups) between the groups with an  $\alpha$  level of 0.05 and a  $\beta$  level of 0.9 (i.e. power of 90%). To account for participant dropout and potentially unusable data in some subjects, we chose to recruit 20 subjects in each group. IBM SPSS v.

19.0 (Chicago, IL, USA) for Windows was used to compute the results. All the data were expressed as mean  $\pm$  SD values, and analysis included descriptive and frequency statistics. All data were normally distributed and independent sample *t*-tests were used to evaluate between-group differences. The association between variables was assessed using the Pearson correlation coefficient, and Pearson's chi-squared test of independence was used to evaluate the association between categorical variables. For all the comparisons, a *P* value < 0.05 was taken to indicate statistical significance.

## **Results**

### **Clinical and metabolic assessment**

A total of 20 control subjects (13 men and seven women) and 20 subjects with IGT (16 men and four women; eight were on simvastatin 40 mg daily) were assessed. Age and height were matched between groups, but weight (*P*=0.002) and BMI (*P*=0.008) were significantly higher in subjects with IGT than in control subjects. HbA<sub>1c</sub> level (*P*=0.006) was significantly higher and cholesterol (*P*=0.04) and LDL (*P*=0.002) were lower in subjects with IGT compared with the control subjects (Table 1).

### **Neuropathy assessment**

We found that NDS (*P*=0.01), VPT (*P*=0.001) and warm threshold (*P*=0.01) were higher and peroneal motor nerve conduction velocity (*P*=0.05), peroneal motor nerve amplitude (*P*=0.01), corneal nerve fibre density (*P*=0.001), corneal nerve branch density (*P*=0.001) and corneal nerve fibre length (*P*=0.007) were significantly lower in subjects with IGT compared with the control group (Table 1).

## Muscle strength and volume

Knee extensor muscle strength ( $P=0.20$ ) and knee extensor ( $P=0.80$ ) and flexor ( $P=0.97$ ) volumes did not differ between the IGT and the control group (Table 2). Ankle plantar flexor muscle strength ( $P=0.04$ ) was significantly lower in the IGT group compared with the control group (Table 2 and Fig. 1). There was no difference in ankle plantar flexor ( $P=0.62$ ) and dorsiflexor ( $P=0.70$ ) muscle volume between the groups (Table 2).

## Intramuscular non-contractile tissue

Figure 3 shows the cross-sectional MRI images at the mid thigh and mid-tibial levels, illustrating the increase in IMNCT in subjects with IGT. There was no significant difference in IMNCT in the knee extensor and flexor muscles between groups (Table 2). IMNCT was significantly higher in the lateral gastrocnemius ( $P=0.03$ ) and ankle dorsiflexors ( $P=0.03$ ) in the IGT group than in the control group (Table 2 and Fig. 2).

## Relationship to neuropathy

When the subjects in the IGT group were categorized into those with plantar flexor muscle strength  $<2$  vs  $>2$  SD from those in the control group, there were no significant differences in any measure of neuropathy. There was a significant correlation between ankle plantar flexor muscle strength and corneal nerve fibre density ( $r=0.53$ ;  $P=0.01$ ) among subjects with IGT. There was no significant correlation between any other measure of neuropathy and ankle plantar and dorsiflexor and knee extensor and flexor muscle volume; however, knee extensor and flexor muscle volume correlated significantly with sensory nerve conduction velocity ( $r=-0.49$ ;  $P=0.03$ ;  $r=0.465$ ;  $P=0.04$ ) and sural sensory nerve amplitude ( $r=-0.54$ ;  $P=0.01$ ,  $r=-$

0.52;  $P=0.02$ ), respectively. There was a significant correlation between IMNCT in the soleus muscle and intraepidermal nerve fibre density ( $r= 0.51$ ;  $P=0.03$ ), IMNCT in the dorsiflexors with NDS ( $r= 0.65$ ;  $P= 0.003$ ), VPT ( $r= 0.69$ ;  $P= 0.001$ ) and warm threshold ( $r= 0.48$ ;  $P=0.04$ ) and IMNCT in the knee extensors with intraepidermal nerve fibre density ( $r=-0.49$ ;  $P=0.04$ ) and VPT ( $r=0.49$ ;  $P=0.03$ ). There was no significant correlation between the different measures of neuropathy and vitamin D.

### **Vitamin D**

There was no significant difference in knee extensor muscle strength ( $1.6 \pm 0.4$  Nm/kg vs  $1.7 \pm 0.5$  Nm/kg;  $P=0.80$ ) or knee extensor ( $1153 \pm 199$  cm<sup>3</sup> vs  $1167 \pm 546$  cm<sup>3</sup>;  $P= 0.93$ ) and flexor ( $643 \pm 161$  cm<sup>3</sup> vs  $678 \pm 319$  cm<sup>3</sup>;  $P=0.76$ ) muscle volume in subjects with IGT with 25(OH)D levels  $<25$  nmol/l ( $n=4$ ) compared with those with levels  $>25$  nmol/l ( $n=16$ ). Ankle plantar flexor strength was significantly lower, however, in subjects with IGT with 25(OH)D levels  $<25$  nmol/l than in those with 25(OH)D levels  $>25$  nmol/l ( $0.6 \pm 0.1$  Nm/kg vs  $0.9 \pm 0.2$  Nm/kg, respectively;  $P=0.02$ ). There was no significant difference between low compared with normal 25(OH)D groups in the ankle plantar flexor ( $683 \pm 226$  cm<sup>3</sup> vs  $717 \pm 340$  cm<sup>3</sup>;  $P=0.81$ ) or dorsiflexor ( $226 \pm 70$  cm<sup>3</sup> vs  $263 \pm 68$  cm<sup>3</sup>;  $P=0.3$ ) muscle volume.

### **Discussion**

We have shown that people with IGT have lower distal plantar flexor strength, but preserved proximal knee extensor muscle strength compared with an age-matched healthy control group. Despite the lower ankle plantar flexor strength in subjects with IGT, we found no difference in distal or proximal lower limb muscle volume between subjects with IGT and

control subjects. This is in contrast to the demonstration of quite marked distal muscle atrophy, particularly in patients with Type 2 diabetes and symptomatic neuropathy [12]. Weakness and atrophy of the distal muscles has been shown in several previous studies and is related to the severity of neuropathy in patients with Type 2 diabetes [1,2,13–15]. In the present study, we found evidence of an early reduction in distal muscle strength and early neuropathy in subjects with IGT. The latter finding is in keeping with several previous studies showing neuropathy in people with IGT [16–18]. Indeed, we have also shown significant small fibre neuropathy in people with IGT [19], particularly in those who later develop Type 2 diabetes [20]. This early small fibre neuropathy appears to be reversible, as supervised exercise has been shown to improve intraepidermal nerve fibre density in both patients without diabetes with metabolic syndrome [21] and in patients with diabetes without neuropathy [22]. Previous studies in patients with Type 2 diabetes have shown a significant relationship between both proximal and distal muscle strength and the severity of neuropathy [23]. In the present study, ankle plantar flexor strength did correlate with corneal nerve fibre density, a measure of small fibre neuropathy. Despite the fact that there was no significant reduction in knee extensor and flexor volumes, both correlated with sural nerve conduction velocity and amplitude, suggesting a relationship to the severity of distal neuropathy.

In addition to atrophy, MRI can show an alteration in signal intensity indicating fibrous and fatty tissue. In the present study, we observed an overall higher signal intensity value in the distal lower limb muscles in subjects with IGT compared with control subjects. The overall signal intensity is derived from a spectrum between low MRI signal intensity indicating connective tissue and high MRI signal intensity indicating fat; therefore, this indicates increased distal intramuscular fat in subjects with IGT. Increased intramuscular fat has been previously associated with obesity [24], which is consistent with the findings of the present study, as the IGT group had a significantly greater BMI. Increased intramuscular fat has also

been associated with increased insulin resistance, which is present in people with IGT [25]. The accumulation of intramuscular fat can alter glucose consumption and fat oxidation in obese people with IGT [24] and may affect motor function and strength [6,26]. In a recent study, the accumulation of intramuscular lipids has been associated with a significant reduction in the maximum force production in distal muscles of the mouse lower limb as a result of impaired  $\text{Ca}^{2+}$  release and force production [26]. Increased IMNCT also correlates with a range of measures of neuropathy, including intraepidermal nerve fibre density, suggesting a link with neuropathy rather than its occurrence as a consequence of muscle atrophy.

Low levels of vitamin D are associated with a decrease in muscle strength [27], and vitamin D supplementation has been shown to improve muscle strength and gait, with a reduction in falls [28]. Severe vitamin D deficiency can lead to a reduction in proximal muscle strength and size [8] as well as increased IMNCT [7,29]. Several randomized studies have recently shown significant improvements in both muscle volume and strength after treatment with vitamin D [30]. In the present study, subjects with IGT and vitamin D deficiency had preserved proximal muscle strength and volume but a reduction in plantar flexor muscle strength. This does not appear to be mediated via muscle atrophy or neuropathy, as there was no difference in muscle volume in those with low and normal vitamin D and there was no relationship between vitamin D levels and the severity of neuropathy.

This is the first detailed quantitative study to examine the relationship between lower limb muscle strength and structure in relation to neuropathy and vitamin D deficiency in participants with IGT. Although the study was adequately powered to detect a difference in the variables assessed, potential confounders, such as differences in gender, ethnicity and BMI between subjects with IGT and control subjects may well influence the outcomes. The

main finding was a reduction in distal plantar flexor strength with increased distal intramuscular fat, which is related to neuropathy in participants with IGT. These data suggest that distal motor weakness may be an early feature in people with IGT and may be associated with small fibre neuropathy before the development of Type 2 diabetes. Whilst all subjects with IGT had insufficient levels of vitamin D, those with deficiency showed a further reduction in distal flexor muscle strength. This merits further study to explore the benefits of vitamin D replacement on distal muscle strength in people with IGT.

In conclusion, people with IGT had a distal reduction in muscle strength, which was associated with elevated intramuscular fat levels and vitamin D deficiency.

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#### **Competing interests**

None declared.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Supplementary methods.

**Table 1** Clinical and demographic characteristics of the participants

Variable	Control	IGT	<i>P</i>
Subjects, <i>n</i> (men/women)	20 (13/7)	20 (16/4)	0.28
Age, years	61.5 ± 6.0	62.7 ± 11.1	0.67
Height, m	1.7 ± 0.9	1.7 ± 0.07	0.15
Weight, kg	78.1 ± 11.5	94.9 ± 18.7	<b>0.002</b>
BMI, kg/m <sup>2</sup>	27.2 ± 3.9	31.5 ± 5.5	<b>0.008</b>
Ethnicity: Asian/European, <i>n</i>	9/11	5/15	0.21
25(OH)D, nmol/l	78.9 ± 48.8	50.8 ± 34.8	<b>0.04</b>
25(OH)D/BMI	2.9 ± 1.9	1.6 ± 1.2	<b>0.01</b>
25(OH)D/ body surface area	1.9 ± 0.17	2.1 ± 0.23	<b>0.002</b>
HbA <sub>1c</sub> , mmol/mol	38.0 ± 2.1	42.4 ± 6.3	<b>0.007</b>
HbA <sub>1c</sub> , %	5.6 ± 0.1	6.0 ± 0.57	<b>0.006</b>
Cholesterol, mmol/l	5.5 ± 0.8	4.7 ± 1.4	<b>0.04</b>
HDL, mmol/l	2.1 ± 3.0	1.2 ± 0.4	0.18
LDL, mmol/l	3.2 ± 0.7	2.1 ± 1.2	<b>0.002</b>
Triglycerides, mmol/l	1.7 ± 0.69	2.2 ± 1.3	0.22
NDS (0–10)	1.1 ± 1.2	3.3 ± 3.4	<b>0.01</b>
VPT, Hz	6.4 ± 3.1	16.9 ± 11.7	<b>0.001</b>

Cold threshold, °C	27.5 ± 2.0	24.9 ± 5.8	0.06
Warm threshold, °C	38.6 ± 2.7	41.2 ± 3.7	<b>0.01</b>
Sensory nerve conduction velocity, m/s	48.6 ± 4.5	45.8 ± 13.6	0.38
Sural sensory nerve amplitude, µV	13.7 ± 7.2	14.6 ± 14.8	0.82
Peroneal motor nerve conduction velocity, m/s	46.6 ± 4.7	41.4 ± 10.7	<b>0.05</b>
Peroneal motor nerve amplitude, mV	5.3 ± 1.8	3.8 ± 1.8	<b>0.01</b>
Corneal nerve fibre density, number/mm <sup>2</sup>	35.9 ± 5.1	27.6 ± 8.2	<b>0.001</b>
Corneal nerve branch density, number/mm <sup>2</sup>	94.9 ± 33.6	55.7 ± 35.8	<b>0.001</b>
Corneal nerve fibre length, mm/mm <sup>2</sup>	26.7 ± 3.7	21.8 ± 6.5	<b>0.007</b>
Corneal nerve fibre tortuosity, TC	16.4 ± 2.7	18.6 ± 6.5	0.16
Intraepidermal nerve fibre density, number/mm	7.7 ± 2.0	6.7 ± 3.4	0.28

25(OH)D; 25-hydroxyvitamin D; IGT, impaired glucose tolerance; NDS, Neuropathy Disability Score; VPT, vibration perception threshold.

Values are mean ± SD, unless otherwise indicated.

**Table 2** Muscle volume (cm<sup>3</sup>) with percentage difference and statistical differences between subjects with impaired glucose tolerance and control subjects

Variables	Control	IGT	<i>P</i>	% difference
Vastus medialis	342 ± 99	407 ± 119	0.08	+18
Vastus intermedius	342 ± 106	400 ± 95	0.60	+5
Vastus lateralis	369 ± 107	402 ± 97	0.31	+9
Rectus femoris	149 ± 48	122 ± 36	0.06	-17
<b>Knee extensors</b>	1202 ± 323	1164 ± 492	0.77	-3
Semi-membranosus	228 ± 58	268 ± 88	0.11	+17
Biceps femoris	288 ± 77	310 ± 84	0.41	+7
Semi-tendinosus	152 ± 51	167 ± 60	0.43	+9
<b>Knee flexors</b>	669 ± 173	671 ± 291	0.97	+0.3
Soleus	419 ± 115	485 ± 116	0.08	+15

Medial gastrocnemius	185 ± 53	198 ± 72	0.52	+7
Lateral gastrocnemius	106 ± 36	106 ± 46	0.98	0
<b>Ankle plantar flexors</b>	710 ± 187	747 ± 275	0.62	+5
<b>Ankle dorsiflexors</b>	218 ± 50	255 ± 68	0.06	+16

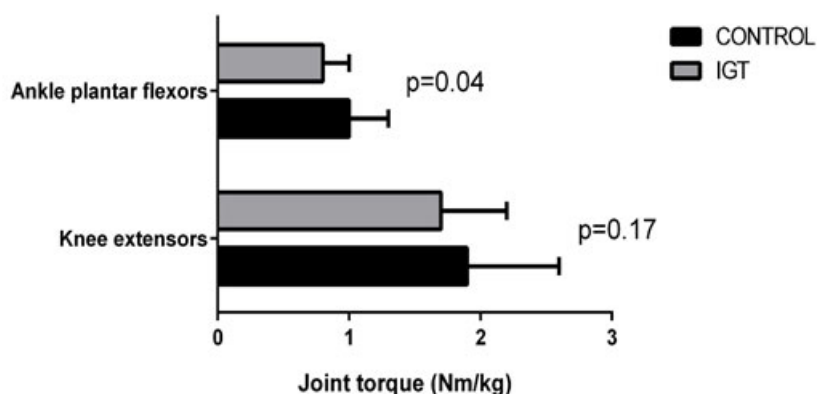
IGT, impaired glucose tolerance.

**FIGURE 1** Muscle strength of knee extensors and ankle plantar flexors, in control subjects and subjects with impaired glucose tolerance (IGT).

**FIGURE 2** Magnetic resonance imaging (MRI) signal intensities of intramuscular non-contractile tissue (IMNCT) in knee extensors and flexors and ankle plantar and dorsiflexor muscles in control subjects and subjects with impaired glucose tolerance (IGT).

**FIGURE 3** Representative lower limb magnetic resonance imaging (MRI) images from a healthy 54-year-old control (a) and (c) and a 69-year-old participant with impaired glucose tolerance [IGT (b) and (d)]. Images are from the mid-thigh level (a) and (b) and mid-tibia level (c) and (d). Note substantial increase in intramuscular non-contractile tissue (dark areas inside the muscle cross-sections are connective tissue) in images from participant with IGT. Note also thick subcutaneous fat layer in participant with IGT, especially in (b). VM, vastus medialis; VI, vastus intermedius; VL, vastus lateralis; RF, rectus femoris; BF, biceps femoris; ST, semitendinosus; SM, semimembranosus; DF, Dorsiflexors; SOL, soleus; LG, lateral gastrocnemius; MG, medial gastrocnemius. Scale-bar along the bottom or the left side of each image = 10 cm.

## Muscle strength of knee extensors and ankle plantar flexors in controls and IGT



## IMNCT in muscles of lower extremities in controls and IGT

